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# Selective oxidative dearomatization of angular tetracyclic phenols by controlled irradiation under air: synthesis of an angucyclinone-type double peroxide with anticancer properties

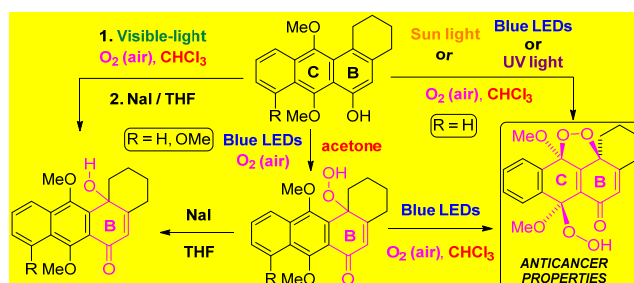
María J. Cabrera-Afonso,<sup>a</sup> Silvia R. Lucena,<sup>b</sup> Ángeles Juarranz,<sup>b</sup> Antonio Urbano,<sup>\*a,c</sup> M. Carmen Carreño<sup>\*a,c</sup>

<sup>a</sup>Departamento de Química Orgánica, Universidad Autónoma de Madrid (UAM), Cantoblanco, 28049-Madrid, Spain.

<sup>b</sup>Departamento de Biología, UAM, Cantoblanco, 28049-Madrid, Spain.

<sup>c</sup>Institute for Advanced Research in Chemical Sciences (IAdChem), UAM, Cantoblanco, 28049-Madrid, Spain.

Supporting Information Placeholder

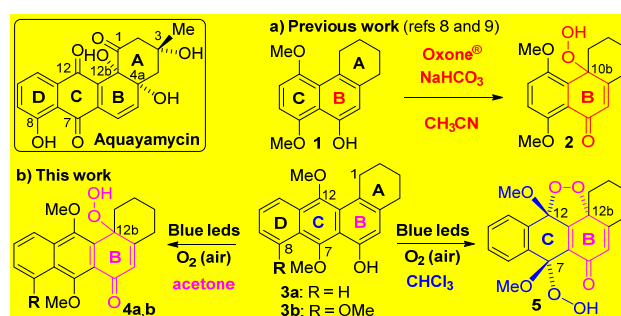


**ABSTRACT:** Angular tetracyclic *p*-peroxyquinols, *p*-quinols and a pentacyclic double peroxide, showing anticancer properties, were synthesized from the corresponding phenols by an environmentally friendly solvent- and wavelength-controlled irradiation under air in the absence of an external photosensitizer.

Angucyclines and their aglucones, named angucyclinones, are a group of natural quinones of poliketide origin, which exhibit a wide range of biological properties.<sup>1</sup> Common features of their structure are a benz[*a*]anthracene ABCD angular tetracyclic skeleton with a methyl group at C-3 and oxygen functionalities at C-1, C-7, C-8 and C-12. Due to biological activity and challenging structures, extensive studies on their synthesis<sup>1,2</sup> have been reported. Among them, there is a subgroup incorporating oxygenated substituents at the angular 4a and 12b positions such as Aquayamycin (Scheme 1). The presence of hydroxyls at the junction of the A and B rings still represents a significant synthetic challenge.<sup>3</sup> Only a few synthetic model studies,<sup>4</sup> together with several total syntheses<sup>5,6</sup> have been reported so far.

As part of our continuing efforts toward the synthesis of angucyclinones,<sup>7</sup> we have reported a novel synthetic approach to tricyclic models of derivatives with angular hydroxyls based on the oxidative dearomatization mediated by the system Oxone®/NaHCO<sub>3</sub>/CH<sub>3</sub>CN, as a source of singlet oxygen, of an angular tricyclic phenol such as **1** (Scheme 1a).<sup>8,9</sup> This key step allowed selective access to *p*-peroxyquinol **2**, a common precursor of six differently substituted oxygenated tricyclic core models of natural angucyclinones. These results led us to consider a similar route to tetracyclic models of angucyclinones starting from adequately substituted angular tetracyclic phenols such as **3** (Scheme 1b).

**Scheme 1. Oxidative dearomatization towards tricyclic and tetracyclic models of Angucyclinones.**

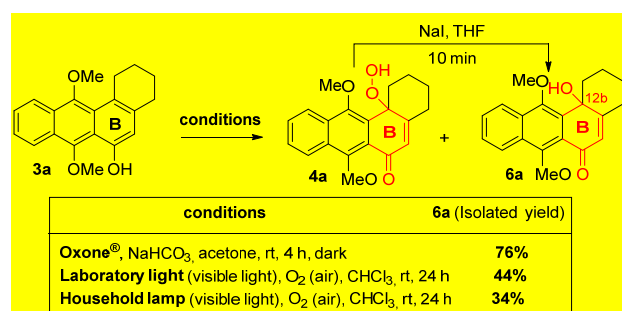


Herein, we report the oxidative dearomatization of tetracyclic phenols **3** into *p*-peroxyquinols **4** using our previous methodology with Oxone®. We also describe a new environmentally friendly photooxidation process without any added photosensitizer to achieve the same oxidative dearomatization process and the unprecedented and selective formation of a pentacyclic double peroxide **5** from **3a** by judicious choice of the solvent and the wavelength of the light source. Biosynthetic studies carried out by Rohr on aquayamycin<sup>10</sup> by culturing the *Streptomyces* precursor under <sup>18</sup>O<sub>2</sub> atmosphere, revealed incorporation of the heavy isotope at the C<sub>12b</sub> position. The oxygen-

ated groups introduced at C<sub>12b</sub> in our angucyclinone-type derivatives **4** and **5** were thus incorporated in a similar manner from molecular oxygen. Considering that peroxides can be exploited for therapeutic benefits against cancer,<sup>11</sup> the cytotoxicity of **5** was tested in selected cancer cell lines. Other angucyclinones,<sup>12</sup> have demonstrated antitumoral activity, producing apoptosis in various cancer cell lines (MDA-MB-231, A549, and HT29).

When tetracyclic phenol **3a** (see Supporting Information (SI) for the synthesis) was submitted to the oxidative dearomatization under our previous conditions (Oxone®/NaHCO<sub>3</sub>/CH<sub>3</sub>CN-H<sub>2</sub>O),<sup>8,9</sup> no evolution occurred. Changing the solvent to acetone and working in the dark (phenol **3a** was shown to be photosensitive), a 50:50 mixture of *p*-peroxyquinol **4a** and *p*-quinol **6a** resulted (Scheme 2), which after treatment with NaI allowed us to obtain *p*-quinol **6a**, with the angular OH at C<sub>12b</sub> (76% overall yield from **3a**).

**Scheme 2.** Oxidative dearomatization of phenol **3a** with Oxone®/NaHCO<sub>3</sub>/acetone or irradiation with visible light.



The observation that phenol **3a** was photosensitive and evolved into mixtures of products upon exposure to light (see SI), encouraged us to investigate this photochemical process in depth. Thus, the exposition of a CHCl<sub>3</sub> solution of phenol **3a** to irradiation by the laboratory fluorescent light under air for 24 h gave rise to an unseparable 75:25 mixture of **4a** and **6a**, whose reduction with NaI afforded tetracyclic *p*-quinol **6a** (44% isolated yield from **3a**, Scheme 2). When a household lamp was used as the light source under identical conditions, a slightly lower 34% isolated yield of *p*-quinol **6a** was obtained.

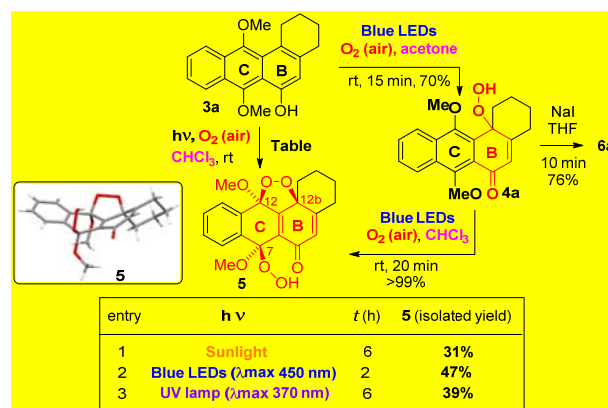
More interestingly, when phenol **3a** was exposed to sunlight, in CHCl<sub>3</sub> under air, a new oxidized product, the pentacyclic double peroxide **5**, was obtained after 6 h, in 31% isolated yield (Scheme 3). In this case, a double oxidative dearomatization at rings B and C of **3a** had taken place, with incorporation of two molecules of oxygen and formation of three stereogenic centers in a highly stereoselective manner. The structure of **5** was confirmed by X-ray diffraction (see SI), evidencing that both oxygen molecules had been incorporated from the same face.

The double oxidative dearomatization of rings B and C of **3a** upon irradiation with sunlight (Scheme 3) contrasted with that obtained with visible-light (Scheme 2), where only ring B of phenol **3a** was oxidized. We reasoned that such difference could be due to the range of UV wavelength provided by sunlight. Then, we decided to evaluate other light sources with maximum emissions at the near UV to achieve the double oxidative dearomatization process. Thus, irradiation of phenol **3a** with blue LEDs (λ<sub>max</sub> 450 nm) in CHCl<sub>3</sub> under air furnished, in only 2 h, the double peroxide **5** in an improved 47% yield (Scheme 4). A similar result was obtained upon irradiation under air of **3a** with an UV lamp (λ<sub>max</sub> 370 nm) in CHCl<sub>3</sub> for 6 h, which gave rise to derivative **5**, in 39% yield. Surprisingly, when phenol **3a**

was irradiated with blue LEDs under air using acetone instead of CHCl<sub>3</sub>, the *p*-peroxyquinol **4a** was formed in only 15 min (70% yield, Scheme 4). This result, as well as the formation of *p*-peroxyquinol **4a** in CHCl<sub>3</sub> by irradiation with visible light, suggested that *p*-peroxyquinol **4a** could be the intermediate in the double oxidative dearomatization of phenol **3a** leading to double peroxide **5**. This was confirmed by irradiation of *p*-peroxyquinol **4a** in CHCl<sub>3</sub>, with blue LEDs under air affording compound **5** in only 20 min and quantitative yield. Further treatment of *p*-peroxyquinol **4a** with NaI in THF gave *p*-quinol **6a** in 76% yield.

The system Oxone®/NaHCO<sub>3</sub>/CH<sub>3</sub>CN had been shown to provide singlet oxygen (<sup>1</sup>O<sub>2</sub>) which, upon reaction with *p*-alkyl phenols led to *p*-peroxyquinols through the corresponding endoperoxides.<sup>9</sup> We reasoned that *p*-peroxyquinol **4a** and double peroxide **5** could be also formed by reaction with singlet oxygen generated by aerobic irradiation of phenol **3a**, which could act as self-sensitizer.

**Scheme 3.** Oxidative dearomatization of **3a** by a solvent- and wavelength-controlled irradiation under air.

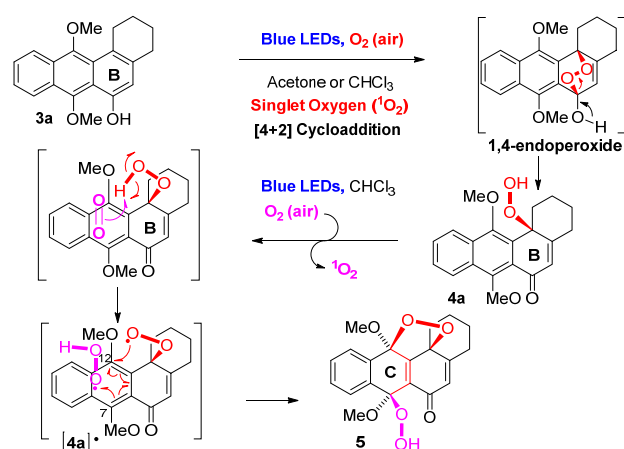


To gain insight into the reaction mechanism, we carried out several studies (see SI). The presence of <sup>1</sup>O<sub>2</sub> in the reactions effected under our photochemical conditions was confirmed by a trapping experiment with 9,10-dimethylanthracene, which afforded the corresponding endoperoxide.<sup>13</sup> The role of <sup>1</sup>O<sub>2</sub> as the oxidant in the synthesis of peroxide **5** from **3a** was supported by the increased reaction rate (15 min vs 2 h) observed when irradiation with blue LEDs of **3a** was performed in a deuterated solvent (see SI), which is known to increase the lifetime of <sup>1</sup>O<sub>2</sub>.<sup>14</sup> The presence of <sup>1</sup>O<sub>2</sub> in the formation of **4a** from **3a** was also supported by addition of the <sup>1</sup>O<sub>2</sub> quencher 1,4-diazabicyclo[2.2.2]octane (DABCO),<sup>15</sup> which greatly reduced the product yield (a 64% of starting phenol **3a** remained unchanged, see SI). We also checked the influence of DABCO and the radical scavenger TEMPO in the formation of **5** from **4a**. When *p*-peroxyquinol **4a** was irradiated in CHCl<sub>3</sub> in the presence of DABCO or TEMPO, the starting hydroperoxide **4a** remained unchanged (see SI). These results suggested that <sup>1</sup>O<sub>2</sub> could be the oxidant responsible of the formation of the *p*-peroxyquinol **4a** which could later evolve into double peroxide **5** by a radical pathway, with <sup>1</sup>O<sub>2</sub> having an essential role.

Generation of <sup>1</sup>O<sub>2</sub> under our conditions could only occur if **3a** was acting as photosensitizer, after excitation by light. UV spectra of **3a** indicated bands at λ 330-450 nm, almost overlapping with the blue LEDs emission (λ 370-490 nm, see SI). Accordingly, upon blue LEDs irradiation, phenol **3a** could itself act as photosensitizer producing the photoexcited <sup>1</sup>O<sub>2</sub>.<sup>16</sup>

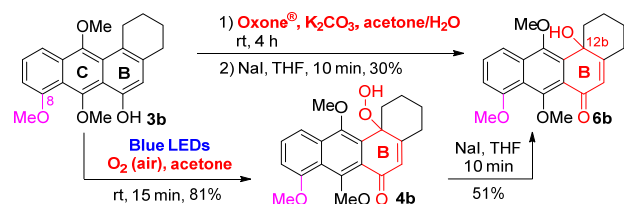
Based on these results, we can advance a possible mechanism for the formation of **4a** and **5** from **3a** (Scheme 4). First, reaction of phenol **3a** with  $^1\text{O}_2$ , through a [4+2] cycloaddition, selectively occurred at the most electron rich *p*-alkyl substituted phenol B ring, leading to the corresponding 1,4-endoperoxide which evolved into *p*-peroxyquinol **4a**. Once **4a** was formed, a radical process favored by irradiation in the chlorinated solvent and triggered by  $^1\text{O}_2^{17}$  through abstraction of the hydrogen of the hydroperoxide **4a**, afforded the peroxy radicals **[4a]** and  $\text{HOO}\cdot$ . Intramolecular attack of **[4a]** to the C<sub>12</sub> position from the face containing the peroxy radical, gave the cyclic peroxide moiety. This attack triggers the movement of electrons represented, favoring reaction at C-7 with the  $\text{HOO}\cdot$  situated on the same face to give the *bis*-peroxide **5** and explaining the exclusive formation of the diastereomer with the *cis* relative configuration of both peroxides.<sup>18</sup>

**Scheme 4. Mechanistic proposal for the aerobic photooxidation of 3a into 4a and 5.**



We also performed the oxidative dearomatization of 8-methoxy-substituted phenol **3b** (see SI for the synthesis) with Oxone®/K<sub>2</sub>CO<sub>3</sub>/acetone-H<sub>2</sub>O followed by reduction with NaI, giving *p*-quinol **6b** with a moderate 30% global yield for the two steps (Scheme 5). Fortunately, irradiation of phenol **3b** with blue LEDs under air in acetone for 15 min furnished *p*-peroxyquinol **4b** in 81% yield. Further reduction of **4b** with NaI, gave *p*-quinol **6b** in 51% yield.

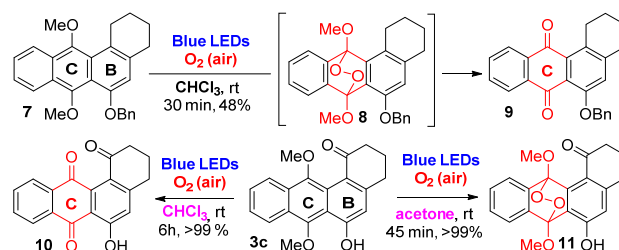
**Scheme 5. Oxidative dearomatization of phenol 3b with Oxone® or photooxidation with blue LEDs.**



The interest of such photooxidation, led us to extend the study to other analogues (Scheme 6). Thus, irradiation of the benzyl-protected derivative **7** (see SI for the synthesis) with blue LEDs (450 nm) under air in CHCl<sub>3</sub> gave quinone **9** (30 min, 48% yield), resulting from the exclusive oxidation of the more electron rich C ring of **7**, probably through the endoperoxide intermediate **8**, which could not be detected. On the other hand, irradiation of the 1-oxo substituted tetracyclic phenol **3c** (see SI for the synthesis) under similar conditions gave quinone

**10** (6 h, 99% yield) resulting from the oxidation of the more electron rich *p*-dimethoxy substituted C ring. When such irradiation was effected in acetone, the endoperoxide **11** could be isolated in almost quantitative yield. Formation of **10** in CHCl<sub>3</sub> could be probably due to a radical homolytic cleavage of the O-O bond on the undetected endoperoxide **11**.

**Scheme 6. Aerobic Photooxidation of 7 and 3c.**



The anticancer properties of peroxide **5** were tested in three established human cell lines, larynx Hep-2, breast MDA-MB and cervix HeLa cells. First, we evaluated the cytotoxicity of the new angucyclinone-type derivative **5** in these cell lines by using two different conditions of treatment: long-incubation period (24 h) with a low concentration of drug ( $10^{-8}$  M) and a short-term incubation (5 h) with higher drug concentration ( $10^{-7}$  M,  $5 \times 10^{-7}$  M,  $2.5 \times 10^{-7}$  M and  $10^{-6}$  M). The final concentration of acetone in the culture medium was always lower than 5%.

When treating cells with  $10^{-8}$  M for 24 h, only Hep-2 and MDA-MB lines significantly decreased their survival compared with the untreated control cells (see SI). The most sensible line was MDA-MB, with a percentage of 25% of lethality. When incubating cells for 5 h, the survival rate was dependent on the drug concentration. No effects were seen for DMEM / acetone 5%. The lower concentration of the drug tested ( $10^{-7}$  M) did not cause a relevant effect in any of the lines; the induced toxicity was lower than 10%. However, the rest of the tested concentrations were highly effective, inducing more than 90% of toxicity for all cell lines, with the exception of Hep-2 cells treated with concentrations of  $2.5 \times 10^{-7}$  M in which only 70% of cell death was detected. Thus, with long-term incubation, the most sensitive cell line in terms of survival was MDA-MB and Hep-2 was the most resistant (Figure 1a,b).

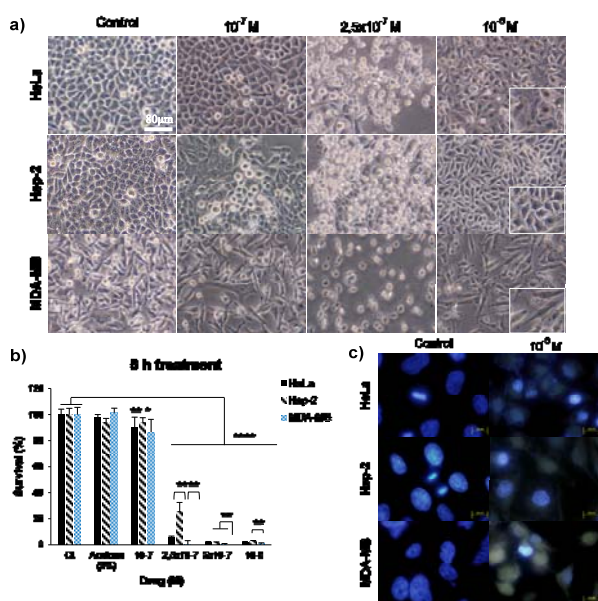
This drug demonstrates an extremely high effectivity at short-term treatment from a concentration of  $2.5 \times 10^{-7}$  M, being even more effective (in terms of lethality) than Doxorubicin (Doxo), whose use is extended in patients with cancer. Doxo presented an IC<sub>50</sub> of  $5 \times 10^{-7}$  M in two mammary cancer cell lines (MDA-MB-231 and MCF-7)<sup>19</sup> and it was even higher in other studies, presenting an IC<sub>50</sub> of  $1.84 \times 10^{-5}$  M in MDA-MB-231,<sup>20</sup> between  $9.16 \times 10^{-6}$  M and  $5.46 \times 10^{-6}$  M for Hep-2, HeLa and MCF-7 cell lines.<sup>21</sup> Comparing the efficiency of Doxo with four angucyclinones of new synthesis employed for the treatment of the breast MCF-7 cell line, again, peroxide **5** was more lethal at lower concentrations, presenting these four compounds an IC<sub>50</sub> between  $3.4 \times 10^{-7}$  M and  $51.3 \times 10^{-7}$  M.<sup>22</sup>

We have also evaluated the changes induced in the cell and nuclear morphology after treatments. Control HeLa and Hep-2 cells present a polygonal morphology, typical of keratinocytes, while the features of the MDA-MB cells were more spindled. These morphologies were maintained in cultures treated with low drug concentrations and in control acetone (5%). In incubations with drug concentrations of  $2.5 \times 10^{-7}$  M, cells become rounded and detach from the substrate. Conversely, concentrations of  $10^{-6}$  M, cells remained attached to the well but exhibited



an irregular shape. In this case, the 3D structure of the cells seemed to be lost. Nuclear morphology was evaluated 5 h after treatment with the highest concentrations ( $5 \times 10^{-7}$  M and  $10^{-6}$  M). Whereas control cells showed rounded nuclei and brilliant blue fluorescent chromatin (after DAPI staining), treated cultures showed nuclei with chromatin irregularly distributed forming highly fluorescent discrete aggregates or with a very low or null blue fluorescence; indicating a **loss** of the DNA content of the nucleus (Figure 1c).

**Figure 1.** Effect of peroxide **5** on larynx Hep-2, breast MDA-MB and cervix HeLa after a short-incubation period with variable concentrations of drug: a) morphological changes induced in the tumoral cells; b) cell survival after treatments evaluated by the MTT assays; c) morphology of cell nuclei after treatments evaluated under the fluorescence microscopy.



In summary, we have discovered that the angular tetracyclic phenols **3a-b** can be directly transformed into the  $C_{12b}$ -substituted hydroperoxides **4a-b** by irradiation with blue LEDs under air in acetone without the need of any added photosensitizer. **When irradiation of phenol 3a** was effected in  $CHCl_3$ , a double oxidative dearomatization took place incorporating two molecules of oxygen into the tetracyclic structure of phenol **3a**, in a very selective way, giving rise to the double peroxide **5**, whose anticancer properties were evaluated. Our in vitro studies indicate the high ability of peroxide **5** to induce lethality in different carcinoma cell lines.

## ASSOCIATED CONTENT

### Supporting Information

Synthetic procedures, characterization data, copies of  $^1H$ - and  $^{13}C$ -NMR spectra, and X-ray data for **5** (CCDC 1832265).

## AUTHOR INFORMATION

### Corresponding Author

\*E-mail: antonio.urbano@uam.es.

\*E-mail: carmen.carrenno@uam.es.

### ORCID

Antonio Urbano: 0000-0003-2563-1469

M. Carmen Carreño: 0000-0002-1721-9936

María J. Cabrera-Afonso: 0000-0002-3736-3206

Ángeles Juarranz: 0000-0002-6574-2887

Silvia R. Lucena: 0000-0002-9936-372X

## Notes

The authors declare no competing financial interest.

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