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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

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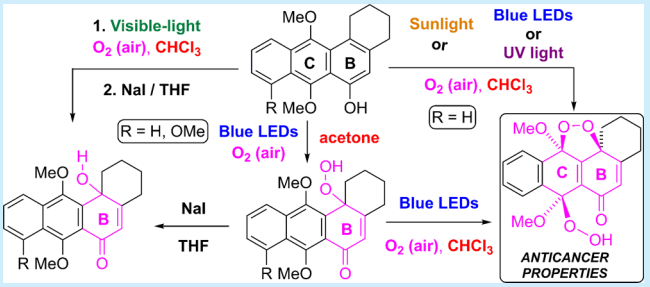
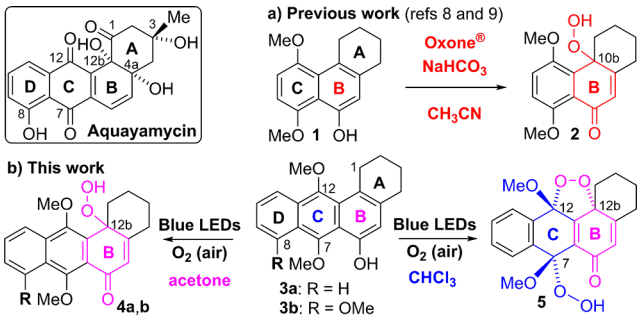
Supporting Information

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.[1] 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[1,2] 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.[3] Only a few synthetic model studies,[4] together with several total syntheses,[5,6] have been reported so far.

Scheme 1. Oxidative Dearomatization toward Tricyclic and Tetracyclic Models of Angucyclinones



As part of our continuing efforts toward the synthesis of angucyclinones,[7] 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/$NaHCO_3$/CH_3CN, as a source of singlet oxygen, of an angular tricyclic phenol such as **1** (Scheme 1a).[8,9] 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).

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[10] by culturing the *Streptomyces* precursor under a $^{18}O_2$ atmosphere, revealed incorporation of the heavy isotope at the C_{12b} position. The oxygenated groups introduced at C_{12b} 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,[11] the

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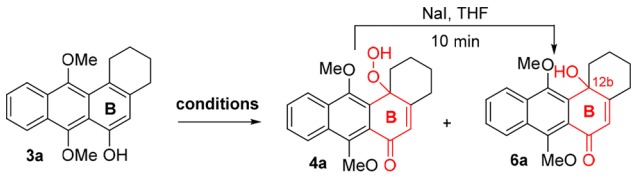
cytotoxicity of **5** was tested in selected cancer cell lines. Other angucyclinones[12] have demonstrated antitumoral activity, producing apoptosis in various cancer cell lines (MDA-MB-231, A549, and HT29).

When tetracyclic phenol **3a** (see Supporting Information (SI) for the synthesis) was submitted to the oxidative dearomatization under our previous conditions (Oxone/$NaHCO_3$/CH_3CN-H_2O),[8,9] no evolution occurred. By 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_{12b} (76% overall yield from **3a**).

Scheme 2. Oxidative Dearomatization of Phenol 3a with Oxone/$NaHCO_3$/Acetone or Irradiation with Visible Light

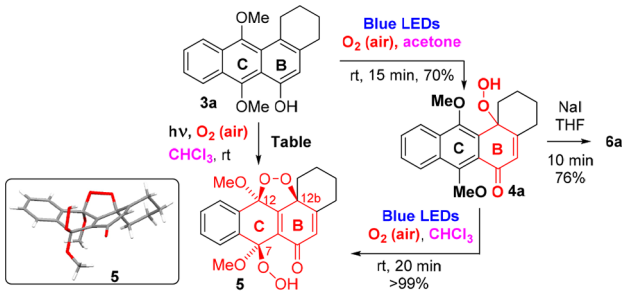


conditions	**6a** (Isolated yield)
Oxone®, $NaHCO_3$, acetone, rt, 4 h, dark	**76%**
Laboratory light (visible light), O_2 (air), $CHCl_3$, rt, 24 h	**44%**
Household lamp (visible light), O_2 (air), $CHCl_3$, rt, 24 h	**34%**

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 $CHCl_3$ solution of phenol **3a** to irradiation by 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 $CHCl_3$ 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

Scheme 3. Oxidative Dearomatization of 3a by a Solvent- and Wavelength-Controlled Irradiation under Air



entry	h ν	*t* (h)	**5** (isolated yield)
1	Sunlight	6	**31%**
2	Blue LEDs (λmax 450 nm)	2	**47%**
3	UV lamp (λmax 370 nm)	6	**39%**

in a highly stereoselective manner. The structure of **5** was confirmed by X-ray diffraction (CCDC 1832265), 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 a 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_{max} 450 nm) in $CHCl_3$ under air furnished, in only 2 h, the double peroxide **5** in an improved 47% yield (Scheme 3). A similar result was obtained upon irradiation under air for **3a** with a UV lamp (λ_{max} 370 nm) in $CHCl_3$ 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 $CHCl_3$, the *p*-peroxyquinol **4a** was formed in only 15 min (70% yield, Scheme 3). This result, as well as the formation of *p*-peroxyquinol **4a** in $CHCl_3$ 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 $CHCl_3$, 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/$NaHCO_3$/CH_3CN had been shown to provide singlet oxygen ($^1O_2$) which, upon reaction with *p*-alkyl phenols, led to *p*-peroxyquinols through the corresponding endoperoxides.[9] We reasoned that *p*-peroxyquinol **4a** and double peroxide **5** could be also formed by reaction with $^1O_2$ generated by aerobic irradiation of phenol **3a**, which could act as a self-sensitizer.

To gain insight into the reaction mechanism, we carried out several studies (see SI). The presence of $^1O_2$ in the reactions effected under our photochemical conditions was confirmed by a trapping experiment with 9,10-dimethylanthracene, which afforded the corresponding endoperoxide.[13] The role of $^1O_2$ 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 $^1O_2$.[14] The presence of $^1O_2$ in the formation of **4a** from **3a** was also supported by addition of the $^1O_2$ quencher 1,4-diazabicyclo[2.2.2]octane (DABCO),[15] 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 $CHCl_3$ in the presence of DABCO or TEMPO, the starting hydroperoxide **4a** remained unchanged (see SI). These results suggested that $^1O_2$ could be the oxidant responsible for the formation of the *p*-peroxyquinol **4a** which could later evolve into double peroxide **5** by a radical pathway, with $^1O_2$ having an essential role.

Generation of $^1O_2$ 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 LED irradiation, phenol **3a** could

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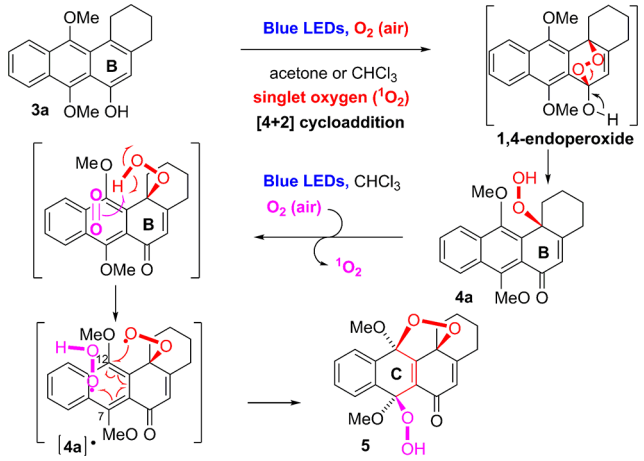
itself act as a photosensitizer producing the photoexcited $^1O_2$.[16]

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 $^1O_2$, through a [4 + 2]

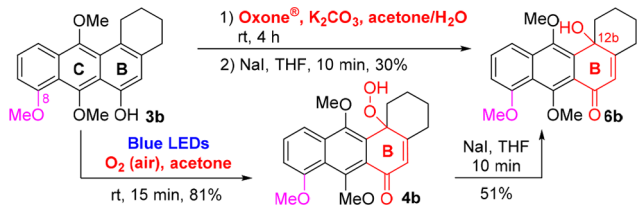
Scheme 4. Mechanistic Proposal for the Aerobic Photooxidation of 3a into 4a and 5



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 $^1O_2$[17] through abstraction of the hydrogen of the hydroperoxide **4a** afforded the peroxy radicals [**4a**]· and HOO·. Intramolecular attack of [**4a**]· to the C_{12} 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 HOO· 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.[18]

We also performed the oxidative dearomatization of 8-methoxy-substituted phenol **3b** (see SI for the synthesis) with Oxone/K_2CO_3/acetone−H_2O 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**

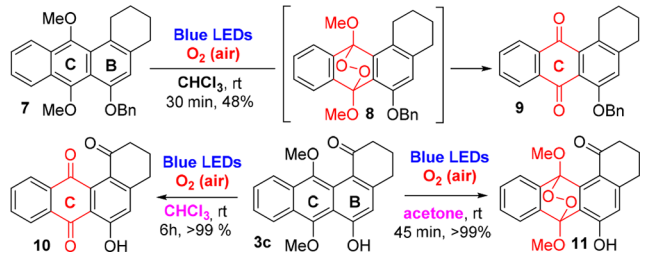
Scheme 5. Oxidative Dearomatization of Phenol 3b with Oxone or Photooxidation with Blue LEDs



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.

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 $CHCl_3$ gave quinone **9** (30 min, 48% yield), resulting from the exclusive oxidation of the

Scheme 6. Aerobic Photooxidation of 7 and 3c



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 $CHCl_3$ could likely be due to a radical homolytic cleavage of the O−O bond on the undetected endoperoxide **11**.

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, 5×10^{-7} M, 2.5×10^{-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 2.5×10^{-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 2.5×10^{-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_{50} of 5×10^{-7} M in two mammary cancer cell lines (MDA-MB-231 and MCF-7),[19] and it was even higher in other studies, presenting an IC_{50} of 1.84×10^{-5} M in MDA-MB-231,[20] between 9.16×10^{-6} M and 5.46×10^{-6} M for Hep-2, HeLa, and MCF-7 cell lines.[21] From comparison of 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 observed to be more lethal at lower concentrations, presenting these four compounds with an IC_{50} between 3.4×10^{-7} M and 51.3×10^{-7} M.[22]

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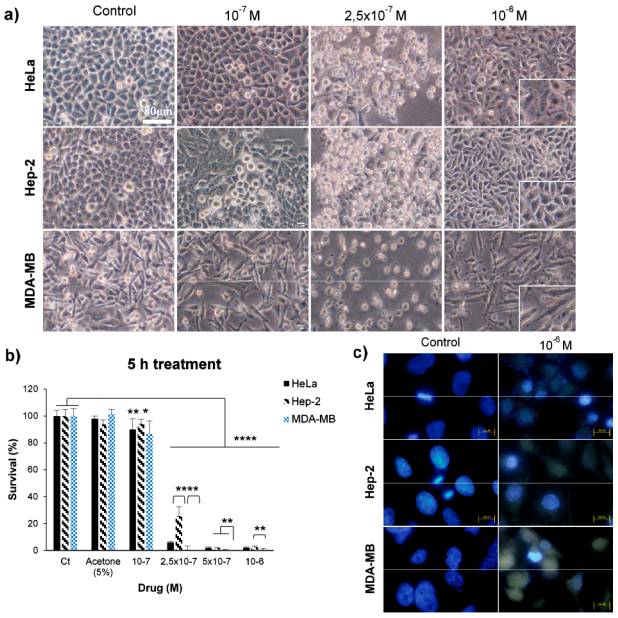

Figure 1. Effect of Peroxide **5** on larynx Hep-2, breast MDA-MB, and cervix HeLa after a short incubation period with variable concentrations of drug: (a) morphological changes induced in the tumoral cells; (b) cell survival after treatments evaluated by the MTT assays; (c) morphology of cell nuclei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

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 2.5×10^{-7} M, cells become rounded and detach from the substrate. Conversely, with 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 (5×10^{-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).

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

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02515.

Synthetic procedures, characterization data, and copies of ^{1}H and ^{13}C NMR spectra (PDF)

Accession Codes

CCDC 1832265 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

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Notes

The authors declare no competing financial interest.

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