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Synthesis and Biological Evaluation of Indoloquinolines and Pyridocarbazoles: A New Example of Unexpected Photoreduction Accompanying Photocyclization

Pierre-Jean Aragon, Ange-Désiré Yapi, Frédéric Pinguet, Jean-Michel Chezal, Jean-Claude Teulade, and Yves Blache

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Indoloquinoline alkaloid cryptolepine and pyridocarbazole alkaloid ellipticine are of great interest because in vitro and in vivo studies revealed their good cytotoxic properties. In order to obtain some biologically active analogs of these compounds, we developed a synthesis based on the photocyclization of tertiary N-substituted enamines derived from 1,3-cyclohexandione and 3 or 6-aminoquinoline. The angular cyclized compounds thus obtained were tested in vitro on K 562 cells and A 2780 doxorubicin sensitive and resistant cells. All compounds were less effective than doxorubicin in sensitive cells but their activity wasn’t decreased by MDR resistance.

Key words indoloquinoline; pyridocarbazole; photochemistry; cytotoxicity

Interest in the chemistry of pyridocarbazoles and indoloquinolines has increased this last decade since these skeletons are present in a large number of alkaloids of biological interest. For example, the indoloquinoline type alkaloid cryptolepine 1 (Fig. 1) extracted from the roots of Cryptolepis sanguinolenta has been shown to exhibit antimalarial, antibacterial and cytotoxic properties. This last activity is due to cryptolepine intercalation into DNA and subsequent inhibition of DNA religation by topoisomerase II. Another example concerns the pyridocarbazole type alkaloid ellipticine 2 (Fig. 1) extracted from the leaves of Ochrosia elliptica which has exhibited cytotoxic properties by different mechanisms: intercalation into DNA, inhibition of topoisomerase II, formation of covalent adducts with DNA after bioactivation, stimulation of apoptosis. Different linear and angular analogs of these compounds have already been synthetized by several groups but the series of indolo[2,3-c]quinoline and pyrido[2,3-c]carbazole haven’t been studied yet.

Therefore, as a part of our program concerning the elaboration of analogs of natural products, we are interested in the chemistry as well as in the biological activities of these angular heterocycles. Our methodology resides in the photocyclization of enamines derived from 3 or 6-aminoquinoline (Fig. 1). Enammines represent convenient tools in heterocyclic chemistry since they can be implicated in the elaboration of carbazoles, α-carbolines, β-carbolines and δ-carbolines. The photocyclization can occur through an electrocyclization for tertiary enamines or through a radical process for halogenated enamines. Furthermore, in our recent works, we obtained N-benzylpyridocarbazoles and indoloquinolines from tertiary N-benzylaminones and non N-substituted compounds from secondary iodinated enamines. In the present work, we investigate the reactivity of tertiary enamines carrying an alkyl or a dialkylaminoalkyl side chain 3 and report the results of in vitro cytotoxicity studies of the obtained cyclized compounds 4.

Chemistry

Firstly, the reactivity of tertiary enamines derived from 3-aminoquinoline was studied. According to previously described procedures, the secondary enamine 7 is obtained by condensation of 3-aminoquinoline 5 and 1,3-cyclohexandione 6 in toluene with catalytic amounts of p-toluensulfonic acid. Then, a methyl or a diethylaminoethyl side chain was introduced on the enamine nitrogen by treating secondary iodinated enamines with methyl iodide or by the hydrochloride salt of diethylaminoethylchloride to give 8 and 9. The dimethylamino propyl side chain couldn’t be introduced following this protocol. However, the tertiary enamine 10 was obtained by treating compound 7 with the hydrochloride salt of dimethylaminoethylchloride in presence of sodium hydride in dimethylformamide (DMF) (Fig. 2).

The methyl substituent was chosen because structure–activity relationships studies realized on ellipticine and cryptolepine linear or angular analogs show the contribution of the N-methyl function to the cytotoxicity and a frequent loss of activity with bigger alkyl chains. Dialkylaminoalkyl chains were chosen because they’re often described as cytotoxicity enhancers, probably through a stabilisation of the drug–DNA interaction.
Irradiation of enaminones 8, 9, 10 to give respectively 11, 12, 13, were performed using a Pyrex well apparatus and a medium pressure mercury UV lamp (150 W) under a set of conditions (Fig. 3, Table 1).

These photocyclizations were regioselective on the C-4 position of the quinolinic nucleus since only angular compounds were obtained. Structure of 11, 12, 13 was easily determined by 1H-NMR, and by comparison with our previous results. More precisely the 1H-NMR spectrum of 11, 12, 13 showed characteristic signals of H-1 shifted downfield due to the proximity of the carbonyl group and appeared as doublets (δ 9.68 for 11, δ 9.81 for 12, δ 9.74 for 13). The N-substituents don’t change the regioselectivity previously observed with N-benzylated enaminones and with secondary iodinated enaminones. Yields of photocyclization were close to those previously obtained: Compound 11 was obtained with the best yield (80%) with methanol as solvent, while the substituted indoloquinolines 12 and 13 were obtained with 60% and 35% yield (other conditions led to massive decomposition of the enaminone and poor yields of cyclized compounds). Compounds 11, 12 and 13 were retained for biological studies.

Then, so as to study the influence of the position of the quinolinic nitrogen on the reactivity of such enaminones, the secondary enaminone 15 was prepared by condensation of 6-aminoquinoline 14 and 1,3-cyclohexandione 6 in toluene with p-toluenesulfonic acid. Then, tertiary enaminones 16, 17 and 18 were obtained by treatment of 15 with sodium hydride in toluene and then, by methyl iodide, ethyl iodide or diethylaminoethyl hydrochloride. Tertiary enaminone 19 was also obtained by treatment of 15 with dimethylaminopropylchloride hydrochloride in DMF in presence of sodium hydride (Fig. 4). The N-ethyl derivative 17 was prepared because the first biological results showed a better activity of the 6-aminoquinoline derivatives, so we wanted to test other alkyl side chains, with the hope of increasing cytotoxicity.

Then, these four tertiary enaminones were subjected to irradiation in a pyrex reactor under different conditions (Fig. 5, Table 2).

The results of these irradiations were quite surprising, since we obtained a mixture of the expected 8,9,10,11-tetrahydropridocarbazoles 24, 25, 26, and 27 and of 5,6,8,9,10,11-hexahydropyrdocarbazoles 20, 21, 22 and 23. In all cases, only angular compounds, identified easily thanks to the chemical shift of proton H-1, were obtained, confirming the perfect regioselectivity of this photocyclization. The global yields of photocyclization were smaller than those obtained with 3-aminoquinoline derivatives and confirm the lower reactivity of 6-aminoquinoline derivatives, previously described. As the influence of solvent is a well-known phenomenon in photochemistry, it was hypothesised that irradiation in another solvent such as toluene could modify the

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**Table 1. Irradiation of Tertiary Enaminones 8, 9, 19**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Conditions</th>
<th>Yield (%) of compd.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8 R=CH₃</td>
<td>Pyrex, methanol, 4 h</td>
<td>11 (80)</td>
</tr>
<tr>
<td>2</td>
<td>8 R=CH₃</td>
<td>Pyrex, toluene, 4 h</td>
<td>11 (55)</td>
</tr>
<tr>
<td>3</td>
<td>8 R=CH₃</td>
<td>Pyrex, methanol/toluene 50/50, 4 h</td>
<td>11 (70)</td>
</tr>
<tr>
<td>4</td>
<td>9 R=CH₃N(Et)₂</td>
<td>Pyrex, methanol, 4 h</td>
<td>12 (60)</td>
</tr>
<tr>
<td>5</td>
<td>10 R=CH₃N(Me)₂</td>
<td>Pyrex, methanol, 4 h</td>
<td>13 (35)</td>
</tr>
</tbody>
</table>

**Table 2. Irradiation of Tertiary Enaminones 16, 17, 18, 19**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Substrate</th>
<th>Conditions</th>
<th>Yield (%) of compd.</th>
<th>Global yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 R=CH₃</td>
<td>Pyrex, methanol, 4 h</td>
<td>20 (56) and 24 (44)</td>
<td>40</td>
</tr>
<tr>
<td>2</td>
<td>17 R=CH₃CH₃</td>
<td>Pyrex, methanol, 4 h</td>
<td>21 (85) and 25 (15)</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>18 R=CH₃N(Et)₂</td>
<td>Pyrex, methanol, 4 h</td>
<td>22 (95) and 26 (5)</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>19 R=CH₃N(Et)₂</td>
<td>Pyrex, methanol, 4 h</td>
<td>23 (80) and 27 (20)</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>16 R=CH₃</td>
<td>Pyrex, toluene, 4 h</td>
<td>20 (40) and 24 (60)</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>17 R=CH₃CH₃</td>
<td>Pyrex, toluene, 4 h</td>
<td>21 (5) and 25 (95)</td>
<td>40</td>
</tr>
<tr>
<td>7</td>
<td>18 R=CH₃N(Et)₂</td>
<td>Pyrex, toluene, 4 h</td>
<td>22 (10) and 26 (90)</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>19 R=CH₃N(Et)₂</td>
<td>Pyrex, toluene, 4 h</td>
<td>23 (25) and 27 (75)</td>
<td>60</td>
</tr>
</tbody>
</table>
ratio hexahydro/terahydro compound. So, compounds 16, 17, 18 and 19 were irradiated in a pyrex reactor in toluene for 4 h. The ratios reduced/non reduced compound were reversed and non-reduced compounds were largely predominant, except in the case of the N-methyl derivative, whose photocyclization seemed to be poorly influenced by the solvent of irradiation.

This C5–C6 bond reduction had already been established when photocyclization of secondary iodinated enamine derived from 6-aminouquinoline was conducted in a pyrex reactor using acetonitrile with 4 ml of triethylamine as solvent.\textsuperscript{26} Only the hexahydro compound was obtained. Furthermore, this reduction could be understood as the result of a photoinduced electron transfer from triethylamine to the aromatic system, followed by abstraction of a proton and a hydrogen radical from the solvent, as described by Yang et al.\textsuperscript{26} But, in the present case, the reaction mixture didn’t contain any triethylamine, so this phenomenon can’t explain the photoreduction. 6π electron mechanism is implicated in the photocyclization of such tertiary enamines. According to this mechanism, described by Grellman et al.\textsuperscript{33} and Chapman et al.,\textsuperscript{34} a zwitterionic species is initially formed, which gives a hexahydro compound, which can eventually undergo a dehydrogenation process to give a tetrahydro compound. Furthermore, Grellman et al.\textsuperscript{35} have irradiated the N-methyl-2-anilinonaphtalene 28 in different solvents and have obtained a mixture of 5-methylbenzo[c]carbazole 29 and 5-methyl-6,7-dihydrobenzo[c]carbazole 30 (2:1 ratio). A hypothesis was proposed by the authors to explain the formation of those two compounds: after the electrocyclication, the zwitterionic intermediate 31 undergoes a suprafacial 1,4 hydrogen shift. The resulting compound 32 can either undergo an internal rearrangement to give 30 or undergo dehydrogenation to give 29 (Fig. 6).

This hypothesis can be applied to our compounds and therefore may explain the results presented above: irradiation of tertiary enamines 16, 17, 18, 19 should give the intermediary hexahydro derivatives 33, 34, 35, 36. Toluene induces dehydrogenation to give 24, 25, 26, 27 whereas methanol induces rearrangement to give 20, 21, 22, 23, as shown in Fig. 7.

A second phenomenon which happens after cyclization and dehydrogenation might explain C₅–C₆ bond reduction. Some authors\textsuperscript{36–38} described the reduction of one bond of aromatic compounds when they underwent irradiation in proton donors solvents, particularly aliphatic alcohols. Methanol could contribute to the reduction via this pathway. However, this phenomenon doesn’t explain the formation of reduced compounds when the N-substituted derivatives were irradiated in toluene alone. Yet we can reasonably suppose that solvent influence on the rearrangement or the dehydrogenation is the most important phenomenon and that photoreduction of tetrahydro cyclized compound by methanol may contribute to the formation of reduced compounds.

Finally, we investigated the strategy which consist in treating reduced compounds by Pd/C 10% in diphenylether.\textsuperscript{39} This reaction was successful in the case of the non-N-substituted pyridocarbazole 37\textsuperscript{26} which gave the compound 38\textsuperscript{26} with a 30% yield. N-Methyl and N-ethyl compounds 24 and 25 were obtained from 20 and 21 with a 30% yield. But, unfortunately, compounds 22 and 23 underwent massive degradation (Fig. 8).

**Biological Results**

In continuation of our previous works\textsuperscript{40,41} concerning the antiproliferative activity of tetracyclic nitrogenous heterocycles, and more precisely, their activity against MDR+ cancer cells lines, the cytotoxicity of the compounds 11, 12, 13, 14, 24, 25, 26, 37, 38 and 39, as the non N-substituted indoloquinoline previously prepared\textsuperscript{26} was evaluated by a cell growth inhibition assay against two human cell lines: K 562 (leukemia), and A 2780 (ovarian cancer) doxorubicin-sensitive and resistant (MDR+) and was compared to the cytotoxicity of doxorubicin. The resistant subline A 2780 R was established by the continuous exposure of cells to gradually increasing concentrations of doxorubicin. The resistant subline K 562 R wasn’t tested because of the poor activity of our products against K 562 S cells. IC₅₀ (concentration inhibiting 50% of the cell proliferation) expressed in mol/l and resistance factor (IC₅₀ on A2780 resistant cells(IC₅₀ on A2780 sensitive cells) of each compound are recapitulated in Table 3.

All the compounds are 10 to 1000 fold less effective than doxorubicin in all sensitive cells whereas compounds 11, 24 and 26 are as active as doxorubicin on A 2780 resistant cells.
with IC$_{50}$ varying between 2.8 and 8.3 × 10$^{-6}$ mol/l. The other compounds are only 10 fold less active than doxorubicin on A 2780 R cells. The resistance factor of all those compounds varies between 1 and 2.9 (doxorubicin: 121), which indicates that these compounds, as most synthetic compounds, are not concerned by the multidrug resistance phenomenon.

Comparison of IC$_{50}$ values of the hexahydropyrimidine 37 and the tetraphenyl compound 38 shows this reduction doesn’t seem to influence the biological activity, in spite of the twisted structure of the reduced compound. Whatever the substituent is, IC$_{50}$ of 3-aminoquinoline derivatives are, in most cases, equal or higher than IC$_{50}$ of 6-aminoquinoline concerned by the multidrug resistance phenomenon.

Experimental

Table 3. IC$_{50}$ and Resistance Factor of Tested Compounds and Doxorubicin

<table>
<thead>
<tr>
<th>Compds.</th>
<th>K 562 cells (mol/l)</th>
<th>A 2780 sensitive cells (mol/l)</th>
<th>A 2780 resistant cells (mol/l)</th>
<th>RF$^{(a)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>4.6 × 10$^{-3}$ ± 2.8 × 10$^{-6}$</td>
<td>8.0 × 10$^{-4}$ ± 6.3 × 10$^{-6}$</td>
<td>8.3 × 10$^{-4}$ ± 4.0 × 10$^{-6}$</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>2.1 × 10$^{-3}$ ± 1.0 × 10$^{-6}$</td>
<td>4.0 × 10$^{-5}$ ± 2.6 × 10$^{-5}$</td>
<td>9.0 × 10$^{-5}$ ± 2.8 × 10$^{-5}$</td>
<td>2.3</td>
</tr>
<tr>
<td>13</td>
<td>1.5 × 10$^{-3}$ ± 1.0 × 10$^{-6}$</td>
<td>3.8 × 10$^{-5}$ ± 2.1 × 10$^{-5}$</td>
<td>9.5 × 10$^{-5}$ ± 1.1 × 10$^{-5}$</td>
<td>2.5</td>
</tr>
<tr>
<td>24</td>
<td>3.3 × 10$^{-5}$ ± 2.0 × 10$^{-6}$</td>
<td>2.0 × 10$^{-5}$ ± 1.1 × 10$^{-6}$</td>
<td>5.8 × 10$^{-5}$ ± 2.4 × 10$^{-6}$</td>
<td>2.9</td>
</tr>
<tr>
<td>25</td>
<td>7.8 × 10$^{-10}$ ± 1.8 × 10$^{-10}$</td>
<td>1.4 × 10$^{-3}$ ± 5.9 × 10$^{-10}$</td>
<td>1.7 × 10$^{-3}$ ± 1.0 × 10$^{-9}$</td>
<td>1.2</td>
</tr>
<tr>
<td>26</td>
<td>2.5 × 10$^{-3}$ ± 3.0 × 10$^{-6}$</td>
<td>2.8 × 10$^{-5}$ ± 1.7 × 10$^{-7}$</td>
<td>2.8 × 10$^{-5}$ ± 1.8 × 10$^{-7}$</td>
<td>1</td>
</tr>
<tr>
<td>37</td>
<td>8.8 × 10$^{-10}$ ± 1.3 × 10$^{-10}$</td>
<td>2.2 × 10$^{-5}$ ± 1.1 × 10$^{-7}$</td>
<td>2.8 × 10$^{-5}$ ± 2.0 × 10$^{-7}$</td>
<td>1.3</td>
</tr>
<tr>
<td>38</td>
<td>2.3 × 10$^{-3}$ ± 1.2 × 10$^{-6}$</td>
<td>1.4 × 10$^{-5}$ ± 7.8 × 10$^{-8}$</td>
<td>2.5 × 10$^{-5}$ ± 4.6 × 10$^{-8}$</td>
<td>1.8</td>
</tr>
<tr>
<td>39</td>
<td>9.3 × 10$^{-5}$ ± 2.6 × 10$^{-5}$</td>
<td>3.3 × 10$^{-5}$ ± 2.4 × 10$^{-5}$</td>
<td>4.2 × 10$^{-5}$ ± 5.5 × 10$^{-6}$</td>
<td>1.3</td>
</tr>
<tr>
<td>Dox$^{(b)}$</td>
<td>1.6 × 10$^{-7}$ ± 3.3 × 10$^{-3}$</td>
<td>2.4 × 10$^{-5}$ ± 4.0 × 10$^{-5}$</td>
<td>2.9 × 10$^{-5}$ ± 4.9 × 10$^{-6}$</td>
<td>121</td>
</tr>
</tbody>
</table>

$^{(a)}$ resistance factor, $^{(b)}$ dox = doxorubicin.
a gradient of methanol as eluent to give 11 as a white powder: 80% (best yield), mp 238—240°C. 1H-NMR (CDCl 3) δ: 2.08 (2H, m), 2.35 (2H, t, J = 6.2 Hz), 2.54 (2H, t, J = 6.3 Hz), 3.16 (3H, s), 7.63 (2H, m), 8.12 (1H, dd, J = 6.7, 1.5 Hz), 8.52 (1H, s), 9.68 (1H, dd, J = 6.6, 1.5 Hz).13C-NMR (CDCl 3) δ: 22.2, 22.5, 29.9, 39.0, 114.7, 123.3, 125.9, 126.2, 127.4, 128.9, 129.5, 132.9, 143.3, 152.7, 193.4. Anal. Calc. for C16H16N2O: C, 76.75; H, 5.62; N, 11.17.

7-[(N-(6-Quinolinyl)ethoxy]-8,9,10,11-tetrahydroindolino[2,3-c]quinolin-11-one 12 Compound 9 (0.3 g, 0.9 mmol) underwent irradiation for 2 h in a pyrex reactor using methanol as solvent. Then, the solvents were evaporated under reduced pressure. The crude product was chromatographed on silica gel using dichloromethane and also washed with water. The organic layers were dried over sodium sulfate and washed with water. The organic layers were dissolved in dichloromethane and also washed with water. The organic layers were dried under vacuum.

3-[(6-Quinolinyl)methyl]indolinopropylamino)cyclohex-2-en-1-one 17 Compound 18 (0.23 g, 0.7 mmol) underwent irradiation under different conditions (see Tables 2, 3). Then, the solvents were evaporated under reduced pressure. The crude product was chromatographed on alumina gel using dichloromethane and a gradient of methanol as eluent to give 12 as a yellow powder: 40% (best yield).1H-NMR (CDCl 3) δ: 1.81 (2H, m), 2.06 (2H, m), 2.09 (6H, s), 2.15 (2H, t, J = 6.4 Hz), 2.57 (2H, t, J = 6.1 Hz), 2.76 (2H, t, J = 6.2 Hz), 4.02 (2H, t, J = 7.5 Hz), 7.58 (2H, m), 8.08 (1H, m), 8.86 (1H, s), 9.74 (1H, m).13C-NMR (CDCl 3) δ: 22.9, 23.1, 38.2, 39.4, 41.8, 45.6, 55.8, 115.3, 123.7, 126.4, 126.7, 127.5, 128.4, 129.5, 130.1, 136.0, 144.7, 152.7, 193.5. Anal. Calc. for C17H13N3O: C, 74.77; H, 7.17; N, 13.08. Found: C, 74.80; H, 7.20; N, 12.99.

5-(6-Quinolinyl)diethylamino)cyclohex-2-en-1-one 19 Compound 19 (0.4 g, 1.2 mmol) underwent irradiation under different conditions (see Tables 2, 3). Then, the solvents were evaporated under reduced pressure. The crude product was chromatographed on alumina gel using dichloromethane and a gradient of methanol as eluent to give 21 as a yellow oil: 38% (best yield).1H-NMR (CDCl 3) δ: 0.89 (6H, d, J = 7.2 Hz), 2.08 (2H, m), 2.47 (6H, m), 2.54 (2H, t, J = 7.2 Hz), 2.74 (4H, m), 3.09 (2H, t, J = 7.5 Hz), 3.80 (2H, t, J = 6.9 Hz), 7.07 (1H, m), 8.13 (1H, dd, J = 4.8, 1.6 Hz), 8.79 (1H, dd, J = 4.2, 1.6 Hz).13C-NMR (CDCl 3) δ: 22.9, 25.2, 36.4, 47.6 (2C), 70.1, 70.2, 105.1, 114.8, 126.8, 128.7, 131.5, 136.2, 142.7, 154.3, 164.7, 197.9. Anal. Calc. for C17H17N3O: C, 74.78; H, 8.01; N, 12.46. Found: C, 74.84; H, 8.03; N, 12.49.
was chromatographed on silica gel using dichloromethane as eluent to eliminate Pd/Carbon, which was washed with a hot mixture of dichloromethane and methanol (50/50). Dichloromethane and methanol were evaporated in vacuo and the diphénylèther containing the final product was chromatographed on silica gel using dichloromethane as eluent to eliminate diphénylèther and dichloromethane and a gradient of methanol to give 24 as a yellow powder: 30%, mp 214—216°C.

Method 2: Compound 16 (0.14 g, 0.6 mmol) underwent irradiation under different conditions (see Tables 2, 3). Then, the solvents were evaporated under reduced pressure. The crude product was chromatographed on alumina gel using dichloromethane and a gradient of methanol as eluent to give 25 (best yield). 1H-NMR

Method 1: Compound 20 (80 mg, 0.3 mmol) was added to a suspension of Pd/carbon 10% (100 mg) in diphenylether (20 ml) and the reaction mixture was refluxed under nitrogen for 2 h. Still hot, the reaction mixture was filtered in order to eliminate Pd/Carbon, which was washed with a hot mixture of dichloromethane and methanol (50/50). Dichloromethane and methanol were evaporated in vacuo and the diphénylèther containing the final product was chromatographed on silica gel using dichloromethane as eluent to eliminate diphénylèther and dichloromethane and a gradient of methanol to give 24 as a yellow powder: 30%, mp 214—216°C.

Method 2: Compound 16 (0.14 g, 0.6 mmol) underwent irradiation under different conditions (see Tables 2, 3). Then, the solvents were evaporated under reduced pressure. The crude product was chromatographed on alumina gel using dichloromethane and a gradient of methanol as eluent to give 25 (best yield). 1H-NMR

This method was used in this study. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylether dication (MTP) was provided by Sigma (St Quentin Fallavier, France). All other reagents were of analytical grade and were obtained from commercial sources.

Cytotoxicity Assay: In all the experiments, K 562 and A 2780 cells were seeded at a final density of 5000 cells/well in 96 well microtiter plates and were treated with drugs (doxorubicin and compounds 11, 12, 13, 20, 21, 22, 37, 38 and 39). Seven dilutions were used for each drug. After 96 h of incubation, 10 μl of MTT solution in PBS (5 mg/ml, phosphate-buffer saline pH 7.3) were added to each well and the wells were exposed to 37°C for 4 h. This colorimetric assay is based on the ability of live and metabolically unimpaired tumor-cells to reduce MTT to a blue formazan product. Then, 100 μl of a mixture of isopropanol and 1 M hydrochloric acid (96/4, v/v) were added to each well. After 10 min of vigorous shaking so as to solubilize formazan crystals, the absorbance was measured on a microculture plate reader (Dynatech MR 5000; France) at 570 nm. For each assay, at least three experiments were performed in triplicate. The resistance factor was calculated from the ratio between the IC[50] values (IC[50] values) recorded from A 2780 R and A 2780 S cells, respectively, for the following tested drugs: doxorubicin, compounds 11, 12, 13, 20, 21, 22, 37, 38 and 39.

References


