Supplementary Material

Design, Synthesis, Pharmacological Evaluation, QSAR Analysis, Molecular Modeling and ADMET of Novel Donepezil-Indolyl Hybrids as Multipotent Cholinesterase/Monoamine Oxidase Inhibitors for the Potential Treatment of Alzheimer’s Disease

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Table 1S. Results of the 3D-QSAR(MAO A) modeling [22-24]

<table>
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<tr>
<th>Training Set</th>
<th>pIC$_{50}$ (MAO A)</th>
<th>Pred pIC$_{50}$ (MAO A)</th>
<th>3D-QSAR(MAO A) parameters</th>
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Figure 1S. The variables such as: v136: TIP-TIP, v162: TIP-TIP, v173: TIP-TIP, v271: DRY-TIP, v293: DRY-TIP, and v365: O-TIP are positively correlated with the MAO A inhibiting activity, while variables v154: TIP-TIP, v317: O-N1, v400: N1-TIP, and v405: N1-TIP, are negatively correlated with the MAO A inhibiting activity (Figure 1S).

HBD properties of amino group creates crucial favorable interactions (v365: O-TIP) with butynyl group (FA-66, Figure 2aS), indol moiety (PF9601N, Figure 2aS), and benzyl group (Donz-D5/Donz-D7, Figure 2bS). The propargylamino moiety creates few favorable interactions (v136: TIP-TIP, v162: TIP-TIP, v173: TIP-TIP) with benzyloxy group (FA-66/PF9601N, Figure 2aS), and para/meta positions of the benzyl group (Donz-D5/Donz-D7, Figure 2bS). The hydrophobic part of propargylamino moiety creates one very specific favorable interactions (v293: DRY-TIP) with para position of the benzyl group (Donz-D5, Figure 2bS). The 3D-QSAR study confirmed previous experimental findings [15-17] that propargylamino moiety has essential positive influence on the MAO A inhibiting activity. Substitution at para/meta positions of the
benzyl moiety (Donz-D5/Donz-D7, Figure 2bS) with bulky groups could enhance MAO A inhibiting activity of the examined compounds by facilitating the favorable interactions (v293: DRY-TIP, v136: TIP-TIP, v162: TIP-TIP, v173: TIP-TIP).

Finally, HBA O-bridge forms very specific unfavorable interaction v317: O-N1 with HBD-amino group of propargylamino moiety (Figure 2aS and 2bS). Furthermore, the O-bridge forms unfavorable interactions (v400: N1-TIP and v405: N1-TIP) with benzyl/piperidinyl moiety (Figure 2aS and 2bS). Therefore, substitution of the oxygen in the bridge with other atoms could enhance MAO A inhibiting activity.

The MBA group of compounds doesn’t have propargylamino moiety to form essential pharmacophores (v365: O-TIP, 136: TIP-TIP, v162: TIP-TIP, v173: TIP-TIP) for MAO A inhibiting activity (Figure 2cS).

**Figure 2S.** (a) 3D-map of interaction energies of FA-66 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favorable/unfavorable interactions of the MAO A inhibiting activity are marked in squares.
Figure 2S. (a) 3D-map of interaction energies of PF9601N with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favorable/unfavorable interactions of the MAO A inhibiting activity are marked in squares.

Figure 2S. (b) 3D-map of interaction energies of Donz-D5 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favorable/unfavorable interactions of the MAO A inhibiting activity are marked in squares.
Figure 2S. (b) 3D-map of interaction energies of Donz-D7 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favorable/unfavorable interactions of the MAO A inhibiting activity are marked in squares.
Figure 2S. (c) 3D-map of interaction energies of 11 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the MAO A inhibiting activity are marked in squares.
**Figure 2S.** (c) 3D-map of interaction energies of 6 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the MAO A inhibiting activity are marked in squares.

Predictive potential of the developed 3D-QSAR (MAO A) model was tested by use of leave-one-out cross validation of the training set ($Q^2$: 0.66, $R^2_{\text{Observed vs. Predicted}}$: 0.908, and RMSEE: 0.433) and verification set ($R^2_{\text{Observed vs. Predicted}}$: 0.809 and RMSEP: 0.617) (Table 1S). The obtained statistical parameters indicated that the created 3D-QSAR (MAO A) model has good prognostic capacity for MAO A inhibiting activity.
Table 2S. Results of the 3D-QSAR(MAO B) modeling [22-24].

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The optimal 3D-QSAR (MAO B) model with two significant components (A=2), R$^2$: 0.97, and Q$^2$: 0.85, was formed (Table 2S). Coefficient plot with the most significant variables for the MAO B inhibiting activity, such as: v136: TIP-TIP, v150: TIP-TIP, v181: DRY-O, v275: O-TIP, v356: O-TIP, v361: O-TIP, v48: O-O, v96: N1-N1, v167: TIP-TIP and v399: N1-TIP is depicted on Figure 3S. The variables such as: v136: TIP-TIP, v150: TIP-TIP, v181: DRY-O, v275: DRY-TIP, v356: O-TIP and v361: O-TIP, are positively correlated with the MAO B inhibiting activity, while variables v48: O-O, v96: N1-N1, v167: TIP-TIP, and v399: N1-TIP, are negatively correlated with the MAO B inhibiting activity (Figure 3S).
Figure 3S. The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (MAO B) model. The specific favourable/unfavourable interactions of the MAO B inhibiting activity are marked in squares.

HBD properties of amino group creates crucial favorable interactions (\(v356: \text{O-TIP}, v361: \text{O-TIP}\)) with unsaturated back chain (PF9601N/FA-97, Donz-D3/Donz-D5, Figure 4aS/4bS). The propargylamino group creates favorable interaction (\(v275: \text{DRY-TIP}\)) with indol moiety (PF9601N/FA-97, Figure 4aS). Thus substitution with methyl or ethyl group in the benzen ring of the indol moiety could increase MAO B activity of the FA-derivatives. The hydrophobic part of propargylamino moiety creates one very specific favorable interactions (\(v181: \text{DRY-O}\)) with N-CH\(_3\)-group of the indol moiety (Donz-D5, Figure 4bS). The 3D-QSAR study confirmed previous experimental findings [15-17] that propargylamino moiety has essential positive influence on the MAO B inhibiting activity.

HBA O-bridge forms unfavorable interactions (\(v399: \text{N1-TIP}, v48: \text{N1-N1}\)) with benzyl moiety (PF9601N/FA-97, Donz-D3, Figure 4aS/4bS). Therefore, substitution of the oxygen in the bridge with other atoms could enhance MAO B inhibiting activity of the examined compounds.
The propargylamino moiety creates unfavorable interaction (v167: TIP-TIP) with \textit{para/meta} positions of the benzyl group (Donz-D3/Donz-D5, 8/11, Figure 4bS/4cS). This unfavorable TIP-TIP interaction indicated that bulky groups of a ligand have negative influence on inhibition of MAO B enzyme.

The new amines and amides don’t have propargylamino moiety to form essential pharmacophores (v181: DRY-O, v275: DRY-TIP, v356: O-TIP and v361: O-TIP) for MAO B inhibiting activity (Figure 4cS).

\textbf{Figure 4S.} (a) 3D-map of interaction energies of PF9601N with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the MAO B inhibiting activity are marked in squares.
Figure 4S. (a) 3D-map of interaction energies of FA-97 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the MAO B inhibiting activity are marked in squares.
Figure 4S. (b) 3D-map of interaction energies of Donz-D3 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the MAO B inhibiting activity are marked in squares.
Figure 4S. (b) 3D-map of interaction energies of Donz-D5 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the MAO B inhibiting activity are marked in squares.
Predictive capacity of the developed 3D-QSAR (MAO B) model was tested by use of leave-one-out cross validation of the training set ($Q^2$: 0.85, $R^2_{\text{observed vs. predicted}}$: 0.957, and RMSEE: 0.303) and verification set ($R^2_{\text{observed vs. predicted}}$: 0.787 and RMSEP: 0.598) (Table 2S). The presented statistical parameters indicated on good prognostic potential of the formed 3D-QSAR (MAO B) model.

The optimal 3D-QSAR (AChE) model with three significant components ($A=3$), $R^2$: 0.87, and $Q^2$: 0.61, was created. Coefficient plot with the most significant variables for the AChE inhibiting activity, such as: $v_{52}$: O-O, $v_{96}$: N1-N1, $v_{146}$: TIP-TIP, $v_{283}$: DRY-TIP, $v_{324}$: O-N1, $v_{434}$: N1-TIP, $v_{33}$: DRY-DRY, $v_{62}$: O-O, $v_{223}$: DRY-N, and $v_{415}$: N1-TIP is depicted on Figure 5S. The variables such as: $v_{52}$: O-O, $v_{96}$: N1-N1, $v_{146}$: TIP-TIP, $v_{283}$: DRY-TIP, $v_{324}$: O-N1, and $v_{434}$: N1-TIP are positively correlated with the AChE
inhibiting activity, while variables $v_{33}$: DRY-DRY, $v_{62}$: O-O, $v_{223}$: DRY-N, and $v_{415}$: N1-TIP, are negatively correlated with the AChE inhibiting activity (Figure 5S).

**Figure 5S.** The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (AChE) model. The specific favourable/unfavourable interactions of the AChE inhibiting activity are marked in squares.

HBD properties of N-atom in the piperidine ring and *ortho* positions of the benzyl group create crucial favorable interactions ($v_{52}$: O-O) for AChE inhibition (Donepezil and Donz-D3, Figure 6a/6bS). Thus an electron withdrawing substituent in the benzyl and piridinyl moiety could enhance AChE inhibiting activity of donepezil derivatives.

HBA feature of methoxy substituent of the benzene ring in donepezil structure creates crucial favorable interactions with the other methoxy group ($v_{96}$: N1-N1) and also with N-atom of the piperidine ring ($v_{324}$: O-N1) (Donepezil, Figure 6aS). Therefore, new electron donating substituents in benzene ring could enhance AChE inhibiting activity of donepezil derivatives.

Opposite of MAO activity, O-bridge forms very specific favorable interaction ($v_{96}$: N1-N1) with benzyl moiety and with propagylamino group ($v_{324}$: N1-O) (Donz-D3, Figure 6bS).
Figure 6S. (a) 3D-map of interaction energies of donepezil with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the AChE inhibiting activity are marked in squares.
Figure 6S. (b) 3D-map of interaction energies of Donz-D3 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the AChE inhibiting activity are marked in squares.

Predictive potential of the developed 3D-QSAR (AChE) model was tested by use of leave-one-out cross validation of the training set ($Q^2$: 0.61, $R^2_{\text{Observed vs. Predicted}}$: 0.873, and RMSEE: 0.431) and verification set ($R^2_{\text{Observed vs. Predicted}}$: 0.539 and RMSEP: 0.661). The obtained statistical parameters indicated that the created 3D-QSAR (AChE) model has good prognostic capacity for AChE inhibiting activity.

The optimal 3D-QSAR (BuChE) model with two significant components ($A=2$), $R^2$: 0.92, and $Q^2$: 0.72, was created. Coefficient plot with the most significant variables for the BuChE inhibiting activity, such as: v173: TIP-TIP, v448: N1-TIP, v21: DRY-DRY, v165: TIP-TIP, V204: DRY-O, V246/v251: DRY-N, V304: DRY-TIP, and V438/v425: N1-TIP is depicted on Figure 7S. The variables v173: TIP-TIP, v448: N1-TIP are positively correlated with the BuChE inhibiting activity, while variables v21: DRY-DRY, v165: TIP-TIP, V204: DRY-O, V246/v251:
DRY-N, V304: DRY-TIP, and V438/v425: N1-TIP are negatively correlated with the BuChE inhibiting activity (Figure 7S).

Figure 7S. The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (BuChE) model. The specific favourable/unfavourable interactions of the AChE inhibiting activity are marked in squares.

Steric and hydrophobic groups of the indol (N-CH₃) and benzyl moieties form two very specific unfavorable interactions (v165: TIP-TIP, v304: DRY-TIP) (Donz-D5, Figure 8S). Therefore, substitution with bulky substituent at para position of the benzyl moiety could decrease BuChE inhibiting activity of Donz-D derivatives, while N-demethylation of the indol group could enhance BuChE inhibiting activity of Donz-D derivatives.
HBA O-bridge forms one favorable interaction (v448: N1-TIP) with benzyl moiety (Donz-D5, Figure 8S), and two unfavorable interactions with indol moiety (v251: DRY-N1) and with benzyl group (v425: N1-TIP).

Figure 8S. 3D-map of interaction energies of Donz-D5 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). The specific favourable/unfavourable interactions of the BuChE inhibiting activity are marked in squares.

Predictive potential of the developed 3D-QSAR (BuChE) model was tested by use of leave-one-out cross validation of the training set ($Q^2$: 0.72, $R^2_{\text{Observed vs. Predicted}}$: 0.922, and RMSEE: 0.261) and verification set ($R^2_{\text{Observed vs. Predicted}}$: 0.657 and RMSEP: 0.521). The obtained statistical parameters indicated that the created 3D-QSAR (BuChE) model has good prognostic capacity for BuChE inhibiting activity.
Experimental Part. Methods

The inhibiting MAO A/MAO B and AChE/BuChE activities (IC\textsubscript{50}) of 30 indole derivatives were used for the QSAR study [22-24]. Negative logarithm of their IC\textsubscript{50} i.e. (pIC\textsubscript{50}) values were calculated.


The pKa calculation and selection of dominant molecules/cations at physiological pH 7.4 was performed for the examined compounds using the MarvinSketch 5.5.1.0 program [ChemAxon MarvinSketch 5.5.1.0 program, Budapest, Hungary (2011) \url{www.chemaxon.com/products.html}]. Dominant forms at pH 7.4, were used for the 3D-QSAR study.

The 3D-QSAR studies of the indole derivatives were performed by use of the Pentacle 1.0.6 program [Pentacle, Version 1.0.6.; Molecular Discovery Ltd, Perugia, Italy (2009) \url{http://www.moldiscovery.com/soft_pentacle.php}].

The Pentacle is advanced software tool for obtaining alignment-independent 3D quantitative structure-activity relationships. The 3D-QSAR starts from computing highly relevant 3D maps of interaction energies (GRID based Molecular Interaction Fields-MIFs) between the examined molecule and four chemical probes: DRY (which represent hydrophobic interactions), O (sp\textsuperscript{2} carbonyl oxygen, representing H-bond acceptor), N1 (neutral flat NH, like in amide, H-bond
donor), and the TIP probe (molecular shape descriptor). The grid spacing was set to 0.5 Å and the MACC2 smoothing window to 1.6 (for 3D-QSAR (ChE) models) and the CLACC smoothing window to 1.6 (for 3D-QSAR (MAO) models). The number of filtered nodes was set to 100 with 50% relative weights within the ALMOND discretization.

The interaction energy between the probe and the target molecule is calculated at each point as the sum of Lennard-Jones (\(E_{lj}\)), hydrogen bond (\(E_{hb}\)), electrostatic interactions (\(E_{el}\)), and an entropic term:

\[ E_{int} = \sum E_{lj} + \sum E_{hb} + \sum E_{el} + S \]


The obtained maps are encoded into GRID Independent Descriptors (GRIND and GRIND2 descriptors) which are independent of the alignment of the series [M. Pastor, G. Cruciani, I. McLay, S. Pickett, S. Clementi, J. Med. Chem. 43 (2000) 3233-3243]. The GRIND approach aims to extract the information enclosed in the MIFs and compress it into new types of variables whose values are independent of the spatial position of the molecule studied by using an optimization algorithm with the intensity of the field at a node and the mutual node–node distances between the chosen nodes as a scoring function. Such variables constitute a matrix of descriptors that are analyzed using multivariate techniques, such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression analysis. The Principal Component Analysis was used for inspection of our series and for obtaining a map of our compounds describing their similarities and differences. The variables were used for development of 3D-QSAR models by use of the PLS regression [L. Eriksson, E. Johansson, N. Kettaneh-Wold, J. Trygg, C. Wikstrom, S. Wold, (Eds.) Multi-and Megavariate Data Analysis. Basic Principles and Applications I, 2nd ed, Umetrics Academy, Umeå, 2001.].

Based on the Score Plots (t1 vs. t2 and t1 vs. u1) the data set of 30 MAO A/B inhibitors and data set of 35 AChE/BuChE inhibitors is divided on Training Set (23-29 compounds for QSAR models building) and Verification set (6-9 compounds for QSAR models validation) [A. Tropsha, Mol. Inf. 29 (2010) 476–488]. The most important pharmacophores (GRID descriptors),
responsible for the MAO A, MAO B, AChE, and BuChE inhibition, were selected by use of the PLS regression and used for the 3D-QSAR (MAO A, MAO B, AChE, BuChE) models building (Pentacle 1.0.6 program). External validation of the formed 3D-QSAR (MAO A, MAO B, AChE, BuChE) models was performed by use of Test set (Table 1S).

Quality of the obtained 3D-QSAR (I$_1$-IR) models (3D-QSAR (I$_1$-IR agonists) model and 3D-QSAR (I$_1$-IR antagonists) model) was examined by use of: leave-one-out cross-validation ($Q^2$), correlation coefficient ($R^2$ Observed vs. Predicted), Root Main Squared Error of Estimation (RMSEE), and external validation (Root Main Squared Error of Prediction (RMSEP)) [S. Wold, E. Johansson, M. Cocchi, 3D-QSAR in drug design, theory, methods, and applications. H. Kubinyi Ed., ESCOM Science Publishers: Leiden, 1993, pp 523–550, A. Tropsha, Mol. Inf. 29 (2010) 476–488].

Predictive power of the model is determined by $Q^2$, which is leave-one-out cross-validated version of $R^2$. A model is fitted to the data leaving one compound out, selects the best variables, and predicts Y for the left–out compound. This procedure is repeated until all compounds have been left out, which result in $n$ parallel models. The difference between observed and the predicted Y values are calculated ($e_{(i)}$) for each model. In this setting were defined PRESS (Predicted Sum of Squares), RMSEP and $Q^2$ as:

$$PRESS = \sum_{i=1}^{n} e_{(i)}^2$$ (1)

$$RMSEP = \sqrt{\frac{PRESS}{n}}$$ (2)

$$Q^2 = 1 - \frac{PRESS}{SSTo}$$ (3)

$SSTo$ - Variation, Sum of Squares (Total)

PLS models with $Q^2 \geq 0.5$ can be considered to have good predictive capability [D.M. Allen, Technometrics, 16 (1974) 125-127, S. Wold, E. Johansson, M. Cocchi, 3D-QSAR in drug design, theory, methods, and applications. H. Kubinyi Ed., ESCOM Science Publishers: Leiden, 1993, pp 523–550].
References


### Table 3S. Calculated physicochemical and ADMET properties for structures 2-16.\textsuperscript{a,b}

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\textsuperscript{a} AMET Predictor, v.6.5. \textsuperscript{b} ACD/Percepta 14.0. \textsuperscript{c} Moriguchi model (see Ref. [46]). \textsuperscript{d} According to the classification made by Ma (see [52]): High absorption to CNS: logBB more than 0.3; Middle absorption to CNS: logBB 0.3 ~ -1.0; Low absorption to CNS: logBB less than -1.0. \textsuperscript{e} Other estimated parameters related to brain penetration were used to classify the compounds as CNS permeable or non-permeable: rate of brain penetration (LogPS) is the rate of passive diffusion/permeability; brain/plasma equilibration rate (Log(PS*fu, brain)); fu,brain – fraction unbound in plasma. / Human intestinal absorption is the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile, and feces. A value between 0 and 20% indicates poor absorption, 20–70% shows moderate absorption, and 70–100% indicates good absorption. g Caco-2 cells are derived from human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. A value <4 indicates low permeability, 4–70 shows middle permeability, and >70 indicates high permeability. h The MDCK cell system may be used as a good tool for rapid permeability screening. A value <25 indicates low permeability, 25–500 shows middle permeability, and >500 indicates high permeability. i The percent of drug binds to plasma protein. A value <90% indicates weak binding, and >90% indicates strong binding to plasma proteins.
NMR spectra for compounds 3-16

4.1.3. *N*-Benzyl-5-methoxy-1-methyl-1*H*-indole-2-carboxamide (3).

\[
\text{MeO} \quad \text{O} \\
\text{N} \quad \text{H} \\
\text{Me} \quad \text{Ph}
\]

\(^1\text{H} \text{ NMR (500 MHz, CDCl}_3\text{)} \text{ Spectrum of compound 3.}\)

\[^{13}\text{C} \text{ NMR (126 MHz, CDCl}_3\text{)} \text{ Spectrum of compound 3.}\]
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 3.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 3.
$^1$H-$^1$C g-HMBC (CDCl₃) spectrum of compound 3.

4.1.4. *N*-Benzyl-1-(5-methoxy-1-methyl-1H-indol-2-yl)methanamine (4).

$^1$H NMR (500 MHz, CDCl₃) Spectrum of compound 4.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 4

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 4.
1H-13C g-HSQC (CDCl₃) spectrum of compound 4.

1H-13C g-HMBC (CDCl₃) spectrum of compound 4.

4.1.5. Ethyl 5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indole-2-carboxylate (5).
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 5.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 5.
$^{1}H^{1}H$ g-COSY (500 MHz, CDCl$_3$) spectrum of compound 5.

$^{1}H^{13}C$ g-HSQC (CDCl$_3$) spectrum of compound 5.
1H-13C g-HMBC (CDCl₃) spectrum of compound 5.

4.1.6. 5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indole-2-carboxylic acid (6).
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 6.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 6.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 6.
1H-13C g-HSQC (CDCl₃) spectrum of compound 6.

1H-13C g-HMBC (CDCl₃) spectrum of compound 6.

4.1.9. N-Benzyl-5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indole-2-carboxamide (7).
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 7.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 7.
$^1$H-$^1$H g-COSY (500 MHz, CDCl₃) spectrum of compound 7.

$^1$H-$^{13}$C g-HSQC (CDCl₃) spectrum of compound 7.
$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 7.

4.1.10. 5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-N-phenyl-1H-indole-2-carboxamide (8).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 8.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 8.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 8.
$^1$H-$^{13}$C g- HSQC (CDCl$_3$) spectrum of compound 8.

$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 8.
4.1.11. (5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)(morpholino)methanone (9).

\[ \text{S43} \]

\[^1\text{H NMR (500 MHz, CDCl}_3\text{)} \text{ Spectrum of compound 9.} \]

\[^{13}\text{C NMR (126 MHz, CDCl}_3\text{)} \text{ Spectrum of compound 9.} \]
$^1$H-$^1$H-COSY (500 MHz, CDCl$_3$) spectrum of compound 9

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 9.
$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 9.

4.1.12. 5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-N-(prop-2-yn-1-yl)-1H-indole-2-carboxamide (10).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 10.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 9.

$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 10.
$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 10.

$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 10.
4.1.13. (5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methanol (11).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 11.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 11.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 11.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 11.
4.1.15. *N*-Benzyl-1-(5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1*H*-indol-2-yl)methanamine (12).

$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 11.

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 12.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 12.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 12.
$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 12.

$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 12.

4.1.16. $N$-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1$H$-indol-2-yl)methyl)aniline (13).
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 13.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 13.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 13.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 13.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 13.

4.1.17. 4-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)morpholine (14).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 14.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 14.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 14.
$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 14.

$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 14.

$N$-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1$H$-indol-2-yl)methyl)prop-2-yn-1-amine (15).
$^{1}$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 15.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 15.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 15.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 15.
$^1$H-$^1$C g-HMBC (CDCl$_3$) spectrum of compound 15.

$N$-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)prop-2-en-1-amine (16).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 16.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 16.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 16.
$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 16.

$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 16.
SUPPORTING INFORMATION

Synthesis, Biological Evaluation and Molecular Modeling of Donepezil +Propargylamine+8-Hydroxyquinoline Hybrids as New Multipotent Chelator, ChE and MAO Inhibitors for the Treatment of Alzheimer’s Disease

Li Wang#a, Gerard Esteban, #b Masaki Ojima,a Oscar M. Bautista-Aguilera,c Tsutomu Inokuchi,*,a Isabel Iriepa,d Mercedes Unzeta,b Abdelouahid Samadi,c Moussa B. H. Youdim,e and José Marco-Contelles*ec

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e Eve Topf Centers of Excellence for Neurodegenerative Diseases Research and Department of Pharmacology, Rappaport Family Research Institute, Technion-Faculty of Medicine, Haifa, 31096, Israel

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1. Chemistry. Experimental Part .............................................S2-S11
2. NMR and IR spectra............................................................S12-S32
3. Biochemistry.................................................................S33- S34
4. Molecular modeling..........................................................S35-S53
1. Chemistry. Experimental Part

5-(Chloromethyl) quinolin-8-ol (8). Synthesized as reported in Moret, V.; Laras, Y.; Cresteil, T.; Aubert, G.; Ping, D. Q.; Di, C.; Barthélémy-Requin, M.; Béclin, C.; Peyrot, V.; Allegro, D.; Rolland, A.; De Angelis, F.; Gatti, E.; Pierre, Ph.; Pasquini, L.; Petrucci, E.; Testa, U.; Kraus, J-L. *Eur. J. Med. Chem.* 2009, 44, 558-567. To a cooled solution of 8-hydroxyquinoline (14.6 g, 100 mmol) in conc. HCl (44 mL) at 0 °C, a 37% aqueous formaldehyde solution (20 mL) was added. Then HCl gas was bubbled through the solution with stirring for 2 h. The mixture was allowed to warm to rt with further stirring for 6 h and without stirring for 2 h more. The product was filtered and the solid was rinsed with conc. HCl, giving product 8 (19.9 g, 77.5%) as light yellow solid: $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 5.30 (s, 2H), 7.51 (d, $J$ = 8.0 Hz, 1H), 7.86 (d, $J$ = 8.0 Hz, 1H), 8.13 (dd, $J$ = 8.7, 5.2 Hz, 1H), 9.12 (dd, $J$ = 5.1, 1.3 Hz, 1H), 9.24 (dd, $J$ = 8.8, 1.3 Hz, 1H).

1-Benzyl-N-(prop-2-ynyl)aminopiperidine (9). To a solution of commercial 1-benzyl-4-piperidone (1.3 g, 7.0 mmol) and propargylamine (580 mg, 10.5 mmol) in MeOH (22 mL) at 0 °C, was added a small amount of CF₃CO₂H (5 drops). After being stirred for 1 h, NaBH₃CN (1.3 g, 19.7 mmol) was added to the solution. The mixture was stirred at 0 °C overnight and then quenched with aqueous saturated NaHCO₃. The mixture was concentrated in vacuo and extracted with AcOEt. The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by column chromatography (SiO₂, AcOEt/MeOH = 4:1 v/v) to give amine 9 (500 mg, 31.2%) as an orange oil: $R_f$ = 0.19 (AcOEt/MeOH = 4:1); $^1$H NMR (400 MHz, CDCl₃) $\delta$ 1.34–1.49 (m, 2H), 1.76–1.88 (m, 2H), 2.00–2.15 (m, 3H), 2.17–2.22 (t, $J$ = 2.3 Hz, 1H), 2.65–2.76 (m, 1H), 2.81–2.90 (m, 2H), 3.41–3.46 (d, $J$ = 2.3 Hz, 1H), 3.52 (s, 2H), 7.20–7.35 (m, 5H); $^{13}$C NMR (100 MHz, CDCl₃) $\delta$ 31.8, 34.9, 51.8, 62.7, 62.8, 71.1, 82.0,126.8, 128.0, 128.9, 138.0.

5-((1-Benzylpiperidin-4-yl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (1) To a solution of compound 9 (475 mg, 2.1mmol) and quinoline 8 (387 mg,1.5 mmol) in CH₂Cl₂ (12 mL), was added Et₃N (0.84 mL, 6.0 mmol) at rt. After being stirred overnight,
the mixture was quenched with aqueous saturated NaHCO₃. The product was extracted with CH₂Cl₂. The organic layer was dried over MgSO₄ and concentrated in vacuo. The product was purified by column chromatography (SiO₂, hexane/AcOE by increasing the gradient from 5:1 to 3:1 v/v). Further purification was achieved by recrystallization (hexane/AcOE= 1:1) to give compound 1 (196 mg, 33.8%) as a white solid: mp 145.5 °C; IR (KBr) ν 3289, 2958, 2914, 2805, 2754, 1582, 1510, 1422, 1360, 1281, 1229, 1186, 1152, 1107, 1069, 1001 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.60–1.76 (m, 2 H), 1.90–2.02 (m, 4 H), 2.17 (t, J= 2.3 Hz, 1H), 2.48–2.60 (m, 1H), 2.91 (d, J= 11.5 Hz, 2 H), 3.16 (d, J= 2.3 Hz, 2 H), 3.46 (s, 2H), 4.00 (s, 2H), 7.00 (d, J= 7.6 Hz, 1 H), 7.15–7.29 (m, 5H), 7.31–7.40 (m, 2H), 8.60 (dd, J= 8.5, 1.6 Hz, 1 H), 8.69 (dd, J= 4.3, 1.5 Hz, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 29.6, 38.0, 51.3, 53.0, 58.5, 63.0, 73.1, 80.0, 108.8, 121.4, 124.9, 127.1, 128.2, 129.2, 129.3, 133.9, 138.7, 147.5, 151.8. Anal. Calcd for C₂₅H₂₇N₃O: C, 77.89; H, 7.06; N 10.90. Found: C, 77.88; H, 7.10; N 10.89.

\[ N-[(1-benzylpiperidin-4-yl)methyl]prop-2-yn-1-aminen (10) and (1-benzylpiperidin-4-yl)(prop-2-yn-1-ylamino)acetonitrile (11). \]

To a cooled solution of 1-benzyl-4-piperidinecarboxaldehyde (Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *Bioorg. Med. Chem. Lett.* **1992**, 2, 871-6) (407 mg, 2.0 mmol) and propargylamine (165 mg, 3.0 mmol) in MeOH (6 mL) at 0 °C, a small amount of CF₃CO₂H (5 drops) was added. After being stirred for 1 h, NaBH₃CN (189 mg, 2.9 mmol) was added to the solution portionwise. The mixture was stirred at rt overnight and then quenched with aqueous saturated NaHCO₃. The mixture was concentrated in vacuo and then extracted with AcOEt. The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure to give a mixture that was purified by column chromatography (SiO₂, eluting solvent was changed from hexane/AcOEt= 5:1 to AcOEt and then AcOEt/MeOH= 4:1 v/v) to give compound 10 (232 mg, 50.2%), as an orange oil, and product 11 (165 mg, 30.9%) as an orange oil. *N*-[(1-benzylpiperidin-4-yl)methyl]prop-2-yn-1-amine (10): \( R_f = 0.23 \) (AcOEt/MeOH= 4:1); IR (film) ν 3287, 3028, 2918, 2801, 2758, 1495, 1454, 1366, 1342, 1121, 1078 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.15–1.55 (m, 3 H), 1.72 (d, J= 12.5 Hz, 2 H),
1.95–2.11 (m, 2 H), 2.20 (t, $J = 2.3$ Hz, 1 H), 2.57 (d, $J = 6.6$ Hz, 2 H), 2.94 (d, $J = 11.7$ Hz, 2 H), 3.33–3.65 (m, 4 H), 7.19–7.40 (m, 5 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 30.1, 35.8, 38.4, 53.4, 54.5, 63.1, 71.4, 82.2, 127.3, 128.3, 129.4, 137.3.

(1-Benzylpiperidin-4-yl)(prop-2-yn-1-ylamino) acetonitrile (11): $R_f$ = 0.78 (AcOEt/MeOH= 4:1); IR (film) $\nu$ 3296, 2940, 2920, 2803, 2760, 1732, 1452, 1368, 1246, 1223, 1072 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 1.37–1.72 (m, 4 H), 1.76–1.88 (m, 2 H), 1.92–2.02 (m, 2 H), 2.26–2.33 (m, 1 H), 2.87–2.98 (m, 2 H), 3.44–3.69 (m, 5 H), 7.17–7.44 (m, 5 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 28.4, 29.0, 36.8, 39.0, 53.0, 54.5, 63.0, 72.8, 80.1, 118.7, 127.1, 128.2, 129.1, 138.3.

5-((((1-Benzylpiperidin-4-yl)methyl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (3). To a stirring solution of compound 10 (638 mg, 2.6 mmol) and chloride 8 (694 mg, 2.7 mmol) in CH$_2$Cl$_2$ (12 mL), Et$_3$N (1.09 mL, 5.2 mmol) was added at rt. After being stirred overnight, the mixture was quenched with water and the product was extracted with CH$_2$Cl$_2$, dried over Mg$_2$SO$_4$, concentrated in vacuo, and purified by column chromatography (SiO$_2$, hexane/AcOEt by increasing the gradient from 5:1 to 1:1 v/v) to give amine 3 (572 mg, 55%) as a light brown solid: mp 128.5 $^o$C; IR (KBr) $\nu$ 3320, 3248, 2946, 2914, 2754, 2365, 1734, 1580, 1506, 1478, 1422, 1366, 1279, 1229, 1192, 1180, 1065 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 0.97–1.14 (m, 2 H), 1.32–1.48 (m, 1 H), 1.60 (d, $J =$ 12.5 Hz, 2 H), 1.84 (t, $J =$ 11.0 Hz, 2 H), 2.19 (s, 1 H), 2.36 (d, $J =$ 7.2 Hz, 2 H), 2.76 (d, $J =$ 11.5 Hz, 2 H), 3.15 (d, $J =$ 1.9 Hz, 2 H), 3.39 (s, 2 H), 3.83 (s, 2 H), 6.98 (d, $J =$ 7.8 Hz, 1 H), 7.12–7.37 (m, 7 H), 8.53 (dd, $J =$ 8.5, 1.4 Hz, 1 H), 8.68 (dd, $J =$ 4.2, 1.4 Hz, 1 H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 30.8, 33.5, 40.8, 53.6, 56.6, 59.1, 63.5, 73.4, 78.4, 108.7, 121.3, 124.9, 126.9, 127.8, 128.1, 129.1, 129.2, 134.1, 138.5, 138.7, 147.5, 151.9. Anal. Calcd for C$_{26}$H$_{29}$N$_3$O: C, 78.16; H, 7.32; N, 10.52. Found: C, 78.05; H, 7.36; N, 10.40.

2-(1-Benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-ynyl)amino)acetonitrile (2). To a solution of compound 11 (70 mg, 0.29 mmol) and Et$_3$N (0.085 mL, 0.58 mmol) in CH$_2$Cl$_2$ (2 mL), chloride 8 (70 mg, 0.25 mmol) was added. After stirring for 30 min at rt, brine was added to the mixture. The product was extracted with CH$_2$Cl$_2$, the extract was dried over Mg$_2$SO$_4$ and then concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt by increasing the gradient from 5:1 to 1:1 v/v), to give compound 2 (99 mg, 80.6%) as a white solid: IR (KBr) $\nu$ 3443, 3300, 3026, 2949, 2814, 1580, 1504, 1476, 1454, 1424, 1371, 1269, 1229, 1193, 1150, 1072 cm$^{-1}$; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 0.68–0.70 (m, 1 H), 1.17–1.22 (m, 1 H), 1.65–1.95 (m, 5 H), 2.41 (s, 1 H), 2.65 (s, 1 H), 2.81–2.83 (m, 1 H), 3.23–3.29 (m, 2 H), 3.34 (s, 1 H), 3.45–3.48 (m, 1 H), 3.68 (d, $J =$ 13.2 Hz, 1 H), 4.63 (d, $J =$ 13.8 Hz, 1 H), 7.1–7.20 (m, 1 H), 7.21–7.28 (m, 5 H), 7.40–7.44 (m, 2 H), 8.60 (d, $J =$ 8.4 Hz, 1 H), 8.76 (d, $J =$ 4.2 Hz, 1 H); $^{13}$C NMR (150 MHz, CDCl$_3$) $\delta$ 29.4, 30.7, 36.0, 40.0, 52.5, 54.2, 57.7, 63.1, 74.0, 78.8, 84.7, 108.9, 111.8, 116.0, 121.7, 122.2, 127.2, 127.5, 128.2, 129.3, 130.0, 134.0, 138.8, 148.9, 152.6. Anal. Calcd for C$_{27}$H$_{28}$N$_3$O: C, 76.39; H, 6.65; N, 13.20. Found: C, 76.45; H, 6.75; N 13.17.
Diethyl(cyanomethyl)phosphonate. Diethyl(cyanomethyl)phosphonate was prepared by heating triethylphosphite (1.0 equiv) and chloroacetonitrile (1.0 equiv) at 150 °C for 3.5 h. The crude product was directly used in the next reaction.

(1-Benzylpiperidin-4-ylidene)acetonitrile (12) (Eckhardt, W.; Grob, C. A. Helv. Chim. Acta 1974, 57, 2339-45; Contreras, J.-M.; Rival, Y. M.; Chayer, S.; Bourguignon, J.-J.; Wermuth, C. G. J. Med. Chem. 1999, 42, 730-741). A solution of diethyl (cyanomethyl)phosphonate (2.13 g, 12 mmol) and K₂CO₃ (1.39 g, 10 mmol) in dry THF (5 mL) was stirred for 15 min at rt. Then the mixture was heated to reflux for 20 min. After cooling down to rt, 1-benzyl-4-piperidone (1.90 g, 10 mmol) was added dropwise to this solution. Then the mixture was heated at reflux for 12 h. After cooling down to rt, 10% K₂CO₃ aqueous solution was added. The reaction mixture was extracted with AcOEt, and the organic layers were dried over MgSO₄ and concentrated in vacuo to give the crude product 12 [2.7 g, >99.0 %; ¹H NMR (300 MHz, CDCl₃) δ 2.40 (t, J = 5.1 Hz, 2 H), 2.51–2.63 (m, 6 H), 3.55 (s, 2 H), 5.10 (s, 1 H), 7.26–7.34 (m, 5 H)], which was directly used in next step.
(1-Benzylpiperidin-4-yl)acetonitrile (13) (Shao, D.; Zou, C.; Luo, C.; Tang, X.; Li, Y. Bioorg. Med. Chem. Lett. 2004, 14, 4639-4642). To a solution of nitrile 12 (2.7 g, 10 mmol) in MeOH (100 mL), Mg (4.6 g, 191 mmol) and infinitesimal quantity of I2 was added. The mixture was stirred until it became gray gel. After conc. HCl was added, the mixture became clear solution. Then it was treated with 10 N NaOH to alkaline. The precipitate was filtered and washed with large amount of EtOAc. The filtrate was extracted with AcOEt, and the combined organic layers were dried over MgSO4 and concentrated in vacuo to give the product 13 (1.59 g, 74.5 %): 1H NMR (300 MHz, CDCl3) δ 1.48 (m, 2 H), 1.68 (m, 1 H), 1.78 (m, 2 H), 2.00 (t, J = 52.77 Hz, 2 H), 2.28 (d, J = 6.60 Hz, 2 H), 2.93 (d, J = 11.8 Hz, 2 H), 3.55 (s, 2 H), 7.24–7.34 (m, 5 H).

(1-Benzylpiperidin-4-yl)acetaldehyde (14) (Rodríguez-Franco, M. I.; Fernández-Bachiller, M. I.; Pérez-C., Castro, A.; Martinez, A. Bioorg. Med. Chem. 2005, 13, 6795-6802). To an oven-dried and argon-purged flask were added the nitrile 13 (1.29 g, 6.0 mmol) and THF (13 mL). The mixture was cooled to -78 °C, and DIBAL-H (6.22 mL, 1 mmol/mL) was added to the reaction via syringe. The reaction was stirred at -78 °C for 1 h, and then quenched with aqueous saturated NaHCO3. The precipitate was filtered and washed with large amount of EtOAc. The filtrate was extracted with EtOAc. The combined organic layers were washed with brine and dried over MgSO4. After concentrated in vacuum, the crude product was purified by chromatography (SiO2, CH2Cl2/MeOH =20:1 v/v) to give product 10 (365 mg, 27.8%) as an orange oil: 1H NMR (300 MHz, CDCl3) δ 1.20–1.50 (m, 2 H), 1.60–2.15 (m, 5 H), 2.36 (dd, J = 6.7, 1.76 Hz, 2 H), 2.88 (d, J = 11.2 Hz, 2 H), 3.51 (s, 2 H), 7.17–7.42 (m, 5 H), 9.76 (t, J = 1.9 Hz, 1 H).

N-[2-(1-benzylpiperidin-4-yl)ethyl]prop-2-yn-1-amine (15) and 3-(1-benzylpiperidin-4-yl)-2-(prop-2-yn-1-ylamino)propanenitrile (16). A solution of 14 (365 mg, 1.68 mmol) and propargylamine (187 mg, 3.4 mmol) in MeOH (10 mL) was stirred at 0 °C for 1 h; then NaBH3CN (214 mg, 3.4 mmol) was added. The mixture was stirred at rt overnight. Water was added to the mixture and MeOH was removed under reduced pressure. Then aqueous saturated NaHCO3 was added, and the mixture was extracted with AcOEt. The separated organic layers were dried over MgSO4 and concentrated in vacuo to give the crude product. Further purification was achieved by column chromatography (SiO2, eluting solvent was changed with gradient from hexane/AcOEt 5:1 to 1:5v/v and then change from AcOEt to MeOH/AcOEt 4:1 v/v) to give compound 15 (168 mg, 38.9%), as an orange oil, and product 16 (190 mg, 40.2%) as an orange oil. N-[2-(1-Benzylpiperidin-4-yl)ethyl]prop-2-yn-1-amine (15): RF = 0.11 (MeOH/AcOEt= 1:4); IR (film) ν 3302, 2922, 2945, 2801, 2758, 1493, 1454, 1366, 1148, 1121, 1078, 1028 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 1.15–1.45 (m, 5H), 1.61 (d, J = 10.5 Hz, 2 H), 1.92 (t, J = 11.1 Hz, 1 H), 2.13 (t, J = 2.1 Hz, 1 H), 2.63 (t, J = 7.2 Hz, 2 H), 2.84 (d, J = 11.5 Hz, 2 H), 3.30–3.55 (m, 4 H), 7.12–7.33 (m, 5 H); 13C NMR (100 MHz, CDCl3) δ 32.1, 33.5, 36.4, 38.2, 46.0, 53.7, 63.3, 71.4, 82.2, 127.0, 128.1, 129.3, 129.4, 137.6, 1493, 1454, 1388, 1343, 1125, 1074 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 1.15–1.38 (m, 2 H), 1.43–1.73 (m, 5 H), 1.92 (t,
\( J = 11.5 \text{ Hz, 2 H}, 2.21 (t, J = 2.3 \text{ Hz, 1 H}), 2.82 (d, J = 10.7 \text{ Hz, 2 H}), 3.35–3.64 (m, 4 \text{ H}), 3.66–3.78 (m, 1H), 7.13–7.33 (m, 5 H) \); 13C NMR (100 MHz, CDCl3) δ 31.7, 31.9, 32.2, 36.4, 39.9, 46.9 53.2, 63.2, 72.7, 79.8, 119.6, 126.9, 128.0, 129.0, 129.1, 138.1.

5-(((2-(1-Benzylpiperidin-4-yl)ethyl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (5). To a cooled mixture of 15 (46 mg, 0.18 mmol) and Et3N (0.053 mL, 0.38 mmol) in CH2Cl2 (2.5 mL) at 0 °C, chloride 8 (47 mg, 0.18 mmol) was added. After being stirred at rt overnight, the reaction was quenched with aqueous 5% NaHCO3. The product was extracted with CH2Cl2, and extracts were dried over Mg2SO4 and then concentrated in vacuo. The crude product was purified by column chromatography (hexane/AcOEt by increasing the gradient from 5:1 to 1:1 v/v) to give compound 5 (40 mg, 53.5%) as a yellow oil: IR (film) ν 3254, 2945, 2915, 2805, 2758, 1578, 1508, 1478, 1420, 1375, 1350, 1279, 1231, 1194, 1148, 1121 cm−1; 1H NMR (400 MHz, CDCl3) δ 1.24 (s, 3 H), 1.44 (d, J = 33.20 Hz, 4 H), 1.82 (m, 2 H), 2.67 (t, J = 2.40 Hz, 1 H), 2.58 (t, J = 7.20 Hz, 2 H), 2.83 (d, J =11.20 Hz, 2 H), 3.26 (d, J = 2.40 Hz, 2 H), 3.48 (s, 2 H), 3.92 (s, 2 H), 7.08 (d, J = 8.00 Hz, 1 H), 7.30–7.44 (m, 7 H), 8.61–8.61 (m, 1 H), 8.76–8.77 (m, 1 H); 13C NMR (100 MHz, CDCl3) δ 32.2, 33.5, 33.9, 41.0, 50.3, 53.7, 55.9, 63.3, 73.3, 78.3, 108.7, 121.4, 124.9, 127.0, 127.9, 128.1, 129.2, 129.3, 134.0, 138.7, 147.5, 151.8. HRMS. Calcd for C27H32N3O (MH+): 414.2545. Found: 414.2570 (MH+).

3-(1-Benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-ynyl)amino)propanenitrile (4). To a cooled mixture of 16 (84 mg, 0.3 mmol) and Et3N (0.093 mL, 0.67 mmol) in CH2Cl2 (6 mL), chloride 8 (77 mg, 0.3 mmol) was added at 0 °C. After being stirred at rt overnight, the reaction was quenched with aqueous 5% NaHCO3. The reaction mixture was extracted with CH2Cl2, and extracts were dried with Mg2SO4 and concentrated in vacuo. The crude product was purified by column chromatography (hexane:AcOEt by increasing the gradient from 5:1 to 1:1 v/v) to give compound 4 (91.4 mg, 69.5%) as an orange oil: IR (film) ν 3298, 2934, 2807, 1580, 1505, 1476, 1370, 1273, 1233, 1198, 1148, 1072, 1028 cm−1; 1H NMR (400 MHz, CDCl3) δ 0.81–0.93 (m, 1 H), 0.93–1.07 (m, 1 H), 1.12–1.32 (m, 2 H), 1.38–1.62 (m, 3 H), 1.69 (dd, J = 14.1, 8.8, 5.5 Hz, 1 H), 1.76–1.89 (m, 1 H), 2.39–2.47 (m, 1 H), 2.58 (d, J = 11.3 Hz, 1 H), 2.80 (d, J = 11.5 Hz, 1 H), 3.21–3.32 (m, 1 H), 3.36–3.53 (m, 3 H), 3.65–3.75 (m, 2 H), 4.60 (d, J = 13.1 Hz, 1 H), 7.06–7.11 (m, 1 H), 7.21–7.33 (m, 5 H), 7.40–7.47 (m, 2 H), 8.58–8.65 (m, 1 H), 8.75–8.81 (m, 1 H); 13C NMR (100 MHz, CDCl3) δ 30.6, 31.5, 37.2, 39.8, 49.6, 52.7, 53.0, 53.6, 62.8, 74.1, 78.6, 108.9, 117.0, 121.6, 122.3, 127.2, 127.5, 128.1,129.3, 129.8, 133.6, 138.6, 147.7, 152.5. HRMS. Calcd for C29H32N4O (MH+): 439.2428. Found: 439.2532 (MH+).
(2E)-3-(1-benzylpiperidin-4-yl)prop-2-enenitrile (17) (Takasugi, H.; Kuno, A.; Ohkubo, M. WO 9313083). A mixture of diethyl (cyanomethyl)phosphonate (2.2 g, 12 mmol) and K$_2$CO$_3$ (1.4 g, 10 mmol) in dry THF (100 mL) was stirred at rt for 15 min, and then heated at reflux for 20 min. After cooling down to rt, 1-benzyl-4-piperidinecarboxaldehyde (Sugimoto, H.; Iimura, Y.; Yamanishi, Y.; Yamatsu, K. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 871-6) (2.0 g, 10 mmol) was added. The mixture was heated to reflux for 3 h. Aqueous 10% K$_2$CO$_3$ water (100 mL) was added after cooling down to rt. The product was extracted with AcOEt and dried over MgSO$_4$. After concentrated in vacuo, the crude product was purified by column chromatography (SiO$_2$, hexane/AcOEt from 5:1 to 1:1 v/v) to give nitrile 17 (1.0 g, 44.5% yield, $R_f$ = 0.70) and (0.74 g, 32.6%) as white solid: $R_f$ = 0.30 (hexane/AcOEt = 1:2); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 1.40–1.60 (m, 2 H), 1.62–1.77 (m, 2 H), 1.93–2.14 (m, 2 H), 2.51–2.71 (m, 1 H), 2.89 (d, $J$ = 11.7 Hz, 2 H), 3.50 (s, 2 H), 5.23 (d, $J$ = 10.9 Hz, 1 H), 6.31 (t, $J$ = 10.4 Hz, 1 H), 7.19–7.38 (m, 5 H).

3-(1-Benzylpiperidin-4-yl)propanenitrile (18) [(a) Takasugi, H.; Kuno, A.; Ohkubo, M. WO 9313083; (b) Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Kawakami, Y.; Nakamura, T.; et al. *J. Med. Chem.* **1990**, *33*, 1880-7]. To a solution of 17 (1.74 g, 7.70 mmol) in MeOH (33 mL) at rt,
turning of Mg (3.70 g, 154 mmol) and infinitesimal quantity of I2 was added to the mixture. The mixture was stirred until it became gray gel. After conc. HCl was added, the mixture became clear solution. Then it was treated with 10 N NaOH to alkaline. The precipitates were filtered and washed with large amount of EtOAc. The filtrate was extracted with AcOEt, and the combined organic layers were dried over MgSO4 and concentrated in vacuo to give the crude product 18 (1.76 g, 99.9%) as an orange oil: 1H NMR (300 MHz, CDCl3) δ 1.17–1.52 (m, 3 H), 1.54–1.73 (m, 4 H), 1.96 (td, J= 11.5, 1.9 Hz, 2 H), 2.35 (t, J= 7.3 Hz, 2 H), 2.89 (d, J= 11.8 Hz, 2 H), 3.50 (s, 2 H), 7.19–7.37 (m, 5 H).

3-(1-Benzylpiperidin-4-yl)propanal (19) (Kitbunnadaj, R.; Zuiderveld, O. P.; De Esch, I. J. P.; Vollinga, R. C.; Bakker, R.; Lutz, M.; Spek, A. L.; Cavoy, E.; Deltent, M.-F.; Menge, W. M. P. B.; et al. J. Med. Chem. 2003, 46, 5445-5457). To an oven-dried and argon-purged flask were added the nitrile 18 (1.00 g, 4.4mmol) and THF (10 mL). The reaction was cooled to -78 °C, and DIBAL-H (4.54 mL, 1mmol/mL) was added to the reaction via syringe. The mixture was stirred at -78 °C for 1 h, and then quenched with aqueous saturated NaHCO3. The precipitates were filtered and washed with large amount of EtOAc. The filtrate was extracted with EtOAc. The combined organic layers were washed with brine and dried over MgSO4. After concentrated in vacuum, the crude product was purified by chromatography(SiO2, CH2Cl2/ MeOH =20:1 v/v), to give aldehyde 19 (427 mg, 42.7%) as orange oil: 1H NMR (400 MHz, CDCl3) δ 1.16–1.35 (m, 3 H), 1.52–1.72 (m, 4 H), 1.92 (t, J= 11.8 Hz, 2 H), 2.43 (td, J= 7.5, 1.6 Hz, 2 H), 2.87 (d, J= 11.1 Hz, 2 H), 3.44–3.53 (m, 2 H), 7.19–7.38 (m, 5 H), 9.76 (t, J= 1.6 Hz, 1 H).

N-[3-(1-Benzylpiperidin-4-yl)propyl]prop-2-yn-1-amine (20) and 4-(1-benzylpiperidin-4-yl)-2-(prop-2-yn-1-ylamino)butanenitrile (21). A solution of aldehyde 19 (427 mg, 1.9 mmol) and propargylamine (204 mg, 3.7 mmol) in MeOH (6 mL) was stirred at 0 °C for 1 h, then NaBH3CN (1.3 g, 2.0 mmol) was added. The mixture was stirred at rt overnight. Water was added to the mixture and MeOH was removed under reduced pressure. Then aqueous saturated NaHCO3 was added, and the mixture was extracted with AcOEt. The separated organic layers were dried over MgSO4 and concentrated in vacuo to give the crude products. Further purification was achieved by column chromatography (SiO2, eluting solvent was changed with gradient from hexane/AcOEt 5:1 to 1:5v/v and then change from AcOEt to AcOEt/MeOH 4:1 v/v) to give products 20 (221 mg, 44.2%), as a yellow oil, and 21 (234 mg, 42.7%), as an yellow oil. N-[3-(1-Benzylpiperidin-4-yl)propyl]prop-2-yn-1-amine 20: Rf = 0.19 (MeOH/AcOEt= 1:4); IR (film) v 3304, 3028, 2922, 2847, 2794, 2758, 1495, 1452, 1386, 1343, 1119, 1028 cm⁻¹; 1H NMR (600 MHz, CDCl3) δ 1.10–1.26 (m, 5 H), 1.33–1.46 (m, 2 H), 1.58 (d, J= 9.9 Hz, 2 H), 1.87 (br s, 2 H), 2.13 (t, J= 2.3 Hz, 1 H), 2.31–2.39 (m, 1 H), 2.59 (t, J= 7.1 Hz, 1 H), 2.81 (d, J= 10.8 Hz, 2 H), 3.35 (d, J= 2.3 Hz, 2 H), 3.43 (s, 2 H), 7.13–7.29 (m, 5 H); 13C NMR (150 MHz, CDCl3) δ 24.6, 27.0, 32.2, 32.3, 34.1, 35.6, 38.0, 48.8, 53.8, 63.4, 71.1, 82.2, 126.7, 128.0, 129.1, 138.4. 4-(1-Benzylpiperidin-4-yl)-2-(prop-2-yn-1-ylamino)butanenitrile 21: Rf = 0.59 (MeOH/AcOEt= 1:4); IR (film) v 3296, 2911, 2845, 2801, 2780, 1493, 1452, 1368, 1343,
In order to confirm the structure of the unexpected α-aminonitrile 21, its unequivocal synthesis has been achieved from aldehyde 19 via Strecker-type reaction (Ventosa-Andres, P.; García-López, M. T.; Herranz, R. A study on the induction of stereoselectivity in the Strecker synthesis of basic amino acid-derived α-amino nitriles *Tetrahedron: Asymmetry* 2012, 23, 1198-1205) as follows:

4-(1-Benzylpiperidin-4-yl)-2-(prop-2-yn-1-ylamino)butanenitrile (21).

3-(1-Benzylpiperidin-4-yl)propanal (19) (136 mg, 0.59 mmol), prop-2-yn-1-amine (47 μL, 0.74 mmol, 1.25 equiv) and trimethylsilyl cyanide (TMSCN) (0.14 mL, 1.1 mmol, 1.85 equiv) were mixed and submitted to microwave irradiation at 125 °C for 10 min. The crude reaction was purified by column chromatography (SiO₂, hexane/AcOEt from 9:1 to 1:1 v/v) to give compound 21 (104 mg, 60 %) as a yellow oil, that showed identical spectroscopic data to the compound isolated in the Mannich reductive amination of aldehyde 19. α-Aminonitrile 21 was also transformed into target compound 6 (see below).

5-(((3-(1-Benzylpiperidin-4-yl)propyl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (7). To a cooled mixture of amine 20 (189 mg, 0.70 mmol) and Et₃N (0.51 ml, 2.5 mmol) in CH₂Cl₂ (15 mL) at 0 °C, chloride 8 (186 mg, 0.73 mmol) was added. After being stirred at rt overnight, the reaction was quenched with aqueous 5% NaHCO₃. The product was extracted with CH₂Cl₂, and extracts were dried over MgSO₄ and then concentrated in vacuo. The crude products were purified by column chromatography (SiO₂, hexane/AcOEt by increasing the gradient from 5:1 to 1:1 v/v) to give compound 7 (162 mg, 54.3%) as a yellow oil: IR (film) ν 3381, 3293, 2911, 2801, 1738, 1580, 1505, 1476, 1425, 1373, 1289, 1238, 1198, 1047 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.99–1.33 (m, 5 H), 1.42–1.66 (m, 4 H), 1.86 (t, J= 11.3 Hz, 2 H), 2.29 (t, J= 2.1 Hz, 1 H), 2.56 (t, J= 7.1 Hz, 2 H), 2.86 (d, J= 11.4 Hz, 2 H), 3.26 (d, J= 2.2 Hz, 2 H), 3.48 (s, 2 H), 3.93 (s, 2 H), 7.09 (d, J= 7.6 Hz, 1 H), 7.20–7.37 (m, 5 H), 7.37–7.49 (m, 2 H), 8.65 (dd, J= 8.5, 1.3 Hz, 1 H), 8.77 (dd, J= 4.1, 1.3 Hz, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 24.4, 32.2, 34.0, 35.3, 40.9, 53.2, 53.8, 55.8, 63.4, 73.4, 78.4, 108.9, 121.4, 124.9, 127.0, 127.9, 128.2, 129.2,
4-(1-Benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-yny)amino)butanenitrile (6). To a mixture of amine 21 (89 mg, 0.3 mmol) and Et3N (0.09 mL, 0.7 mmol) in CH2Cl2 (6 mL) at 0 °C, chloride 8 (77 mg, 0.3 mmol) was added. After being stirred at rt overnight, the reaction was quenched with aqueous 5% NaHCO3 and the product was extracted with CH2Cl2. The extracts were dried MgSO4, and concentrated in vacuo. The crude products were purified by column chromatography (SiO2, hexane/AcOEt by increasing the gradient from 5:1 to 1:1 v/v), to give compound 6 (89 mg, 65.4%) as a yellow oil: IR (film) ν 3295, 2931, 2812, 1578, 1505, 1476, 1371, 1271, 1203, 1196, 1148, 1123, 1072, 1028 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 0.99-0.77 (m, 1 H), 1.40-1.38 (m, 6H), 1.53-1.40 (m, 1 H), 1.84-1.60 (m, 5 H), 2.42 (t, J = 2.4 Hz, 1H), 2.77 (ddt, J= 12.0, 8.3, 2.1 Hz, 1H), 3.28 (dd, J = 16.8, 2.4 Hz, 1H, 1 H), 3.45 (s, 2 H), 3.58 (dd, J = 16.8, 2.4 Hz, 1H, 1 H), 3.75 (d, J = 13.1 Hz, 1 H), 4.60 (d, J = 13.1 Hz, 1H), 7.11 (d, J = 7.6 Hz, 1 H), 7.32–7.22 (m, 5 H), 7.57-7.39 (m, 2H), 8.64 (dd, J = 8.5, 1.6 Hz, 1 H), 8.78–8.80 (m, 1 H); 13C NMR (100 MHz, CDCl3) δ 28.1, 31.5, 31.9, 32.0, 34.4, 39.8, 52.3, 53.4, 53.5, 53.7, 63.3, 74.2, 78.6, 108.9, 117.2, 121.8, 122.5, 127.1, 127.6, 128.2, 129.3, 129.9, 133.7, 138.8, 147.9, 152.6. HRMS. Calcd for C29H32N4O (MH+): 453.2654. Found: 452.2526 (MH+).
2. NMR and IR spectra

5-(Chloromethyl) quinolin-8-ol (8)
1-Benzyl-N-(prop-2-ynyl)amino-piperidine (9)
5-(((1-Benzylpiperidin-4-yl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (1)
*N*-[(1-Benzylpiperidin-4-yl)methyl]prop-2-yn-1-amine (10)
(1-Benzylpiperidin-4-yl)(prop-2-yn-1-ylamino)acetonitrile (11)

n=1 (-1) CN

ppm

28.163 28.754 36.617 38.810 52.759 52.797 54.248 62.835 72.604 76.677 76.995 77.314 79.883 118.540 126.860 128.039 128.858 138.060
5-(((1-Benzylpiperidin-4-yl)methyl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (3)
2-(1-Benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-ynyl)amino)acetonitrile (2)
(1-Benzylpiperidin-4-ylidene)acetonitrile (12)
(1-Benzylpiperidin-4-yl)acetonitrile (13)
(1-Benzylpiperidin-4-yl)acetaldehyde (14)
N-[2-(1-Benzylpiperidin-4-yl)ethyl]prop-2-yn-1-amine (15)
3-(1-Benzylpiperidin-4-yl)-2-(prop-2-yn-1-ylamino)propanenitrile (16)
5-(((2-(1-Benzylpiperidin-4-yl)ethyl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (5)
3-(1-Benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-ynyl)amin) propanenitrile (4)

n=2 (final) CN
(E)-3-(1-Benzylpiperidin-4-yl)prop-2-enenitrile (17)

n=3 (-4) b
3-(1-Benzylpiperidin-4-yl)propanenitrile (18)

\[
\text{CN}
\]

\[
\text{n=3 (-3)}
\]
3-(1-Benzylpiperidin-4-yl)propanal (19)
$N\text{-}[3\text{-(1-Benzylpiperidin-4-yl)propyl}]\text{prop-2-yn-1-amine (20)}$
4-(1-Benzylpiperidin-4-yl)-2-(prop-2-yn-1-ylamino)butanenitrile (21)
5-(((3-(1-Benzylpiperidin-4-yl)propyl)(prop-2-ynyl)amino)methyl)quinolin-8-ol (7)
4-(1-Benzylpiperidin-4-yl)-2-(((8-hydroxyquinolin-5-yl)methyl)(prop-2-ynyl)amino)butanenitrile (6)
3. Biochemistry

3.1. Monoamine Oxidase (A and B) and SSAO/VAP-1 Inhibitory Activities. The inhibitory activity of the derivatives 1-7 towards MAO A, MAO B and SSAO/VAP-1 was determined as previously described (Fowler, C. J.; Tipton, K. F. Concentration dependence of the oxidation of tyramine by the two forms of rat liver mitochondrial monoamine oxidase. *Biochem. Pharmacol.* **1981**, 30, 3329–3332.), using [14-C]-labelled substrates (Perkin Elmer, USA). Mitochondria from rat liver homogenates were used as source of MAO activities (Gómez N.; Unzeta M.; Tipton K.F.; Anderson M.C.; O’Carroll A.M. *Biochem. Pharmacol.* **1986**, 33, 4467-4472) and microsomes from bovine lung as SSAO/VAP-1 source (Lizcano J.M; Fernández de Arriba A.; Tipton K.F.; Unzeta M. Inhibition of bovine lung semicarbazide-sensitive amine oxidase (SSAO) by some hydrazine derivatives. *Biochem. Pharmacol.* **1996**, 52, 187-195). MAO A activity was determined towards 100µM (0.5mCi/mmol) [14-C]-(5-hydroxytryptamine) (5-HT) allowing the enzymatic reaction for 20 minutes. MAO B activity was determined towards 20µM (2.5mCi/mmol) [14-C]-phenylethylamine (PEA) allowing the enzymatic reaction for 4 minutes. SSAO/VAP-1 activity was measured towards 100µM (2mCi/mmol) [14-C]-benzylamine (Bz) following a 30-min incubation. Inhibition curves were made by pre-incubating the enzyme with several concentrations of each compound for 30 minutes in 50mM phosphate buffer (pH 7.4). An inhibitor-free sample was always present to determine the 100% of enzyme activity. Enzymatic reactions were carried out by adding 25µl of substrate in a final volume of 225µl and stopped by the addition of 2M citric acid. Radiolabelled-aldehyde products were extracted into toluene/ethylacetate (1:1, v/v) containing 0.6% (w/v) 2,5-diphenyloxazole prior to liquid scintillation counting (Tri-Carb 2810TR). The inhibition curves were expressed in percentage of total activity and IC$_{50}$ values were calculated by using the GraphPad ‘PRISM’ software (version 3.0). Total protein was measure by the method of Bradford (1976) using bovine-serum albumin as standard. Data are the mean ± SEM of at least three different experiments performed in triplicate.

3.2. Cholinesterase Inhibitory Activities. Cholinesterase activities were assessed following a spectrophotometric method (Ellman, G. L.; Courtney, K. D.; Andres, V.; Featherstone, R. M. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.* **1961**, 7, 88–95), using AChE from *Electrophorus electricus* (type V-S) and BuChE from equine serum (lyophilized powder) (Sigma-Aldrich, Madrid, Spain). Donepezil was used as standard compound. Enzymatic reactions took place in 96-well plates in a final volume of 300 µL containing 0.1M phosphate buffer (pH 8.0), 0.035 U/mL AChE or 0.05 U/mL BuChE and 0.35 mM of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB, Sigma-Aldrich, Madrid, Spain). Inhibition curves were made by pre-incubating this mixture with serial dilutions of each compound for 20 minutes. The activity in absence of compounds was always performed to determine the 100% of enzyme activity. After the pre-incubation period, 50µl of substrate were added to a final concentration of 0.35mM acetylthiocholine iodide or 0.5 mM butyrylthiocholine iodide (Sigma-Aldrich). Enzymatic reactions were allowed for 5 min
incubation for AChE and 25 min for BuChE while the DNTB produces the yellowish anion 5-thio-2-nitrobenzoic acid along with the enzymatic degradation of both substrates. Changes in absorbance were detected at 405 nm in a spectrophotometric plate reader (FluoStar OPTIMA, BMG Labtech). Color generation is reduced as the enzymatic activities are inhibited by the compounds and IC50 values were calculated at the compound concentration that produces 50% of enzymatic activity inhibition by using the GraphPad ‘PRISM’ software (version 3.0). Data are expressed as mean ± SEM of at least three different experiments performed in triplicate.

3.3. Kinetic studies of AChE. In order to estimate the mechanism of action of DPH6 on AChE activity, reciprocal plots of 1/V versus 1/[S] were constructed at different substrate concentrations. Substrate concentrations ranging 0.1-5 µM of ASCh for AChE, as previously described. The plots were assessed by a weighted least-squares analysis. Data analysis was performed with GraphPad ‘PRISM’ software (version 3.0). Slopes of the reciprocal plots were plotted against the concentration of DPH 6 (0-5 µM) for AChE to determine Ki values.

3.4. Reversibility and Time-dependence inhibition studies. Reversibility inhibition studies exerted by DPH6 towards MAO A and MAO B were determined by incubating the enzyme in the presence and in the absence of the inhibitor before and after three consecutive washings with buffer. MAO A and MAO B samples were pre-incubated for 30 min at 37 ºC with 10 µM DPH6 and 20 µM DPH6, respectively. A sample of 40 nM clorgyline and 20 nM l-deprenyl was also used as control of irreversible MAO A and MAO B inhibition. Samples were washed with 50 mM phosphate buffer (pH 7.4) and centrifuged at 25000 g for 10 min at 4 ºC consecutively three times. Total protein was measured by the Bradford method and MAO activity determined as described above. Time-dependence inhibition of DPH6 towards MAO A and MAO B was evaluated by pre-incubating varying concentrations of the inhibitor with the enzyme at different times (0-180 minutes). IC50 values were accordingly determined for each time as described above.

3.5. Metal-Chelating Properties. The complexing studies were made in distilled water at room temperature using a UV-vis spectrophotometer (Lambda 25, PerkinElmer). The absorption spectra of 10 µM DPH6 alone or in the presence of varying concentrations of CuSO4 or Fe2(SO)3 were recorded in a 1 cm quartz cell. The stoichiometry of the complexes DPH6-Cu(II) and DPH6-Fe(III) were determined by the Job’s method (Huang C.Y. Determination of binding stoichiometry by the continuous variation method: the Job plot. Bioorg. Methods Enzymol. 1982, 87, 509–521). A series of 21 different solutions containing DPH6 and CuSO4 or Fe2(SO4)3 in distilled water were prepared at a final sum of concentrations of both species of 10µM. Proportions of both components varied between 0 and 100%. Absorbance at 257 nm was plotted versus the mole fraction of DPH6 for each metal.
4. Molecular modeling DPH6

4.1. Inhibition of AChE

A modeling study was carried out through docking simulations for the purpose of gaining insights on the nature and spatial location of the key interactions of the \((R)-\) and \((S)-\)enantiomers of DPH6 modulating the inhibitory activity of AChE and BuChE. As in our previous studies, we have chosen the 3D structure of the enzyme species (EeAChE and eqBuChE) used for the kinetic studies.

Ligand docking studies were performed with Autodock Vina\(^1\) using a single catalytic sub-unit of EeAChE (PDB: 1C2B). This docking procedure allows the docking of ligands on the entire protein surface, without prior specification of the binding site. As in previous studies, the recognition process between \((R)-\) and \((S)-\)enantiomers of DPH6 was theoretically investigated by flexible docking experiments. Flexible torsions in the ligands were assigned and protein side chain flexibility was incorporated allowing the rearrangement of the side chains of eight residues, Trp286, Tyr124, Tyr337, Tyr72, Asp74, Thr75, Trp86 and Tyr341. These residues delineate the shape of the gorge entry and lining and their motion may significantly enlarge the gorge to facilitate bulky ligand access to the catalytic site.\(^2,3\)

As shown in Figures S1 and S2, it appears that both enantiomers of DPH6 interact simultaneously with both catalytic and peripheral site of EeAChE thanks to a linker of appropriate length showing a strong correlation with the observations we have from
4.1.1. Docking studies of (R)-DPH6 with EeAChE

Computational docking studies of (R)-DPH6 with EeAChE yielded four major binding modes at the enzyme binding site. In Figure 1, the four most favored binding modes are presented along with the first shell of residues surrounding (R)-DPH6.
Figure S1. Binding mode of inhibitor \((R)-\text{DPH6}\) at the active site of \(EeAChE\). (a) Mode I, compound \((R)-\text{DPH6}\) is illustrated in green. (b) Mode II, compound \((R)-\text{DPH6}\) is illustrated in blue. (c) Mode III, compound \((R)-\text{DPH6}\) is illustrated in red. (d) Mode IV, compound \((R)-\text{DPH6}\) is illustrated in violet. Ligands are rendered as sticks and the side chains conformations of the mobile residues are illustrated in the same color light as the ligand. Different subsites of the active site were colored: catalytic triad (CT) in green, oxyanion hole (OH) in pink, anionic sub-site (AS) in orange, except Trp86, acyl binding pocket (ABP) in yellow, and peripheral anionic subsite (PAS) in blue. Black dashed lines are drawn among atoms involved in hydrogen bond interactions.
Mode I and Mode II (Figures S1a and S1b) placed the quinoline moiety near the opening of the binding pocket. Mode III and Mode IV (Figures S1c and S1d) placed the quinoline deep into the binding pocket next to the residues known to be involved in catalysis. The classical catalytic triad is shaded in green in all four panels.

A close examination of (R)-DPH6 in Mode I revealed that the interaction with the AChE peripheral site involved a face to face π-π stacking between the indole ring of Trp286 and the quinoline moiety and a T-shaped π-π stacking between the phenyl ring of Tyr72 and the quinoline moiety. Besides, in this complex, a bifurcated hydrogen bond was formed between the nitrogen atom of the cyano group and Arg296-NH and Phe295-OH. The benzyl moiety pointed towards the bottom of the gorge and established edge-to-face π-π interactions with Trp86 and face-to-face π-π interactions with Tyr337.

In Mode II, the benzyl and the quinoline moieties interacted with Trp86 of the catalytic pocket and Trp286 of the peripheral site, respectively. In the middle of the gorge, the nitrogen atom of the cyano group forms a hydrogen bond with the hydroxyl group of the Phe295. At the top of the gorge, the hydroxyl group of the quinoline moiety forms a hydrogen bond with the carboxylate group of Asp74.

As can be seen in Figure S1c (Mode III), (R)-DPH6 has several interactions along the active-site gorge of EeAChE. At the top of the gorge, the benzyl ring and Trp286 indole ring formed a favorable T-shaped π-π interaction. Near the bottom of the gorge the quinoline moiety stacked against the Trp86 indole ring. In this region, the nitrogen atom of the quinoline moiety was hydrogen bonded to the hydroxyl group of Tyr133.

Comparison of Mode IV (Figure S1d) and Mode III (Figure S1c) revealed a broadly similar interaction but with two key differences: a) a significant movement of the Trp286 indole and benzyl rings in order to establish a face-to-face π-π interaction, and b) the rotation of the hydroxyl group of the quinoline moiety to form a hydrogen bond with the carbonyl group of Gly120.

In both poses (Mode III and Mode IV), the three methylene units in the spacer of (R)-DPH6 was long enough to allow a proper interaction between (R)-DPH6 and both sites of the enzyme. The linker was lodged in a narrow cavity described by Asp74, Tyr124, Phe297, Tyr337, Phe338, and Tyr341. The protonated nitrogen was favorably interacting
with the residues of a kind of “electrostatic cage” (Tyr124, Asp74, Tyr337 and Tyr341 side chains).

4.1.2. Docking studies of (S)-DPH6 with EeAChE

Docking studies of (S)-DPH6 with EeAChE yielded three major binding modes at the enzyme binding-site. In figure S2 the three most favored binding modes are presented along with the first shell of residues surrounding (S)-6.

Mode I and Mode II (Figure S2a and Figure S2b) placed the quinoline moiety near the opening of the binding pocket. Mode III (Figure S2c) placed the quinoline deep into the binding pocket next to the residues involved in catalysis.

In Mode I (Figure S2a), the main stabilizing factors that keep stable the (S)-DPH6-AChE complex were found to be the hydrophobic contacts, \( \pi-\pi \) interactions and hydrogen bonding interactions. Compound (S)-DPH6 can simultaneously bind at both the peripheral anionic site (PAS) and the catalytic active site (CAS) of EeAChE. (S)-DPH6 is able to bind in the PAS by face-to-face and edge-to-face \( \pi-\pi \) interactions between the quinoline moiety of the ligand and the Trp286 indole ring and the Tyr72 phenyl ring, respectively. Other interactions like a bifurcated hydrogen bond between the nitrogen atom of the cyano group and Arg296-NH and Phe295-OH could also play an important role in positioning and stabilizing the ligand inside the active site gorge. The benzene ring of (S)-6 formed a \( \pi-\pi \) stacking interaction with Trp86.

In Mode II (Figure S2b), compound (S)-DPH6 can also adopt a similar binding conformation to that in Mode I. The ligand had an orientation along the active-site gorge, extending from the anionic sub-site of the active site at the bottom, to the peripheral anionic site at the top, via aromatic stacking interactions with Trp86, Trp286 and Tyr72 residues (Mode II, Figure S2b). Conversely, in this orientation, compound (S)-DPH6 was not able to form hydrogen bonds but a stronger stacking interaction between benzene ring and Trp86.

Mode III (Figure S2c) also placed the ligand along the active-site gorge. The quinoline ring formed a \( \pi-\pi \) interaction with the indole ring of Trp86 and also established three additional hydrogen bonds, which have an important contribution to the binding
potency of the ligand. The hydroxyl group of Tyr133 formed two hydrogen bonds with the nitrogen and the hydroxyl group of the quinoline moiety, the later group is also hydrogen bonded to the carbonyl oxygen from the backbone of Gly120. On the other hand, at the peripheral anionic site (PAS), the benzene ring of the ligand form edge-to-face $\pi-\pi$ interaction with the indole ring of Trp286 and with the benzene ring of Tyr72. In comparison with the other poses, the cyano residue is involved in the interactions with the catalytic triad of the active site gorge. This group is able to bind by mean of hydrogen bond with the Ser203 side chain.

On the basis of the similar orientation and the set of interactions, which were identified between the (R)-enantiomer (Modes I-III, Figures S1a, S1b and S1c) and (S)-enantiomer (Modes I-III, Figures S2a, S2b and S2c) and the protein residues within the gorge, it can be hypothesized that both enantiomers of DPH6 could simultaneously bind at both the peripheral anionic site (PAS) and the catalytic active site (CAS) of EeAChE.
Figure S2. Binding mode of inhibitor (S)-DPH6 at the active site of hAChE. (a) Mode I, compound (S)-DPH6 is illustrated in grey. (b) Mode II, compound (S)-DPH6 is illustrated in brown. (c) Mode III, compound (S)-DPH6 is illustrated in pink. Ligands are rendered as sticks and the side chains conformations of the mobile residues are illustrated in the same light color as the ligand. Different subsites of the active site were colored: catalytic triad (CT) in green, oxyanion hole (OH) in pink, anionic sub-site (AS) in orange, except Trp86, acyl binding pocket (ABP) in yellow, and peripheral anionic subsite (PAS) in blue. Black dashed lines are drawn among atoms involved in hydrogen bond interactions.
4.2. Inhibition of BuChE

With respect to BuChE, in the absence of X-ray structure of eqBuChE, a homology model was used. The modeling of the 3D structure was performed by an automated homology-modeling program (SWISS-MODEL). A putative three-dimensional structure of eqBuChE has been created based on the crystal structure of human BuChE (pdb: 2PM8), as these two enzymes exhibited 89% sequence identity.

In order to simulate the binding of both enantiomers of compound DPH6 to eqBuChE, docking experiments were performed as blind dockings following the same computational protocol used for EeAChE.

The best-ranked docking solutions revealed that BuChE can effectively accommodate both enantiomers of DPH6 inside the active site gorge and two major binding modes can be proposed (Figure S3).

Modes I and II (Figures S3a and S3b) for both enantiomers placed the quinoline moiety into the binding pocket next to the residues involved in catalysis. In this orientation, the phenyl moiety interacts with Trp231 by means of hydrophobic interactions and Trp82 allowed a π-π stacking interaction with the quinoline ring of the ligand.

In Mode I (Figure S3a), a close examination of the first shell of residues surrounding (R)-DPH6 and (S)-DPH6 revealed that in both enantiomers the hydroxyl group of the quinoline ring formed one hydrogen bond with the carboxylate group of Glu197 and another one with the hydroxyl group of the Tyr128. Moreover, the cyano group for the (R)-DPH6 enantiomer, formed a hydrogen bond with the NH2 of the side chain of Asn83, this bond was bifurcated in the case of (S)-DPH6 enantiomer. In this orientation, hydrophobic interactions with the catalytic triad residues, Ser198 and His438 were found.

In mode II (Figure S3b), only one hydrogen bond was observable for the (R)-enantiomer, it was formed between the cyano group and the hydroxyl group of Thr120. The (S)-enantiomer preserved the cyanide bifurcated hydrogen bond already observed in Mode I, and the hydroxyl group of the quinoline ring established a hydrogen bond with the catalytic triad residue His438.
Figure S3. Complex of compound (R)-DPH6 and (S)-DPH6 and eqBuChE homology built 3D-model. (a) Mode I, compound (R)-DPH6 is illustrated in orange and compound (S)-DPH6 in green. (b) Mode II, compound (R)-DPH6 is illustrated in yellow and compound (S)-DPH6 in blue. The compounds are rendered as sticks.
Analysis of the intermolecular interactions indicated key residues responsible for ligand binding. The cyanide group is likely to be an important feature for these derivatives to exhibit BuChE inhibitory activity.

4.3. Inhibition of MAO A and MAO B

In order to explore the nature of the ligand-receptor interactions, the ligand was docked to the active site of both MAO A and MAO B isoforms using the program Autodock Vina.\(^1\) We have focused on DPH\(^6\), which showed the best both MAO A (IC\(_{50} = 1.77 \pm 0.1 \mu M\)) and MAO B (IC\(_{50} = 1.63 \pm 0.25 \mu M\)) inhibitory activities, with significant \(EeAChE\) and eqBuChE inhibitory potencies.

Although rat MAO A (rMAO A) and rat MAO B (rMAO B) are \~90% identical in sequence with human enzymes, their functional properties are similar but not identical to those of human enzymes. Given that MAO inhibition assays were carried out on rat brain mitochondria, docking experiments were carried out using the X-ray structure of rMAO A (PDB ID: 1O5W) and the homology model of rMAO B developed from a human MAO B (hMAO B) crystallographic structure (PDB ID: 1S3E),\(^7\) as previously described for eqBuChE.

The recognition process between (R)- and (S)-enantiomers of DPH\(^6\) (chosen as reference compound) was theoretically investigated by blind docking experiments, in accordance with a protocol previously defined by us and well validated.\(^8\) The enzyme-inhibitor interactions might allow a theoretical evaluation of which enantiomer of the inhibitor could be better accommodated into the catalytic site of MAO A and MAO B.

Results from several studies have shown that it must be the neutral amine that reaches the active site of MAO A and MAO B to allow the chemistry.\(^9\)\(^\text{--12}\) Therefore the docking simulations were done using both enantiomers of DPH\(^6\) as neutral species despite of at physiological pH, most of the piperidine rings would be in the protonated, positively charged form.

In docking with MAO A, during each run, the side chains of twenty-one residues (Tyr 69, Leu97, Gln99, Ala111, Phe112, Tyr124, Trp128, Phe173, Leu176, Phe177,
Ile180, Asn181, Ile207, Phe208, Gln215, Cys323, Ileu325, Ileu335, Phe352, Tyr407 and Tyr444) were allowed to relax with the ligand, while the remainder of the enzyme was fixed in 3-D space.

Six water molecules labeled as w72, w193, w11, w23, w15, and w53 in accordance with the numbering reported for the hMAO B crystallographic structure (PDB ID: 1S3E) located near the FAD cofactor were considered as integral components of the protein structure during the docking simulation.

Docking of DPH6 was performed for both enantiomers and a sole binding mode per enantiomer was found. Figures S4a and S4b illustrated the binding modes of (R)-DPH6 and (S)-DPH6 enantiomers into the hMAO A binding cavity. Both enantiomers showed a relatively similar localization for the hydroxy-quinoline and benzyl moieties, with the rest of the molecule easily accommodated in the relatively large cavity of MAO A.

4.3.1. Inhibition of MAO A by (R)- and (S)-DPH6

Figure S4a showed that the optimal position for the (R)-DPH6 enantiomer placed the quinoline ring in an “aromatic cage” formed by Tyr407, Tyr444 side chains, as well as the isoalloxazine FAD ring. (R)-DPH6 forms π-π stacking interactions with Tyr407 and the carbonyl group of Gln215 residue. The hydroxy-quinoline moiety was involved in two hydrogen bonds between the OH and the carbonyl oxygen of the FAD and the 193w molecule. The benzyl group is located in a hydrophobic core delimited by residues Phe173, Phe208, Ileu325, Ala111 Phe112 and Leu176. The cyano group is also able to form a hydrogen bond with Cys323 side chain.

Compound (S)-DPH6 showed a binding geometry very similar to that displayed by (R)-DPH6 as for the hydroxy-quinoline and benzyl moieties (see Figure S4b), which displayed a set of intermolecular interactions described before in the case of the (R)-enantiomer. The main difference with respect to the recognition of (R)-DPH6 was in the spatial orientation of the propargyl, piperidine and cyano groups, the later accommodated this time to establish hydrogen bond with Thr336 residue.

The docking studies rationalized the relevant inhibitory activity of DPH6 towards
MAO A, as due to the formation of several favorable interactions with the catalytic site of the enzyme.

Figure S4. Docking poses of inhibitor DPH6 into rMAO A. (a) (R)-DPH6 (purple sticks). (b) (S)-DPH6 (blue sticks). Amino acid residues of the binding site are color-coded. The flavin adenine dinucleotide cofactor (FAD) and the six water molecules are represented as an integral part of the MAO A structure model and are rendered as yellow sticks and red balls, respectively. Green dashed lines are drawn among atoms involved in hydrogen bond interactions.

The importance of the cyano group in properly positioning the ligand by H-bond
formation is pointed out. It is worth noting that compounds lacking this CN, like in the case of DPH7, showed the inhibitory activity drastically lowered.

Figure S5. Docking pose of inhibitor DPH6 into rMAO B. (a) (R)-DPH6 (green sticks). (b) (S)-DPH6 (pink sticks). Amino acid residues of the binding site are color-coded. The flavin adenine dinucleotide cofactor (FAD) and the six water molecules are represented as an integral part of the MAO B structure model and are rendered as yellow sticks and red balls, respectively.
4.3.2. Inhibition of MAO B by (R)- and (S)-DPH6

To rationalize the selectivity towards MAO A and MAO B of (R)- and (S)-DPH6, blind docking studies of DPH6 into the MAO B were done. Up to date, a reliable 3-D structure of rMAO B is not available and we used a 3D homology model of rMAO B for the docking studies. The six structural water molecules selected for rMAO A were also included in the model.

For MAO B, the inhibitor (R)-DPH6 crosses both cavities, presenting the piperidine nucleus located between the “entrance” and “catalytic” cavities, separated by the residues Ile199 and Tyr326. This complex was stabilized by hydrophobic contacts of quinoline ring with Phe103, Pro104, Trp119, His90, Val316 and Tyr115. Besides, the phenyl ring is hosted into the “aromatic cage” framed by Tyr398, Phe343, Tyr435, and the FAD aromatic ring, where it forms a number of $\pi$-$\pi$ interactions also including Gln206. No intermolecular H-bonds between ligand and enzyme were observed (Figure S5a).

For MAO B, the quinoline system of the inhibitor (S)-DPH6 was hosted in the entrance cavity made up by lipophilic residues Phe103, Pro104, Trp119, His90, Ileu164, Val316 and Tyr115. The phenyl ring occupied the substrate cavity and was in direct contact with the Tyr398, Phe343, Tyr435, and the FAD aromatic ring. The compound established a H-bond with Tyr115 OH hydrogen by its OH oxygen (Figure S5b).

The study confirmed the selectivity of DPH6 for MAO A isoform. Selectivity is likely due to the orientation of the quinoline and phenyl moieties of DPH6 in MAO A and in MAO B. For MAO B, the quinoline system was hosted in the entrance cavity and for MAO A this system occupied the substrate cavity. In this disposition the quinoline moiety interacted directly with the FAD aromatic ring.

4.4. Predicted Binding Affinities

AutoDock Vina provides a computed binding affinity for each docking mode predicted. Binding affinity data for both enantiomers with the four enzymes are summarized in Table S1. The more negative the value, the tighter the predicted bonding.

For the (R)-DHP6 interaction with the AChE, predicted binding affinities were very similar for the four modes (I-IV), ranging from -11.0 to -12.3 kcal/mol.
Predicted binding affinities for (R)-DHP6 with BuChE (-10.4 and -10.7 kcal/mol) were slightly lower in range than those for the AChE complex (Table S1).

Data for (S)-DHP6 interactions gave very similar predicted binding than that for (R)-DHP6 interactions: predicted binding energies of -11.5 to -12.4 kcal/mol for the AChE and -9.9 and -9.7 kcal/mol for the BuChE.

Consistent with biological trends, both enantiomers were predicted to have similar binding interactions with AChE and BuChE.

The weakest predicted binding affinities were computed for the both enantiomers interactions with the MAO A and MAO B enzymes. Very similar binding affinity values were also observed for both enantiomers with MAO A and MAO B.

Based on these results we propose that the active species should be possibly the major non-chiral DPH6 iminium cyanides in quick equilibrium interchange with the free base (Scheme S1, see page S35) and it is the reason for why we have not tried to separate or synthesize both pure enantiomers of DPH6.

### Table S1. Predicted binding affinities (kcal/mol)

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4.5. Experimental Methods

4.5.1. Molecular docking into AChE and BuChE. (R)-DPH6 and (S)-DPH6 were assembled as hydrochlorides and free bases within Discovery Studio, version 2.1,
software package, using standard bond lengths and bond angles. With the CHARMM force field\textsuperscript{13} and partial atomic charges, the molecular geometries of (\textit{R})-DPH\textsubscript{6} and (\textit{S})-DPH\textsubscript{6} were energy-minimized using the adopted-based Newton-Raphson algorithm. Structures were considered fully optimized when the energy changes between iterations were less than 0.01 kcal/mol.\textsuperscript{14}

\subsection*{4.5.2. Molecular docking of (\textit{R})-DPH\textsubscript{6} and (\textit{S})-DPH\textsubscript{6} into \textit{EeAChE}.} The coordinates of \textit{Electrophorus electricus} AChE (PDB ID: 1C2B), were obtained from the Protein Data Bank (PDB). For docking studies, initial protein was prepared by removing all water molecules, heteroatoms, any co-crystallized solvent and the ligand. Proper bonds, bond orders, hybridization and charges were assigned using protein model tool in Discovery Studio, version 2.1, software package. CHARMM force field was applied using the receptor-ligand interactions tool in Discovery Studio, version 2.1, software package. Docking calculations were performed with the program Autodock Vina.\textsuperscript{1} AutoDockTools (ADT; version 1.5.4) was used to add hydrogens and partial charges for proteins and ligands using Gasteiger charges. Flexible torsions in the ligands were assigned with the AutoTors module, and the acyclic dihedral angles were allowed to rotate freely. Trp286, Tyr124, Tyr337, Tyr72, Asp74, Thr75, Trp86, and Tyr341 receptor residues were selected to keep flexible during docking simulation using the AutoTors module. Because VINA uses rectangular boxes for the binding site, the box center was defined and the docking box was displayed using ADT. For \textit{Electrophorus electricus} AChE (PDB ID: 1C2B) the docking procedure was applied to whole protein target, without imposing the binding site ("blind docking"). A grid box of 60 x 60 x 72 with grid points separated 1 Å, was positioned at the middle of the protein (x= 21.5911; y= 87.752; z= 23.591). Default parameters were used except num_modes, which was set to 40. The AutoDock Vina docking procedure used was previously validated.\textsuperscript{2}

\subsection*{4.5.3. Molecular docking of inhibitors (\textit{R})-DPH\textsubscript{6} and (\textit{S})-DPH\textsubscript{6} into eqBuChE.} The horse BuChE model has been retrieved from the SWISS-MODEL Repository. This is a database of annotated three-dimensional comparative protein structure models generated by the fully automated homology-modeling pipeline SWISS-MODEL. A putative three-dimensional structure of eqBuChE has been created based on the crystal structure
of hBuChE (PDB ID: 2PM8), these two enzyme exhibited 89% sequence identity. Proper
bonds, bond orders, hybridization and charges were assigned using protein model tool in
Discovery Studio, version 2.1, software package. CHARMM force field was applied
using the receptor-ligand interactions tool in Discovery Studio, version 2.1, software
package. Docking calculations were performed following the same protocol described
before for EeAChE. All dockings were performed as blinds dockings where a cube of
75Å with grid points separated 1 Å, was positioned at the middle of the protein (x=29.885;
y=-54.992; z= 58.141). Default parameters were used except num_modes, which was set
to 40. The lowest docking-energy conformation was considered as the most stable
orientation. Finally, the docking results generated were directly loaded into Discovery
Studio, version 2.1.

4.5.4. Molecular docking of compounds (R)-DPH6 and (R)-DPH7 into rat MAO A/B.
Compounds (R)-DPH6 and (R)-DPH7 were assembled as non-protonated amine within
Discovery Studio, version 2.1, software package, following the procedure described
before for cholinesterases. The crystal structure of rat MAO A in complex with its
irreversible inhibitor MLG-709 was obtained from the Protein Data Bank (PDB ID
1O5W). The rat MAO B model has been retrieved from the SWISS-MODEL Repository.
A putative three-dimensional structure of rat MAO B has been created based on the
crystal structure of hMAO B (PDB ID: 1S3E), these two enzymes exhibited 89%
sequence identity. For docking studies initial proteins were prepared. First, in the PDB
crystallographic structure 1O5W (rat MAO A), any co-crystallized solvent and the ligand
were removed; it is not necessary in the PDB MAO B model. Then, proper bonds, bond
orders, hybridization and charges were assigned using protein model tool in Discovery
Studio, version 2.1, software package. CHARMM force field was applied using the
receptor-ligand interactions tool in Discovery Studio, version 2.1, software package. Six
water molecules located around the FAD cofactor were considered in the docking
experiments because of their well-known role into the MAO’s inhibition. Finally, atoms
of the FAD cofactor were defined in their oxidized state. Docking calculations were
performed following the same protocol described before for EeAChE. In docking with
MAO A, Tyr 69, Leu97, Gln99, Ala111, Phe112, Tyr124, Trp128, Phe173, Leu176,
Phe177, Ile180, Asn181, Ile207, Phe208, Gln215, Cys323, Ileu325, Ileu335, Phe352, Tyr407 and Tyr444 receptor residues were selected to keep flexible during docking simulation using the AutoTors module. All dockings were performed as blind dockings where a cube of 40Å with grid points separated 1 Å, was centered on the FAD N5. Default parameters were used except num_modes, which was set to 40. According to Vina best scored poses, the most stable complex configurations were considered. At the end of the docking, the best poses were analyzed using Discovery Studio.

4.6. References


(6) Peitsch, M. C. Protein modeling by E-mail, Bio/Technology 1995, 13, 658-660.


(8) Samadi, A.; de los Ríos, C.; Bolea, I.; Chioua, M.; Iriepa, I.; Moraleda, I.; Bartolini, M.; Andrisano, V.; Gálvez, E.; Valderas, C.; Unzueta, M.; Marco-Contelles, J. Multipotent MAO and cholinesterase inhibitors for the


SUPPLEMENTARY MATERIAL

Multipotent cholinesterase/monoamine oxidase inhibitors for the treatment of Alzheimer’s disease: design, synthesis, biochemical evaluation, ADMET, molecular modeling and QSAR analysis of novel donepezil-pyridyl hybrids

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1. 3D-QSAR study

General

The inhibiting MAO-A/MAO-B and AChE/BuChE activities (IC$_{50}$) of 37 donepezil-indol and donepezil-pyridine derivatives were used for the QSAR study.\textsuperscript{22-24} Negative logarithm of their IC$_{50}$ i.e. (pIC$_{50}$) values were calculated. Geometry optimization for the donepezil-indol and donepezil-pyridine derivatives was performed by \textit{ab initio} Hartree-Fock/3-21G method\textsuperscript{60} included in the Gaussian 98 program.\textsuperscript{61} The selected Gaussian basis set methods have proven to be a very good choice for geometry optimization of related aromatic and organic compounds.\textsuperscript{62-66} The pKa calculation and selection of dominant molecules/cations at physiological pH 7.4 was performed for the examined compounds using the MarvinSketch 5.5.1.0 program.\textsuperscript{67} Dominant forms at pH 7.4, were used for the 3D-QSAR study. The 3D-QSAR studies of the donepezil-indol and donepezil-pyridine derivatives were performed by use of the Pentacle 1.0.6 program\textsuperscript{68} and Schrödinger-Phase software\textsuperscript{69} included in Maestro 2011 program.\textsuperscript{70} The Pentacle is advanced software tool for obtaining alignment-independent 3D quantitative structure-activity relationships. The 3D-QSAR starts from computing highly relevant 3D maps of interaction energies (GRID based Molecular Interaction Fields-MIFs) between the examined molecule and four chemical probes: DRY (which represent hydrophobic interactions), O (sp$^2$ carbonyl oxygen, representing H-bond acceptor), N1 (neutral flat NH, like in amide, H-bond donor), and the TIP probe (molecular shape descriptor). The grid spacing was set to 0.5 Å and the MACC2 smoothing window to 0.8 (for 3D-QSAR (ChE) models) and the CLACC smoothing window to 0.8 (for 3D-QSAR (MAO) models). The number of filtered nodes was set to 100 with 50% relative weights within the ALMOND discretization.
The interaction energy between the probe and the target molecule is calculated at each point as the sum of Lennard-Jones ($E_{lj}$), hydrogen bond ($E_{hb}$), electrostatic interactions ($E_{el}$), and an entropic term: \[ E_{tot} = \sum E_{lj} + \sum E_{hb} + \sum E_{el} + S. \]

The obtained maps are encoded into GRID Independent Descriptors (GRIND and GRIND2 descriptors) which are independent of the alignment of the series. The GRIND approach aims to extract the information enclosed in the MIFs and compress it into new types of variables whose values are independent of the spatial position of the molecule studied by using an optimization algorithm with the intensity of the field at a node and the mutual node–node distances between the chosen nodes as a scoring function. Such variables constitute a matrix of descriptors that are analyzed using multivariate techniques, such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression analysis. The Principal Component Analysis was used for inspection of our series and for obtaining a map of our compounds describing their similarities and differences. The variables were used for development of 3D-QSAR models by use of the PLS regression.

Based on the Score Plots (t1 vs. t2 and t1 vs. u1) the data set of 37 MAO-A/B and 26 AChE/BuChE inhibitors is divided on Training Set (21-30 compounds for QSAR models building) and Verification set (3-7 compounds for QSAR models validation). The most important pharmacophores (GRID descriptors), responsible for the MAO-A, MAO-B, AChE, and BuChE inhibition, were selected by use of the PLS regression and used for the 3D-QSAR (MAO-A, MAO-B, AChE, BuChE) models building. The formed 3D-QSAR (MAO-A, MAO-B, AChE, BuChE) models and corresponding 3D-pharmacophores were used for design and selection of novel donepezil-pyridine hybrids as promising multipotent ligands.

Quality of the obtained 3D-QSAR (MAO-A, MAO-B, AChE, BuChE) models was examined by use of: leave-one-out cross-validation ($Q^2$), correlation coefficient ($R^2$...
Observed vs. Predicted), Root Main Squared Error of Estimation (RMSEE), and external validation (Root Main Squared Error of Prediction (RMSEP)).\textsuperscript{72,73}

Predictive power of the model is determined by $Q^2$, which is leave-one-out cross-validated version of $R^2$. A model is fitted to the data leaving one compound out, selects the best variables, and predicts $Y$ for the left–out compound. This procedure is repeated until all compounds have been left out, which result in $n$ parallel models. The difference between observed and the predicted $Y$ values are calculated ($e_{(i)}$) for each model. In this setting were defined PRESS (Predicted Sum of Squares), RMSEP and $Q^2$ as:

$$PRESS = \sum_{i=1}^{n} e_{(i)}^2$$  \hspace{1cm} (1)

$$RMSEP = \sqrt{\frac{PRESS}{n}}$$  \hspace{1cm} (2)

$$Q^2 = 1 - \frac{PRESS}{SSTo}$$  \hspace{1cm} (3)

$SSTo$ - Variation, Sum of Squares (Total)

PLS models with $Q^2 \geq 0.5$ can be considered to have good predictive capability.\textsuperscript{72,74}
Experimental


Chart 2S. Structures for Donepezil and compounds DonzD-3, DonzD-4, DonzD-5 (= ASS234), DonzD-6, DonzD-7, DonzD-8 and DonzD-9.
The optimal 3D-QSAR (MAO-A) model with two significant components (A= 2), $R^2$: 0.97, and $Q^2$: 0.87, was created by use of the Pentacle 1.0.6 program (see Table 1S). Coefficient plot with the most significant variables for the MAO-A inhibiting activity, such as: $v_{287}$: TIP-TIP, $v_{342}$: TIP-TIP, $v_{892}$: N1-TIP, $v_{301}$: TIP-TIP, $v_{325}$: TIP-TIP, $v_{332}$: TIP-TIP, $v_{783}$: O-TIP, $v_{854}$: N1-TIP, and $v_{868}$: N1-TIP is depicted on Figure 1. The variables such as: $v_{287}$: TIP-TIP, $v_{342}$: TIP-TIP, $v_{892}$: N1-TIP, are positively correlated with the MAO-A inhibiting activity, while variables $v_{301}$: TIP-TIP, $v_{325}$: TIP-TIP,
TIP-TIP, v332: TIP-TIP, v783: O-TIP, v854: N1-TIP, and v868: N1-TIP, are negatively correlated with the MAO-A inhibiting activity (Figure 1S).

**Figure 1S.** The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (MAO-A) model. The specific favourable/unfavourable interactions of the MAO-A inhibiting activity are marked in squares.
Figure 2S. a) 3D-map of interaction energies of Donz-D4 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the MAO-A inhibiting activity are marked in squares.

Figure 2. b) 3D-map of interaction energies of Donz-D9 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the MAO-A inhibiting activity are marked in squares.
## Table 1S. Results of the 3D-QSAR(MAO-A) and 3D-QSAR(MAO-B) modelling.

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### Model Performance Metrics:

- **R^2**: 0.97
- **Q^2**: 0.87
- **RMSE**: 0.229
- **R^2_{Obs vs Pred}**: 0.977
- **RMSEP**: 0.578
- **R^2Obs vs Pred**: 0.804
The optimal 3D-QSAR (MAO-B) model with two significant components (A=2), $R^2$: 0.93, and $Q^2$: 0.82, was formed by use of the Pentacle 1.0.6 program (Table 1S). Coefficient plot with the most significant variables for the MAO-B inhibiting activity, such as: $v_{287}$: TIP-TIP, $v_{295}$: TIP-TIP, $v_{314}$: TIP-TIP, $v_{349}$: TIP-TIP, $v_{765}$: O-TIP, $v_{775}$: O-TIP, $v_{477}$: DRY-N1, $v_{527}$: DRY-N1, $v_{662}$: O-N1 and $v_{858}$: N1-TIP is depicted on Figure 3S. The variables such as: $v_{287}$: TIP-TIP, $v_{295}$: TIP-TIP, $v_{314}$: TIP-TIP, $v_{349}$: TIP-TIP, $v_{765}$: O-TIP, $v_{775}$: O-TIP, are positively correlated with the MAO-B inhibiting activity, while variables $v_{477}$: DRY-N1, $v_{527}$: DRY-N1, $v_{662}$: O-N1, and $v_{858}$: N1-TIP, are negatively correlated with the MAO-B inhibiting activity (Figure 3S).

![Figure 3S. The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (MAO-B) model. The specific favorable/unfavorable interactions of the MAO-B inhibiting activity are marked in squares.](image-url)
Figure 4S. a) 3D-map of interaction energies of Donz-D3 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the MAO-B inhibiting activity are marked in squares.

Figure 4S. b) 3D-map of interaction energies of Donz-D5 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the MAO-B inhibiting activity are marked in squares.

The optimal 3D-QSAR (AChE) model with three significant components (A=3), R²: 0.99, and Q²: 0.87, was created by use of the Pentacle 1.0.6 program (Table 1S). Coefficient plot with the most significant variables for the AChE inhibiting activity, such as: v289: TIP-TIP, v603: DRY-TIP, v688: O-N1, v785: O-TIP, v895: N1-TIP, v133: O-O, v334: TIP-TIP, v678: O-N1, v884: N1-TIP, and v909: N1-TIP is depicted on Figure 5S. The variables such as: v289: TIP-TIP, v603: DRY-TIP, v688: O-N1,
v785: O-TIP, and v895: N1-TIP, are positively correlated with the AChE inhibiting activity, while variables v133: O-O, v334: TIP-TIP, v678: O-N1, v884: N1-TIP, and v909: N1-TIP, are negatively correlated with the AChE inhibiting activity (Figure 5S).

**Figure 5S.** The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (AChE) model. The specific favorable/unfavorable interactions of the AChE inhibiting activity are marked in squares.
Figure 6S. a) 3D-map of interaction energies of DPH8 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the AChE inhibiting activity are marked in squares.

Figure 6S. b) 3D-map of interaction energies of DPH8 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the AChE inhibiting activity are marked in squares.
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The optimal 3D-QSAR (BChE) model with two significant components (A=2), R^2: 0.92, and Q^2: 0.78, was formed by use of the Pentacle 1.0.6 program (Table 2S). Coefficient plot with the most significant variables for the AChE inhibiting activity, such as: v317: TIP-TIP, v344: TIP-TIP, v351: TIP-TIP, v625: DRY-TIP, v690: O-N1, v811: O-TIP, v894: N1-TIP, v525: DRY-N1, v884: N1-TIP, and v907: N1-TIP is
depicted on Figure 7S. The variables such as: v317: TIP-TIP, v344: TIP-TIP, v351: TIP-TIP, v625: DRY-TIP, v690: O-N1, v811: O-TIP, and v894: N1-TIP, are positively correlated with the AChE inhibiting activity, while variables N1-TIP, v525: DRY-N1, v884: N1-TIP, and v907: N1-TIP, are negatively correlated with the AChE inhibiting activity (Figure 7S).

Figure 7S. The PLS-Coefficient plot and the most important variables of the created 3D-QSAR (BChE) model. The specific favorable/unfavorable interactions of the BChE inhibiting activity are marked in squares.
Figure 8S. a) 3D-map of interaction energies of DPH3 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the BChE inhibiting activity are marked in squares.

Figure 8S. b) 3D-map of interaction energies of DPH4 with DRY-probe (yellow), TIP-probe (green), O-probe (red), and N1-probe (blue). Favorable interactions are depicted with red lines, while unfavorable interactions are presented with blue lines. The specific favorable/unfavorable interactions of the BChE inhibiting activity are marked in squares.
References


2. Molecular Modeling

*Molecular docking into AChE and BuChE*

Compound DPH14 was assembled within Discovery Studio, version 2.1, software package, using standard bond lengths and bond angles. With the CHARMM force field\(^7\) and partial atomic charges, the molecular geometry of DPH14 was energy-minimized using the adopted-based Newton-Raphson algorithm. Structures were considered fully optimized when the energy changes between iterations were less than 0.01 kcal/mol.\(^7\)

*Molecular docking of compound DPH14 into EeAChE*

The coordinates of *Electrophorus electricus* AChE (PDB ID: 1C2B), were obtained from the Protein Data Bank (PDB). For docking studies, initial protein was prepared by removing all water molecules, heteroatoms, any co-crystallized solvent and the ligand. Proper bonds, bond orders, hybridization and charges were assigned using protein model tool in Discovery Studio, version 2.1, software package. CHARMM force field was applied using the receptor-ligand interactions tool in Discovery Studio, version 2.1, software package. Docking calculations were performed with the program Autodock Vina.\(^3\) AutoDockTools (ADT; version 1.5.4) was used to add hydrogens and partial charges for proteins and ligands using Gasteiger charges. Flexible torsions in the ligands were assigned with the AutoTors module, and the acyclic dihedral angles were allowed to rotate freely. Trp286, Tyr124, Tyr337, Tyr72, Asp74, Thr75, Trp86, and Tyr341 receptor residues were selected to keep flexible during docking simulation using the AutoTors module. Because VINA uses rectangular boxes for the binding site, the box center was defined and the docking box was displayed using ADT. For *Electrophorus electricus* AChE (PDB ID: 1C2B) the docking procedure was applied to whole protein target, without imposing the binding site (“blind docking”). Using the GridBox option, the 3-dimensional parameters for docking the ligand to the protein were determined; the
grid center coordinates were x = 21.5911, y = 87.752, z = 23.591 and the size coordinates were x = 60, y = 60, z = 72 with grid points separated 1 Å. Default parameters were used except num_modes, which was set to 40. The AutoDock Vina docking procedure used was previously validated.77

*Molecular docking of inhibitor DPH14 into eqBuChE*

The eqBuChE model has been retrieved from the SWISS-MODEL Repository. This is a database of annotated three-dimensional comparative protein structure models generated by the fully automated homology-modeling pipeline SWISS-MODEL. A putative three-dimensional structure of eqBuChE has been created based on the crystal structure of hBuChE (PDB ID: 2PM8), these two enzyme exhibited 89% sequence identity. Proper bonds, bond orders, hybridization and charges were assigned using protein model tool in Discovery Studio, version 2.1, software package. CHARMM force field was applied using the receptor-ligand interactions tool in Discovery Studio, version 2.1, software package. Docking calculations were performed following the same protocol described before for EeAChE. All dockings were performed as blinds dockings where a cube of 75 Å with grid points separated 1 Å, was positioned at the middle of the protein (x = 29.885; y = -54.992; z = 58.141). Default parameters were used except num_modes, which was set to 40. The lowest docking-energy conformation was considered as the most stable orientation. Finally, the docking results generated were directly loaded into Discovery Studio, version 2.1.

*Molecular docking of compound DPH14 into hMAO A/B*

Compound DPH14 was assembled as non-protonated amine within Discovery Studio, version 2.1, software package, following the procedure described before for cholinesterases. The crystal structures of human MAO A in complex with harmine (PDB ID 2Z5X) and human MAO B in complex with safinamide (PDB ID 2V5Z) were obtained from the Protein Data Bank. For docking studies initial proteins were prepared.
First, in the PDB crystallographic structures any co-crystallized solvent and the ligand were removed. Then, proper bonds, bond orders, hybridization and charges were assigned using protein model tool in Discovery Studio, version 2.1, software package. CHARMM force field was applied using the receptor-ligand interactions tool in Discovery Studio, version 2.1, software package. Six water molecules located around the FAD cofactor were considered in the docking experiments because of their well-known role into the MAO’s inhibition. Finally, atoms of the FAD cofactor were defined in their oxidized state. Docking calculations were performed following the same protocol described before for EeAChE. For MAO A, the grid center coordinates were x= 54.181, y= 160.270, z= 22.052 and the size coordinates were x= 50, y = 40, z = 40 with grid points separated 1 Å; for MAO B, the grid center coordinates were x= 55.743, y= 161.175, z= 19.420 and the size coordinates were x= 46, y = 40, z = 40 with grid points separated 1 Å. Default parameters were used except num_modes, which was set to 40. According to Vina best scored poses, the most stable complex configurations were considered. The docked ligand output files was viewed and atomic distances and interactions were analyzed using Discovery Studio.

References


3. ADMET analysis

Some relevant ADME (Absorption, Distribution, Metabolism and Excretion) properties have been calculated, with special emphasis on the requirements of the central nervous system (CNS). The drugs used for neurological disorder treatment, such as AD, are generally CNS acting drugs, so factors that are important to the success of CNS drugs were analyzed. In particular, the new molecules should present a good CNS penetration profile and low toxic effects. Computer predictions were performed with ADMET Predictor 6.5\textsuperscript{45} and ACD/Percepta 14.0.0\textsuperscript{46} software packages.

References

46. ACD/Percepta 14.0.0, Advanced Chemistry Development, 2013.
Table 3S. Predicted ADMET properties for compounds DPHs1-6, 9-16

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\textsuperscript{a} AMET Predictor, v. 6.5; \textsuperscript{b} ACD/Percepta 14.0; \textsuperscript{c} Moriguchi model (ref.[48]); \textsuperscript{d} According to the classification made by Ma et al [51]: High absorption to CNS: logBB more than 0.3; Middle absorption to CNS: logBB 0.3 ~ -1.0; Low absorption to CNS: logBB less than -1.0. \textsuperscript{e} Other estimated parameters related to brain penetration were used to classify the compounds as CNS permeable or non-permeable: rate of brain penetration (LogPS) is the rate of passive diffusion/permeability; brain/plasma equilibration rate (Log(PS*fu, brain)) = LogPS*fu,brain – fraction unbound in plasma.
4. Synthesis of the intermediate and target molecules

General procedure for the preparation of hydrochlorides. Hydrochlorides were prepared by dissolving the compounds in a minimum quantity of ethyl ether, and adding dropwise a saturated solution of HCl(g) in ethyl ether. A white solid was formed immediately, that was separated by filtration, washed with ether and dried.

3-(1-Benzylpiperidin-4-yl)propan-1-amine (21).[^30] DPPA (1.39 mL, 6.428 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-en DBU (0.961 mL, 6.428 mmol) were added dropwise to a cooled (0 °C) solution of 1-benzyl-4-(4-hydroxyalkyl)piperidine[^59] (0.5 g, 2.143 mmol) in dry DMF (20 mL), under argon. After 30 min, NaN₃ (0.417 g, 6.428 mmol) was added. Then, the cooling bath was removed and the resulting solution was heated at 100 °C for 2 h. After cooling at room temperature, the reaction was diluted with CH₂Cl₂, washed with water, and the organic layer was washed with brine. After drying over sodium sulphate, filtration, and evaporation, the crude was purified by column chromatography (hexane/EtOAc, 4/1 to 1/1) to give compound 4-(3-azidopropyl)-1-benzylpiperidine (0.39 g, 70%) as colorless oil: IR (KBr) ν 3338, 2928, 2095, 1491, 1453, 1270 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.33-7.21 (m, 5H, NCH₂C₆H₅), 3.47 (s, 2H, NC₆H₂C₆H₅), 3.22 (t, J = 7.0 Hz, 2H, CH₂N₃), 2.86 [d, J= 11.5 Hz, 2H, BnN(CH₅(CH₆)₂(CH₂)₂CH)], 1.91 [t, J = 10.9 Hz, 2H, BnN(CH₅(CH₆)₂(CH₂)₂CH)], 1.64-1.53 (m, 4H). 1.31-1.16 (m, 5H); ¹³C NMR (100 MHz, CDC₁₃) δ 138.4 (C₁', C₆H₅), 129.5 (2xCH, C₆H₅), 128.0 (2xCH, C₆H₅), 126.8 (C₄', C₆H₅), 63.4 (NCH₂C₆H₅), 53.7 [2C, BnN(CH₂)₂(CH₂)₂CH], 51.6 (CH₂N₃), 35.3 [CH₂(CH₂)₂N₃], 33.5 [BnN[(CH₂)₂(CH₂)₂CH]], 32.2 [2C, BnN(CH₂)₂(CH₂)₂CH], 26.1 [(CH₂)₂CH₂(CH₂)N₃]; MS (IE) m/z (%): 91 (100) [PhCH₂]^+, 202 (15) [M-CH₂N₃]^+, 216 (19) [M-N₃]^+, 230 (8) [M-N₂]^+, 258 (2) [M]^+. HRMS (ESI): Calcd for C₁₅H₂₃N₄ ([M+H]^+): 259.1923. Found: 259.1910. Water (0.163 mL, 9.06 mmol, 6 equiv), and triphenylphosphine PPh₃ (0.6 g, 2.26 mmol, 1.5 equiv) were added to a solution of 4-(3-azidoalkyl)-1-benzylpiperidine (0.390 g, 1.51 mmol) in dry THF (10 mL). The reaction mixture was heated at reflux for 2 h 30 min. After complete reaction, the solvent was evaporated and the residue was purified by column chromatography using CH₂Cl₂/MeOH (5/1) then CH₂Cl₂/Methanol/TEA (100/20/1) to give compound 21[^30] (0.300 g, 86%) as a colorless oil: IR (KBr) ν 2917, 2799, 1574, 1494, 1454 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.29-7.21 (m, 5H, NCH₂C₆H₅), 3.46 (s, 2H, NCH₂C₆H₅), 2.85 [d, J= 10.7 Hz, 2H, BnN(CH₅(CH₆)₂(CH₂)₂CH)], 2.65 (t, J= 7.1 Hz, 2H, CH₂NH₂), 2.26 (br s, 2H, NH₂) 1.89
(t, J = 10.7 Hz, 2H, BnN(CH$_2$H$_{eq}$)$_2$(CH$_2$)$_2$CH], 1.63-1.61 (m, 2H), 1.47-1.39 (m, 2H),
1.23-1.20 (m, 5H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 138.3 (C1’, C$_6$H$_5$), 129.1 (2xCH,
C$_6$H$_5$), 126.8 (2xCH, C$_6$H$_5$), 126.7 (C4’, C$_6$H$_5$), 63.4 (NCH$_2$C$_6$H$_5$), 53.8 [2C, BnN(CH$_2$)$_2$(CH$_2$)$_2$CH], 42.1 (CH$_2$NH$_2$), 35.5 [BnN (CH$_2$)$_2$(CH$_2$)$_2$CH], 33.9 [CH$_2$-
(CH$_2$)$_2$NH$_2$], 32.2 [2C, BnN(CH$_2$)$_2$(CH$_2$)$_2$CH], 30.4 (CH$_2$-CH$_2$NH$_2$); MS (EI) m/z (%):
91 (100) [PhCH$_2$]+, 141 (33) [M-Bn]$^+$, 188 (10) [M-(CH$_2$)$_2$NH$_2$]$^+$, 202 (11) [M-
CH$_2$NH$_2$]$^+$, 216 (3) [M-NH$_2$]$^+$, 232 (10) [M]$^+$.

General procedure for the synthesis of DPHs9-16, and compounds 19, 20, 23 and
25. 2,6-Dichloropyridine-3,5-dicarbonitriles $^{17-25}$ and $^{18-26}$ were suspended in a mixture
of THF/EtOH (2:1). Then, the corresponding amines $^{21,30}$ $^{22,29}$ and $^{24-31}$ followed by
triethylamine (1.5 equiv), were added, and the mixture was refluxed. After cooling, the
solvent was removed and the resulting crude submitted to flash column chromatography, to give the desired compounds.

2-Chloro-6-(methyl(prop-2-yn-1-yl)amino)-4-phenylpyridine-3,5-dicarbonitrile
(19). Following the General Procedure, reaction of 2,6-dichloro-4-phenylpyridine-3,5-
dicarbonitrile (17) (0.766 g, 2.795 mmol) with N-methylprop-2-yn-1-amine (0.232 mL,
2.795 mmol) and triethylamine (0.85 mL, 5.6 mmol) in EtOH/THF (30 mL), after 5 h, and column chromatography (hexane:EtOAc, 8:1) gave unreacted starting material 17
(68.3 mg) and product 19 (0.692 g, 87%) as yellow solid ($R_f$ = 0.22, hexane/AcOEt, 4:1):
mp 116-8 °C; IR (KBr) ν 3253, 2953, 2220, 2114, 1562, 1530, 1489, 1415, 1340, 1260,
1210 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.56-7.45 (m, 5H, C$_6$H$_5$), 4.55 (d, J = 2.5 Hz,
2H, NCH$_2$C≡CH), 3.51 (s, 3H, NCH$_3$), 2.33 (t, J = 2.5 Hz, 1H, NCH$_2$C≡CH); $^{13}$C NMR
(100 MHz, CDCl$_3$) δ 163.2 (C4), 158.2 (C6), 154.8 (C2), 133.2 (C1’, C$_6$H$_5$), 131.1
(C4’, C$_6$H$_5$), 129.0 (2xCH, C$_6$H$_5$), 128.5 (2xCH, C$_6$H$_5$), 115.5 and 114.2 (2xCN), 99.3
and 90.2 (C3, C5), 77.2 (C≡CH), 73.3 (CH≡C), 41.5 (NCH$_2$C≡CH), 38.8 (NCH$_3$); MS
(IE): 291.00 (37) [M-CH$_3$]$^+$, 305.05 (100) [M-H]$^+$, 306.10 (41) [M]$^+$. Anal. Calcd. for
C$_{17}$H$_{11}$ClN$_4$: C, 66.56; H, 3.61; N, 18.26; Cl, 11.56. Found: C, 66.84; H, 3.67; N, 18.31;
Cl, 11.65.

2-Chloro-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile
(20). Following the General Procedure, reaction of 2,6-dichloropyridine-3,5-dicarbonitrile
(18) (0.89 g, 4.495 mmol) with N-methylprop-2-yn-1-amine (0.37 mL, 4.495 mmol) and
triethylamine (1.36 mL, 8.99 mmol) in EtOH/THF (45 mL), after 90 min, and column chromatography (hexane/AcOEt, from 9:1, to 8:2) gave product 20 and 2,6-bis(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile. 20 (white solid; 0.677 g, 66%): Rf = 0.27 (hexane/AcOEt, 4/1); mp 71-3 °C; IR (KBr) ν 3293, 3264, 2227, 1595, 1548, 1494, 146, 1348, 1284, 1264, 1124 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.97 [s, 1H, C(4)H], 4.54 (d, J= 2.49 Hz, 2H, NCH₂C≡CH), 3.51 (s, 3H, NCH₃), 2.33 (t, J= 2.48 Hz, 1H, NCH₂C=CH); ¹³C NMR (100 MHz, CDCl₃) δ 157.1 (C₆), 154.7 (C₂), 150.3 (C₄), 116.3, 116.4 (2xCN), 98.4, 89.9 (C₅, C₃), 77.2 (HC=CH), 73.7 (CH=CH), 41.8 (NCH₂C=CH), 38.4 (NCH₃); MS (EI) m/z (%): 162 (21) [M-CH₃NCH₂C≡CH]+, 191 (18) [M-CH₂C=CH]+, 215 (100) [M]+. Anal. Calcd. for C₁₁H₇ClN₄: C, 57.28; H, 3.06; Cl, 15.37; N, 24.29. Found: C, 57.43; H, 3.12; Cl, 14.73; N, 24.02.

2,6-Bis(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (white solid; 0.263 g, 21%): Rf = 0.20 (hexane/AcOEt, 4/1); mp 92-5 °C; IR (KBr) ν 3291, 2208, 1600, 1555, 1514, 1412, 1347 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.80 (s, 1H, CH₄), 4.40 (d, J= 2.4 Hz, 4H, 2xNC₂H₄C≡CH), 3.42 (s, 6H, 2xNCH₃), 2.24 (t, J= 2.4 Hz, 2H, 2xNCH₂C=CH); ¹³C NMR (100 MHz, CDCl₃) δ 156.8 (2C, C₂, C₆), 152.7 (C₄), 81.5 (C₅, C₃), 78.4 (2C, CH=CH), 72.1 (2C, CH=CH), 40.8 (2C, NCH₂C=CH), 37.9 (2C, NCH₃); MS (EI) m/z (%): 224 (53) [M-CH₂C≡CH]+, 248 (45) [M-Me]+, 262 (100) [M-H]+, 263 (81) [M]+. Anal. Calcd. for C₁₅H₁₃N₅: C, 68.42; H, 4.98; N, 26.60. Found: C, 68.19; H, 4.75; N, 26.37.

2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-6-(methyl(prop-2-yn-1-yl)amino)-4-phenylpyridine-3,5-dicarbonitrile (DPH9). Following the General Procedure, reaction of 2-chloro-6-(methyl(prop-2-yn-1-yl)amino)-4-phenylpyridine-3,5-dicarbonitrile (19) (0.144 g, 0.47 mmol) with 4-(1-benzylpiperidin-4-yl)butan-1-amine (22) (0.11 mg, 0.47 mmol) and triethylamine (0.130 mL) in EtOH/THF (8 mL), after 10 h, and column chromatography (from 1.5% to 10% MeOH/CH₂Cl₂), gave product DPH9 (0.213 g, 97%) as yellow oil: Rf = 0.35 (CH₂Cl₂/MeOH), 10/1, v/v); IR (KBr) ν 3348, 3303, 2926, 2204, 1566, 1510, 1403 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.52–7.44 (m, 5H, C₆H₅), 7.33–7.22 (m, 5H, C₆H₅), 5.63 (d, J= 5.6 Hz, 1H, NH), 4.42 (d, J= 2.4 Hz, 2H, NCH₂C=CH), 3.53-3.48 (m, 4H, CH₂NH + NCH₂C₆H₅), 3.46 (s, 3H, NCH₃), 2.89 [d, J= 11.1 Hz, 2H, BnN(CH₃Heq)₂(CH₂)₂CH], 2.25 (t, J= 2.4 Hz, 1H, NCH₂C=CH), 1.93 [t, J= 10.8 Hz, 2H, BnN(CH₃Heq)₂(CH₂)₂CH], 1.67-1.60 (m, 4H), 1.42-1.32 (m, 2H), 1.31-1.21 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 161.9 (C₄), 160.3 (C₆), 157.5 (C₂), 157.2 (C₆), 154.3 (C₂), 150.3 (C₄), 116.3, 116.4 (2xCN), 98.4, 89.9 (C₅, C₃), 77.2 (HC=CH), 73.7 (CH=CH), 41.8 (NCH₂C=CH), 38.4 (NCH₃); MS (EI) m/z (%): 162 (21) [M-CH₃NCH₂C≡CH]+, 191 (18) [M-CH₂C=CH]+, 215 (100) [M]+. Anal. Calcd. for C₁₅H₁₃N₅: C, 68.42; H, 4.98; N, 26.60. Found: C, 68.19; H, 4.75; N, 26.37.
138.2 (C1’, NCH2C6H5), 134.7 (C1’, C6H5), 130.2 (C4’, C6H5), 129.2 (2xCH, C2’ and C6’, NCH2C6H5), 128.6 (2xCH, C6H5), 128.4 (2xCH, C6H5), 128.0 (2xCH, C3’ and C5’, NCH2C6H5), 126.8 (C4’, NCH2C6H5), 117.8 and 116.4 (2xCN), 82.9 (C3), 80.8 (C5), 78.7 (NCH2C≡CH), 71.9 (NCH2C≡CH), 63.4 (NCH2C6H5), 53.8 [2C, BnN(CH2)2(CH2)2CH], 41.7 (CH2NH), 41.0 (NCH2C≡CH), 38.7 (NCH3), 36.1 [CH2(CH2)2NH], 35.5 [BnN(CH2)2(CH2)2CH], 29.4 (CH2CH2NH), 24.1 [CH2(CH2)2NH]. HRMS (ESI): Calcd for C33H37N6 ([M+H]+): 517.3080. Found: 517.3073. 

DPH9.HCl: Anal. Calcd. for C33H37ClN6: C, 71.66; H, 6.74; N, 15.19; Cl, 6.41. Found: C, 71.73; H, 6.69; N, 15.21; Cl, 6.30.

2-Chloro-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (23). Following the General Procedure, reaction of 2,6-dichloro-4-phenylpyridine-3,5-dicarbonitrile (17) (0.81 g, 2.96 mmol) with prop-2-yn-1-amine (0.095 mL, 1.48 mmol) and triethylamine (0.81 mL, 5.92 mmol) in EtOH/THF (10 mL), after 16 h, and column chromatography (hexane:EtOAc, 8:1) to give product 23 (0.76 g, 88%) as yellow solid: 

Rf = 0.32 (hexane/AcOEt, 4:1); mp 190-2 °C; IR (KBr) v 3435, 3346, 3301, 2916, 2223, 1574, 1551, 1506, 1453, 1318 cm⁻¹; ¹H NMR (300 MHz, CDCl3) δ 7.77-7.38 (m, 5H, C6H5), 6.04 (br s, 1H, NH), 4.41 (dd, J = 5.3, 2.5 Hz, 2H, NCH2C≡CH), 2.36 (t, J = 2.5 Hz, 1H, CH2C≡CH). HRMS (ESI): Calcd for C16H9N4Cl ([M+H]⁺): 293.0589. Found: 293.0600.

2-((2-(1-Benzylpiperidin-4-yl)ethyl)amino)-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH10). Following the General Procedure, reaction of 2-chloro-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (23) (0.1 g, 0.37 mmol) with 2-(1-benzylpiperidin-4-yl)ethan-1-amine (24) (82 mg, 0.37 mmol) and triethylamine (0.10 mL) in EtOH/THF (10 mL), after overnight, and column chromatography (from 1.5% to 10% MeOH/CH2Cl2), gave product DPH10 (151 mg, 85%) as yellow solid: 

Rf = 0.33 (CH2Cl2/MEOH, 10/1); mp 166-170 °C; IR (KBr) v 3428, 3027, 2925, 2202, 1586, 1568, 1532, 1451, 1344 cm⁻¹; ¹H NMR (500 MHz, CDCl3) δ 7.52-7.46 (m, 5H, C6H5), 7.35-7.29 (m, 5H, C6H5), 5.75 (t, J = 5.4 Hz, 1H, NHCH2C≡CH), 5.65 (t, J = 5.7 Hz, 1H, NHCH2), 4.32-4.27 (m, 2H, NCH2C≡CH), 3.62-350 (m, 4H, CH2NH + NCH2C6H5), 2.93 [2H, BnN(CHaxHeq)2(CH2)2CH], 2.27 (t, J = 2.5 Hz, 1H, NCH2C≡CH), 1.72 (d, J = 9.8 Hz, 2H, BnN(CHaxHeq)2(CH2)2CH), 1.65-1.59 (m, 4H), 1.43-1.33 (m, 3H); ¹³C NMR (126 MHz, CDCl3) δ 159.3 (C6), 158.9 (C4), s28
158. (C2), 134.2, 130.5, 129.38, 128.85, 128.25 (NCH₂C₆H₅, C₆H₅), 116.6 and 116.4
(2xCN), 81.6 (C5), 81.0 (C3), 79.2 (NCH₂C≡CH), 71.8 (NCH₂C≡CH), 63.2
(NCH₂C₆H₅), 53.5 [2C, BnN(CH₂)₂(CH₂)₂CH], 39.3 (CH₂NH), 35.8 (CH₂-CH₂NH),
33.2 [BnN(CH₂)₂(CH₂)₂CH], 31.9 [2C, BnN(CH₂)₂(CH₂)₂CH], 31.1 (NCH₂C≡CH).

**DPH10.HCl**: Calcd. for C₃₀H₃₁ClN₆.5/4H₂O: C, 67.53; H, 6.33; N, 15.75. Found: C,
67.47; H, 6.14; N, 15.90.

2-((3-(1-Benzylpiperidin-4-yl)propyl)amino)-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH11). Following the General Procedure, reaction of 2-chloro-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile 23 (0.16 g, 0.56 mmol) with 3-(1-benzylpiperidin-4-yl)propan-1-amine 21 (130 mg, 0.56 mmol) and triethylamine (0.16 mL) in EtOH/THF (15 mL), after 17 h, and column chromatography (from 1.5% to 10% MeOH/CH₂Cl₂), gave product **DPH11** (0.21 g, 89%): mp 134-6⁰C; IR (KBr) ν 3746, 3351, 2927, 2799, 2758, 2202, 1568, 1532, 1465,
1343, 1291, 1145 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.53-7.47 (m, 5H, C₆H₅), 7.34-
7.28 (m, 5H, C₆H₅), 5.76 (t, J= 5.4 Hz, 1H, NHCH₂C≡CH), 5.70 (t, J= 5.7 Hz, 1H,
NHCH₂), 4.30 (dd, J= 5.4 Hz, 2.5 Hz, 2H, NHCH₂C≡CH), 3.66-3.40 (m, 4H, C₆H₅NH +
NCH₂C₆H₅), 2.90 (d, J= 10.5 Hz, 2H, BnN(CH₂H₃)₂(CH₂)₂CH], 2.26 (t, J= 2.5 Hz,
1H, NCH₂C≡CH), 2.01-1.90 (m, 2H, BnN(CH₂H₃)₂(CH₂)₂CH], 1.74–1.58 (m, 4H),
1.40-1.13 (m, 5H); ¹³C NMR (126 MHz, CDCl₃) δ 159.4 (C₆), 158.9 (C₄), 158.8 (C₂), 138.3, 134.2, 130.4, 129.2, 128.8, 128.2, 128.1 (C₆H₅, NCH₂C₆H₅), 116.6 and
116.5 (2xCN), 81.6 (C5), 80.9 (C3), 79.21 (NCH₂C≡CH), 71.8 (NCH₂C≡CH), 63.4
(NCH₂C₆H₅), 53.7 [2C, BnN(CH₂)₂(CH₂)₂CH], 42.0 (CH₂NH), 35.5
[BnN(CH₂)₂(CH₂)₂CH], 33.7 [CH₂CH₂CH₂NH], 32.2 [2C, BnN(CH₂)₂(CH₂)₂CH], 31.1
(NCH₂C≡CH), 26.6 [CH₂(CH₂)₂NH]. HRMS (ESI): Calcd for C₃₁H₃₂N₆ ([M+H]+):
70.11; H, 6.39; N, 15.82. Found: C, 70.21; H, 6.24; N, 16.06.

2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH12). Following the General Procedure, reaction of 2-chloro-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile 23 (0.16 g, 0.57 mmol) with 4-(1-benzylpiperidin-4-yl)butan-1-amine 22 (142 mg, 0.57 mmol) and triethylamine (0.16 mL) in EtOH/THF (12 mL), after overnight, and column chromatography (from 1.5% to 10% MeOH/CH₂Cl₂), gave product **DPH12** (0.27 g,
96%) as yellow solid: \( R_f = 0.42 \) (hexane/AcOEt, 4/1); mp 157-9 °C; IR (KBr) ν 3348, 3303, 2926, 2204, 1566, 1510, 1403 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 7.53-7.45 (m, 5H, C\(_6\)H$_5$), 7.34-7.28 (m, 5H, C\(_6\)H$_5$), 5.74 (t, \( J = 5.4 \) Hz, 1H, NHCH$_2$C≡CH), 5.68 (t, \( J = 5.7 \) Hz, 1H, NHCH$_2$C≡CH), 4.31 (dd, \( J = 5.4, 2.5 \) Hz, 2H, NCH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 3.61-3.42 (m, 4H, C\(_6\)H$_2$NH + C\(_6\)H$_2$CH$_2$), 2.89 [d, \( J = 10.0 \) Hz, 2H, BnN(CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 1.95 (m, 2H, BnN(CH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 1.64-1.53 (m, 2H), 1.45-1.33 (m, 4H), 1.34-1.16 (m, 5H); \(^1\)C NMR (126 MHz, CDCl\(_3\)) δ 159.4 (C\(_6\)H$_5$), 158.9 (C\(_4\)H$_4$), 158.8 (C\(_2\)H$_2$), 138.3, 134.2, 130.5, 129.2, 128.8, 128.2, 128.1, 126.9 (C\(_6\)H$_5$, NCH$_2$C\(_6\)H$_5$), 116.6 and 116.5 (2 x CN), 81.6 (C5), 80.9 (C3), 79.2 (NCH$_2$C≡CH), 71.8 (NCH$_2$C≡CH), 63.4 (NCH$_2$C\(_6\)H$_5$), 53.8 [2C, BnN(CH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 41.7 (CH$_2$N), 36.2 [CH$_2$(CH$_2$)$_3$N], 35.5 [BnN(CH$_2$)$^2$(CH$_2$)$_2$CH], 32.2 (2C, BnN(CH$_2$)$^2$(CH$_2$)$_2$CH)], 31.18 (NCH$_2$C≡CH), 29.5 (CH$_2$CH$_2$NH), 24.1 [CH$_2$+(CH$_2$)$_3$NH]. HRMS (ESI): Calcd for C$_{32}$H$_{34}$N$_6$ ([M+H]$^+$): 503.2918. Found: 503.2920. **DPH12.HCl** Anal. Calcd. for C$_{32}$H$_{35}$ClN$_6$.H$_2$O: C, 68.99; H, 6.69; N, 15.08. Found: C, 68.84; H, 6.48; N, 15.37.

**2-((3-(1-Benzylpiperidin-4-yl)propyl)amino)-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (DPH13).** Following the General Procedure, reaction of 2-chloro-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (20) (0.13 g, 0.56 mmol) with 3-(1-benzylpiperidin-4-yl)propan-1-amine (21) (130 mg, 0.56 mmol) and triethylamine (0.16 mL) in EtOH/THF (8 mL), after 27 h, and column chromatography (from 1.5% to 10% MeOH/CH$_2$Cl$_2$), gave product **DPH13** (0.21 g, 93%) as yellow solid: \( R_f = 0.42 \) (hexane/AcOEt, 4/1); mp 157-9 °C; IR (KBr) ν 3365, 3281, 2932, 2851, 2757, 2210, 1575, 1514, 1409, 1310 cm\(^{-1}\); \(^1\)H NMR (500 MHz, CDCl\(_3\)) δ 7.69 (s, 1H), 7.32-7.28 (m, 5H, C\(_6\)H$_5$), 5.46 (t, \( J = 5.7 \) Hz, 1H, NH), 4.41 (d, \( J = 2.0 \) Hz, 2H, BnN(CH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 3.52-3.44 (m, 4H, C\(_6\)H$_2$NH + NCH$_2$Ph), 3.44 (s, 3H, NCH$_3$), 2.88 [d, \( J = 11.1 \) Hz, 2H, BnN(CH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 2.23 (t, \( J = 2.4 \) Hz, 1H, NCH$_2$C≡CH), 1.99-1.87 [m, 2H, BnN(CH$_2$CH$_2$CH$_2$CH$_2$C≡CH), 1.69-1.59 (m, 4H), 1.35-1.27 (m, 5H); \(^1\)C NMR (126 MHz, CDCl\(_3\)) δ 158.71 (C\(_2\)), 157.44 (C\(_6\)), 149.20 (C\(_4\)), 138.2 (C1, Bn), 129.24 (2 x CH, C2 and C6, Bn), 128.14 (2 x CH, C3 and C5, Bn), 126.96 (C4, Bn), 118.55 and 116.18 (2 x CN), 81.76 (C5), 79.72 (C3), 78.48 (C=CH), 72.16 (C=CH), 63.40 (CH$_2$Ph), 53.78 (2C, 2CH$_2$), 41.92 (CH$_2$NH), 40.73 (CH$_2$-C=), 38.06 (NCH$_3$), 35.52 (CH), 33.79 (CH$_2$-(CH$_2$)$_2$NH), 32.25 (2C, 2CH$_2$), 26.61 (CH$_2$-CH$_2$-CH$_2$NH). HRMS (ESI): Calcd for C$_{26}$H$_{30}$N$_6$ ([M+H]$^+$): 427.2625. Found: 427.2612.
DPH13.HCl: Anal. Calcd. for C_{26}H_{30}N_{6}HCl.1/5 H_{2}O: C, 66.92; H, 6.78; N, 18.01.
Found: C, 66.91; H, 6.60; N, 18.19.

2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (DPH14). Following the General Procedure, reaction of 2-chloro-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (20) (0.13 g, 0.56 mmol) with 4-(1-benzylpiperidin-4-yl)butan-1-amine (22) (140 mg, 0.56 mmol) and triethylamine (0.15 mL) in EtOH/THF (8 mL), after 42 h, and column chromatography (from 1.5% to 10% MeOH/CH2Cl2), gave product DPH14 (0.2 g, 81%) as yellow solid: $R_f$ = 0.42 (hexane/AcOEt, 4/1); mp 82-4 ºC; IR (KBr) $\tilde{\nu}$ 3348, 3303, 2926, 2204, 1566, 1510, 1403 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.69 (s, 1H, $H_4$), 7.34-7.19 (m, 5H, C$_6$H$_5$), 5.43 (t, $J$ = 5.9 Hz, 1H, NH), 4.41 (d, $J$= 2.5 Hz, 2H, NCH$_2$C≡CH), 3.55-3.45 (m, 4H, C$_6$H$_2$NH + NCH$_2$C$_6$H$_5$), 3.44 (s, 3H, NCH$_3$), 2.87 (d, $J$ = 10.7 Hz, 2H, BnN(CH$_2$Heq)$_2$(CH$_2$)$_2$CH), 2.23 (t, $J$= 2.4 Hz, 1H, NCH$_2$C≡CH), 1.92 (m, 2H, BnN(CH$_2$Heq)$_2$(CH$_2$)$_2$CH), 1.67–1.54 (m, 4H), 1.36 (m, 2H), 1.30–1.15 (m, 5H); $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 158.7 (C$_6$), 157.4 (C$_2$), 149.1 (C4), 129.2 (2 CH, C2’, C6’, NCH$_2$C$_6$H$_5$), 128.1 (2 CH, C3’, C5’, NCH$_2$C$_6$H$_5$), 126.8 (C4’, NCH$_2$C$_6$H$_5$), 118.5, 116.1 (2C, 2xCN), 81.7 (C3), 79.7 (C5), 78.4 (NCH$_2$C≡CH), 72.1 (NCH$_2$C≡CH), 63.5 (NCH$_2$C$_6$H$_5$), 53.8 [2C, BnN(CH$_2$)$_2$(CH$_2$)$_2$CH], 41.6 (CH$_2$NH), 40.7 (NCH$_2$C≡CH), 38.0 (NCH$_3$), 36.2 [CH$_2$(CH$_2$)$_2$NH], 35.6 [BnN(CH$_2$)$_2$(CH$_2$)$_2$CH], 32.3 [2C, BnN(CH$_2$)$_2$(CH$_2$)$_2$CH], 29.4 (CH$_2$CH$_2$NH), 24.1 [CH$_2$-(CH$_2$)$_2$NH]. HRMS (ESI): Calcd for C$_{27}$H$_{32}$N$_6$([M+H]$^+$): 441.2761. Found: 441.2752.

2-Chloro-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (25). Following the General Procedure, reaction of 2,6-dichloropyridine-3,5-dicarbonitrile (18) (0.63 g, 3.18 mmol) with prop-2-yn-1-amine (0.20 mL, 3.18 mmol) and triethylamine (0.88 mL, 6.36 mmol) in EtOH/THF (10 mL), after 30 min, and column chromatography (hexane/AcOEt, from 9:1 to 8:2) gave product 25 and 2,6-bis(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile. 25 (white solid; 0.68 g, 80%): $R_f$ = 0.35 (hexane/AcOEt, 4/1); mp 188-190 ºC; IR (KBr) $\nu$ 3435, 3307, 3280, 3074, 2232, 1604, 1576, 1390, 1350, 1327, 1157 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.93 (d, $J$= 1.5 Hz, 1H, H4), 5.93 (t, $J$= 5.3 Hz, 1H, NH), 4.39 (dd, $J$= 5.3, 2.6 Hz, 2H, NCH$_2$C≡CH), 2.37 (t, $J$= 2.6 Hz, 1H, NCH$_2$C≡CH). HRMS (ESI): Calcd for C$_{10}$H$_{8}$N$_4$Cl ([M+H]$^+$): 217.0276. Found: 217.0266. 2,6-Bis(prop-2-yn-1-ylamino)pyridine-3,5-
dicarbonitrile (white solid (0.29 g, 40%): $R_f = 0.35$ (hexane/AcOEt, 4/1, v/v); mp 255-7 °C; IR (KBr) ν 3325, 3284, 2325, 2211, 1605, 1542, 1407, 1331 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ 7.69 (s, 1H, H$4$), 5.68 (t, $J = 5.3$ Hz, 2H, 2xNH), 4.31 [dd, $J = 5.3$, 2.5 Hz, 4H, 2xNC$_2$H$_2$C≡CH]], 2.29 [t, $J = 2.5$ Hz, 2H, 2xNCH$_2$C≡C]], HRMS (ESI): Calcd for C$_{13}$H$_9$N$_5$ ([M+H]$^+$): 236.0931. Found: 236.0932.

2-((3-(1-Benzylpiperidin-4-yl)propyl)amino)-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH15). Following the General Procedure, reaction of 2-chloro-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile 25 (0.12 g, 0.56 mmol) with 3-(1-benzylpiperidin-4-yl)propan-1-amine (21) (130 mg, 0.56 mmol) and triethylamine (0.16 mL) in EtOH/THF (12 mL), after 23 h, and column chromatography (from 1.5% to 10% MeOH/CH$_2$Cl$_2$), gave product DPH15 (186 mg, 81%) as yellow solid: $R_f = 0.42$ (hexane/AcOEt, 4/1); mp 170-1 °C; IR (KBr) ν 3348, 3303, 2926, 2204, 1566, 1510, 1403 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$) δ 7.62 (s, 1H, H$4$), 7.31-7.22 (m, 5H, C$_6$H$_5$), 5.62 (t, $J = 5.4$ Hz, 1H, NHCH$_2$C≡CH), 5.56 (t, $J = 5.7$ Hz, 1H, NHCH$_2$), 4.26 (dd, $J = 5.4$, 2.5 Hz, 2H, NCH$2$C≡CH), 3.49 (4H: td, $J = 7.6$, 5.8 Hz, 2H, CH$_2$NH; s, 2H, NCH$_2$C≡CH), 2.88 [d, $J = 11.6$ Hz, 2H, BnN(CH$_2$CH$_2$)$_2$(CH$_2$)$_2$CH], 2.25 (t, $J = 2.5$ Hz, 1H, NCH$_2$C≡CH), 1.93-187 [m, 2H, BnN(CH$_2$CH$_2$)$_2$(CH$_2$)$_2$CH], 1.64 (m, 4H), 1.37-1.15 (m, 5H); $^{13}$C NMR (126 MHz, CDCl$_3$) δ 159.0 (C$_6$), 158.4 (C$_2$), 145.5 (C$4$), 138.4 (C$1'$, C$_6$H$_5$), 129.2 (2xCH, C$2'$, C$6'$, C$_6$H$_5$), 128.1 (2xCH, C$3'$, C$5'$, C$_6$H$_5$), 126.9 (C$4'$, C$_6$H$_5$), 116.3 and 116.2 (2xCN), 80.8 (C$5$), 80.1 (C$3$), 79.0 (NCH$_2$C≡CH), 71.9 (NCH$_2$C≡CH), 63.4 (NCH$_2$C$_6$H$_5$), 53.7 [2C, BnN(CH$_2$CH$_2$)$_2$(CH$_2$)$_2$CH], 41.9 (CH$_2$NH), 35.5 [BnN(CH$_2$CH$_2$)$_2$(CH$_2$)$_2$CH], 33.7 [CH$_2$-(CH$_2$)$_2$NH], 32.3 [2C, BnN(CH$_2$CH$_2$)$_2$(CH$_2$)$_2$CH], 31.0 (NCH$_2$C≡CH), 26.58 (CH$_2$CH$_2$NH). HRMS (ESI): Calcd for C$_{25}$H$_{28}$N$_6$ ([M+H]$^+$): 413.2448. Found: 413.2456. **DPH15.HCl**: Anal. Calcd. for C$_{25}$H$_{29}$ClN$_6$.1/2H$_2$O: C, 65.56; H, 6.60; N, 18.35. Found: C, 65.22; H, 6.49; N, 18.08.

2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH16). Following the General Procedure, reaction of 2-chloro-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile 25 (0.12 g, 0.56 mmol) with 4-(1-benzylpiperidin-4-yl)butan-1-amine (22) (140 mg, 0.56 mmol) and triethylamine (0.15 mL) in EtOH/THF (10 mL), after overnight, and column chromatography (from 1.5% to 10% MeOH/CH$_2$Cl$_2$), gave product DPH16 (0.21 g, 88%) as yellow solid: $R_f = 0.42$ (hexane/AcOEt, 4/1); mp 142-4 °C; IR (KBr) ν 3348, 3303, 2926, 2204, 1566, 1510,
1403 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.62 (s, 1H, H₄), 7.35-7.27 (m, 5H, C₆H₅), 5.62 (t, J = 5.4 Hz, 1H, NHCH₂C≡CH), 5.54 (t, J = 5.7 Hz, 1H, NHCH₂), 4.32-4.16 (m, 2H, NCH₂C≡CH), 3.50 (4H: dt, J = 7.2, 5.4 Hz, 2H, NCH₂), 2.98-2.77 [m, 2H, BnN(CH₂)₂(CH₂)₂CH], 2.25 (t, J = 2.5 Hz, 1H, NCH₂C≡CH), 1.94 [t, J = 11.1 Hz, 2H, BnN(CH₂)₂(CH₂)₂CH], 1.71-1.53 (m, 4H), 1.37 (m, 2H), 1.32-1.17 (m, 5H); ¹³C NMR (126 MHz, CDCl₃) δ 159.0 (C₆), 158.4 (C₂), 145.5 (C₄), 129.3, 128.1, 126.9 (NCH₂C₆H₅), 116.3 and 116.2 (2xCN), 80.8 (C₅), 80.0 (C₃), 79.0 (NCH₂C≡CH), 71.9 (NCH₂C≡CH), 63.3 (NCH₂C₆H₅), 53.7 [2C, BnN(CH₂)₂(CH₂)₂CH], 41.6 (CH₂NH), 36.1 [CH₂(CH₂)₂NH], 35.5 [BnN(CH₂)₂(CH₂)₂CH], 32.2 [2C, BnN(CH₂)₂(CH₂)₂CH], 31.0 (NCH₂C≡CH), 29.4 (CH₂CH₂NH), 24.1 [CH₂(CH₂)₂NH].


References


5. NMR spectra for compounds DPHs9-16

2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-6-(methyl(prop-2-yn-1-yl)amino)-4-phenylpyridine-3,5-dicarbonitrile (DPH9).

$^1$H NMR (400 MHz, CDCl$_3$) Spectrum of compound DPH9.

$^{13}$C NMR (100 MHz, CDCl$_3$) Spectrum of compound DPH9.
$^1$H-$^1$H g-COSY (400 MHz, CDCl$_3$) spectrum of compound DPH9.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound DPH9.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound **DPH9**.
2-((2-(1-Benzylpiperidin-4-yl)ethyl)amino)-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH10).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound DPH10.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound DPH10.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound DPH10.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound DPH10.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound DPH10.
2-((3-(1-Benzylpiperidin-4-yl)propyl)amino)-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH11).

$^{1}$H NMR (500 MHz, CDCl₃) Spectrum of compound DPH11.

$^{13}$C NMR (126 MHz, CDCl₃) Spectrum of compound DPH11.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound DPH11.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound DPH11.
$^1$H-$^{13}$C g-HMBC (CDCl₃) spectrum of compound **DPH11**.
2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-4-phenyl-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH12).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound DPH12.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound DPH12.
$^{1}$H–$^{1}$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound DPH12.

$^{1}$H–$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound DPH12.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound DPH12.
2-((3-(1-Benzylpiperidin-4-yl)propyl)amino)-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (DPH13).

\[ \text{1H NMR (500 MHz, CDCl}_3\text{) Spectrum of compound DPH13.} \]

\[ \text{13C NMR (126 MHz, CDCl}_3\text{) Spectrum of compound DPH13.} \]
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound DPH13.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound DPH13.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound DPH13.
2-((4-(1-Benzylpiperidin-4-yl)butyl)amino)-6-(methyl(prop-2-yn-1-yl)amino)pyridine-3,5-dicarbonitrile (DPH14).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound DPH14.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound DPH14.
$^1$H-1H g-COSY (500 MHz, CDCl₃) spectrum of compound DPH14.

$^1$H-13C g-HSQC (CDCl₃) spectrum of compound DPH14.
$^{1}H-^{13}C$ g-HMBC (CDCl$_3$) spectrum of compound **DPH14**.
2-((3-(1-Benzylpiperidin-4-yl)propyl)amino)-6-(prop-2-yn-1-ylamino)pyridine-3,5-dicarbonitrile (DPH15).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound DPH15.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound DPH15.
Supporting Information

N-Methyl-N-((1-methyl-5-(3-(1-(2-methylbenzyl)piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine, a New Cholinesterase and Monoamine Oxidase Dual Inhibitor

Oscar M. Bautista-Aguilera, Abdelouahid, Samadi, Mourad Chioua, Katarina Nikolic, Slavica Filipic, Danica Agbaba, Elena Soriano, Stefano Alcaro, Rona R. Ramsay, Francesco Ortuso, Matilde Yañez, and José Marco-Contelles

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**QSAR Methods**

The inhibiting MAO A/ B and AChE/BuChE activities (IC$_{50}$) of 30 indole derivatives were used for the QSAR study [(a) Bolea, I.; Gella, A.; Monjas, L.; Pérez, C.; Rodríguez-Franco, M. I.; Marco-Contelles, J. L.; Samadi, A.; Unzeta, M. The multipotent, permeable drug ASS234 inhibits Aβ aggregation, possesses antioxidant properties and protects from Aβ-induced apoptosis, *Curr. Alzheimer Res.* **2013**, 9, 797-808; (b) V. Pérez, V.; Marco, J. L.; Fernández-Álvarez, E.; Unzeta, M. Relevance of benzyloxy group in 2-indolyl methylamines in the selective MAO-B inhibition, *Br. J. Pharmacol.* **1999**, 127, 869-876]. Negative logarithm of their IC$_{50}$ i.e. (pIC$_{50}$) values were calculated.

The pKa calculation and selection of dominant molecules/cations at physiological pH 7.4 was performed for the examined compounds using the MarvinSketch 5.5.1.0 program [ChemAxon MarvinSketch 5.5.1.0 program, Budapest, Hungary (2011) www.chemaxon.com/products.html]. Dominant forms at pH 7.4, were used for the 3D-QSAR study.

The 3D-QSAR studies of the indole derivatives were performed by use of the Pentacle 1.0.6 program [Pentacle, Version 1.0.6.; Molecular Discovery Ltd, Perugia, Italy (2009) http://www.moldiscovery.com/soft_pentacle.php].

The Pentacle is advanced software tool for obtaining alignment-independent 3D quantitative structure-activity relationships. The 3D-QSAR starts from computing highly relevant 3D maps of interaction energies (GRID based Molecular Interaction Fields-MIFs) between the examined molecule and four chemical probes: DRY (which represent hydrophobic interactions), O (sp² carbonyl oxygen, representing H-bond acceptor), N1 (neutral flat NH, like in amide, H-bond donor), and the TIP probe (molecular shape descriptor). The grid spacing was set to 0.5 Å and the MACC2 smoothing window to 1.6 (for 3D-QSAR (ChE) models) and the CLACC smoothing window to 1.6 (for 3D-QSAR (MAO) models). The number of filtered nodes was set to 100 with 50% relative weights within the ALMOND discretization.

The interaction energy between the probe and the target molecule is calculated at each point as the sum of Lennard-Jones (\(E_{lj}\)), hydrogen bond (\(E_{hb}\)), electrostatic interactions (\(E_{el}\)), and an entropic term: 

\[
E_{xyz} = \sum E_{lj} + \sum E_{el} + \sum E_{hb} + S
\]

[Pastor, M.; Cruciani, G.; McLay, I.; Pickett, S.; Clementi, S.; G Rid-INdependent descriptors]
The maps obtained are encoded into GRID Independent Descriptors (GRIND and GRIND2 descriptors) which are independent of the alignment of the series [Pastor, M.; Cruciani, G.; McLay, I.; Pickett, S.; Clementi, S.; GRid-INdependent descriptors (GRIND): A novel class of alignment-independent three-dimensional molecular descriptors. *J. Med. Chem.* **2000**, *43*, 3233-3243]. The GRIND approach aims to extract the information enclosed in the MIFs and compress it into new types of variables whose values are independent of the spatial position of the molecule studied by using an optimization algorithm with the intensity of the field at a node and the mutual node–node distances between the chosen nodes as a scoring function. Such variables constitute a matrix of descriptors that are analyzed using multivariate techniques, such as Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression analysis. The Principal Component Analysis was used for inspection of our series and for obtaining a map of our compounds describing their similarities and differences. The variables were used for development of 3D-QSAR models by use of the PLS regression [Eriksson, L.; Johansson, E.; Kettaneh-Wold, N.; Trygg, J.; Wikstrom, C.; Wold, S. (Eds.) Multi-and Megavariate Data Analysis. Basic Principles and Applications I, 2nd ed, Umetrics Academy, Umeå, 2001].

Based on the Score Plots (t1 vs. t2 and t1 vs. u1) the data set of 30 MAO A/B inhibitors and data set of 35 AChE/BuChE inhibitors is divided on Training Set (23-29 compounds for QSAR models building) and Verification set (6-9 compounds for QSAR models validation) [Tropsha, A. Best practices for QSAR model development, validation, and exploitation. *Mol. Inf.* **2010**, *29*, 476–488]. The most important pharmacophores (GRID descriptors), responsible for the MAO A, MAO B, AChE, and
BuChE inhibition, were selected by use of the PLS regression and used for the 3D-QSAR (MAO A, MAO B, AChE, BuChE) models building (Pentacle 1.0.6 program).

Quality of the obtained 3D-QSAR (I₁-IR) models (3D-QSAR (I₁-IR agonists) model and 3D-QSAR (I₁-IR antagonists) model) was examined by use of: leave-one-out cross-validation ($Q^2$), correlation coefficient ($R^2_{\text{Observed vs. Predicted}}$), Root Main Squared Error of Estimation (RMSEE), and external validation (Root Main Squared Error of Prediction (RMSEP)) [(a) Wold, S.; Johansson, E.; Cocchi, M. 3D-QSAR in drug design, theory, methods, and applications. H. Kubinyi Ed., ESCOM Science Publishers: Leiden, 1993, pp 523–550; (b) Tropsha, A. Best practices for QSAR model development, validation, and exploitation. *Mol. Inf.* **2010**, **29**, 476–488]. Predictive power of the model is determined by $Q^2$, which is leave-one-out cross-validated version of $R^2$.

ADMET descriptors

Table S1. Calculated physicochemical and ADMET properties for structures ASS234 and MBA236.\textsuperscript{a,b}

<table>
<thead>
<tr>
<th></th>
<th>Molecular weight</th>
<th>No. of H-bond donors</th>
<th>No. of H-bond acceptor</th>
<th>No. of Rotatable Bonds</th>
<th>logP (Moriguchi)\textsuperscript{a,c}</th>
<th>logP\textsuperscript{d}</th>
<th>TPSA (in Å\textsuperscript{2})</th>
<th>No. violations Lipinski’s rule</th>
<th>LogBB\textsuperscript{a}</th>
<th>LogBB\textsuperscript{b}</th>
<th>Permeability (cm/s x 10\textsuperscript{4})</th>
<th>Human intestinal absorption (%)\textsuperscript{e}</th>
<th>In vitro Caco-2 perm (nm/sec)\textsuperscript{f}</th>
<th>MDCK (cm/s x 10\textsuperscript{7})\textsuperscript{g}</th>
<th>% Plasma protein binding (in vitro)\textsuperscript{h}</th>
<th>Toxicity\textsuperscript{i}</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASS234</td>
<td>443.64</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>3.83</td>
<td>5.88</td>
<td>20.64</td>
<td>1</td>
<td>0.25</td>
<td>0.31</td>
<td>5.63</td>
<td>100</td>
<td>32.71</td>
<td>216.59</td>
<td>60.53</td>
<td>hERG</td>
</tr>
<tr>
<td>MBA236</td>
<td>457.66</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>4.17</td>
<td>6.19</td>
<td>20.64</td>
<td>1</td>
<td>0.39</td>
<td>0.54</td>
<td>5.71</td>
<td>100</td>
<td>34.52</td>
<td>225.08</td>
<td>64.99</td>
<td>hERG</td>
</tr>
</tbody>
</table>

\textsuperscript{a} AMET Predictor, v.6.5. \textsuperscript{b} ACD/Percepta 14.0. \textsuperscript{c} Moriguchi model. \textsuperscript{d} High absorption to CNS: logBB more than 0.3; Middle absorption to CNS: logBB 0.3 – -1.0; Low absorption to CNS: logBB less than -1.0. \textsuperscript{e} Human intestinal absorption is the sum of bioavailability and absorption evaluated from ratio of excretion or cumulative excretion in urine, bile, and feces. A value between 0 and 20% indicates poor absorption, 20–70% shows moderate absorption, and 70–100% indicates good absorption. \textsuperscript{f} Caco-2 cells are derived from human colon adenocarcinoma and possess multiple drug transport pathways through the intestinal epithelium. A value <4 indicates low permeability, 4–70 shows middle permeability, and >70 indicates high permeability. \textsuperscript{g} The MDCK cell system may be used as a good tool for rapid permeability screening. A value <25 indicates low permeability, 25–500 shows middle permeability, and >500 indicates high permeability. \textsuperscript{h} The percent of drug binds to plasma protein. A value <90% indicates weak binding, and >90% indicates strong binding to plasma proteins. \textsuperscript{i} hERG = hERG liability.
ADMET predictions.

These predictions prompted us to carry out the virtual ADMET analysis of hybrid 2 by comparing it with ASS234. The lipophilicity (expressed as logP) predicted for both ASS234 and 2 is slightly higher than the traditionally cutoff value of 5 of the Lipinski’s rules used in drug design (logP < 5 and/or mlogP < 4.1). CNS drugs have significantly reduced molecular weights compared with other therapeutics, and it has been suggested that molecular weight (MW) should be kept below 450 to facilitate brain penetration and to be lower than that for oral absorption. According to this, the structures show limit values (MW ≈ 450). The computed values predict a brain penetration sufficient for CNS activity, showing 2 with a better penetration profile than ASS234. The structures show an adequate permeability to be good candidates (Peff > 0.1, MDCK > 25), and should be well absorbed compounds (% HIA). In addition, a middle Caco-2 cell permeability is suggested. Regarding toxicity, the structures lack hepatotoxicity, and show hERG liability. In summary, it can be concluded that hybrid 2 presents similar good drug-like characteristics and ADMET properties as ASS234, and a slightly better brain penetration ability (Table S1, Supporting Information).
Chemistry

Structure of the synthesized compounds
Experimental procedures

**General Methods.** Melting points were determined in a Koffler apparatus, and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded at room temperature in CDCl$_3$ or DMSO-$d_6$ at 300, 400 or 500 MHz and at 75.4, 100.6 or 125.6 MHz, respectively, using solvent peaks [CDCl$_3$: 7.27 (D), 77.2 (C) ppm and DMSO-$d_6$ 2.50 (D) and 39.7 (C) ppm] as internal references. The assignment of chemical shifts is based on standard NMR experiments ($^1$H, $^{13}$C, $^1$H-$^1$H COSY, $^1$H-$^{13}$C HSQC, HMBC, DEPT). Mass spectra were recorded on a GC/MS spectrometer with an API-ES ionization source. Elemental analyses were performed at the IQOG (CSIC, Spain). Tlc analyses were performed on silica F254 and detection by UV light at 254 nm, or by spraying with phosphomolybdic-H$_2$SO$_4$ dyeing reagent. Column chromatographies were performed on silica Gel 60 (230 mesh). “Chromatotron” separations were performed on a Harrison Research Model 7924. The circular disks were coated with Kieselgel 60 PF254 (E. Merck). The chlorydrate salts were prepared by solubilising the compound in a minimum of ether and a solution of ether saturated with HCl(g) was added dropwise. A white solid was formed immediately. The precipitated hydrochloride was separated by filtration, washer with ether and dried.

$N$-Methyl-$N$-((1-methyl-5-(3-(piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine (1)
3-(Piperidin-4-yl)propan-1-ol hydrochloride (MBA160). To a solution of commercial 3-(pyridin-4-yl)propan-1-ol (A3-17) (1.04 g, 7.6 mmol) in dry ethanol (40 mL), chlorhydric acid in dioxane (1.4 mL, 4N), PtO₂ (0.208 gr), and Pd/C 20% (0.104 g) were added. The mixture was hydrogenated at rt and 45 psi for 48 h. After complete reaction (tlc analysis), the reaction mass was filtered over Celite, washed with methanol, and the solvent eliminated under vacuum, to give pure MBA160 (1.30 g, 95%) as a yellow solid (Rf= 0.22, DCM/MeOH, 20%) (Engbertson, M. S.; Chang, C. T.-C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, Jr., J. L.; Lynch, R. J.; Manno, P. D.; Naylor, A. M.; Prugh, J. D.; Ramjit, D. R.; Sitko, G. R.; Smith, R. S.; Turchi, L. M.; Zhang, G. Non-Peptide Fibrinogen Receptor Antagonists. 2. Optimization of a Tyrosine Template as a Mimic for Arg-Gly-Asp, J. Med. Chem. 1994, 37, 2537-2551).

tert-Butyl 4-(3-hydroxypropyl)piperidine-1-carboxylate (MBA163). To a solution of 3-(piperidin-4-yl)propan-1-ol hydrochloride (MBA160) (530 mg, 2.6 mmol) in dioxane (10 mL), NaOH 3N (3.6 mL), di-tert-butyl dicarbonate (569 mg, 2.6 mmol) was added, and the mixture was stirred overnight at rt. After complete reaction (tlc analysis), the solvent was eliminated, ethyl ether (10 mL) was added, and the mixture was treated with aqueous 10% KH₂SO₄ (5 mL). After work-up, the organic phase was dried (Na₂SO₄), filtered and submitted to chromatography (hexane/AcOEt, 1:1) to give compound MBA163 (566 mg, 78%) as an oil (Rf= 0.30, DCM/MeOH, 2%) (1994JMC2546).
**tert-Butyl 4-(3-chloropropyl)piperidine-1-carboxylate (MBA177).** To a solution of tert-butyl 4-(3-hydroxypropyl)piperidine-1-carboxylate (MBA163) (180 mg, 0.74 mmol) and PPh₃ (388 mg, 1.48 mmol) in dry DCM (5 mL), CCl₄ (106 µL, 1.11 mmol) was added. The mixture was stirred at rt overnight. Next, the solvent was removed and the residue was purified by chromatography (hexane/AcOE, 5%) to yield MBA177 (155 mg, 80%) as an oil (R_f= 0.28, hexane/AcOEt, 10%) (ref. J2004MC711 : Baraldi, P. G.; Romagnoli, R.; Núñez, M. C.; Perretti, M.; Paul-Clark, M. J.; Ferrario, M.; Govoni, M.; Benedini, F.; Ongini, E. Synthesis of nitro esters of prednisolone, new compounds combining pharmacological properties of both glucocorticoids and nitric oxide, *J. Med. Chem.* 2004, 47, 711-719).

**tert-Butyl 4-(3-(1-methyl-2-((methyl(prop-2-ynyl)amino)methyl)-1H-indol-5-ylxy)propyl)piperidine-1-carboxylate (MBA184).** To a solution of tert-butyl 4-(3-chloropropyl)piperidine-1-carboxylate (MBA177) (152 mg, 0.58 mmol) and 1-methyl-2-[((methyl(prop-2-ynyl)amino)methyl)-1H-indol-5-ol (MBA176) (132 mg, 0.58 mmol) (ref. 1991EJMC33: Cruces, M. A.; Elorriaga, C.; Fernández-Álvarez, E. Acetylenic and allenic derivatives of 2-(5-benzoxindolyl) and 2-(5-hydroxyindolyl)methylamines: synthesis and in vitro evaluation as monoamine oxidase inhibitors, *Eur. J. Med. Chem.* 1991, 26, 33-41), in dry DMF (10 mL), under argon, at rt, NaH (41.7 mg, 1.7 mmol, 60% dispersion in mineral oil) was slowly added, and the mixture stirred at rt overnight. Then, the solvent was removed, water (15 mL) was added, and the mass was extracted with DCM several times. The organic organic phase was dried (Na₂SO₄), filtered and submitted to chromatography (hexane/AcOEt, 10%) to afford indole MBA184 (189 mg, 72%) as a white solid (R_f= 0.52, hexane/AcOEt, 40%): mp 88-90 ºC; IR (KBr) ν 3433, 3257, 2929, 1692, 1489, 1160, 1019 cm⁻¹; ¹H NMR (500 MHz, CD₃Cl) δ 7.17 (d, J= 8.8 Hz, 1H, H7-indole), 7.02 (d, J= 2.5 Hz, 1H, H4), 6.84 (dd, J= 8.8, 2.5 Hz, 1H, H6), 6.32 (s, 1H, H3), 4.06 (br s, 2H, [BocN(CHEq₂CH₂)₂CH]), 3.97 [t, J= 6.3 Hz, 2H, C(5)OCH₂CH₂CH₂], 3.73 [s, 3H, N(1)CH₃], 3.67 [br s, 2H, indoleCH₂NMe], 3.31 [d, J= 2.4 Hz, 2H, MeNCH₂C=CH], 2.67 [br s, 2H, BocN(CHax₂CH₂)₂CH], 2.33 (s, 3H, CH₃NCH₂C=CH), 2.28 [t, 1 H, MeNCH₂C=CH], 1.84-1.81 [m, 2H, C(5)OCH₂CH₂CH₂], 1.79-1.67 [m, 2H, BocN(CH₂CHEq₂)₂CH], 1.57 [m, 1H, BocN(CH₂CH₂)₂CH]], 1.45 [s, 9H, C(CH₃)₃], 1.44-1.40 [m, 2H, C(5)OCH₂CH₂CH₂], 1.25-1.10 (m, 2H, H₂N+(CH₂CHax₂)₂CH]; ¹³C
NMR (125 MHz, CD3Cl) δ 154.9 [NCO2C(CH3)3], 153.2 (C5), 137.0 (C7a), 133.4 (C2), 127.5 (C3a), 112.0 (C6), 109.6 (C7), 103.4 (C4), 102.0 (C3), 79.1 [MeNCH2C≡CH], 78.4 [MeNCH2C≡CH], 73.4 [NCO2C(CH3)3], 68.9 [C(5)OCH2CH2CH2], 51.8 [indoleCH2NMe], 44.7 [MeNCH2C≡CH], 41.5 [CH3NCH2C≡CH], 35.8 [C(5)OCH2CH2CH2], 32.9 [3C, BocN(CH2CH2)2CH], 29.8 [N(1)CH3], 28.4 [H2N+(CH2CH2)2CH]; MS (EI) m/z (%): 453 (48) [M]+, 397 (22), 386 (29), 353 (25), 330 (100), 311 (11), 283 (38), 227 (16), 187 (12), 160 (98); HRMS (ESI): Calcd for C27H39N3O3: 453.2987. Found: 453.2991.

Anal. Calcd for C27H39N3O3.1/2H2O: C, 70.10; H, 8.71; N, 9.08. Found: 70.19; H, 8.48; N, 8.96.

**N-Methyl-N-{(1-methyl-5-(3-(piperidin-4-yl)propoxy)-1H-indol-2-yl)prop-2-yn-1-amine dihydrochloride (1.2HCl).** A saturated solution of AcOEt/HCl (4 mL) was slowly added to a solution of tert-butyl[3-(1-methyl-2-((methyl(prop-2-ynyl)amino)methyl)-1H-indol-5-yloxy)propyl]piperidine-carboxylate (MBA184) (24 mg, 0.053 mmol) in AcOEt (5 mL), cooled at 0 °C. The mixture was cooled in the freezer overnight. Then, the solid was filtered, washed with cold AcOEt and dried to afford compound 1.2HCl (22 mg, 99%), as a white solid (Rf= 0.44, DCM/MeOH, 20%): mp 220-3 ºC; IR (KBr) ν 3435, 3189, 2934, 2505, 1485, 1469, 1250, 1209, 1161 cm⁻¹; ¹H NMR (400 MHz, CD3OD) δ 7.35 (d, J= 9.0 Hz, 1H, H7), 7.08 (d, J= 2.4 Hz, 1H, H4), 6.92 (dd, J= 9.0, 2.4 Hz, 1H, H6), 6.74 (s, 1H, H3), 4.67 [br s, 2H, indoleCH2N+(H)Me], 4.16 [br s, 2 H, Me(H)N+CH2C≡CH], 4.00 [t, J= 6.3 Hz, 2H, C(5)OCH2CH2CH2], 3.82 [s, 3H, N(1)CH3], 3.49 [t, J= 2.5 Hz, 2H, Me(H)N+CH2C≡CH], 3.38 (d, J= 12.8 Hz, 2H, [H2N+(CHeq2CH2)2CH], 2.98 [m, 2H, H2N+(CHax2CH2)2CH], 2.82 (s, 3H, CH3(H)N+CH2C=CH), 1.97 (m, 2H, H2N+(CH2Ceqq2)2CH), 1.82 [m, 2H, C(5)OCH2CH2CH2], 1.68 [m, 1H, H2N+(CHax2CH2)2CH], 1.52 [m, 2H, C(5)OCH2CH2CH2], 1.42 (m, 2H, H2N+(CH2Ceqq2)2CH); ¹³C NMR (100 MHz, CD3OD) δ 154.1 (C5), 134.1 (C7a), 127.6 (C2)*, 127.5 (C3a)*, 114.4 (C6), 110.8 (C7), 106.5 (C3), 103.1 (C4), 80.6 [Me(H)N+CH2C≡CH], 71.8 [Me(H)N+CH2C≡CH], 68.3 [C(5)OCH2CH2CH2], 49.8 [indoleCH2N+(H)Me], 44.2 [Me(H)N+CH2C≡CH], 44.1 [H2N+(CH2CH2)2CH], 38.9 [CH3(H)N+CH2C≡CH], 33.5 [H3N+(CH2CH2)2CH], 32.5 [C(5)OCH2CH2CH2], 29.5 [N(1)CH3], 28.8 [H2N+(CH2CH2)2CH], 26.2 [C(5)OCH2CH2CH2]; MS (EI) m/z (%): 353 (61) [M]+, 284 (61), 227 (20), 161 (100), 126 (84); HRMS (ESI): Calcd for
C$_{22}$H$_{31}$N$_3$O: 353.2481. Found: 392.2467. Anal. Calcd for C$_{22}$H$_{31}$N$_3$O.2HCl.½ H$_2$O: C, 60.68; H, 7.87; N, 9.65. Found: 60.68; H, 7.62; N, 9.80.

*N*-Methyl-*N*-((1-methyl-5-(3-(1-(2-methylbenzyl)piperidin-4-yl)propoxy)-1$H$-indol-2-yl)methyl)prop-2-yn-1-amine (2 = MBA236).

4-((4-((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1$H$-indol-5-yl)oxy)propyl)piperidin-1-yl)methyl)benzonitrile (3).

**General method for the *N*-alkylation of indole 1.** To a solution of compound 1 in dry CH$_3$CN, under argon, at 0 °C, di-isopropylethylamine (DIPEA) (4 equiv) and the corresponding 1-(bromomethyl)benzene derivative (1.08 equiv) were added, and refluxed overnight. After complete reaction (tlc analysis), the solvent was removed and purified by chromatography using hexane/AcOEt mixtures, to give the corresponding indole derivative.

*N*-Methyl-*N*-((1-methyl-5-(3-(1-(2-methylbenzyl)piperidin-4-yl)propoxy)-1$H$-indol-2-yl)methyl)prop-2-yn-1-amine (MBA236). Following the General method, compound 1 (85 mg, 0.24 mmol) in CH$_3$CN (3.5 mL), was treated with DIPEA (0.17 mL, 0.96 mmol) and 1-(bromomethyl)-2-methylbenzene (36 µL, 0.26 mmol), to give MBA236 (70 mg, 63%), as an oil (R$_{f}$ = 0.32, hexane/AcOEt, 20%), after chromatography (hexane/AcOEt, 10-50%): $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.26 (m, 1H, o-CH$_3$-C$_6$H$_4$-CH$_2$N), 7.16 (d, $J$ = 8.8 Hz, 1H, H7), 7.13-7.12 (m, 3H, o-CH$_3$-C$_6$H$_4$-CH$_2$N), 7.01 (d, $J$ = 2.4 Hz, 1H, H4), 6.84 (dd, $J$ = 8.8, 2.4 Hz, 1H, H6), 6.31 (s, 1H, H3),

Scheme 2. Reagents and conditions: a) RBr, DIPEA, CH$_3$CN, reflux.
3.95 (t, J = 6.6 Hz, 2H, OCH₂CH₂CH₂), 3.72 [s, 3H, N(1)CH₃], 3.65 [s, 2H, C(2)CH₂N(Me)], 3.40 [s, 2H, NCH₂(o-CH₃-C₆H₄), 3.30 [d, J = 2.2 Hz, 2H, N(Me)CH₂C=CH], 3.24 [s, 3H, N(CH₂)₂C=CH], 2.84 (d, J = 11.6 Hz, 2H, N(CH₂)₂(CH₂)₂CH), 2.32 (s, 3H, o-CH₃-C₆H₄-CH₂N), 2.27 (t, J = 2.2 Hz, 1H, N(Me)CH₂C=CH), 1.94 [t, J = 11.6 Hz, 2H, N(CH₂)₂(CH₂)₂CH], 1.81-1.77 [m, 2H, OCH₂CH₂CH₂], 1.66 [d, J = 9.9 Hz, 2H, N(CH₂)₂CH₂eqCH], 1.41-1.36 (m, 2H, OCH₂CH₂CH₂), 1.29-1.24 [m, 3H, N(CH₂)₂CH₂eqCH]; ¹³C NMR (126 MHz, CDCl₃) δ 153.5 (C₅), 137.6 (2C, C₁', C₂'), o-CH₃-C₆H₄CH₂N), 137.2 (C₂), 133.5 (C₇a), 130.3 (C₃', o-CH₃-C₆H₄CH₂N), 129.8 (C₆', o-CH₃-C₆H₄CH₂N), 127.7 (C₃a), 126.9, 125.6 (2C, C₄', C₅'), o-CH₃-C₆H₄CH₂N), 112.2 (C₆), 109.8 (C₇), 103.5 (C₄), 102.2 (C₃), 78.6 (NCH₂C=CH), 73.6 (NCH₂C=CH), 69.3 (OCH₂CH₂CH₂), 61.3 (o-CH₃-C₆H₄CH₂N), 54.3 [2CH₂, N(CH₂)₂(CH₂)₂CH], 52.0 [C(2)CH₂N(CH₃)CH₂C≡CH], 44.9 [C(2)CH₂NCH₂C=CH], 41.8 [C(2)CH₂N(CH₃)CH₂C=CH], 35.9 [N(CH₂)₂(CH₂)₂CH], 33.2 (OCH₂CH₂CH₂), 32.7 [2C, N(CH₂)₂(CH₂)₂CH], 30.1 [N(1)CH₃], 27.0 (OCH₂CH₂CH₂), 19.5 (o-CH₃-C₆H₄CH₂N); MS (ESI) m/z (%): 458 (M+1)⁺. The bis-chlorhydrate was prepared as usual to give compound MBA236.2HCl: mp 200-5 ºC; IR (KBr) ν 3433, 2927, 2510, 1485, 1468, 1210 cm⁻¹; MS (ESI) m/z (%): 458 (M+1)⁺. Anal. C₃₀H₃₉N₃O.2HCl 1/₂H₂O: C, 66.78; H, 7.85; N, 7.79. Found: C, 66.59; H, 7.59; N, 7.99.

4-((4-(3-((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)propyl)piperidin-1-yl)methyl)benzonitrile (3). Following the General method, compound 1 (200 mg, 0.56 mmol) in CH₃CN (4 mL), was treated with DIPEA (0.38 ml, 2.24 mmol), and 4-(bromomethyl)benzonitrile (133 mg, 0.67 mmol), to afford compound 3 (98 mg, 45%), as a white solid (Rₚ= 0.30, hexane/AcOEt, 40%), after chromatography (hexane/AcOEt, 30-70%): mp 75-8 ºC; IR (KBr) ν 3445, 3255, 2908, 2231, 1607, 1488, 1203, 1026 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.59 (d, J = 8.3 Hz, 2H, H₂', H₆'), CN-C₆H₄-CH₂N), 7.59 (d, J = 8.3 Hz, 2H, H₃', H₅'), CN-C₆H₄-CH₂N), 7.17 (d, J = 8.8 Hz, 1H, H₇), 7.01 (d, J = 2.5 Hz, 1H, H₂), 6.84 (dd, J = 8.8, 2.5 Hz, 1H, H₆), 6.32 (s, 1H, H₃), 3.97 (t, J = 6.6 Hz, 2H, OCH₂CH₂CH₂), 3.73 [s, 3H, N(1)CH₃], 3.67 [s, 2H, C(2)CH₂N(Me)CH₂C=CH], 3.51 (s, 2H, N(CH₂)₂-C₆H₄-CN), 3.31 [d, J = 2.2 Hz, 2H, C(2)CH₂N(Me)CH₂C=CH], 2.81 (d, J = 11.8 Hz, 2H, N(CH₂)₂eq), 2.34 [s, 3H, C(2)CH₂N(CH₃)CH₂C=CH], 2.28 (t, J = 2.2 Hz, 1H, C(2)CH₂N(Me)CH₂C=CH), 1.97 (tm, J = 11.8 Hz, 2H, N(CH₂)₂ax), 1.81-1.78 [m, 2H, OCH₂CH₂CH₂], 1.70 [dm, J = 9.8 Hz, 2H, N(CH₂)₂(CH₂)eq], 1.43-1.41 (m, 2H, OCH₂CH₂CH₂), 1.29-1.25 [m, 3H,
$^{13}$C NMR (126 MHz, CDCl$_3$) δ 153.2 (C5), 144.8 [C1’, NCC$_6$H$_4$CH$_2$N], 137.0 (C2), 133.3 (C7a), 132.0 [2C, C2’, C6’, NCC$_6$H$_4$CH$_2$N], 129.4 [2C, C3’, C5’, NCC$_6$H$_4$CH$_2$N], 127.5 (C3a), 119.0 (CN), 112.0 (C6), 110.6 [C4’, NCC$_6$H$_4$CH$_2$N], 109.5 (C7), 103.4 (C4), 100.2 (C3), 78.4 (NCH$_2$C=CH), 73.4 (NCH$_2$C=CH), 69.0 (OCH$_2$CH$_2$CH$_2$), 62.9 [NCC$_6$H$_4$CH$_2$N], 54.0 [2CH$_2$, N(CH$_2$)$_2$(CH$_2$)$_2$CH], 51.8 [C(2)CH$_2$N(CH$_3$)CH$_2$C=CH], 44.7 [C(2)CH$_2$NCH$_2$C=CH], 41.5 [C(2)CH$_2$N(CH$_3$)CH$_2$C=CH], 35.4 [N(CH$_2$)$_2$(CH$_2$)$_2$CH], 32.9 [2C, N(CH$_2$)$_2$(CH$_2$)$_2$CH], 29.8 [N(1)CH$_3$], 26.7 (OCH$_2$CH$_2$CH$_2$); MS (ESI) m/z (%): 469 (M+1$^+$). Calcd for C$_{30}$H$_{36}$N$_4$O: C, 76.89; H, 7.74; N, 11.96. Found: C, 76.69; H, 7.62; N, 12.01.

5-(((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)methyl)quinolin-8-yl dimethylcarbamate (14).

N-((5-(8-Methoxyquinolin-5-yl)methoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methylprop-2-yn-1-amine (13).

N-((5-(3-((8-Methoxyquinolin-5-yl)methyl)piperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methylprop-2-yn-1-amine (4).

5-((4-(3-((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)propyl)piperidin-1-yl)methyl)quinolin-8-yl dimethylcarbamate (5).

5-(Chloromethyl)quinolin-8-ol hydrochloride (MBA150). To a solution of quinolin-8-ol (7.3 g, 50.3 mmol) and concentrated chlorhydric acid (22 mL), cooled at 0 °C,
aqueous formaldehyde (37%) (10 mL) was added, and HCl(g) was bubbled into the solution during 2 h at 0 °; then, the mixture was stirred at rt for 2 h more. The solid was filtered, washed with cc HCl, and dried to give compound MBA150 (11.4 g, 99%) as a yellow solid (Rf= 0.40, DCM/MeOH, 5%) (Li, L.; Xu, B. Synthesis and characterization of 5-substituted 8-hydroxyquinoline derivatives and their metal complexes, *Tetrahedron* 2008, 64, 10986–10995).

**5-(Hydroxymethyl)quinolin-8-ol (MBA190).** To a solution of 5-(hydroxymethyl)quinolin-8-ol (MBA150) (2.0 g, 8.7 mmol) in water (10 mL), aqueous NH3 (30%) was added until pH 9-10. The mixture was stirred for 15 min, and the solid was washed with water, and filtered. Further purification by chromatography (DCM/methanol, 1-5 %, with 2% NH3) gave compound MBA190 (1.52 g, 99%) as a white solid (Rf= 0.35, DCM/ methanol, 10%, with 2.5% NH3) (Li, L.; Xu, B. Synthesis and characterization of 5-substituted 8-hydroxyquinoline derivatives and their metal complexes, *Tetrahedron* 2008, 64, 10986–10995).

**(8-Methoxyquinolin-5-yl)methanol (MBA191).** To a solution of MBA190 (500 mg, 2.8 mmol) in dry DMF (5 mL), cooled at 0 °C, under argon, NaH (75 mg, 3.1 mmol, 60% dispersion in mineral oil) and MeI (0.20 mL, 3.4 mmol) were added. The reaction mixture was stirred at rt for 6 h; then, the solvent was removed, the residue was suspended in water and extracted with DCM. The organic layer was dried (Na2SO4), dried and purified by chromatography (DCM/AcOEt, 50-70%) to give product MBA191 (378 mg0, 70%), as white solid (Rf= 0.40, AcOEt) (Dimsdale, M. J. The formation of 2-alkoxyquinolines from quinoline N-oxides in alcoholic media, *J. Heterocyclic Chem*. 1979, 16, 1209-11).

**5-(Hydroxymethyl)quinolin-8-yl dimethylcarbamate (MBA217).** To a solution of MBA190 (510 mg, 2.9 mmol) in dry DMF (6 mL), under argon at 0 °C, NaH (84 mg, 3.5 mmol, 60% dispersion in mineral oil). After 10 min, dimethyl carbamoyl chloride (374 mg, 3.49 mmol) was added, and the mixture was stirred overnight. After complete reaction (tlc analysis), the solvent was removed and the residue suspended in water and extracted with DCM several times. The organic fraction was dried (Na2SO4), filtered and submitted to chromatography (DCM/methanol, 2%) to give compound MBA217 (523 mg, 73%), as an amorphous solid (Rf= 0.38, DCM/methanol, 5%): IR (KBr) ν 3419, 2931, 1703, 1598, 1503, 1388, 1248, 1170, 1070, 1022 cm⁻¹; 1H NMR (500 MHz,
CD$_3$Cl) $\delta$ 8.90 (dd, $J= 1.5$, 3.9 Hz, 1H, H2), 8.44 (dd, $J= 1.5$, 8.5 Hz, 1H, H4), 7.46 7 (d, $J= 7.9$ Hz, 1H, H6), 7.41 (dd, $J= 3.9$, 8.5 Hz, 1H, H3), 7.37 (d, $J= 7.9$ Hz, 1H, H7), 5.00 (d, $J= 5.8$ Hz, 2H, CH$_2$OH), 3.28, 3.06 [s, s, 6H, OC(O)N(CH$_3$)$_2$], 2.23 (t, $J= 5.8$ Hz, 1H, OH); $^{13}$C NMR (100 MHz, CD$_3$Cl) $\delta$ 155.2 [(CH$_3$)$_2$NO$_2$C], 150.1 (C2), 148.0 (C8), 142.2 (C8a), 134.3 (C4a), 132.5 (C4), 127.6 (C5), 125.5 (C6), 121.4 (C7), 120.9 (C3), 62.8 (CH$_2$OH), 36.8 [(CH$_3$)$_2$NO$_2$C]; MS (EI) $m/z$ (%): 247 (M+1)$^+$.  

**General Method for chlorination with SOCl$_2$**. To a solution of the alcohol in dry DCM, under argon, at rt, SOCl$_2$ (1.58 equiv) was added, and the mixture was stirred at rt overnight. Then, the solvent was evaporated, and the solid was washed with dry DCM to isolate the corresponding chloride.

**5-(Chloromethyl)-8-methoxyquinoline hydrochloride (MBA207)**. Following the **General Method for chlorination with SOCl$_2$**, (8-methoxyquinolin-5-yl)methanol MBA191 (377 mg, 1.9 mmol) in dry DCM (4.5 mL) was treated with SOCl$_2$ (0.21 mL, 3.0 mmol), to give compound MBA207 (413 mg, 85%) as a yellow solid: $R_f= 0.05$ AcOEt/methanol, 10%; $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$ 9.10-9.06 (m, 2H, H2, H4), 8.03 (m, 1H, H3), 7.91 (d, $J= 8.0$ Hz, 1H, H6), 7.47 (d, 1H, H7), 5.32 (s, 2H, CH$_2$Cl), 4.09 (s, 3H, OCH$_3$). [Himmelsbach, F.; Langkopf, E.; Eckhardt, M.; Mark, M.; Maier, R.; Lotz, R. R. H.; Tadayyon, M. Preparation of 8-[3-aminopiperidin-1-yl]xanthines as dipeptidylpeptidase-IV (DPP-IV) inhibitors. PCT Int. Appl. (2004), WO 2004018468 A2 20040304].

**5-(Chloromethyl)quinolin-8-yl dimethylcarbamate hydrochloride (MBA219)**. Following the **General Method for chlorination with SOCl$_2$**, alcohol MBA217 (303 mg, 1.23 mmol) in dry DCM (3 mL) was reacted with SOCl$_2$ (0.18 mL, 2.5 mmol) to give chloride MBA219 (337 mg, 91%), as a beige solid ($R_f= 0.35$, DCM/methanol, 5%): mp 165-170 °C, IR (KBr) v 3544, 3443, 2930, 2499, 1737, 1550, 1385, 1296, 1249, 1162, 1022 cm$^{-1}$; $^1$H NMR (500 MHz, CD$_3$Cl) $\delta$ 9.12 (br s, 1H, H2), 8.69 (br d, $J= 4.9$ Hz, 1H, H4), 7.66-7.65 (m, 2H, H3, H6), 7.53 (d, $J= 7.8$ Hz, 1H, H7), 5.00 (s, 2H, CH$_2$Cl), 3.34, 3.08 [s, s, 6H, OC(O)N(CH$_3$)$_2$]; $^{13}$C NMR (126 MHz, CD$_3$Cl) $\delta$ 154.3 [(CH$_3$)$_2$NOCO], 148.7 (C2), 147.2 (C8), 135.4 (C8a), 130.9 (2C, C4, C4a), 128.9 (C6), 126.8 (CH$_2$OH), 36.8 [(CH$_3$)$_2$NOCO]; MS (EI) $m/z$ (%): 247 (M+1)$^+$.
127.7 (C5), 122.8 (C7), 121.8 (C3), 42.8 (CH₂Cl), 37.2, 37.1 [(CH₃)₂NOCO]; MS (EI) m/z (%): 265 (M+1)⁺, 267 (M+3)⁺.

**Scheme 4.** Reagents conditions: for 13: (a) MBA207 [5-(chloromethyl)-8-methoxyquinoline], NaH, dry DMF, rt; for 14: (a) MBA219 [5-(chloromethyl)quinolin-8-yl dimethylcarbamate], NaH, dry DMF, rt;

**General Method for the O-alkylation of indole MBA176.** To a solution of compound MBA176 in dry DMF, under argon, at 0 °C, NaH (3 equiv, 60% dispersion in mineral oil) was added, and after 5 min, MBA207 or MBA219 (1 equiv) was added. The mixture was left stirring overnight at rt. The solvent was removed, and the residue was submitted to chromatography to afford the corresponding derivative.

**N-((5-((8-Methoxyquinolin-5-yl)methoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methylprop-2-yn-1-amine (13).** Following the **General method for the O-alkylation**, MBA176 (134 mg, 0.58 mmol) in DMF (4 mL) was treated with NaH (42 mg, 1.74 mmol), and MBA207 (122 mg, 0.58 mmol) to give compound 13 (125 mg, 54%), as a white solid (Rf= 0.26, AcOEt), after chromatography (hexane/AcOEt, 50-90%): mp 165-9 °C, IR (KBr) ν 3435, 3301, 2939, 2912, 2834, 2793, 1617, 1572, 1505, 1487, 1470, 1401, 1374, 1313, 1205, 1014 cm⁻¹; ¹H NMR (400 MHz, CD₃Cl) δ 8.94 (dd, J= 1.6, 4.2 Hz, 1H, H2’), 8.43 (dd, J= 1.6, 8.6 Hz, 1H, H4’), 7.54 (d, J= 7.9 Hz, 1H, H6’), 7.45 (dd, J= 4.2, 8.6 Hz, 1H, H3’), 7.20-7.18 (m, 2H, H4, H7), 6.99 (d, J= 7.9 Hz, 1H, H7’), 6.90 (dd, J= 2.4, 9.0 Hz, 1H, H6), 6.34 (s, 1H, H3), 5.38 [s, 2H, (C5’)CH₂O], 4.08 [s, 3H, C(8’)OCH₃], 3.73 [s, 3H, C(1)NCH₃], 3.67 [s, 2H, CH₂N(Me)CH₂C≡CH], 3.29 [d, J= 2.4 Hz, 2H, CH₂N(Me) CH₂C≡CH], 2.33 [s, 3H, CH₂N(CH₃)CH₂C≡CH], 2.27 [t, J= 2.4 Hz, 1H, CH₂N(Me)CH₂C≡CH]; ¹³C NMR (100 MHz, CD₃Cl) δ 156.0 (C5), 153.1 (C8’), 149.3 (C2’), 140.8 (C2), 137.5 (C8a’), 133.9 (C5’), 133.1 (C4’), 128.4
(C4a’), 128.2 (C6’), 127.2 (C3a)*, 125.1 (C7a)*, 122.1 (C3’), 112.4 (C6), 109.9 (C7), 108.7 (C7’), 104.2 (C4), 102.3 (C3), 78.6 [CH2N(Me)CH2C=CH], 73.7 [CH2N(Me)CH2C=CH], 69.4 [(C5’)CH2O], 56.2 [C(8’)OCH3], 52.0 [CH2N(Me)CH2C=CH], 44.9 [CH2N(Me)CH2C=CH], 41.8 [CH2N(CH3)CH2C=CH], 30.5 [N(1)CH3]; MS (EI) m/z (%): 172 (100) [M-C14H15N2O]+, 399 (4) [M]+. Anal. Calcd. for C25H25N3O2.H2O: C, 71.92; H, 6.52; N, 10.06. Found: 71.72; H, 6.47; N, 9.80.

5-(((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)methyl)quinolin-8-yl dimethylcarbamate (14). Following the General method for the O-alkylation, MBA176 (228 mg, 1.0mmol) in DMF (4 mL), was treated with NaH (120 mg, 3.0 mmol) and MBA219 (316 mg, 1.2 mmol) to afford 14 (260 mg, 57%), as a white solid, after chromatography (hexane/AcOEt, 90%): Rf= 0.32, AcOEt; mp 145-8 ºC; IR (KBr) ν 3267, 2872, 1710, 1618, 1484, 1158 cm⁻¹; ¹H NMR (500 MHz, CD3Cl) δ 8.95 (dd,  J  =  1.5, 4.0 Hz, 1H, H2’), 8.46 (dd, J=  1.5, 8.3 Hz, 1H, H4’), 7.62 (d, J=  7.4 Hz, 1H, H6’), 7.45-7.43 (m, 2H, H3’, H7’), 7.25-7.20 (m, 2H, H4, H7), 6.92 (dd, J=  2.5, 8.8 Hz, 1H, H6), 6.36 (s, 1H, H3), 5.45 [s, 2H, (C5’)CH2O] , 3.75 [s, 3H, C(1)NCH3], 3.69 [s, 2H, CH2N(Me)CH2C=CH], 3.31 [d, J=  2.4 Hz, 2H, CH2N(Me)CH2C=CH], 3.29, 3.07 [s, 8H, (CH3)2NOCO], 2.35 [s, 3H, CH2N(CH3)CH2C=CH], 2.29 [t, J=  2.4 Hz, 1H, CH2N(Me)CH2C=CH]; ¹³C NMR (126 MHz, CD3Cl) δ155.2 [(CH3)2NOCO], 152.8 (C5), 150.2 (C2’), 148.4 (C8’), 142.2 (C8a’), 137.3 (C2), 133.7 (C7a), 132.7 (C4’), 131.0 (C5’), 128.1 (C4a’), 127.5 (C3a), 126.9 (C6’), 121.5 (C3’)*, 120.9 (C7’)*, 112.1 (C6), 109.7 (C7), 104.7 (C4), 102.2 (C3), 78.3 [CH2N(Me)CH2C=CH], 73.4 [CH2N(Me)CH2C=CH], 69.0 [(C5’)CH2O], 51.8 [CH2N(Me)CH2C=CH], 44.7 [CH2N(Me)CH2C=CH], 41.5 [CH2N(CH3)CH2C=CH], 36.9 [2C, OCON(CH3)2], 29.9 [N(1)CH3]; MS (ESI) m/z (%): 457 (M+1)⁺. Anal. Calcd. for C27H28N4O3.1/3H2O C, 71.01; H, 6.25; N, 12.11. Found: C, 70.04; H, 6.08; N, 12.26
Following the General method for the N-alkylation of indoles, product 1 (80 mg, 0.18 mmol) in CH$_3$CN (3.0 mL), was treated with DIPEA (95 µl, 0.54 mmol) and MBA207 (38 mg, 0.18 mmol) to give compound 4 (62 mg, 58%), as a white solid (R$_f$ = 0.30, DCM/methanol, 5%) after chromatography (DCM/methanol, 1%): mp 117-120 ºC, IR (KBr) $\nu$ 3301, 2934, 2849, 2793, 1620, 1574, 1504, 1487, 1475, 1454, 1205, 1161, 1103 cm$^{-1}$; $^1$H NMR (400 MHz, CD$_3$Cl) $\delta$ 8.90 (dd, $J$ = 1.7, 4.1 Hz, 1H, H$_2$'), 8.66 (dd, $J$ = 1.7, 8.6 Hz, 1H, H4'), 7.42 (dd, $J$ = 4.1, 8.6 Hz, 1H, H3'), 7.32 (d, $J$ = 7.9 Hz, 1H, H6'), 6.92 (d, $J$ = 7.9 Hz, 1H, H7'), 6.91 (dd, $J$ = 2.4, 8.8 Hz, 1H, H6), 6.29 (s, 1H, H3), 4.06 [s, 3H, C(8')OCH$_3$], 3.93 [t, $J$ = 6.7 Hz, 2H, C(5)OCH$_2$C$_2$H$_2$CH$_2$], 3.74 [s, 2H, (C5')CH$_2$N], 3.71 [s, 3H, C(1)NCH$_3$], 3.64 [s, 2H, CH$_2$N(Me)CH$_2$C≡CH], 3.28 [d, $J$ = 2.4 Hz, 2H, CH$_2$N(Me)CH$_2$C≡CH], 2.84 (d, $J$ = 11.5 Hz, 2H, [N(CH$_2$Eq$_2$)CH$_2$]$_2$CH], 2.31 [s, 3H, CH$_2$N(CH$_3$)CH$_2$C≡CH], 2.26 [t, $J$ = 7.4 Hz, 1H, CH$_2$N(Me)CH$_2$C≡CH], 1.95 [dd, $J$ = 9.9, 11.5 Hz, 2H, N(CH$_{ax}$x)CH$_2$CH$_2$], 1.79-1.75 [m, 2H, C(5)OCH$_2$CH$_2$CH$_2$], 1.66-1.62 [m, 3H, N(CH$_2$CH$_2$)$_2$CH], 1.39-1.34 [m, 2H, C(5)OCH$_2$CH$_2$CH$_2$], 1.23-1.16 [m, 2H, N(CH$_2$CH$_{ax}$x)CH$_2$] $^{13}$C NMR (100 MHz, CD$_3$Cl) $\delta$ 155.1 (C5), 153.5 (C8'), 149.0 (C2'), 140.7 (C2), 137.2 (C8a'), 133.9 (C4'), 133.5 (C5'), 128.9 (C4a'), 127.9 (C6'), 127.7 (C3a)*, 126.8 (C7a)*, 121.4 (C3'), 112.2 (C6), 109.8 (C7), 106.5 (C7'), 103.5 (C4), 102.2 (C3), 78.6 [CH$_2$N(Me)CH$_2$C≡CH], 73.6 [CH$_2$N(Me)CH$_2$C≡CH], 69.2 [OC(5)H$_2$CH$_2$CH$_2$], 61.2 [(C5')CH$_2$N], 56.1 [C(8')OCH$_3$], 54.2 [N(CH$_2$CH$_2$)$_2$CH], 52.0 [CH$_2$N(Me)CH$_2$C≡CH], 44.9 [CH$_2$N(Me)CH$_2$C≡CH], 41.7 [CH$_2$N(CH$_3$)CH$_2$C≡CH], 35.9 [N(CH$_2$CH$_2$)$_2$CH], 33.1 [C(5)OCH$_2$CH$_2$CH$_2$], 32.6 [N(CH$_2$CH$_2$)$_2$CH], 30.1 [N(1)CH$_3$], 27.0 [C(5)OCH$_2$CH$_2$CH$_2$]; MS (EI) $m/z$ (%): 172 (100) [M-C$_{11}$H$_{16}$NO]+, 283 (15) [M-C$_{18}$H$_{23}$N$_2$O]+, 442 (3) [CH$_2$N(Me)CH$_2$C≡CH]+, 456 (6) [N(Me)CH$_2$C≡CH]+, 485 (10)
Following the General method for the N-alkylation of indoles, compound 1 (66 mg, 0.15 mmol) in CH₃CN (3.5 mL) was reacted with DIPEA (0.1 mL, 0.6 mmol) and MBA219 (41 mg, 0.15 mmol) to give product 5 (63 mg, 70%), as a white solid (Rₚ= 0.38, DCM/methanol 5%), after chromatography (DCM/methanol, 1%): mp 115-8 ºC, IR (KBr) ν 3434, 3281, 2915, 1722, 1486, 1390, 1170, 1072 cm⁻¹; ¹H NMR (400 MHz, CD₃Cl) δ 8.89 (dd, J = 1.7, 4.0 Hz, 1H, H2'), 8.69 (dd, J = 1.7, 8.4 Hz, 1H, H4'), 7.40-7.37 (m, 2H, H3', H6'), 7.33 (d, J = 7.7 Hz, 1H, H7'), 7.15 (d, J = 8.8 Hz,1H, H7), 7.00 (d, J = 2.4 Hz, 1H, H4), 6.82 (dd, J = 2.4, 8.8 Hz, 1H, H6), 6.30 (s, 1H, H3), 3.94 [t, J = 6.6 Hz, 2H, (C5)OC₃H₂CH₂CH₂], 3.78 [s, 2H, (C5')CH₂N], 3.71 [s, 3H, C(1)NCH₃], 3.65 [s, 3H, CH₂N(Me)CH₂C≡CH], 3.28 [d, J = 11.5 Hz, 2H, (C(5)OC₃H₂), 2.32 [s, 3H, CH₂N(CH(2)₃)₂C≡CH], 2.26 [t, J = 2.4 Hz, 1H, CH₂N(Me)CH₂C≡CH], 1.95 [td, J = 2.2, 11.5 Hz, 2H, N(CH₂C≡C₂)₂CH], 1.81-1.73 [m, 2H, C(5)OCH₂CH₂CH₂], 1.64 [br d, J = 12.5, 2H, N(CH₂C≡C₂)₂CH], 1.40-1.34 [m, 2 H, C(5)OCH₂CH₂CH₂], 1.33-1.24 [m, 1H, N(CH₂CH₂)₂CH], 1.21-1.15 [m, 2H, N(CH₂CH₂)₂CH]; ¹³C NMR (100 MHz, CD₃Cl) δ 155.5 [(CH₃)₂NOCO], 153.5 (C5), 150.2 (C2'), 147.6 (C8'), 142.4 (C5'), 137.2 (C2), 133.9 (C4'), 133.5 (C7a), 133.1 (C8a'), 129.1 (C3a), 127.7 (C4a'), 127.3 (C6'), 121.1 (C3), 120.8 (C7'), 112.2 (C6), 109.8 (C7), 103.5 (C4), 102.2 (C3), 78.6 [CH₂N(Me)CH₂C≡CH], 73.6 [CH₂N(Me)CH₂C≡CH], 69.2 [OC(5)H₂CH₂CH₂], 61.3 [(C5')CH₂N], 54.2 [N(CH₂CH₂)₂CH], 52.0 [CH₂N(Me)CH₂C≡CH], 44.9 [CH₂N(Me)CH₂C≡CH], 41.8 [CH₂N(CH₂)₃CH₂C≡CH], 37.0 [2C, OCON(CH₃)₂], 35.9 [N(CH₂CH₂)₂CH], 33.1 [C(5)OCH₂CH₂CH₂], 32.6 [N(CH₂CH₂)₂CH], 30.1 [N(1)CH₃], 27.0 [C(5)OCH₂CH₂CH₂]; MS (EI) m/z (%): 352 (97) [M-C₃H₆NO₂]⁺, 493 (4) [M-C₃H₆NO₂]⁺, 513 (18) [M-N(Me)CH₂C≡CH]⁺, 542 (26) [M-CH₂C≡CH]⁺, 581 (35) [M]⁺ . Anal. Calcd. for C₃₅H₄₃N₅O₃: C, 72.24; H, 7.45; N, 12.04. Found: C, 72.24; H, 7.20; N, 12.08.

1-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N-((5-methoxy-1-methyl-1H-indol-2-yl)methyl)-N-methylmethanamine (15).
1-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methylmethanamine (9).

1-(5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)-N-methyl-N-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)methanamine (10).

**General Method for the synthesis of triazolindole derivatives.** To a solution of the alkyne and the commercial azide (2 equiv) in a mixture of DMF and water (1:1), a solution of sodium ascorbate (0.4 equiv) and CuSO₄·7H₂O (0.17 equiv) in water was added. The mixture was stirred at 60 °C overnight. Then, the solvents were evaporated, and the residue was purified by chromatography to give the pure molecules.

**Scheme 6.** Reagents and conditions: (a) Benzylazide, sodium ascorbate, CuSO₄·7H₂O, DMF/H₂O, 60 °C.

1-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N-((5-methoxy-1-methyl-1H-indol-2-yl)methyl)-N-methylmethanamine (15). Following the **General Method for the synthesis of triazolindole derivatives**, a solution of ASS20 (100 mg, 0.41 mmol), and benzylazide (1.61 mL, 0.82 mmol) in DMF and water (1:1, 10 mL), were reacted with a solution of sodium ascorbate (32 mg, 0.16 mmol) and CuSO₄·7H₂O (18 mg, 0.07 mmol) in water (1.0 mL), to yield 15 (114 mg, 74%), as a white solid (Rf= 0.22, hexano/AcOEt, 70%), alter chromatography (hexane/AcOEt, 50-70): mp 93-5 °C; IR (KBr) ν 3436, 3130, 2912, 1489, 1209, 1031 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.37-7.35 (m, 3H, H3″, H5″, H4″), 7.27 (s, 1H, H5′), 7.25-7.23 (m, H2″, H6″, 2 H, NCH₂C₆H₅), 7.14 (dd, J= 7.8 Hz, 1H, H7), 7.01 (d, J= 2.5 Hz, 1H, H4), 6.82 (dd, 1H, H6), 6.27 (s, 1H, H3), 5.47 (s, 2H, NCH₂C₆H₅), 3.83 (s, 3H, OCH₃), 3.70 [s, 2H, C(2)CH₂N(CH₃)C₆H₅], 3.62 [s, 3H, N(1)CH₃], 3.61 [s, 2H, C(2)CH₂N(CH₃)CH₂], 2.24 [s, 3H, C(2)CH₂N(CH₃)CH₂]; ¹³C NMR (126 MHz, CDCl₃) δ 153.9 (C5), 145.5 (C4′), 137.4 (C2), 134.7 (C1″), 133.3 (C7a), 129.1 (2C, C3″, C5″, NCH₂C₆H₅), 128.7 (C4″, NCH₂C₆H₅), 127.9 (2C, C2″, C6″, NCH₂C₆H₅), 127.5 (C3a), 122.3 (C5′), 11.3 (C6), S22
109.6 (C7), 102.1 (C3)*, 102.0 (C4)*, 55.9 (OCH3), 54.0 (NCH2C6H5), 53.5 [C(2)CH2N(CH3)CH2], 51.9 [C(2)CH2N(CH3)CH2], 42.3 [C(2)CH2N(CH3)CH2], 29.8 [N(1)CH3]; MS (EI) m/z (%): 203 (100) [M-C10H10N3]+, 174 (52) [M-C11H13N4]+, 375 (38) [M]+; Calcd for C22H25N5O: C, 70.38; H, 6.71; N, 18.65. Found: C, 70.11; H, 6.59; N, 18.58.

Scheme 7. Reagents and conditions: (a) Benzylazide, sodium ascorbate, CuSO4.7H2O, DMF/H2O, 60 ºC.

1-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methylmethanamine (9). Following the General Method for the synthesis of triazolindole derivatives, a solution of MBA138F3 (107 mg, 0.24 mmol) and benzylazide (0.96 mL, 0.48 mmol) in DMF and water (1:1, 10 mL), were reacted with a solution of sodium ascorbate (19 mg, 0.09 mmol) and CuSO4.7H2O (11 mg, 0.04 mmol) in water (1.5 mL), to give 9 (99 mg, 71%) as a white solid [Rf= 0.30, hexane/AcOEt70%, plus triethylamine (TEA) 1%], after chromatography (hexane/AcOEt, 50-70%, TEA 1%): mp 95-8 ºC; IR (KBr) ν 3468, 2935, 1487, 1206, 1016 cm⁻¹; ¹H NMR (500 MHz, CDCl3) δ 7.30-7.27 (m, 2H, NCH2C6H5, triazoleNCH2C6H5), 7.23-7.21 (m, 3H, NCH2C6H5, triazoleNCH2C6H5), 7.20 (s, 1H, H5’’), 7.18-7.15 (m, 5H, NCH2C6H5, triazoleNCH2C6H5), 7.06 (dd, J = 8.8 Hz, 1 H7), 6.92 (d, J = 2.5 Hz, 1H, H4), 6.75 (dd, 1H, H6), 6.18 (s, 1H, H3), 5.40 (s, 2H, triazoleNCH2C6H5), 3.87 (t, J = 6.3 Hz, 2H, OCH2CH2CH2), 3.62 [s, 2H, C(2)CH2N(CH3)CH2], 3.54 [s, 3H, N(1)CH3], 3.53 [s, 2H, C(2)CH2N(CH3)CH2], 3.41 (s, 2H, NCH2C6H5), 2.80 [d, J = 11.2 Hz, 2H, N(CH2)eq(CH2)ax(CH2)2CH], 2.16 [s, 3H, C(2)CH2N(CH3)CH2], 1.86 [t, J = 11.2 Hz, 2H, N(CH2)eq(CH2)ax(CH2)2CH], 1.73-1.70 (m, 2H, OCH2CH2CH2), 1.61 [d, J = 9.3 Hz, 2H, N(CH2)2(CH2)eq(CH2)axCH], 1.35-1.31 (m, 2H, OCH2CH2CH2), 1.26-1.20 [m, 3H, N(CH2)2(CH2)eq(CH2)axCH]; ¹³C NMR (126 MHz, CDCl3) δ 153.3 (C5), 145.5 (C4’’), 138.6 (C1’’, NCH2C6H5), 137.3 (C3a), 134.7 (C1’’’, triazoleNCH2C6H5), 133.3 (C7a), 129.2-127.5 (triazoleNCH2C6H5, NCH2C6H5), 126.8 (C2), 122.3 (C5’’’), 111.9 (C6), 109.5 (C7), 103.3 (C4), 101.9 (C3),
69.1 (OCH₂CH₂CH₂), 63.5 (NCH₂C₆H₅), 54.0 N(CH₂)₂(CH₂)₂CH], 53.9 [C(2)CH₂N(CH₃)CH₂], 53.5 (triazoleNCH₂C₆H₅), 51.9 [C(2)CH₂N(CH₃)CH₂], 42.3 [C(2)CH₂N(CH₃)CH₂], 35.5 [N(CH₂)₂(CH₂)₂CH], 32.9 (OCH₂CH₂CH₂), 32.4 [N(CH₂)₂(CH₂)₂CH], 29.8 [N(1)CH₃], 26.8 (OCH₂CH₂CH₂); MS (EI) m/z: 404 (100) [M-C₁₀H₁₀N₃]+, 374 (74) [M-C₁₁H₁₃N₄]+, 576 (5) [M]+. Calcd for C₃₆H₄₄N₆O: C, 74.97; H, 7.69; N, 14.57. Found: C, 74.72; H, 7.61; N, 14.47.

1-(5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)-N-methyl-N-((1-phenyl-1H-1,2,3-triazol-4-yl)methyl)methanamine (10). Following the General Method for the synthesis of triazolindole derivatives, a solution of MBA138F3 (119 mg, 0.23 mmol) and phenylazide (0.94 ml, 0.46 mmol), in DMF and water DMF and water (1:1, 10 mL), were reacted with a solution of sodium ascorbate (18 mg, 0.09 mmol) and CuSO₄·7H₂O (11 mg, 0.04 mmol) in water (1.5 mL), to give compound 10 (100 mg, 65%), as a oil (Rf= 0.30, hexane/AcOEt, 70%; TEA, 1%), after chromatography (hexane/AcOEt, 50-70%; TEA, 1%), charcterized as the bis-chlorhydrate, prepared as usual: mp 215-218 °C; IR (KBr) ν 3428, 2939, 2504, 1621, 1457, 1208, 1046 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 12.94 (br s, 1H, BnN+Hpiperidine), 12.37 (br s, 1H, triazoleCH2NMeN+H), 8.95 (s, 1H, H5''), 7.78-7.76 (2H), 7.63-7.61 (2H), 7.55-7.52 (2H), 7.49-7.47 (1H), 7.43-7.42 (3H) [m, 10H, NCH2C6H5, triazoleNC6H5], 7.21 (dd, J= 8.8 Hz, 1 H7), 7.01 (d, J= 2.8 Hz, 1H, H4), 6.91 (dd, 1H, H6), 6.87 (s, 1H, H3), 4.55 (br d, J= 13.8 Hz, 2H; 4.45 (br d, J= 11.1 Hz, 1 H; 4.39 (br d, J= 12.7, 1H: C(2)CH2N+H(CH3)C6H5), C(2)CH2N+H(CH3)CH2], 4.12 (d, J= 4.4 Hz, 2H, HN+CH2C6H5), 3.94 (t, J= 6.3 Hz, 2H, OCH2CH2CH2), 3.83 [s, 3H, N(1)CH3], 3.46 [d, J= 11.1 Hz, 2H, N(CH2)2eq(CH2)ax(CH2)2CH], 2.77 [s, 3H, C(2)CH2N(CH3)CH2], 2.58 [dd, J= 11.1 and 10.5 Hz, 2H, N(CH2)eq(CH2)ax(CH2)2CH], 2-06 [dd, J= 11.7 and 14.1 Hz, 2H, N(CH2)2(CH2)eq(CH2)axCH], 1.86 [d, J= 14.1 Hz, 2H, N(CH2)2(CH2)eq(CH2)axCH], 1.80-1.74 (m, 2H, OCH2CH2CH2), 1.53-1.47 [m, 3H, OCH2CH2CH2, N(CH2)2(CH2)eq(CH2)axCH]; ¹³C NMR (126 MHz, CDCl₃) δ 153.7 (C5), 136.4 (C4''), 133.8 (C1', HN'CH2C₆H₅), 131.5 (C3a), 131.0 (C1'''), triazoleNCH₂C₆H₅), 130.08 (C7a), 130.04, 129.8, 129.4, 129.2, 128.1, 127.1 (triazoleNCH₂C₆H₅, NCH₂C₆H₅), 126.2 (C2), 125.6 (C5''), 120.7 (2C, C2''', C6'''''),114.7 (C6), 110.9 (C7), 107.6 (C3), 103.2 (C4), 68.2 (OCH2CH2CH2), 60.9 (N'CHCH2C₆H₅), 52.4 [N(CH2)₂(CH₂)₂CH], 49.8 and 49.6 [2C: C(2)CH2N⁺H(CH3)CH2, C(2)CH2N⁺H(CH3)CH2], 38.8 [C(2)CH2N(CH3)CH2], 34.0 [N(CH2)₂(CH₂)₂CH], 31.9
(OCH₂CH₂CH₂), 30.9 [N(1)CH₃], 28.9 [N(CH₂)₂(CH₂)₂CH], 26.2 (OCH₂CH₂CH₂); MS (El) m/z: 404 (100) [M-C₉H₆N₃]⁺, 374 (85) [M-C₁₀H₁₂N₄]⁺, 216 (32) [M-C₂₀H₂₀N₅O]⁺, 174 (10) [M-C₂₃H₂₆N₅O]⁺. Caled for C₃₅H₄₃N₆O.2HCl.1/2H₂O: C, 65.21; H, 7.04; N, 13.04. Found: C, 65.41; H, 6.88; N, 12.93.

Ethyl 5-(3-(1-benzylpiperidin-4-yl)propoxy)-1H-indole-2-carboxylate hydrochloride (11).

(5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methanol (12).

N-(5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)-N-methylprop-2-yn-1-amine (6).

N-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine (7)

N-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-en-1-amine (8).
Ethyl 5-hydroxy-1H-indole-2-carboxylate (MBA233). To a solution of commercial C-6 (1.02 g, 3.4 mmol) in dry THF (15 mL), Pd/C (15%) (153 mg) and TEA (0.48 mL, 3.4 mmol) were added, and the mixture was hydrogenated at rt for 2 h at 45 psi. Then, the crude was filtered over Celite, the cake was washed with THF, and the solvent was removed, to give pure compound MBA233 (701 mg, 99%; Rf = 0.35, hexane/AcOEt, 30%), as a yellow solid (Buchi, G.; Botkin, J. H.; Lee, G. C. M.; Yakushijin, K. A. Synthesis of methoxatin, J. Am. Chem. Soc. 1985, 107, 5555-6).

1-(tert-Butyl) 2-ethyl 5-hydroxy-1H-indole-1,2-dicarboxylate (MBA237). To a solution of MBA233 (701 mg, 3.41 mmol) in dry dioxane (6 mL), TEA (0.70 mL, 5.10 mmol) and (t-BOC)₂O (1.12 g, 5.10 mmol), dissolved in dry dioxane (3.0 mL), were
added. The mixture was stirred at 70 °C overnight. The solvent was removed, DCM and aqueous HCl (5%) were added till pH 6-7. The work-up was repeated several times and the organic layer was dried (Na$_2$SO$_4$), filtered, and the solvent was evaporated. The residue was submitted to chromatography (hexane/AcOEt, 10%) to yield compound **MBA237** (1.02 g, 98%, RF = 0.31, hexane/AcOEt, 30%) (Akwabi-Ameyaw, A. Caravella, J.A.; Chen, L. Creech, K.L.; Deaton, D.N.; Madauss, K.P.; Marr, H.B.; Miller, A.B.; Navas, F. III; Parks, D.J.; Spearing, P.; Todd, D. Williams, S.P.; Wisely, G. B. Conformationally constrained farnesoid X receptor (FXR) agonists: Alternative replacements of the stilbene, *Bioorg. Med. Chem. Lett.* **2011**, 21, 6154-6160).

3-(1-Benzylpiperidin-4-yl)propan-1-ol (MBA240). To a suspension of **MBA160** (700 mg, 3.91 mmol) in dry DCM (5 mL), TEA (2.16 mL, 15.6 mmol) followed by benzyl bromide (0.58 mL, 4.88 mmol) were added. The reaction mixture was refluxed overnight. Then, the solvent was removed, and the crude was submitted to chromatography (hexane/AcOEt, 10%) to give product **MBA240** (838 mg, 92%) as an oil (RF = 0.28, hexane/AcOEt, 50%) (Kitbunnadaj, R.; Zuiderveld, O. P.; De Esch, I. J. P.; Vollinga, R. C.; Bakker, R.; Lutz, M.; Spek, A. L.; Cavoy, E.; Deltent, M.-F.; Menge, W. M. P. B.; Timmerman, H.; Leurs, R. Synthesis and Structure-Activity Relationships of Conformationally Constrained Histamine H3 Receptor Agonists, *J. Med. Chem.* **2003**, 46, 5445-5457).

Ethyl 5-(3-(1-benzylpiperidin-4-yl)propoxy)-1H-indole-2-carboxylate hydrochloride (11). To a solution of **PPh$_3$** (1.20 g, 4.6 mmol) in dry THF (6 mL), under argon and at 0 °C, DIAD (0.90 mL, 4.6 mmol) was slowly added, and the mixture was stirred for 1 h; then, **MBA240** (527 mg, 2.30 mmol), followed by **MBA247** (700 mg, 2.30 mmol) were added, and stirred for 48 h at rt. The solvent was evaporated, and the crude purified by chromatography (hexane/AcOEt, 10%) affording **1-(tert-butyl) 2-ethyl 5-(3-(1-benzylpiperidin-4-yl)propoxy)-1H-indole-1,2-dicarboxylate (MBA242)** (656 mg, 55%) as an oil: RF = 0.25 (hexane/AcOEt, 40%); $^1$H NMR (300 MHz, CDCl$_3$) δ 7.44 (d, J = 2.5 Hz, 1H, H4), 7.36-7.25 (m, 7H, NCH$_2$C$_6$H$_5$, H7, H3), 7.14 (dd, J = 8.9, 2.5 Hz, 1H, H6), 4.51 [t, J = 7.6 Hz, 2H, OCH$_2$(CH$_2$)$_2$], 4.36 (q, J = 7.1 Hz, 2H, CO$_2$CH$_2$CH$_3$), 3.47 (s, 2H, NCH$_2$C$_6$H$_5$), 2.85 [d, J = 11.1 Hz, 2H, N(CH$_2$)$_{eq}$(CH$_2$)$_{ax}$(CH$_2$)$_2$CH], 1.89 [t, J = 11.1 Hz, 2H, N(CH$_2$)$_{eq}$(CH$_2$)$_{ax}$(CH$_2$)$_2$CH], 1.86-1.55 [m, 5H, N(CH$_2$)$_2$(CH$_2$)$_{eq}$(CH$_2$)$_{ax}CH$, OCH$_2$CH$_2$CH$_3$], 1.59 [s, 9H, NCO$_2$C(CH$_3$)$_3$],
1.39 (t, J = 7.1 Hz, 3H, CO₂CH₂CH₃), 1.28-1.21 [m, 4H, OCH₂CH₂CH₃, N(CH₂)₂(eq)(CH₂)axCH₃]; MS (ESI) m/z (%): 520 (M+H)⁺. To a solution of MBA242 (656 mg, 1.26 mmol) in AcOEt (5 mL), under argon and at 0 °C, a saturated solution of HCl(g) in AcOEt (10 mL) was added. After stirring overnight, the solid was removed, washed with cold AcOEt, to give chlorhydrate 11 as a oil (529 mg, 100%): Rf = 0.25 (DCM/methanol, 10%/TEA,1%); IR (KBr) ν 3208, 2982, 2935, 1703, 1508, 1465, 1375, 1208, 1095, 1038 cm⁻¹; 1H NMR (500 MHz, CD₃OD) δ 7.48-7.35 (m, 5H, NCH₂C₆H₅), 7.31 (d, J = 9.3 Hz, 1H, H7), 7.08 (s, 1H, H₃), 6.94 (d, J = 2.5 Hz, 1H, H₄), 6.89 (dd, J = 9.3, 2.5 Hz, 1H, H₆), 4.52 [t, J = 7.4 Hz, 2H, OC₂H₂(CH₂)₂], 4.31 (q, J = 6.8 Hz, 2H, CO₂C₂H₂CH₃), 4.25 (s, 2H, HN + C₆H₅), 3.44 [d, J = 11.8 Hz, 2H, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃], 2.93 [t, J = 11.8 Hz, 2H, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃], 2.86 (m, 1H, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃), 2.85 (m, 1H, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃), 2.10 (m, 2H, OCH₂C₂H₂CH₃), 1.92 [d, J = 14.2 Hz, 2H, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃], 1.78 (m, 2H, OCH₂C₂H₂CH₃), 15.9-153 [m, 1H, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃], 1.37 (t, J = 6.8 Hz, 3H, CO₂CH₂CH₃), 1.31-1.28 [m, 4H, OCH₂CH₂CH₃, N(CH₂)₂(eq)(CH₂)ax(CH₂)axCH₃]; 13C NMR (126 MHz, CD₃OD) δ 162.0 (CO₂CH₂CH₃), 153.3 (C₅), 134.3 (C₇a) 130.9 (C₄’, NCH₂C₆H₅), 129.8 (2xCH, C’₂, C’₃, C’₅’, NCH₂C₆H₅)*, 128.9 (3C: 2xCH, C’₂’, C’₆’, NCH₂C₆H₅; C’₁’)*, 127.1 (C₃a)*, 126.5 (C₂)**, 115.8 (C₆), 110.8 (C₇), 109.0 (C₃), 104.7 (C₄), 60.3 (HN + C₆H₅), 60.0 (CO₂C₂H₂CH₃), 52.3 [2xCH₂, N(CH₂)₂(eq)(CH₂)axCH₃], 43.8 (CH₂CH₂CH₂O), 33.0 (CH, N(CH₂)₂(eq)(CH₂)axCH₃), 32.3 (CH₂CH₂CH₂O), 29.1 [2xCH₂, N(CH₂)₂(eq)(CH₂)axCH₃], 27.1 (CH₂CH₂CH₂O), 13.2 (CO₂C₂H₂CH₃); MS (EI) m/z (%): 420 (100) [M]⁺, 347 (94) (M-CO₂C₂H₅)⁻, 329 (17) [M-CH₂C₆H₅]⁺, 202 (89) [M-C₁₂H₁₂NO₃]⁺, 174 (15) [M-C₁₄H₁₆NO₃]⁻. Anal. Caled. for C₂₆H₃₃ClN₂O₃: C, 66.24; H, 7.40; Cl, 7.52; N, 5.94. Found: C, 66.27; H, 7.41; N, 6.16.

(5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methanol (12). To a suspension of LAH (54 mg, 1.42 mmol) in dry THF (3 mL), under argon at 0 °C, product 11 (150 mg, 0.35 mmol) was slowly added. Then, the mixture was refluxed 2 h. The reaction was cooled, and water added carefully. The solid was removed, washing the cake with AcOEt, and discarded. The solvent was evaporated and the crude was purified by chromatography (DCM/methanol, 1-5%) giving product 12 (128 mg, 95%; Rf = 0.35, DCM/methanol 10%): mp 135-7°C; IR (KBr) ν 3280, 2922, 1620, 1468, 1372, 1253, 1201, 1182, 1011 cm⁻¹; 1H NMR (500 MHz, CDCl₃) δ 7.30-7.25 (m, 5H, NCH₂C₆H₅), 7.13 (d, J = 8.8 Hz, 1H, H7), 6.95 (d, J = 2.5 Hz, 1H, H₄), 6.95 (dd, J = 8.8, 2.5 Hz, 1H, H₆), 6.30 (s, 1H, H₃), 4.74 (s, 2H, CH₂OH), 4.11 [t, J = 6.6 Hz, 2H,
OCH\(_2\)(CH\(_2\))\(_2\)}, 3.47 (s, 2H, NCH\(_2\)Ph), 2.87 [d, J= 11.7 Hz, 2H, N(CH\(_2\))\(_{2eq}\)], 1.90 [tm, J= 11.7 Hz, 2H, N(CH\(_2\))\(_{2ax}\)], 1.81-1.78 (m, 2H, OCH\(_2\)CH\(_2\)CH\(_2\)), 1.69-1.67 [m, 2H, N(CH\(_2\))\(_2\)(CH\(_2\))\(_{3eq}\)], 1.41-1.39 (m, 2H, OCH\(_2\)CH\(_2\)), 1.28-127 [m, 3H, N(CH\(_2\))\(_2\)(CH\(_2\))\(_{ax}\)C\(_6\)H\(_5\)] (the N(1)H signal was not observed); \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta\) 149.4 (C5-indol), 139.1 (2C, C2a, C1'-Ph), 132.7 (C7a), 129.3 (2C, C2', C6'-Ph), 128.1 (2C, C3', C5'-Ph), 127.8 (C3a), 126.9 (C4'-Ph), 111.7 (C6), 110.1 (C7), 105.1 (C4), 100.7 (C3), 63.4 (NCH\(_2\)Ph), 57.5 (CH\(_2\)OH), 53.7 [2xCH\(_2\), N(CH\(_2\))\(_2\)(CH\(_2\))\(_2\)CH], 44.0 (CH\(_2\)CH\(_2\)C\(_6\)H\(_5\)), 35.5 [CH, N(CH\(_2\))\(_2\)(CH\(_2\))\(_2\)CH], 33.8 [2xCH\(_2\), N(CH\(_2\))\(_2\)(CH\(_2\))\(_2\)CH], 32.1 (CH\(_2\)CH\(_2\)CH\(_2\)), 26.7 (CH\(_2\)CH\(_2\)CH\(_2\)); MS (EI) m/z (%): 378 (53) \([M]^+\), 361 (19) \([M-OH]^-\), 347 (100) \([M-CH_3O.]^-\), 202 (44) \([\text{M-C}_10\text{H}_{10}\text{NO}_2.]^+\). Anal. Calcd. for C\(_{24}\)H\(_{30}\)N\(_2\)O\(_2\).1/6 H\(_2\)O: C, 75.56; H, 8.01; N, 7.34. Found: C, 75.65; H, 7.83; N, 7.50.

5-(3-(1-Benzylpiperidin-4-yl)propoxy)-N-methyl-N-(prop-2-yn-1-yl)-1H-indole-2-carboxamide (MBA316). To a solution of compound 11 (150 mg, 0.36 mmol) in dry THF (5 mL), under argon, N-methylpropargylamine (76 µL, 0.9 mmol) followed by trimethyl aluminium (AlMe\(_3\)) (2.0 M solution in hexanes) (0.80 mL, 1.6 mmol). This mixture was irradiated in a microwave apparatus (Biotage initiator 2.5) at 125 °C for 30 min. The, the reaction mass was treated with some drops of an aqueous solution of HCl (1N), evaporated to dryness. The crude was submitted to column chromatography (DCM/methanol, 1-5%) to provide amine MBA316 (139 mg, 88%) as an oil, R\(_f=\) 0.31, DCM/methanol, 10%); IR (KBr) \(\nu\) 3428, 3291, 2925, 2851, 2802, 2758, 1630, 1526, 1453, 1404, 1374, 1225, 1201, 1071 cm\(^{-1}\); \(^{1}H\) NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.32-7.22 (m, 5H, NCH\(_2\)C\(_6\)H\(_5\)), 7.09 (d, J= 8.8 Hz, 1H, H7), 6.90 (d, J= 2.2 Hz, 1H, H4), 6.76 (dd, J= 8.8, 2.2 Hz, 1H, H6), 6.55 (br s, 1H, H3), 4.34 (d, J= 2.2 Hz, 1H, CONMeCH\(_2\)C=CH), 4.20 [t, J= 7.2 Hz, 2H, OCH\(_2\)(CH\(_2\))\(_2\)], 3.52 (s, 2H, NCH\(_2\)Ph), 3.20 [s, 3H,N(1)CH\(_3\)], 2.90 [d, J= 11.3 Hz, 2H, N(CH\(_2\))\(_{2eq}\)], 2.33 (br s, 1H, CONMeCH\(_2\)C=CH), 1.93 [t, J= 10.9 Hz, 2H, N(CH\(_2\))\(_{2ax}\)], 1.72-1.64 (m, 2H, OCH\(_2\)CH\(_2\)CH\(_2\)), 1.56 [br, J= 10.2 Hz, 2H, N(CH\(_2\))\(_2\)(CH\(_2\))\(_{ax}\)], 1.37-1.17 (m, 5H, OCH\(_2\)CH\(_2\)CH\(_2\), N(CH\(_2\))\(_2\)(CH\(_2\))\(_{ax}\)CH]) (the N(1) H signal was not observed); MS (ESI) m/z (%): 444 (M+H)\(^+\).

5-(3-(1-Benzylpiperidin-4-yl)propoxy)-N-(prop-2-yn-1-yl)-1H-indole-2-carboxamide (MBA315). Following the same method for the synthesis of compound MBA313, product 11 (130 mg, 0.30 mmol) and propargylamine (50 µL, 0.75 mmol) were reacted with AlMe\(_3\) (0.67 mL, 1.35 mmol), to give amine MBA315 (100 mg, 76%) as a solid (R\(_f=\) 0.35, DCM/methanol, 10%) after purification by chromatography
(DCM/methanol, 1-5%): mp 73-6 °C; IR (KBr) ν 3414, 3296, 2924, 2850, 2802, 2758, 1644, 1531, 1455, 1226, 1110 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 7.32-7.23 (m, 5H, NCH₂C₆H₅), 7.15 (d, J= 8.8 Hz, 1H, H7), 6.91 (d, J= 2.4 Hz, 1H, H4), 6.94 (dd, J= 8.8, 2.4 Hz, 1H, H6), 6.40 (s, 1H, H3), 6.41 (t, J= 5.2 Hz, 1H, CONHCH₂C≡CH), 4.43 [t, J= 7.3 Hz, 2H, OCH₂(CH₂)₂], 4.20 (dd, J= 2.4, 5.2 Hz, 1H, CONHCΗ₂C≡CH), 3.52 (s, 2H, NCH₂Ph), 2.90 [d, J= 10.9 Hz, 2H, N(CH₂)₂], 2.26 (t, J= 2.4 Hz, 1H, CONHCH₂C≡CH), 1.94 [t, J= 10.9 Hz, 2H, N(CH₂)₂], 1.81-1.64 (m, 2H, OCH₂CH₂CH₂), 1.58 [brd, J= 9.3 Hz, 2H, N(CH₂)₂(CH₂)₂], 1.37-1.17 (m, 5H, OCH₂CH₂CH₂, N(CH₂)₂(CH₂)₂CH)]. MS (ESI) m/z: 430 (M+H)⁺.

**General Method for the reduction of amides with LAH.** To a suspension of LAH (4.5 equiv) in dry THF (0.18M), under argon and at 0 °C, the amide was slowly added, and refluxed for 1 h. The mixture was cooled at 0 °C, and water was added to destroy the excess of LAH. Next, AcOEt was added and the salts was removed by filtration, and the solvent was evaporated to give a crude that was submitted to chromatography.

**N-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)-N-methylprop-2-yn-1-amine (6).** Following the General Method for the reduction of amides with LAH, a suspension of LAH (36 mg, 0.94 mmol) in THF (3 mL) was reacted with MBA313 (70 mg, 0.16 mmol) to give compound 6 as an oil (50 mg, 74%; Rf= 0.36, DCM/methanol 10%), after purification and separation by chromatography (DCM/methanol 1-5%): IR (KBr) ν 3414, 3292, 3028, 2923, 2851, 2797, 1620, 1480, 1455, 1416, 1360, 1203, 1183 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.34-7.25 (m, 5H, NCH₂C₆H₅), 7.08 (d, J= 8.8 Hz, 1H, H7), 6.91 (d, J= 2.4 Hz, 1H, H4), 6.70 (dd, J= 8.8, 2.4 Hz, 1H, H6), 6.25 (s, 1H, H3), 4.09 [t, J= 7.5 Hz, 2H, OCH₂(CH₂)₂], 3.64 [s, 2H, C(2)CΗ₂NMe], 3.54 (s, 2H, NCΗ₂Ph), 3.28 (d, J= 2.2 Hz, 1H, NMeCH₂C≡CH), 2.93 [d, J= 11.3 Hz, 2H, N(CH₂)₂], 2.32 [s, 3H, NCH₃], 2.28 (t, 1H, NMeCH₂C≡CH), 1.96 [t, J= 11.3 Hz, 2H, N(CH₂)₂], 1.74-1.69 (m, 2H, OCH₂CH₂CH₂), 1.64 [d, J= 10.3 Hz, 2H, N(CH₂)₂(CH₂)₂], 1.35-1.18 (m, 5H, OCH₂CH₂CH₂, N(CH₂)₂(CH₂)₂CH)], (the N(1)H signal was not observed); ¹³C NMR (125 MHz, CDCl₃) δ 149.8 (C5), 137.0, 136.6 (C2), 132.3 (C7a), 129.6 [2C, C2’, C6’, C₆H₅CH₂N] 128.2 [2C, C3’, C5’, C₆H₅CH₂N], 128.0 (C3a), 127.2 (C4’), 111.3 (C6), 109.8 (C7), 104.9 (C4), 101.8 (C3), 78.4 (NCH₂C≡CH), 73.5 (NCH₂C≡CH), 63.2 (C₆H₅CH₂N), 53.6 [2C,N(CH₂)₂(CH₂)₂CH], 51.9 [C₂CH₂NMeCH₂C≡CH], 44.8 [C₂CH₂NMeCH₂C≡CH], 43.8 (OCH₂CH₂CH₂), 41.6 (N(1)CH₃), 35.2 [N(CH₂)₂CH₂CH₂], 33.7 (OCH₂CH₂CH₂), 31.7
[2C,N(CH2)2(CH2)2CH], 27.3 (OCH2CH2CH2); MS (ESI) m/z (%): 430 (M+H)+. The bis-chlorhydrate was obtained as usual. 6.2HCl: mp 140-5 °C; IR (KBr) v 3428, 2930, 2634, 1626, 1456, 1203 cm⁻¹; 1H NMR (300 MHz, D2O) δ 7.35-7.30 (m, 6H, NCH2C6H5, H7), 6.96 (d, J= 2.4 Hz, 1H, H4), 6.78 (dd, J= 8.8, 2.4 Hz, 1H, H6), 6.60 (s, 1H, H3), 4.11 [t, J= 7.5 Hz, 2H, OCH2(CH2)2], 4.06 [s, 2H, C(2)C6H2N+HMe], 3.90 (d, J= 2.2 Hz, 1H, N+HMeCH2CH2C≡CH), 3.25 [d, J= 16.4 Hz, 2H, N(CH2)2eq], 3.05 (m, 4H, OCH2CH2CH2, N(CH2)2(CH2)2ax), 1.68-1.45 (m, 4H, OCH2CH2CH2, N(CH2)2(CH2)2eq), 1.22-0.90 (m, 5H, OCH2CH2CH2, N(CH2)2(CH2)2axCH)], (the signal for N+H2C6H5 was not observed, surprisingly absent). Anal. Calcd for C28H35N3O.2HCl.5/2H2O: C, 61.42; H, 7.73; N, 7.67. Found: C, 61.60; H, 7.54; N, 7.86.

N-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine (7) and N-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-en-1-amine (8). Following the General Method for the reduction of amides with LAH, a suspension of LAH (24 mg, 0.62 mmol) in THF (3.5 mL) was reacted with MBA315 (60 mg, 0.14 mmol) to afford a mixture of compounds that were separated by column chromatography (DCM/methanol, 1-5%) to give pure products 7 as an oil (27 mg, 47%) and 8 as an oil (25 mg, 43%). 7: Rf= 0.33 (DCM/methanol, 10%); 1H NMR (300 MHz, CDCl3) δ 7.31-7.23 (m, 5H, NCH2C6H5), 7.10 (d, J= 8.8 Hz, 1H, H7), 6.93 (d, J= 2.4 Hz, 1H, H4), 6.72 (dd, J= 8.8, 2.4 Hz, 1H, H6), 6.27 (s, 1H, H3), 4.09 [t, J= 7.6 Hz, 2H, OCH2(CH2)2], 3.98 [s, 2H, C(2)CH2NH], 3.48 (s, 2H, NCH2Ph), 3.44 (d, J= 2.4 Hz, 1H, NHCH2C≡CH), 2.86 [d, J= 11.2 Hz, 2H, N(CH2)2ax], 2.27 (t, 1H, NHCH2C≡CH), 1.90 [t, J= 11.2 Hz, 2H, N(CH2)2ax], 1.74-1.70 (m, 2H, OCH2CH2CH2), 1.63-1.60 [m, 2H, N(CH2)2(CH2)2eq], 1.30-1.18 [(m, 5H, OCH2CH2CH2, N(CH2)2(CH2)2axCH)], (the NH signals were not observed); MS (ESI) m/z (%): 416 (M+H)+. The bis-chlorhydrate (7.2HCl) has been prepared as usual: mp 150-160 °C; IR (KBr) ν 3428, 2932, 2726, 1626, 1456, 1199, 1154, 1030 cm⁻¹; 1H NMR (500 MHz, D2O) δ 7.35-7.23 (m, 6H, NCH2C6H5, H7), 6.93 (d, J= 2.2 Hz, 1H, H4), 6.75 (dd, J= 8.8, 2.2 Hz, 1H, H6), 6.48 (s, 1H, H3), 4.39 [s, 2H, C(2)CH2NH2⁺], 4.08-4.6 [4H, (m OCH2CH2CH2, (s) N+H2C6H5Ph), 3.79 (d, J= 2.5 Hz, 2H, N+H2CH2C≡CH), 3.26 [d, J= 12.4 Hz, 2H, N(CH2)2ax], 2.88 (t, 1H, N+H2CH2C≡CH), 2.69 [t, J= 12.4 Hz, 2H, N(CH2)2ax], 1.68-1.54 [m, 4H, OCH2CH2CH2, N(CH2)2(CH2)2ax], 1.17-0.98 [m, 5H, OCH2CH2CH2, N(CH2)2(CH2)2axCH)], 13C NMR (126 MHz, D2O) δ 149.1 (C5), 132.3
(C7a), 130.9 [2C, C2', C6', C6H5CH2N+H], 129.9 (C4'), 129.8 (C2), 129.0 [2C, C3', C5', C6H5CH2N+H], 128.4 (C1'), 127.3 (C3a), 112.6 (C6), 111.6 (C7), 104.7 (C4), 103.2 (C3), 72.9 (N'H2CH2C≡CH), 60.4 (C6H5CH2N+H), 52.2 [2C,N+H(2CH2)2(CH2)2CH], 43.2 (OCH2CH2CH=CH), 35.5 [C(2)C6H2C≡CH], 32.6 [2C,N+H(CH2)2(CH2)2CH], 28.7 [2C,N+H(CH2)2(CH2)2CH], 26.4 (OCH2CH2CH2). Anal. Calcd for C27H33N3O.2HCl.2H2O: C, 62.45; H, 7.67; N, 7.80. Found: C, 62.31; H, 7.59; N, 8.04. 8: Rf = 0.22 (DCM/methanol, 10%); IR (KBr) ν 3429, 3028, 2922, 2851, 2802, 2758, 1621, 1455, 1416, 1367, 1198, 1154 cm⁻¹; ¹H NMR (300 MHz, CDCl3) δ 7.33-7.29 (m, 5H, NCH2C6H5), 7.04 (d, J = 8.4 Hz, 1H, H7), 6.93 (d, J = 3.3 Hz, 1H, H4), 6.69 (dd, J = 8.4, 3.3 Hz, 1H, H6), 6.21 (s, 1H, H3), 5.92 (ddt, J = 6.0, 17.1, 10.3 Hz, 1H, NHCH2CH2H), 5.21 (dq, J = 1.7, 17.1 Hz, 1H, NHCH2CH=CH2), 5.12 (dq, J = 1.6, 10.3 Hz, 1H, NHCH2CH=CH2), 4.06 [t, J = 7.6 Hz, 2H, OCH2(CH2)2], 3.87 [s, 2H, C(2)C6H2NH], 3.58 (s, 2H, N+HC6H2), 3.32 (dt, J = 6.0, 17.1 Hz, 1H, NHCH2CH2H), 2.95 [d, J = 11.8 Hz, 2H, N(CH2)2eq], 2.02 [t, J = 11.8 Hz, 2H, N(CH2)2ax], 1.73-1.64 (m, 2H, OCH2CH2CH2), 1.61 [d, J = 9.4 Hz, 2H, N(CH2)2(fCH2)eq], 1.34-1.23 [(m, 5H, OCH2CH2CH2, 2N(CH2)2(CH2)axCH)]; MS (ESI) m/z (%): 91 (92) [M-Bn]+, 347 (100) [M-C2O4H2]+, 361 (13) [M-NHCH2CH=CH2]+, 376 (7) [M-C6H5CH2=CH2]+, 417 (38) [M]+. The mixed oxalate was prepared as usual to give 8.C2O4H2: mp 140-5 ºC; IR (KBr) ν 3426, 2934, 2852, 1719, 1624, 1456, 1404, 1279, 1204 cm⁻¹; ¹H NMR (300 MHz, D2O) δ 7.35-7.25 (m, 6H, NCH2C6H5, H7), 6.93 (d, J = 2.4 Hz, 1H, H4), 6.75 (dd, J = 8.4, 2.4 Hz, 1H, H6), 6.45 (s, 1H, H3), 5.74 (ddt, J = 6.9, 16.9, 10.3 Hz, 1H, NHCH2CH=CH2), 5.38 (d, J = 16.9 Hz, 1H, NHCH2CH=CH2), 5.36 (d, J = 10.3 Hz, 1H, NHCH2CH=CH2), 4.06 [s, 2H, C(2)CH2N'H2], 4.04 [t, J = 6.8 Hz, 2H, OCH2CH2CH2], 3.59 (d, J = 6.9, 1.4 Hz, 2H, N'H2CH2CH2H), 3.27 [d, J = 12.9 Hz, 2H, N(CH2)2eq], 2.69 [t, J = 12.9 Hz, 2H, N(CH2)2ax]. 1.65 [d, J = 10.4 Hz, 2H, N(CH2)2(CH2)ax], 1.59-1.51 (m, 2H, OCH2CH2CH2), 1.34-0.98 [(m, 5H, OCH2CH2CH2, N(CH2)2(CH2)axCH)]. Anal.Calcd for C27H35N3O.2Oxal.H2O: C, 60.48; H, 6.71; N,6.83. Found: C, 60.36; H, 6.97; N, 6.68.

**Ethyl 5-(((1-benzylpiperidin-4-yl)carbamoyl)oxy)-1H-indole-2-carboxylate (18).**
Ethyl 5-(((4-nitrophenoxy)carbonyl)oxy)-1H-indole-2-carboxylate (MBA329). To a solution of ethyl 5-hydroxy-1H-indole-2-carboxylate (MBA328) (205 mg, 1 mmol) in dry THF (5 mL), under argon, 4-methylmorpholine (0.22 ml, 2 mmol) and 4-nitrophenyl chloroformate (402 mg, 2 mmol) were added, and stirred for 2 h, at rt. The solvent was evaporated and the crude purified by chromatography (hexane/AcOEt, 10-40%) to give carbonate MBA329 (240 mg, 65%), as a white solid: Rf = 0.32 (hexane/AcOEt, 40%); mp 199-202 ºC; IR (KBr) ν 3435, 3336, 3076, 2851, 1765, 1698, 1531, 1351, 1260, 1247, 1200 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 9.02 (br s, 1H, NH), 8.31 (d, J = 9.4 Hz, 2H, H3’, H5’, 4’-NO₂C₆H₄OCO), 7.59 (s, 1H, H3), 7.50 (d, J = 9.4 Hz, 2H, H2’, H6’, 4’-NO₂C₆H₄OCO), 7.45 (d, J = 8.8 Hz, 1H, H7), 7.25-7.21 (m, 2H, H4, H6), 4.42 (q, J = 7.2 Hz, 2H, CO₂CH₂CH₃), 1.42 (q, J = 7.2 Hz, 3H, CO₂CH₂CH₃); ¹³C NMR (126 MHz, CDCl₃) δ 161.5 (CO₂CH₂CH₃), 155.3 (C1’)**, 151.6 (C5’)**, 145.5 (C4’)*, 145.1 (C4’-NO₂C₆H₄OCO)* 134.7 (C2), 129.2 (C3a), 127.5 (C7a), 125.3 (2C, C3’, C5’, 4’-NO₂C₆H₄OCO), 121.7 (2C, C2’, C6’, 4’-NO₂C₆H₄OCO), 118.7 (C6), 113.7 (C3), 112.7 (C7), 108.7 (C4), 61.2 (CO₂CH₂CH₃), 14.3 (CO₂CH₂CH₃); MS (EI) m/z (%): 370 (M⁺, 100), 340 (3), 324 (19), 280 (49), 204 (19), 188 (9), 158 (49), 142 (29), 130 (25) 114 (22), 102 (14); MS (ESI): m/z (%): 371 (M+H⁺).

Ethyl 5-(((1-benzylpiperidin-4-yl)carbamoyl)oxy)-1H-indole-2-carboxylate (18). To a solution of commercial 4-amino-N-benzylpiperidine (60 µL, 0.29 mmol) in dry THF (3.5 mL), 4-dimethylaminopiridine (19 mg, 0.15 mmol) and ethyl 5-(((4-nitrophenoxy)carbonyl)oxy)-1H-indole-2-carboxylate (MBA329) (55 mg, 0.15 mmol) were added, and the mixture was stirred at rt for 1 h, under argon. The solvent was evaporated and the crude purified by chromatography (DCM/methanol, 0-2.5%) giving
compound 18 (62 mg, 99%) as a white solid: Rf= 0.35 (DCM/methanol, 2.5%); mp 180-3 °C; IR (KBr) ν 3325, 3028, 2936, 1701, 1521, 1249, 1204, 1024 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.96 [br s, 1H, N(1)H], 7.38 (d, J= 2.2 Hz, 1H, H4), 7.33-7.31 (m, 6H, NCH₂CH₃, H7), 7.15 (s, 1H, H3), 7.05 (dd, J= 9.0, 2.2 Hz, 1H, H6), 4.95 [br d, J= 7.7 Hz, NHC(O)O], 4.38 (q, J= 7.2 Hz, 2H, CO₂CH₂CH₃), 3.63-3.53 [m, 1H, N(CH₂)₂(CH₂)₂CH₃NHC(O)O], 3.50 (s, 2H, NCH₂C₆H₅), 2.82 [d, J= 11.3 Hz, 2H, N(CH₂)₂(CH₃)₂CH], 2.13 [t, J= 11.3 Hz, 2H, N(CH₂)₂(CH₃)₂CH], 1.58-1.99 [d, J= 11.6 Hz, 2H, N(CH₂)₂(CH₃)₂ax CH], 1.50 [m, 1H, N(CH₂)₂(CH₃)₂ax CH], 1.39 (t, J= 7.2 Hz, 3H, CO₂CH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 161.9 (CO₂CH₂CH₃), 154.6 [HNC(O)O], 145.4 (C5), 138.4 (C7a), 134.6 (C1', NCH₂C₆H₅), 129.3 (2xCH, C'3,C5', NCH₂C₆H₅), 128.8 (C4', NCH₂C₆H₅), 128.4 (3C: 2xCH, C2', C6', NCH₂C₆H₅), 127.8 (C3a),* 127.3 (C2)*, 120.5 (C6), 114.5 (C4), 112.4 (C7), 108.8 (C3), 63.2 (NCH₂C₆H₅), 61.3 (CO₂CH₂CH₃), 52.3 [2xCH₂, N(CH₂)₂(CH₂)₂CH], 48.6 [CH, N(CH₂)₂(CH₃)₂CH], 32.6 [2xCH₂, N(CH₂)₂(CH₂)₂CH], 14.5 (CO₂CH₂CH₃); MS (EI) m/z (%): 368 (2), 236 (4), 217 (37), 205 (44), 159 (100), 139 (22), 125 (18); MS (ESI) m/z (%): 422 (M+1)⁺. Anal. Calcd. for C₂₄H₂₇N₃O₄: C, 68.39; H, 6.46; N, 9.97. Found: C, 68.46; H, 6.73; N, 9.71.

![Scheme 10](image)

**Scheme 10.** Reagents and conditions: (a) BBr₅, CHCl₃, -78 °C; (b) Propargyl bromide, t-BuNH₂, THF, rt; (c) Me₂NCOCl, NaH, THF, 0 °C; (d) Ph(CH₂)₅Br, NaH, DMF, reflux.
1-Methyl-2-(((methylamino)methyl)-1H-indol-5-ol (ASS38). A solution of BBr₃ in CH₂Cl₂ (7.5 mL, 1M) was added to a stirred solution of JL132 (1 g, 4.89 mmol) in anhydrous CHCl₃ (50 mL), cooled at -78 ºC, under argon. When the addition was complete, the reaction mixture was stirred at rt for 48 h, cooled at 0 ºC, and quenched with water, and neutralized by saturated NaHCO₃. The resulting mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na₂SO₄, filtered, and after removing the solvent, the crude was purified by column chromatography (CH₂Cl₂/MeOH/TEA, 40/1/1% to 10/1/1%) to afford ASS38 (0.742 g, 80%) as a white solid: Rᵓ = 0.34 (CH₂Cl₂/MeOH/TEA, 10/1/1 %); ¹H NMR (300 MHz, CDCl₃) δ 2.38 (s, 3H, CH₃-NH), 3.66 (s, 3H, CH₃-N), 3.91 (s, 2H, CH₂), 6.21 (s, 1H, CH), 6.62 (dd, J= 2.3 and 8.7 Hz, 1H, CH), 6.79 (d, J= 2.2 Hz, 1H, CH), 7.18 (d, J= 8.7 Hz, 1H, CH), 8.66 (s, 1H, OH); MS (EI) m/z (%): 160 (100) [M-HNMe]+, 173 (3) [M-OH]+, 190 (48) [M]+.

5-Hydroxy-N-[(1-methyl-1H-indol-2-yl)methyl]-N-methylprop-2-yn-1-amine (ASS39). To a solution of amine ASS38 (0.31 g, 1.629 mmol) and t-BuNH₂ (0.25 mL, 2.44 mmol) in anhydrous THF (10 mL), cooled at 0-5 ºC, a solution of propargyl bromide (0.131 mL, 1.468 mmol) in THF (5 mL) was added. The reaction was stirred at rt overnight. Then, the solvent was removed in vacuum and the solid purified by column chromatography (CH₂Cl₂/EtOAc, 10/1) to afford ASS39 (0.28 g, 75%) as a white solid: Rᵓ = 0.13 (CH₂Cl₂/EtOAc, 10/1); ¹H NMR (300 MHz, CDCl₃) δ 2.21 (s, 3H, CH₃), 3.23 (m, CH), 3.29 (m, CH₂), 3.6 (s, 2H, CH₂), 3.64 (s, 3H, CH₃), 6.13 (s, CH), 6.6 (dd, J= 2.3 and 8.7 Hz, 1H, CH), 6.78 (d, J= 2.2 Hz, 1H, CH), 8.7 (d, J= 8.7 Hz, CH), 8.64 (s, OH); MS (EI) m/z (%): 160 (100) [M-HNMe]+, 228 (22) [M]+.

1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl dimethylcarbamate (17). To an ice-cooled solution of ASS39 (19 mg, 0.083 mmol) in dry CH₃CN (0.5 mL), NaH (3.3 mg, 0.083 mmol, 60% in mineral oil) was added under argon. The mixture was stirred at 0 ºC for 10 min. Then, to this mixture was added Me₂NCOCl (8 μL, 0.085 mmol, 1.02 equiv) in dry CH₃CN (0.5 mL). The reaction was stirred at 0 ºC for 4 h. Then, the reaction was evaporated in vacuum, and water (10 mL) was added. The aqueous layer was extracted with CH₂Cl₂, and the combined extract was dried (Na₂SO₄), evaporated to dryness in vacuum, and the residue was purified by column chromatography (CH₂Cl₂/EtOAc, 10/1) to give compound 17 (24.4 mg, 98%).
$R_f = 0.27$ (CH$_2$Cl$_2$/AcOEt, 5/1); mp 93-5 °C; IR (KBr) v 3283, 2940, 1704, 1486, 1394, 1191, 1174 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.29 (t, $J$= 2.34 Hz, CH), 2.34 (s, 3H, CH$_3$), 3.02 (s, 3H, N-CH$_3$), 3.13 (s, 3H, N-CH$_3$), 3.31 (d, $J$= 2.36 Hz, 2H, CH$_2$), 3.69 (s, 2H, CH$_2$), 3.76 (s, 3H, CH$_3$), 6.38 (s, 1H, CH-3), 6.95 (dd, $J$ = 8.76 and 2.28 Hz, 1H, CH-6), 7.24 (d, $J$= 8.79 Hz, 1H, CH-7), 7.26 (d, $J$= 2.25 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 29.9 (N-CH$_3$), 36.4 (CON-CH$_3$), 36.6 (CON-CH$_3$), 41.4 (N-CH$_3$), 44.6 (CH$_2$-C), 51.7 (CH$_2$-N), 73.4 (C=CH), 78.2 (-C≡C), 102.6 (CH-3), 109.0 (CH-7), 112.5 (CH-4), 115.8 (CH-6), 127.3 (C), 135.6 (C), 137.4 (C), 144.8 (C), 155.9 (CO); MS (EI) $m/z$ (%): 72 (88) [(CH$_3$)$_2$NCO]$^+$, 232 (100) [M-(CH$_3$NCH$_2$C≡CH)+H]$^+$, 256 (6) [M-CH$_3$)$_2$N]$^+$, 299 (22) [M]$^+$. The free base was dissolved in dry ether (2 mL) and a solution of HCl/ether was added dropwise with stirring. The precipitate was separated by filtration, washed with ether and dried in vacuum to afford 17.HCl as a white powder: mp 191-3 °C; IR (KBr) v 3230, 2920, 2619, 2499, 2418, 1722, 1713, 1477, 1390, 1179 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.78 (s, CH$_3$), 2.81 (s, 3H, CH$_3$), 2.97 (s, 3H, H$_3$), 3.02 (m, 1H, CH), 3.75 (s, 3H, CH$_3$), 3.87 (d, $J$= 2.4 Hz, 2H, CH$_2$), 4.44 (s, 2H, CH$_2$), 6.62 (s, 1H, CH), 6.89 (dd, $J$= 9 and 2.4 Hz, 1H), 7.18 (d, $J$= 2.4 Hz, 1H), 7.3 (d, $J$= 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 30.0 (CH$_3$), 36.0 (CON-CH$_3$), 36.3 (CON-CH$_3$), 39.5 (N-CH$_3$), 44.5 (CH$_2$), 49.7 (CH$_2$), 71.6 (C=CH), 80.5 (-C≡C), 106.6 (CH-ind), 111.2 (CH-ind), 113.3 (CH), 117.9 (CH), 126.7 (C), 128.9 (C), 136.1 (C), 145.0 (C), 157.85 (CO). Anal. Calcd. for C$_{17}$H$_{21}$N$_3$O$_2$.HCl: C, 60.80; H, 6.60; N, 12.51. Found: C, 60.98; H, 6.77; N, 12.62.

$N$-Methyl-$N$-((1-methyl-5-(3-phenylpropoxy)-1H-indol-2-yl)methyl)prop-2-yn-$1$-amine (16). A solution of ASS39 (15 mg, 0.0657 mmol) in DMF (0.5 mL) was treated with NaH (4 mg, 0.1 mmol, 60% in mineral oil), and then with 4-phenyl-1-bromopropane (13 mg, 0.0657 mmol) for 30 min at reflux. The reaction mixture was diluted with water, and extracted with EtOAc. The organic phase was washed with brine, dried (MgSO$_4$), and evaporated at reduced pressure to afford compound ASS50 (22.2 mg, 98%). $R_f$ = 0.66 (CH$_2$Cl$_2$/AcOEt, 10/1); mp 74-76 °C; IR (KBr) v 3281, 2953, 2932, 2854, 1618, 1488, 1472, 1396, 1209, 1019 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 2.12 (m, 2H, CH$_2$), 2.28 (t, $J$= 3.0Hz, CH), 2.34 (s, 3H, CH$_3$), 2.84 (t, $J$= 7.3 Hz, CH$_2$), 3.31 (d, $J$= 3.0 Hz, CH$_2$), 3.67 (s, 2H, CH$_2$), 3.74 (s, 3H, CH$_3$), 4.00 (t, $J$= 6.3 Hz, 2H, CH$_2$), 6.32 (s, CH-3), 6.88 (dd, $J$= 2.4 and 8.8 Hz,1H, CH-6), 7.02 (d, $J$= 2.3 Hz, 1H, CH-4), 7.16-7.3 (m, CHind + 5Har); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 29.8 (CH$_3$), 31.0 (CH$_2$), 32.0 (CH$_2$), 36.2 (CH$_2$), 41.3 (N-CH$_3$), 44.4 (N-CH$_3$).
32.2 (CH$_2$), 41.6 (CH$_3$), 44.6 (CH$_2$), 51.7 (CH$_2$), 67.7 (CH$_2$), 73.4 (C), 78.3 (CH), 102.0 (CH-3), 103.4 (CH-4), 109.5 (CH-7), 112.0 (CH-6), 125.7 (CH-Ph), 127.4 (C), 128.3 (2xCH-Ph), 128.3 (2xCH-Ph), 133.3 (C), 137.0 (C), 141.7 (C), 153.2 (C); MS (EI) m/z (%): 160 (73) [M-HNMe – Ph(CH$_2$)$_3$]$^+$, 279 (100) [M- HNMe]$^+$, 346 (36) [M]$^+$. The free base was dissolved in diethyl ether and treated with a solution of ether saturated with HCl with stirring; the precipitate was collected by filtration, triturated with fresh ether, and filtered again. Drying in vacuum afforded 16.HCl as a white powder: mp 185-7 ºC; IR (KBr) ν 3196, 2933, 2561, 2512, 1486, 1473, 1207 cm$^{-1}$. Anal. Calcd. for C$_{23}$H$_{26}$N$_2$O.HCl: C, 72.14; H, 7.11; N, 7.32. Found: C, 72.01; H, 7.08; N, 7.43.

**tert-Butyl 5-(benzoxo)-2-((((1-benzylpiperidin-4-yl)carbamoyl)oxy)methyl)-1H-indole-1-carboxylate (19).**

![Scheme 10](image)

**Scheme 10.** Reagents and conditions: (a) LAH, THF, rt; (b) TBDMSI, imidazole, DCM; (c) t-BOC$_2$O, DMAP, TEA, DCM; (d) AcOH, H$_2$O, THF; (e) 4-Nitrophenyl chloroformate, 4-methylmorpholine, THF; (f) 1-benzylpiperidin-4-amine, DMAP, THF.

**{(5-(Benzylxoy)-1H-indol-2-yl)methanol (MBA331).}** To a suspension of LAH (228 mg, 6 mmol) in dry THF (4 mL), under argon at a 0 ºC, commercial ethyl 5-(benzylxoy)-1H-indole-2-carboxylate C-6 (60 mg, 0.14 mmol) was slowly added. Then, the mixture was refluxed for 2 h, cooled at 0 ºC, and treated with some drops of water. AcOEt was added and the mass was filtered, washed with more AcOEt. The solvent was evaporated, and the residue was purified by chromatography (DCM/methanol, 1-5%) to give compound MBA331 (211 mg, 97%) (Marco, Jose L. Improved preparation

**5-(Benzyloxy)-2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-indole (MBA334).** To a solution of 5-(benzyloxy)-1H-indol-2-ylmethanol (MBA331) (210 mg, 0.83 mmol) in dry DCM (4 mL), tert-butyldimethylsilyl chloride (149 mg, 0.96 mmol) and imidazole (71 mg, 1.05 mmol) were added, and at stirred at rt for 24 h. Then, the solvent was removed, and the crude was submitted to chromatography (hexane/AcOEt, 5-10%) to yield compound MBA334 (198 mg, 65%) \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.22 [br s, 1H, N(1)H], 7.54-7.41 (m, 5H, OCH\(_2\)C\(_6\)H\(_5\)), 7.28 (d, J= 8.8 Hz, 1H, H7), 7.16 (d, J= 2.4 Hz, 1H, H4), 6.96 (dd, J = 8.8, 2.4 Hz, 1H, H6), 6.29 (s, 1H, H3), 5.14 (s, 2H, OCH\(_2\)C\(_6\)H\(_5\)), 4.89 [s, 2H, C(2)CH\(_2\)OTBDMS], 1.00 [s, 9H, Si(CH\(_3\))\(_2\)C(C\(_6\)H\(_3\))], 0.16 [s, 6H, Si(C\(_6\)H\(_3\))\(_2\)C(CH\(_3\))\(_3\)] \[Seehra, Jasbir S.; Kaila, Neelu; McKew, John C.; Lovering, Frank; Bemis, Jean E.; Xiang, Yibin, Preparation of indole derivatives as phospholipase enzyme inhibitors, PCT Int. Appl. (1999), WO 9943651\].

**tert-Butyl 5-(benzyloxy)-2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-indole-1-carboxylate (MBA335).** 5-(Benzyloxy)-2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-indole (MBA334) (198 mg, 0.42 mmol) was dissolved in dry DCM (5 mL) and treated with (tert-BOC)\(_2\)O (114 mg, 0.52 mmol), TEA (88 µL, 0.64 mmol) and DMAP (13 mg, 0.10 mmol) at rt for 72 h. Then, the solvent was evaporated, and the resulting crude purified by column chromatography (hexane/AcOEt, 1-5%) affording compound MBA335 (176 mg, 70%) \( ^1H \) NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.01 (d, J= 9.0 Hz, 1H, H7), 7.50-7.34 (m, 5H, OCH\(_2\)C\(_6\)H\(_5\)), 7.09 (d, J = 2.6 Hz, 1H, H4), 6.97 (dd, J = 9.0, 2.6 Hz, 1H, H6), 6.63 (s, 1H, H3), 5.14 (s, 2H, OCH\(_2\)C\(_6\)H\(_5\)), 5.04 [s, 2H, C(2)CH\(_2\)OTBDMS], 1.70 [s, 9H, N(1)CO\(_2\)C(CH\(_3\))\(_3\)], 1.02 [s, 9H, Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)], 0.18 [s, 6H, Si(CH\(_3\))\(_2\)C(CH\(_3\))\(_3\)] \[Seehra, Jasbir S.; Kaila, Neelu; McKew, John C.; Lovering, Frank; Bemis, Jean E.; Xiang, Yibin, Preparation of indole derivatives as phospholipase enzyme inhibitors, PCT Int. Appl. (1999), WO 9943651\].

**tert-Butyl 5-(benzyloxy)-2-(hydroxymethyl)-1H-indole-1-carboxylate (MBA336).** \textit{tert-Butyl 5-(benzyloxy)-2-(((tert-butyldimethylsilyl)oxy)methyl)-1H-indole-1-carboxylate (MBA335)} (176 mg, 0.38 mmol) was dissolved in a mixture of cc AcOH (5.6 mL), H\(_2\)O (1.88 mL) and THF (1.88 mL) and the mixture was stirred at rt overnight. Then, the solvents were evaporated, and the crude purified by chromatography (hexane/AcOEt, 10%) affording product MBA336 (107 mg, 80%) \( ^1H \)
NMR (300 MHz, CDCl₃) δ 7.88 (d, J= 8.9 Hz, 1H, H7), 7.48-7.34 (m, 5H, OCH₂C₆H₅), 7.06 (d, J= 2.6 Hz, 1H, H4), 6.99 (dd, J= 8.9, 2.6 Hz, 1H, H6), 6.51 (s, 1H, H3), 5.12 (s, 2H, OCH₃C₆H₅), 4.97 [s, 2H, C(2)CH₂OH], 3.62 (br s, 1H, OH), 1.73 [s, 9H, N(1)CO₂C(CH₃)₃] [Seehra, Jasbir S.; Kaila, Neelu; McKew, John C.; Lovering, Frank; Bemis, Jean E.; Xiang, Yibin, Preparation of indole derivatives as phospholipase enzyme inhibitors, PCT Int. Appl. (1999), WO 9943651].

tert-Butyl 5-(benzyl氧)-2-(((4-nitrophenoxycarbonyloxy)methyl)-1H-indole-1-carboxylate (MBA337). To a solution of tert-butyl 5-(benzyl氧)-2-(hydroxymethyl)-1H-indole-1-carboxylate (MBA336) (105 mg, 0.29 mmol) in dry THF (5 mL), 4-methylmorpholine (66 µL, 0.6 mmol) and 4-nitrophenyl chloroformate (119 mg, 0.20 mmol) were added and the mixture was stirred at rt for 2.5 h, under argon. Then, the solvents were evaporated, and the crude purified by chromatography (hexane/AcOEt, 10%) to give compound MBA337 (107 mg, 69%), as a oil: Rf= 0.30 (hexane/AcOEt, 30%); IR (KBr) v 3367, 2923, 1731, 1525, 1452, 1369, 1260, 1212, 1159, 1121, 1095, 1025 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.28 (d, J= 9.3 Hz, 2H, H3', H5', 4'-NO₂C₆H₄OCO), 8.02 (d, J= 9.0 Hz, 1H, H7), 7.48-7.33 (m, 7H: 2H, H2', H6', 4'-NO₂C₆H₄OCO; 5H, OCH₂C₆H₅), 7.08-7.02 (m, 2H, H4, H6), 6.69 (s, 1H, H3), 5.64 [s, 2H, C(2)C₆H₂OC(O)O], 5.12 (C₆H₅OC₆H₂), 1.70 [s, 9H, N(1)COC(CH₃)₃]; MS (EI) m/z (%): 139 (14), 236 (48), 280 (100), 327 (11), 418 (22, M-BOC+H⁺); MS (ESI) m/z (%): 518 (M+23, M+Na⁺).

tert-Butyl 5-(benzyl氧)-2-(((1-benzylpiperidin-4-yl)carbamoyloxy)methyl)-1H-indole-1-carboxylate (19). To a solution of commercial 4-amino-N-benzylpiperidine (84 µL, 0.41 mmol) in dry THF, DMPA (25 mg, 0.20 mmol) and tert-butyl 5-(benzyl氧)-2-(((4-nitrophenoxycarbonyloxy)methyl)-1H-indole-1-carboxylate (MBA337) (107 mg, 0.20 mmol) were added, and the mixture was stirred for 3 h, at rt, under argon. Then, the solvent was evaporated, and the crude purified by chromatography (DCM/methanol, 1%) leading to product 19 (83 mg, 70%) as an oil: Rf= 0.39 (DCM/methanol, 2%); IR (KBr) v 3338, 2928, 1730, 1531, 1476, 1452, 1370, 1123, 1042, 850, 738, 697 cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 7.95 (d, J= 8.8 Hz, 1H, H7), 7.37 (d, J= 7.4 Hz, 1H, aromatic), 7.30 (t, J= 7.3 Hz, 1H, aromatic), 7.25-7.16 (m, 8H, C₆H₅OCH₂, NCH₂C₆H₅), 6.95 (d, J= 2.0 Hz, 1H, H4), 6.90 (dd, J= 8.8, 2.0 Hz, 1H, H6), 6.64 (s, 1H, H3), 5.31 [s, 2H, C(2)CH₂OC(O)NH], 5.02 (C₆H₅OCH₂), 4.63 [br d, J= 7.3 Hz, NHC(O)O], 3.49-3.47 [m, 1H, N(CH₂)₂(CH₂)₂CH]NHC(O)O], 3.44 (s, 2H,
NCH₂C₆H₅), 2.74 [m, 2H, N(CH₂)eq(CH₂)ax(CH₂)₂CH], 2.07 [t, J = 10.3 Hz, 2H, N(CH₂)eq(CH₂)ax(CH₂)₂CH], 1.88 [d, J = 10.8 Hz, 2H, N(CH₂)₂(CH₂)eq(CH₂)axCH], 1.58 [s, 9H, N(1)COC(CH₃)₃], 1.45-1.42 [m, 2H, N(CH₂)₂(CH₂)eq(CH₂)axCH]; ¹³C NMR (126 MHz, CDCl₃) δ 155.2 (C₅), 155.0 [HNC(O)O]*, 149.9 [N(1)C(O)OC(CH₃)₃], 137.2 (C₇a), 136.1 and 131.7 (2C, 2xCH’, C₆H₃), 129.4, 129.1, 128.8, 128.5, 128.2 (10C, CH, C₆H₅CH₂O, NCH₂C₆H₅), 127.4 (C₃a)* 127.1 (C₂)*, 116.4 (C₇), 113.9 (C₆), 109.3 (C₃), 104.1 (C₄), 84.4 [N(1)C(O)OC(CH₃)₃], 70.5 (C₆H₅OCH₂), 62.9 (NCH₂C₆H₃), 61.2 [C(2)CH₂OC(O)NH ], 52.1 [2xCH₂, N(CH₂)₂(CH₂)₂CH], 48.1 [CH, N(CH₂)₂(CH₂)₂CH], 32.3 [2xCH₂, N(CH₂)₂(CH₂)₂CH], 28.1 [N(1)C(O)OC(CH₃)₃]; MS (EI) m/z (%): 233 (100) [M-C₂₁H₂₂NO₃]⁺, 469 (4)[MH-BOC]⁺, 217 (12) [M-C₂₁H₂₂NO₄]⁺. The oxalate was obtained as usual to give compound 19-Oxal: mp 102-5 °C; IR (KBr) ν 3422, 2978, 2550, 1725, 1618, 1453, 1371, 1194, 1125 cm⁻¹; ¹H NMR (300 MHz, DMSO-d₆) δ 7.94 (d, J = 9.1 Hz, 1H, H₇), 7.53 (d, J = 6.5 Hz, 1H, aromatic), 7.46-7.31 (m, 9H, C₆H₅OCH₂, N+HCH₂C₆H₅), 7.18 (d, J = 2.5 Hz, 1H, H₄), 6.98 (dd, J = 9.1, 2.5 Hz, 1H, H₆), 6.64 (s, 1H, H₃), 5.25 [s, 2H, C(2)CH₂OC(O)NH], 5.10 (C₆H₅OCH₂), 3.99 (s, 2H, N⁺HCH₂C₆H₃), 3.54 [m, 1H, N(CH₂)₂(CH₂)₂CH]NHC(O)O], 3.12 [m, 2H, N(CH₂)eq(CH₂)ax(CH₂)₂CH], 2.72 [m, 2H, N(CH₂)eq(CH₂)ax(CH₂)₂CH], 1.88 [d, J = 13.2 Hz, 2H, N(CH₂)₂(CH₂)eq(CH₂)axCH], 1.58 [s, 9H, N(1)COC(CH₃)₃], m, 2H, N(CH₂)₂(CH₂)eq(CH₂)axCH]; MS (EI) m/z (%): 378 (1), 280 (1), 172 (2), 146 (1), 91 (100); MS (ESI) m/z (%): 569 (M+1)⁺. Anal. Calcd for C₃₄H₃₉N₃O₅.Oxal: C, 65.54; H, 6.26; N, 6.37. Found: C, 65.51; H, 6.34; N, 6.33.
$^1$H and $^{13}$C NMR spectra

3-(Piperidin-4-yl)propan-1-ol hydrochloride (MBA160).

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound **MBA160**.

*tert*-Butyl 4-(3-hydroxypropyl)piperidine-1-carboxylate (MBA163).

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound **MBA163**.
**tert-Butyl 4-(3-chloropropyl)piperidine-1-carboxylate (MBA177).**

1H NMR (300 MHz, CDCl₃) Spectrum of compound MBA177.

**tert-Butyl 4-(3-(1-methyl-2-((methyl(prop-2-ynyl)amino)methyl)-1H-indol-5-yloxy)propyl)piperidine-1-carboxylate (MBA184).**

1H NMR (500 MHz, CDCl₃) Spectrum of compound MBA184.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound MBA184.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound MBA184.
$^1$H-$^1$C g-HSQC (CDCl$_3$) spectrum of compound MBA184.

$^1$H-$^1$C g-HMBC (CDCl$_3$) spectrum of compound MBA184.
**N-Methyl-N-[(1-methyl-5-(3-(piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl]prop-2-yn-1-amine dihydrochloride (1.2HCl).**

\[ \text{\textsuperscript{1}H NMR (500 MHz, CDCl}\textsubscript{3} \text{ Spectrum of compound 1.2HCl.} \]

\[ \text{\textsuperscript{13}C NMR (126 MHz, CDCl}\textsubscript{3} \text{ Spectrum of compound 1.2HCl.} \]
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 1.2HCl.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 1.2HCl.
$^1$H-$^1$C g-HMBC (CDCl₃) spectrum of compound 1.2HCl.

$N$-Methyl-$N$-((1-methyl-5-(3-(1-(2-methylbenzyl)piperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine (2= MBA236).

$^1$H NMR (500 MHz, CDCl₃) Spectrum of compound MBA236.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound MBA236.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound MBA236.
\(^1\)H-\(^{13}\)C g-\(^1\)H-\(^{13}\)C spectrum of compound MBA236.

\(^1\)H-\(^{13}\)C g-\(^1\)H-\(^{13}\)C spectrum of compound MBA236.
4-((4-((3-((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)propyl)piperidin-1-yl)methyl)benzonitrile (3).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 3.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 3.
\(^1\)H\(^1\)H \(g\)-COSY (500 MHz, CDCl\(_3\)) spectrum of compound 3.

\(^1\)H\(^1\)C \(g\)-HSQC (CDCl\(_3\)) spectrum of compound 3.
$^1$H-$^1$C g-HMBC (CDCl$_3$) spectrum of compound 3.

5-(Chloromethyl)quinolin-8-ol (MBA150).

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA150.
5-(Hydroxymethyl)quinolin-8-ol (MBA190).

![NMR Spectrum of MBA190](image)

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA190.

(8-Methoxyquinolin-5-yl)methanol (MBA191).

![NMR Spectrum of MBA191](image)

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA191.
5-(Hydroxymethyl)quinolin-8-yl dimethylcarbamate (MBA217).

\[
\begin{align*}
\text{\textsuperscript{1}H NMR (500 MHz, CDCl}_3) \text{ Spectrum of compound MBA217.}
\end{align*}
\]

\[
\begin{align*}
\text{\textsuperscript{13}C NMR (126 MHz, CDCl}_3) \text{ Spectrum of compound MBA217.}
\end{align*}
\]
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound MBA217.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound MBA217.
$^1$H-$^1$C g-HMBC (CDCl$_3$) spectrum of compound MBA217.

5-(Chloromethyl)-8-methoxyquinoline (MBA207).

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA207.
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound MBA219.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound MBA219.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound \textbf{MBA219}.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound \textbf{MBA219}.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound MBA219.

$N$-((5-((8-Methoxyquinolin-5-yl)methoxy)-1-methyl-1H-indol-2-yl)methyl)-$N$-methylprop-2-yn-1-amine (13).

$^1$H NMR (400 MHz, CDCl$_3$) Spectrum of compound 13.
$^{13}$C NMR (100 MHz, CDCl$_3$) Spectrum of compound 13.

$^1$H-$^1$H g-COSY (400 MHz, CDCl$_3$) spectrum of compound 13.
$^{1}H-^{13}C$ g-HSQC (CDCl$_3$) spectrum of compound 13.

$^{1}H-^{13}C$ g-HMBC (CDCl$_3$) spectrum of compound 13.
5-(((1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)methyl)quinolin-8-yl dimethylcarbamate (14)

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 14.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 14.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 14.
$^1$H-$^1$C $g$-HSQC (CDCl$_3$) spectrum of compound 14.

$^1$H-$^1$C $g$-HMBC (CDCl$_3$) spectrum of compound 14.
$N\text{-}((5\text{-}(3\text{-}(1\text{-}((8\text{-}\text{Methoxyquinolin-5-yl})\text{methyl})\text{piperidin-4-yl})\text{propoxy})\text{-}1\text{-}\text{methyl}-1\text{H-indol-2-yl})\text{methyl})\text{-}\text{N-methylprop-2-yn-1-amine}$ (4).

$^1\text{H NMR (400 MHz, CDCl}_3\text{)}$ Spectrum of compound 4.

$^{13}\text{C NMR (100 MHz, CDCl}_3\text{)}$ Spectrum of compound 4.
$^{1}H-^{1}H$ g-COSY (400 MHz, CDCl$_3$) spectrum of compound 4.

$^{1}H-^{13}C$ g-HSQC (CDCl$_3$) spectrum of compound 4.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 4.
5-((4-(3-((1-Methyl-2-((methyl(2-yn-1-yl)amino)methyl)-1H-indol-5-yl)oxy)propyl)piperidin-1-yl)methyl)quinolin-8-yl dimethylcarbamate (5).

$^1$H NMR (400 MHz, CDCl$_3$) Spectrum of compound 5.

$^{13}$C NMR (100 MHz, CDCl$_3$) Spectrum of compound 5.
$^1$H-$^1$H g-COSY (400 MHz, CDCl$_3$) spectrum of compound 5.

$^1$H-$^1$C g-HSQC (CDCl$_3$) spectrum of compound 5.
$^{1}$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 5.

1-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N-((5-methoxy-1-methyl-1H-indol-2-yl)methyl)-N-methylmethanamine (15).

$^{1}$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 15.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 15.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 15.
$^{1}$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 15.

$^{1}$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 15.
1-(1-Benzyl-1H-1,2,3-triazol-4-yl)-N-((5-(3-(1-benzylpiperidin-4-yl)propoxy)-1-methyl-1H-indol-2-yl)methyl)-N-methylmethanamine (9).

\[^1\text{H} \text{NMR} \ (500 \text{ MHz, CDCl}_3) \text{ Spectrum of compound 9.}\]

\[^{13}\text{C} \text{ NMR} \ (126 \text{ MHz, CDCl}_3) \text{ Spectrum of compound 9.}\]
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 9.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 9.
$^{1}$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 9.

1-(5-(3-(1-Benzytpiperidin-4-yl)propoxy)-1-methyl-1$H$-indol-2-yl)-N-methyl-N-((1-phenyl-1$H$-1,2,3-triazol-4-yl)methyl)methanamine hydrochloride (10.2HCl).

$^{1}$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 10.2HCl.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound $^{10.2}\text{HCl}$.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound $^{10.2}\text{HCl}$. 
$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 10.2HCl.

$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 10.2HCl.
$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA233.

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA237.
$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA240.

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA242.
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 11.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 11.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 11.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 11.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 11.

(5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methanol (12).
$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 12.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 12.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 12.
$^1$H-$^{13}$C $g$-HSQC (CDCl$_3$) spectrum of compound 12.

$^1$H-$^{13}$C $g$-HMBC (CDCl$_3$) spectrum of compound 12.
5-(3-(1-Benzylpiperidin-4-yl)propoxy)-N-methyl-N-(prop-2-yn-1-yl)-1H-indole-2-carboxamide (MBA316).

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA316.

5-(3-(1-Benzylpiperidin-4-yl)propoxy)-N-(prop-2-yn-1-yl)-1H-indole-2-carboxamide (MBA315).

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA315.
$N$-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1\textit{H}-indol-2-yl)methyl)-$N$-methylprop-2-yn-1-amine (6).

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound 6.

$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound 6.
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 6.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 6.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 6.

$N$-((5-((3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)-$N$-methylprop-2-yn-1-amine (6.2HCl)

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound 6.2HCl.
\[ \text{N-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine (7).} \]

\[ ^1\text{H NMR (300 MHz, CDCl}_3\text{) Spectrum of compound 7.} \]

\[ \text{N-((5-(3-(1-Benzylpiperidin-4-yl)propoxy)-1H-indol-2-yl)methyl)prop-2-yn-1-amine (7.2HCl).} \]

\[ ^1\text{H NMR (500 MHz, CDCl}_3\text{) Spectrum of compound 7.2HCl.} \]
\[ ^{13}\text{C} \text{ NMR (126 MHz, CDCl}_3 \text{)} \text{ Spectrum of compound } 7.2\text{HCl.} \]

\[ ^{1}\text{H} \text{ } ^{1}\text{H} \text{ } g\text{-COSY (500 MHz, CDCl}_3 \text{)} \text{ spectrum of compound } 7.2\text{HCl.} \]
$^1$H-$^1$C g-HSQC (CDCl$_3$) spectrum of compound 7.2HCl.

$^1$H-$^1$C g-HMBC (CDCl$_3$) spectrum of compound 7.2HCl.
$N\-((5\-(3\-(1\text{-}Benzylpiperidin\text{-}4\text{-}yl)propoxy\)\-1\text{-}H\text{-}indol\text{-}2\text{-}yl)methyl)prop\text{-}2\text{-}en\text{-}1\text{-}amine}$ (8).

$^{1}$H NMR (300 MHz, CDCl$_3$) Spectrum of compound 8.
$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound **8.Oxal**.

$^1$H NMR (500 MHz, CDCl$_3$) Spectrum of compound **MBA329**.
$^{13}$C NMR (126 MHz, CDCl$_3$) Spectrum of compound MBA329.

$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound MBA329.
$^1$H-$^13$C g-HSQC (CDCl$_3$) spectrum of compound MBA329.

$^1$H-$^13$C g-HMBC (CDCl$_3$) spectrum of compound MBA329.
Ethyl 5-(((1-benzylpiperidin-4-yl)carbamoyl)oxy)-1H-indole-2-carboxylate (18).

$^1$H NMR (400 MHz, CDCl$_3$) Spectrum of compound 18.

$^{13}$C NMR (100 MHz, CDCl$_3$) Spectrum of compound 18.
$^1$H-$^1$H g-COSY (400 MHz, CDCl$_3$) spectrum of compound 18.

$^1$H-$^1$C g-HSQC (CDCl$_3$) spectrum of compound 18.
$^1$H-$^{13}$C g-HMBC (CDCl$_3$) spectrum of compound 18.

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA334.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) Spectrum of compound MBA335.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) Spectrum of compound MBA336.
$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound MBA337.
**tert-Butyl 5-(benzylxy)-2-((((1-benzylpiperidin-4-yl)carbamoyl)oxy)methyl)-1H-indole-1-carboxylate (19).**

\[ \text{1H NMR (500 MHz, CDCl}_3\text{) Spectrum of compound 19.} \]

\[ \text{13C NMR (126 MHz, CDCl}_3\text{) Spectrum of compound 19.} \]
$^1$H-$^1$H g-COSY (500 MHz, CDCl$_3$) spectrum of compound 19.

$^1$H-$^{13}$C g-HSQC (CDCl$_3$) spectrum of compound 19.
$^1$H-$^1$C g-HMBC (CDCl$_3$) spectrum of compound 19.

$^1$H NMR (300 MHz, CDCl$_3$) Spectrum of compound 19-Oxal.
1-Methyl-2-((methyl(prop-2-yn-1-yl)amino)methyl)-1\textsubscript{H}-indol-5-yl dimethylcarbamate (17)

\begin{center}
\includegraphics[width=\textwidth]{figure1}
\end{center}

\textsuperscript{1}H-NMR (300 MHz, CDCl\textsubscript{3}) spectrum of compound 17.

\begin{center}
\includegraphics[width=\textwidth]{figure2}
\end{center}

\textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}) spectrum of compound 17
$N$-Methyl-$N$-((1-methyl-5-(3-phenylpropoxy)-1$H$-indol-2-yl)methyl)prop-2-yn-1-amine (16)

$^1$H-NMR (300 MHz, CDCl₃) spectra of compound 16.

$^{13}$C-NMR (100 MHz, CDCl₃) spectra of compound 16.
**Biological Evaluation**

**Table S2.** \( IC_{50} \) values and MAO-B selectivity ratios \([IC_{50} \text{ (MAO-A)}/IC_{50} \text{ (MAO-B)}]\) for the inhibitory effects of test drugs (new compounds 1-19, and reference inhibitors) on the enzymatic activity of hMAO isoforms expressed in baculovirus infected BTI insect cells. \( IC_{50} \) values for the inhibitory effects of test drugs (all new compounds and reference inhibitors) on the enzymatic activity of recombinant acetylcholinesterase (hAChE) and butyrylcholinesterase (hBuChE) expressed in HEK 293 cells.
<table>
<thead>
<tr>
<th>DRUG</th>
<th>hMAO-A</th>
<th>hMAO-B</th>
<th>Ratio</th>
<th>hAChE</th>
<th>hBuChE</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASS234</td>
<td>64.97 ± 1.28 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.15 µM</td>
<td>0.06</td>
<td>3.50 ± 0.16 µM</td>
<td>3.37 ± 0.32 µM</td>
<td>1.03</td>
</tr>
<tr>
<td>1</td>
<td>132.00 ± 16.02 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.03 ± 0.18 µM</td>
<td>0.13</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>2 (MBA236)</td>
<td>9.1 ± 1.2 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>203.8 ± 5.4 nM</td>
<td>0.04</td>
<td>2.77 ± 0.29 µM</td>
<td>5.16 ± 0.34 µM</td>
<td>0.53</td>
</tr>
<tr>
<td>3</td>
<td>431.5 ± 48.7 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.3 ± 1.7 nM</td>
<td>21.2</td>
<td>***</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>4</td>
<td>10.1 ± 1.5 nM</td>
<td>8.2 ± 0.6 nM</td>
<td>1.2</td>
<td>***</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>5</td>
<td>252.2 ± 18.1 nM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>183.8 ± 6.5 nM</td>
<td>1.4</td>
<td>8.50 ± 1.77 µM</td>
<td>5.63 ± 0.62 µM</td>
<td>1.51</td>
</tr>
<tr>
<td>6</td>
<td>8.67 ± 0.93 µM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.91 ± 2.78 µM</td>
<td>0.25</td>
<td>16.23 ± 1.45 µM</td>
<td>6.77 ± 0.48 µM</td>
<td>2.39</td>
</tr>
<tr>
<td>7</td>
<td>17.70 ± 2.64 µM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.80 ± 1.32 µM</td>
<td>0.56</td>
<td>4.76 ± 0.32 µM</td>
<td>7.19 ± 1.61 µM</td>
<td>0.66</td>
</tr>
<tr>
<td>8</td>
<td>42.66 ± 1.58 µM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.27 ± 4.17 µM</td>
<td>1.9</td>
<td>4.45 ± 0.63 µM</td>
<td>40.42 ± 2.21 µM</td>
<td>0.11</td>
</tr>
<tr>
<td>9</td>
<td>***</td>
<td>***</td>
<td></td>
<td>1.57 ± 0.21 µM</td>
<td>4.14 ± 0.67 µM</td>
<td>0.37</td>
</tr>
<tr>
<td>10</td>
<td>***</td>
<td>**</td>
<td></td>
<td>0.78 ± 0.11 µM</td>
<td>2.75 ± 0.37 µM</td>
<td>0.28</td>
</tr>
<tr>
<td>11</td>
<td>881.8 ± 21.3 nM</td>
<td>1037.0 ± 53.3 nM</td>
<td>0.85</td>
<td>9.17 ± 1.08 µM</td>
<td>9.52 ± 0.86 µM</td>
<td>0.96</td>
</tr>
<tr>
<td>12</td>
<td>9.13 ± 0.26 µM</td>
<td>12.71 ± 1.73 µM</td>
<td>0.72</td>
<td>12.24 ± 1.61 µM</td>
<td>36.06 ± 5.71 µM</td>
<td>0.33</td>
</tr>
<tr>
<td>13</td>
<td>594.3 ± 12.5 nM&lt;sup&gt;a&lt;/sup&gt;</td>
<td>158.6 ± 13.2 nM</td>
<td>3.7</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>14</td>
<td>304.6 ± 13.2 µM</td>
<td>272.9 ± 7.6 nM</td>
<td>1.1</td>
<td>**</td>
<td>**</td>
<td>**</td>
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<tr>
<td>15</td>
<td>***</td>
<td>**</td>
<td></td>
<td>**</td>
<td>**</td>
<td>53.82 ± 1.69 µM</td>
</tr>
<tr>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.383 ± 0.099 µM</td>
<td>1.001 ± 0.205 µM</td>
<td>0.7</td>
<td>&gt; 100</td>
<td>&gt; 100</td>
<td>-</td>
</tr>
</tbody>
</table>
| ChE  | MAO: All IC\textsubscript{50} values shown in this table are the mean ± S.E.M. from five experiments. Level of statistical significance: \(^aP < 0.01\) or \(^bP < 0.05\) versus the corresponding IC\textsubscript{50} values obtained against MAO-B, as determined by ANOVA/Dunnett’s. 
* Inactive at 1 mM (highest concentration tested). 
** Inactive at 100 µM (highest concentration tested). 
*** 100 µM inhibits the corresponding MAO activity by approximately 40-50%. At higher concentration the compounds precipitate. 
\# Values obtained under the assumption that the corresponding IC\textsubscript{50} against MAO-A is the highest concentration tested (1 mM). 
\# Values obtained under the assumption that the corresponding IC\textsubscript{50} against MAO-A is the highest concentration tested (1 mM). 
\* We thank Dr. Irene Bolea (UAB, Barcelona) for these analyses. \* EeAChE. \* eqBuChE. |  |  |  |  |  |
<table>
<thead>
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</thead>
<tbody>
<tr>
<td>17(^c)</td>
<td>3.2 ± 0.7 nM</td>
<td>5.2 ± 1.8 nM</td>
<td>1.6</td>
<td>&gt; 100(^d)</td>
<td>&gt; 100(^e)</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>**</td>
<td>***</td>
<td>31.47 ± 4.65 µM</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF9601N</td>
<td>281.26 ± 13.17 nM(^a)</td>
<td>4.31 ± 0.86 nM</td>
<td>65</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Clorgyline</td>
<td>4.46 ± 0.32 nM(^a)</td>
<td>61.35 ± 1.13 µM</td>
<td>0.73\times10^{-2}</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>(-)-Deprenyl</td>
<td>67.25 ± 1.02 µM(^a)</td>
<td>19.60 ± 0.86 nM</td>
<td>3,431</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Iproniazide</td>
<td>6.56 ± 0.76 µM</td>
<td>7.54 ± 0.36 µM</td>
<td>0.87</td>
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<tr>
<td>Moclobemide</td>
<td>361.38 ± 19.3 µM</td>
<td>*</td>
<td>&lt;0.36(^#)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eserine</td>
<td></td>
<td></td>
<td>122.63 ± 4.55 nM</td>
<td>165.57 ± 2.12 nM</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Tacrine</td>
<td></td>
<td></td>
<td>439.81 ± 37.10 nM</td>
<td>151.53 ± 7.68 nM</td>
<td>2.90</td>
<td></td>
</tr>
</tbody>
</table>
Methods

**Determination of MAO isoform activity.** The potential effects of compounds on MAO activity were investigated by measuring their effects on the production of H₂O₂ from p-tyramine, using the Amplex® Red MAO assay kit (Molecular Probes, Inc., Eugene, Oregon, USA) and membrane MAO isoforms prepared from insect cells (BTI-TN-5B1-4) infected with recombinant baculovirus containing cDNA inserts for human MAO-A or MAO-B (Sigma-Aldrich Química S.A., Alcobendas, Spain). The production of H₂O₂ catalysed by MAO isoforms can be detected using 10-acetyl-3,7-dihydroxyphenoxazine (Amplex® Red reagent), a non-fluorescent, highly sensitive and stable probe, which reacts with H₂O₂ in the presence of horseradish peroxidase to produce a fluorescent product, resorufin. MAO activity was evaluated by the above mentioned coupled assay, previously described but with several modifications (Yáñez, M.; Fraiz, N.; Cano, E.; Orallo, F.; *Biochem. Biophys. Res. Commun.* **2006**, *344*, 688-695).

Briefly, 0.1 mL of sodium phosphate buffer (0.05 M, pH 7.4) containing compounds or reference inhibitors and pure MAO-A or MAO-B required to oxidize (in the control group) 165 pmol of p-tyramine/min were incubated for 15 min at 37 °C in corresponding wells from a 96-well plate (BD, NJ, USA) already placed into the dark fluorimeter chamber. After this incubation period, reaction was started by adding (final concentrations) 200 μM Amplex® Red reagent, 1 U/mL horseradish peroxidase and 1 mM p-tyramine as a common substrate for both MAO A/ B. The production of H₂O₂ and, consequently, of resorufin was quantified at 37 °C in an spectrometer FLX800™ Multi-Detection microplate reader (Biotek Instruments, Inc, Vermont, USA) on the basis of the fluorescence generated (excitation 545 nm, emission 590 nm) over a 15 min period, a period in which fluorescence increased linearly from the beginning.
Control experiments were carried out simultaneously by replacing the test drugs with appropriate dilutions of the vehicles. In addition, the possible capacity of the above-mentioned test drugs to directly react with Amplex® Red reagent was determined by adding these drugs to solutions containing only the Amplex® Red reagent in a sodium phosphate buffer.

The specific fluorescence emission (used to obtain the final results) was calculated after subtraction of background activity which was determined from vials containing all components with the exception of the MAO isoforms, which were replaced by a sodium phosphate buffer solution.

For reversibility assays, a 100X concentration of the enzyme used in the above described experiments was incubated with a concentration of inhibitor equivalent to 10-fold its IC₅₀. After 30 min, the mixture was diluted 100-fold into reaction buffer containing Amplex® Red reagent, horseradish peroxidase and p-tyramine and reaction was monitored for 15 min. Control tests were carried out by pre-incubating and diluting in the absence of inhibitor (Copeland, R. A. Evaluation of Enzyme Inhibitors in Drug Discovery, Wiley-Interscience, Hoboken, NJ, 2005).

Enzyme kinetic assays were performed by testing four different concentrations of compound in the presence of four independent amounts of substrate p-tyramine. Slopes achieved in each experiment were registered and the data analyzed by global non linear regression.

Ki values for irreversible and reversible inhibition were determined by measuring the remaining activity after a 15 min incubation time of MBA236 in saturated substrate conditions, or without preincubation at a substrate concentration equal to 2xKm.
Determinations of cholinesterases activities. The cholinesterase assay method of Ellman (Ellman, G. L.; Courtney, K. D.; Andres, B. J.; Featherstone, R. M. Biochem. Pharmacol. 1961, 7, 88) was used to determine the in vitro cholinesterase activity. The activity was measured by the increase in absorbance at 412 nm due to the yellow color produced from the reaction of acetylthiocholine iodide with the dithiobisnitrobenzoate (DTNB) ion. Acetylcholinesterase recombinant expressed in HEK 293 cells and butyrylcholinesterase from human serum was obtained from Sigma. Enzyme activity was measured using a FLUOstar Optima microplate reader. The assay medium contained phosphate buffer, pH 8.0, 20 mM DTNB, 0.165 U/mL of enzyme, 0.75 µM substrate (acetylthiocholine iodide or butyrylthiocholine iodide). The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals for 10 min at 37 °C. In dose-dependent inhibition studies, the substrate was added to the assay medium containing enzyme, buffer, and DTNB with inhibitor after 10 min of incubation time. All experiments were carried out in duplicate and expressed as mean ± SEM. The relative activity is expressed as percentage ratio of enzyme activity in the absence of inhibitor.

Reversibility was checked by measuring the restoration of the enzyme after a quickly dilution into reaction buffer containing acetylthiocholine iodide and DTNB. Control tests were carried out by pre-incubating and diluting in the absence of inhibitor. Enzyme kinetic assays were performed by testing four different concentrations of compound in the presence of four independent amounts of acetylthiocholine iodide. Slopes achieved in each individual experiment were registered and data were analyzed by global non-linear regression.
Statistical assay

Unless otherwise specified, results shown in the text and tables are expressed as mean ± standard error of the mean (S.E.M.) from n experiments. Significant differences between two means were determined by one-way analysis of variance (ANOVA) followed by the Dunnett’s post-hoc test. Graph Pad Prism Software (GraphPad Software, San Diego, California, USA) was used to perform statistical analyses and to calculate IC₅₀ values and kinetic parameters.
Molecular modeling

The hMAO-A and MAO-B enzyme models have been obtained from crystallographic structures deposited in the Protein Data Bank (PDB)\(^1\) with codes 2Z5X\(^2\) and 4CRT\(^3\) respectively. These PDB entries have been selected because the first one represents the highest hMAO-A X-ray resolution model available while the second reports the covalent adduct between a partially resolved structure of ASS234 and the hMAO-B. The molecular modeling study started building a complete model of ASS234 hMAO-B adduct by means of the addition of the benzylpiperidine moiety onto the 4CRT inhibitor structure while, for hMAO-A, the inhibitor, after removing the original ligand, has been manually designed into the catalytic site of the PDB model 2Z5X. The geometries of both theoretical models have been energy minimized. In order to investigate the difference between the interaction of ASS234 and MBA236 with MAO, on the previous optimized structures, one ortho hydrogen atom of the inhibitor benzylpiperidine moiety has been replaced by a methyl group.

A better positioning of the inhibitors into both hMAO-A and MAO–B catalytic sites has been achieved submitting the four covalent adduct models to a conformational search. Three and five possible conformations of ASS234 were highlighted with hMAO-A and hMAO–B, respectively. The Boltzmann population analysis, carried out on the internal energies of above reported structure, revealed probabilities equal to 98.65% and 67.28%, for the existence of the global minimum energy adduct models with hMAO-A and hMAO–B, respectively. In the case of the theoretical models with MBA236, a narrow conformational space appeared; actually only one proposed structure was found for hMAO-A and two for hMAO-B, with a global minimum population equal to 96.11%. The graphical inspection of the conformational search results highlighted similar binding modes of ASS234 and MBA236 in the MAO clefts.
Both inhibitors were observed in folded conformations in hMAO-A, but were linear in MAO–B, respectively (Figure S1).

**Figure S1.** Global minimum energy structures of ASS234 and MBA236 covalently bound to hMAO-A and hMAO-B. The FAD cofactor is shown in green and inhibitors are depicted in white polytube with colored carbons. Interacting residues are showed in white carbons wireframe. Higher energy conformers of the inhibitors are reported in various colored wireframe and superposed to the global minimum one.

The reasons for the differential recognition by the isozymes could be mainly due to the hMAO-A Phe208 replaced by Ile199 in hMAO-B. In hMAO-B, Ile199 allowed the positioning of the benzylpiperidine moiety of the inhibitor into a lipophilic cage delimited by Phe103, His115 and Val106 whereas, in hMAO-A, the bulkier Phe173 directed the same inhibitor moiety toward Glu327. Taking into account the protonation state at pH 7, the Glu327 side chain has established a strong electrostatic interaction to the positively charged nitrogen of the piperidine ring. The conformations of the inhibitors in hMAO-A revealed other productive hydrophobic interaction to Phe173, Ile176 and Ile180. Overall, the interactions between ASS234, MBA236 and the MAOs
are driven by both steric hindrance and hydrophobic contribution. Only in the case of hMAO-A is an additional electrostatic term highlighted.

Taking into account that covalent interactions can be established only after a non bonding recognition, the MC global minimum energy structures have been modified to design the corresponding reversible complexes. In order to improve our investigation, MC global minimum energy covalent adducts and derived reversible complexes have been submitted to 100 ns of molecular dynamics (MD) simulations. The evaluation of the MD trajectories has been carried out considering the structure modification during the simulation. A root means square deviation (RMSd) matrix has been computed comparing, one each other, all sampled structures for each MD run. The RMSd calculation has been applied to both inhibitor and enzyme atoms revealing small perturbation (Figures S2 and S3). The effect of the methyl group, both on the inhibitor and on the enzyme conformation, has been qualitatively correlated to the percentage of MBA236 RMSd matrix included values lower than the corresponding ASS234. As reported in Table S3, in all cases the methyl group has remarkably improved the conformation stability of both inhibitors and targets.

Table S3. Conformation stabilizing effect of MBA236 methyl group as a percentage.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adduct Complex</td>
</tr>
<tr>
<td>hMAO-A</td>
<td>71.83</td>
</tr>
<tr>
<td>hMAO-B</td>
<td>28.28</td>
</tr>
</tbody>
</table>
Figure S2. Molecular dynamics inhibitor atomic fluctuation. RMSd is reported in Å.
Figure S3. Molecular dynamics enzyme atomic fluctuation. RMSd is reported in Å.

Using the target RMSd matrix, each MD trajectory has been clustered in nine groups. The representative structure that reported the lowest RMSd value for that group, has been superposed to the initial conformation. The visual inspection of the ten
structures for each inhibitor with each enzyme clearly indicates the conformation stabilizing effect of the methyl group (Figure S4).

The analysis of MD simulation performed on the non-covalent complexes has provided an estimation of the thermodynamic difference between the recognition of ASS234 and MBA236 by the MAOs. The enzyme-ligand interaction energies have been computed after each 10 ps of MD simulations. Coupled to Boltzman population analysis at 300° K, the energies highlight a good qualitative accord to IC$_{50}$ experimental data (Table S4).

**Table S4.** Boltzman population weighted average MAOs interaction energies in kcal/mol.

<table>
<thead>
<tr>
<th></th>
<th>ASS234</th>
<th>MBA236</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h$MAO-A</td>
<td>-68.91</td>
<td>-75.47</td>
</tr>
<tr>
<td>$h$MAO-B</td>
<td>-63.30</td>
<td>-64.18</td>
</tr>
</tbody>
</table>
Figure S4. Superimposition of inhibitors in MAO: the starting conformation (green polytube) and the representative structures of the nine MD clusters in hMAO-A (yellow) and hMAO-B (light blue).

Both inhibitors reported better interaction energies in the hMAO-A case. The stabilizing effect of the methyl group, suggested by the MD geometry analysis, has been confirmed by thermodynamics data. The role of this substituent can be attributed to its productive contribution to the hydrophobic interaction driving the recognition of ASS234 and MBA236 by both hMAO-A and hMAO-B.

A molecular modeling study on hAChE and hBuChE has been conducted by means of docking experiments. Biochemical experiments have demonstrated that ASS234 and MBA236 are reversible inhibitors of ChEs and their mechanism of action is based on non-covalent interaction with these enzymes. The evident structural similarity with the known AChE inhibitor donepezil suggested that ASS234 and
**MBA236** could analogously interact to ChE targets. The catalytic sites of human AChE and BuChE are quite similar: the main difference is found at the entrance gorge where the hAChE Trp286 is replaced by Ala277 in hBuChE. This mutation enlarges the entrance gorge allowing the recognition of substrates bulkier than acetylcholine. Moreover, this residue is the basis of the donepezil selectivity, because its indanone moiety establishes π-π interaction to the Trp286 sidechain. It is also known that the conformation of a conserved Tyr residue, 341 in AChE and 332 in BuChE, plays a pivotal role for the binding of known inhibitors such as donepezil and tacrine. The Tyr conformation adopted for the interaction with tacrine is not compatible with the donepezil binding mode and *viceversa*.

Aiming to take into account the enzyme conformation flexibility, we decided to consider several receptor models for both targets in docking simulations. The PDB was searched for wild type structures of hAChE and hBuChE. The resulting PDB entries were refined, discarding those models reporting complexes of the enzymes with macromolecules (such as fasciculin). Finally, our receptor models have been built, in the case of AChE, from PDB entries 3LII, 4EY4, 4EY5, 4EY6, 4EY7, 4M0E6 and 4M0F6 and for BuChE, 1P0M, 1P0P, 2J4C, 2PM89 and 4BDS. The docking results analysis revealed similar binding modes of **ASS234** and **MBA236** in both AChE and BuChE receptor models. PDB structures 4EY7 and 1P0P, reporting AChE·donepezil and BuChE·butryrylthiocholine complexes, respectively, have been the better recognized by our ligands. The docking scoring function best values have been in partial accord with the experimental data. In the AChE case, donepezil-like binding modes (Figure S5) are shown by both inhibitors with the docking scores indicating an advantage for the **MBA236** recognition equal to 3.32 kcal/mol with respect to **ASS234**. Such a result can be attributed to the improved π-π stacking of the
Figure S5. Donepezil recognition in human AChE (PDB: 4EY7). The ligand is depicted in green carbons polytube. Trp86 (inner side) and Trp286 (outer side) are reported in spacefill cpk colored notation. Hydrophobically interacting residues are showed in polytube white carbon atoms.

MBA236 o-methyl benzyl moiety to the Trp86 sidechain compared to the ASS234 unsubstituted benzyl ring. The remaining interactions of ASS234 and MBA236 to the AChE can be considered equivalent. In both cases we observed one hydrogen bond between the inhibitors ether oxygen atom and the backbone of Phe295, hydrophobic contacts to Phe338 and Tyr341 have been also reported. In addition, productive electrostatic interaction was observed between the positively charged piperidine moiety of the ligands and the Asp74 side chain (Figure S6).
Figure S6. Cholinesterases recognition of ASS234 and MBA236. Yellow dotted lines indicate hydrogen bonds. Most relevant inhibitor-interacting residues are labeled.

Figure S7. MBA236 and ASS234 docking average score of with respect to all AChE (blue) and BuChE (red) receptor models. The bars indicate the best and worst values.

The docking simulations performed on the BuChE receptor models indicated, for both ligands, a less productive recognition with respect to the previously reported...
AChE. Actually docking score, either computed as average on all receptor models and best and worst values, highlighted BuChE interaction energies higher than the corresponding AChE. (Figure S7).

The poses analysis confirmed the remarkable ligand recognition stabilizing contribution of AChE Trp286. In fact, its replacement with the Ala277 in BuChE produced deleterious effects: A ligand·enzyme recognition favorable \( \pi - \pi \) stacking has been lost and the corresponding increased volume of the entrance gorge has allowed the ligand to assume a folded conformation for reducing its solvent exposition. The best poses of both inhibitors have shown that only hydrogen bonds (HB) provided specific interaction to the target. In particular, \textbf{ASS234} has donated HB either to the Ser287 sidechain and to the backbone of Pro285, while \textbf{MBA236} established this interaction to Leu286 backbone only. The observed folded conformation of both inhibitors, geometrically prevented the stacking between the ligands benzyl moiety and inner site Trp82 (Trp86 in AChE) limiting the recognition contribution of such a residue to a weak generic hydrophobic interaction.

For building the complete hMAO–B \textbf{ASS234} adduct model a multi step procedure has been followed. First of all, the benzylpiperidine moiety not resolved in the X-ray data has been added to the 4CRT PDB entry inhibitor structure. All missing hydrogen atoms have been included and FAD cofactor and ligand bonds order has been fixed. The final structure (Figure S8), including X-ray water molecules, has been energy minimised using the OPLS-2005\textsuperscript{11} force field. Water enviroment effects have been mimicked by means of the implicit solvtion model GB/SA.\textsuperscript{12} In order to prevent unrealistic distorsion of the targets, a costant force equal to 100 kJ/mol·Å\textsuperscript{-1} has been
applied to the enzyme backbone atoms. The optimisation procedure has been carried out using Schrodinger Suite.\textsuperscript{12}

**Figure S8.** Preliminary structure of ASS234 hMAO-B covalent adduct model. FAD cofactor, green carbons colored, and covalently bound inhibitor, are displayed in polytube. The ASS234 manually added moiety is depicted in cyan carbons, the original X-ray in white carbons. The hMAO-B structure is reported as gray cartoon.

A similar approach has been followed for building the hMAO-A ASS234 covalent adduct model: harmine, the orginal X-ray ligand, has been removed from the 2Z5X catalytic site and manually replaced by ASS234 molecule bound to FAD.

The conformational search of ASS234 and MBA236 covalently bound to hMAO-A and hMAO-B has been carried out by means of MonteCarlo (MC) method.\textsuperscript{12} Previously reported optimized models have been considered as starting structures. 2000 inhibitor conformations were randomly generated by moving each ligand rotatable bond in a range equal to ±180°. The conformations generated were energy minimised using the same protocol as previously reported. For each adduct model, the MC optimised
geometries have been compared by superimposition after taking into account the root means square deviation (RMSd) computed, on the atoms other than hydrogen. Structures were considered identical if their RMSd was lower than 0.5 Å.

The design of ASS234 and MBA236 non covalent complexes of the MAOs active sites, started from the corresponding MC global minimum energy structures by removing the covalent bond between the inhibitors and the FAD. The complexes so generated, after fixing FAD and inhibitor bond order and adding the required hydrogen atoms, have been submitted to the energy optimisation procedure previously described (Figure S9).

**Figure S9.** Optimised structures of ASS234 and MBA236 non-convalent complexes with MAO. The FAD cofactor (green) carbons colored, and the inhibitors (white) are displayed in polytube. The enzymes are reported as gray cartoon.

Molecular dynamics experiments have been performed on both covalent adducts and complexes of ASS234 and MBA236 in the hMAO-A and MAO-B catalytic sites. All simulation have been carried out up to 100 ns at 300° K. The pressure was fixed at 1
atm and the integration time step was equal to 2 fs. Trajectories snapshots have been sampled at regular time intervals of 100 ps. The energy evaluation has been based on the OPLS-2005 force field and SPC explicit solvation model has been adopted to take into account water environment effects. All molecular dynamics simulations have been computed by means of Desmond software.\textsuperscript{13-15}

Docking simulations of ASS\textsubscript{234} and MBA\textsubscript{236} with respect to human AChE and BuChE receptor models have been performed using Glide software.\textsuperscript{16-19} All selected PDB structures have been modified by removing co-crystallised water molecules and ligands. According to the force field OPLS-2005, missing hydrogen atoms have been added and energy minimised. For those PDB structures reporting enzyme dimeric forms, both chains have been, separately, taken into account. The binding site core of each receptor model has been defined by means of a regular box of 1000 Å\textsuperscript{3} centered onto the catalytic Ser residue. Ligand flexible docking algorithm has been adopted and theoretical complexes have been evaluated using the extra-precision (XP) Glide scoring function.

**Molecular Modeling references**


