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# Synthesis of dihydroisoindolo[2,1-*a*]quinolin-11-ones, their *in silico* ADMET properties and *in vitro* antitumor activities†

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We evaluated the antitumoral activity of diverse series of 5-aryl-dihydroisoindolo[2,1-*a*]quinolin-11-ones, AIQ (Aryl IsoIndolo-Quinoline, **4a–m**), and 5-vinyl dihydroisoindolo[2,1-*a*]quinolin-11-ones, VIIQ (Vinyl IsoIndolo-Quinoline, **6a–l**), obtained using three component imino Diels–Alder (DA) reaction of anilines, *o*-phthalaldehyde and dienophiles. The first series was obtained in previous work employing isoeugenol and anethole as dienophiles, whereas the vinyl series was synthesized in high yields (75–90%) using isoprene as a dienophile. The cytotoxic activity of both AIQ and VIIQ series was evaluated against four cancer lines, identifying a new lead compound **4h** from the AIQ series, active against MCF-7 (310 nM), SKBR3 (1434 nM), PC3 (210 nM) and HeLa (79 nM) cells with high selectivity. In addition, *in silico* ADMET properties for the two series were assessed and discussed.

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## Introduction

Nitrogen-containing polycyclic aromatic compounds stand out as promising bioactive entities. Molecular architectures such as isoindolo[2,1-*a*]quinolines (IIQ, **I–III**) have been synthesized and these molecules showed important applications in the treatment against cancer and bacterial infection as they have potent Topo II and DNA gyrase inhibitory activities<sup>1,2</sup> (Fig. 1). Recently, a new isoindoloquinoline compound **IV** was identified as a selective ligand of telomeric RNA G-quadruplexes, a potential therapeutic target for cancer treatment.<sup>3</sup>

Furthermore, presence of the tetrahydro and dihydroisoindoloquinoline moieties on secondary metabolites has been highlighted in several reports, including representative compounds like the alkaloid camptothecin, isolated from *Camptotheca acuminata*.<sup>4</sup> Due to that few natural and synthetic examples of isoindoloquinoline models have been identified, their synthesis continues being attractive. Therefore, some methodologies as Grignard reaction,<sup>5,6</sup> *ortho*-aromatic metalation,<sup>7</sup> Suzuki coupling,<sup>8</sup> Friedel–Crafts reaction from

phthalimides,<sup>9</sup> Barbier-type allylation,<sup>10</sup> among others; have been effective for the isoindolo[2,1-*a*]quinoline synthesis. Moreover, phthalimide building block has showed an important role in almost all synthetic tools, becoming easily obtained from anilines and phthalic anhydride, further its derivatization towards IIQ synthesis. The synthetic development of these systems during the period of 1966–2004 has been reviewed.<sup>11</sup>

Another recent approach to access to this isoindolo[2,1-*a*]quinoline topology had been gaining importance. In this case, the imino-Diels–Alder reaction (DAR) (Povarov reaction) in its intramolecular<sup>12</sup> and intermolecular<sup>13</sup> version was the synthetic tool to afford different nitrogen-containing polycyclic heterocycles. One more efficient way to obtain the isoindoloquinolines was proposed by Khadem group using a couple of anilines and phthalaldehydic acid in a multicomponent reaction [4 + 2] catalysed by TFA.<sup>14</sup> Despite of several reports about this core synthesis, the reaction parameters have shown some drawbacks

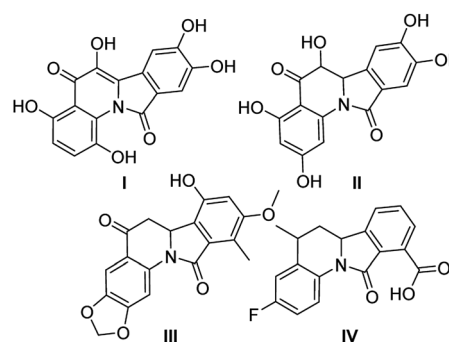


Fig. 1 Isoindoloquinolines with biological activity.

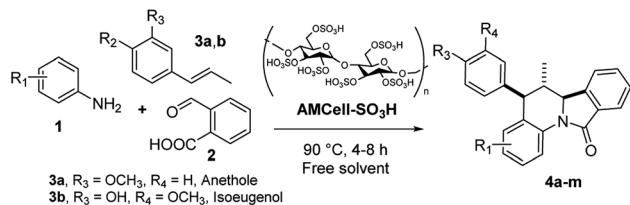
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Scheme 1 Previous work, synthesis of aryl isoindolo[2,1-*a*]quinolin-11-ones used in this research to test as potential antitumoral compounds.

related with high temperatures (200 °C), toxic and harmful solvents (acetonitrile, dichloromethane, xylene, among others), additives and metal (Pd) catalysed conditions. Our group recently reported the free-solvent synthesis of highly functionalized 6,6-dihydroisoindolo[2,1-*a*]quinolin-11(5*H*)-one derivatives, using natural  $\beta$ -styrenes (isoeugenol, anethole and isosafrole) as dienophiles (Scheme 1).<sup>15</sup>

This type of DAR is a bulk synthetic tool since it is possible to use different commercial starting materials, catalysts, promoters, solvents; in general, a great variety of conditions.<sup>16</sup> Moreover, this reaction has shown the importance of several activated alkenes, as their structural features allow to act as dienes or dienophiles, depending on the established reaction conditions and molecular energy barriers. Among the different dienes reported, the 1,3-butadiene derivatives have served as ideal precursors in natural products synthesis.<sup>17</sup> However, the role of this diene on the synthesis of heterocycles with this frame in the final product is reduced, especially when electron-deficient aza-dienes are employed. Some studies in which these dienes are used as dienophiles, have permitted to obtain alkaloid analogues with the 1,2,3,4-tetrahydroquinoline scaffold with a high regio and diastereoselectivity.<sup>18,19</sup> Based on these evidences, in the present work we focused our research towards the cytotoxic evaluation of new 5-methyl-5-vinyl substituted isoindolo[2,1-*a*]quinolin-11-ones (VIIQ series) synthesized using isoprene as dienophile in an imino-DAR. In addition, a 5-aryl-6-methyl substituted isoindolo[2,1-*a*]quinolin-11-ones (AIIQ series) series prepared by our own protocol was evaluated as potential antitumoral agent. We propose its plausible reaction mechanism where a preformed iminium ion is involved to react with the isoprene through of an imino-DAR, instead of mentioned cascade reaction and accessing their ADMET properties for both series.

## Results and discussion

### Chemistry

Synthesis of isoindolo[2,1-*a*]quinolin-11-ones has been preceded for the motivation of their interesting biological activity and rigid polycyclic architecture. Therefore, in this research we focused our task in the construction of this core, using the three component imino-DAR as synthetic tool. As we mentioned above, in a previous work we obtained a series of AIIQ, employing anilines **1**, phthalic aldehyde **2** and (*E*)-prop-1-en-1-ylbenzenes **3**; in the presence of sulfonated amorphous milled cellulose (AMCell-SO<sub>3</sub>H) as promoter (Scheme 1) to synthesize a series of isoindolo[2,1-*a*]quinolin-11-ones **4a-m** (Fig. 2).<sup>15</sup>

These scaffolds were created integrating fragments with potential pharmacophore groups, closely relationship with biological interesting isoindoloquinolines (Fig. 1) and other bioactive molecules reported in our researches.<sup>20</sup> Thus, alkene phenolic molecules **3** available from natural sources, were used to include guaiac oil, 4-hydroxy-3-methoxyphenyl fragment (from isoeugenol **3a**) and anisyl, 4-methoxyphenyl moiety (from anethole **3b**).<sup>20</sup> The desired molecules **4a-m** with C-5 aryl moieties (AIIQ series), were easily prepared without any solvents in good to excellent yields (see ESI†).

Looking for expand our molecular series, we wanted to introduce a vinyl fragment instead of the aryl group on C-5 position to obtain a vinyl substituted derivatives, *i.e.*, VIIQ series **6**. For this aim, we employed the same imino-DAR of arylamines **1** and phthalic aldehyde **2**, with participation of isoprene **5** that allowed generating different and varied molecular diversity (Table 1).

Noteworthy, that isoprene **5** has been used before in normal DAR as diene and dienophile, but poorly employed in imino-DAR as dienophile.<sup>18,19</sup> One of few works on this theme reports that tetrahydroquinoline (THQ) molecules, obtained from aldimines and different dienophiles, including the isoprene, could be used as potential antidiabetic compounds through of modulation of (adenosine monophosphate)-activated protein kinase (AMPK).<sup>21</sup>

On that way, isoprene is an interesting precursor, not explored in imino-DA reaction and could be used to incorporate favourable bioactive groups in IIQ skeleton for our diversity-oriented synthesis. According to the above statements and considering the high volatility of this alkene (bp = 34 °C), the reaction had to be performed at room temperature (25 °C). Based on our group's experience in account and knowing that BF<sub>3</sub>·OEt<sub>2</sub> (10 mol%, previous work),<sup>15</sup> cerium ammonium nitrate (CAN) and AlCl<sub>3</sub>, are commonly used as catalysts for imino-DA reactions,<sup>22</sup> we anticipate good results in the synthesis of VIIQ series. Thus, assuming a possible volatilization of isoprene from the reaction media, we realized a small set of experiments using these catalysts to optimize the reaction conditions (Table 1).

At first, we examined all potential products according with the isoprene reactivity, this kind of butadiene can also produce six members adducts when it acts as diene. According to Kobayashi *et al.*,<sup>23</sup> the diene behaviour of 2,3-dimethyl-1,3-butadiene was observed when a bi-aryl substituted pyridine such as **6'b** was obtained in a 37%. On the other hand, despite isoprene (2-methyl-1,3-butadiene) and derivatives as 2-trimethylsilyloxybutadiene, commonly used in hetero DA reaction, have showed low site selectivity acting as dienophile, obtaining pyridines and THQs as inseparable mixtures;<sup>24</sup> in our research compound **6'b** was not observed. Therefore, we obtained selectively the main product VIIQ structure. However, according to others studies,<sup>25</sup> reaction could be occurring *via* two possible  $\pi$ -sites, the methyl substituted double bond or the second one unsubstituted and two possible products **6a** and **6'a** can be formed. Nevertheless, structure **6'a** was not observed and it can be explained because orbital coefficient contribution to the frontiers orbital (HOMO) of isoprene for the structure *s-trans* are higher on the methylated double bond (16.30, 31.13), than



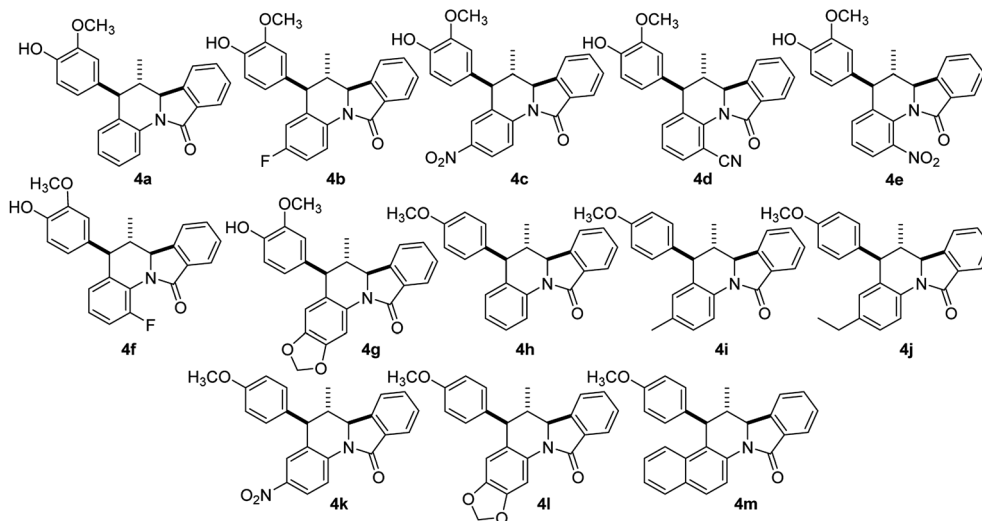


Fig. 2 The 5-aryl-6-methyl isoindolo[2,1-a]quinolin-11-ones (AIIQ series) synthesized in a previous work.<sup>15</sup>

Table 1 Optimization of reaction conditions<sup>a</sup>

| Entry | Catalyst                          | Solvent | Isoprene eq. | Yield (%) |
|-------|-----------------------------------|---------|--------------|-----------|
| 1     | BF <sub>3</sub> ·OEt <sub>2</sub> | MeCN    | 1.2          | 55        |
| 2     | CAN                               | MeCN    | 1.2          | 42        |
| 3     | AlCl <sub>3</sub>                 | MeCN    | 1.2          | 48        |
| 4     | BF <sub>3</sub> ·OEt <sub>2</sub> | DCM     | 1.2          | 52        |
| 5     | BF <sub>3</sub> ·OEt <sub>2</sub> | DCE     | 1.2          | 50        |
| 6     | BF <sub>3</sub> ·OEt <sub>2</sub> | MeCN    | 1.5          | 67        |
| 7     | BF <sub>3</sub> ·OEt <sub>2</sub> | MeCN    | 3            | 80        |

<sup>a</sup> Reaction was carried out for 0.5 mmol of substrate, aniline (0.5 mmol), phthalic aldehyde (0.6 mmol) and isoprene to room temperature; catalyst load was 10% mol using 5 mL of solvent in a 20 mL flask under N<sub>2</sub> atmosphere. Reported yields are from isolated products after chromatography column purification.

the no methylated double bond (17.7, 30.9).<sup>26</sup> We realized a similar approach using a semiempirical basis set as 3-21G by the second-order Møller–Plesset perturbation (MP2) (Fig. 3), where numbers indicate the percentual contribution of all orbital coefficients (see ESI†).<sup>26</sup>

Then, using this theoretical semiempirical analysis for the isoprene, we prove that C1–C2 (methylated carbon) and C3–C4 double bonds have more electronic density for the HOMO and LUMO frontier orbitals (FO). Nevertheless, the HOMO for the isoprene is the directly implied FO in imino DA reaction as an energetic inverse demand reaction. Thus, we concluded that

this highest coefficient contribution for C1 (44.2) and C2 (14.9) for the HOMO\_*s-trans* configuration, explains preliminary the site-selectivity reaction, yielding exclusively the 6a product.

After identification of the final product, we followed the optimization process using mainly the above-mentioned Lewis acids. The best behaviour was observed initially for the BF<sub>3</sub>·OEt<sub>2</sub> in MeCN (Table 1, entry 1), this catalyst has been widely studied,<sup>27</sup> where it proposed interaction with the diene has showed an influence of the HOMO, LUMO energies calculated<sup>28</sup> and this complex has showed good results in our investigations.<sup>29</sup> On the other hand, used solvents haven no influence over reaction yields in this reaction. However, when the number of isoprene equivalents were enlarged, a considerable increase in the reaction yield up to 80% was observed (Table 1, entry 7). Based on these results, three equivalents of isoprene, relative to the starting aniline, were employed for the synthesis of VIIQ products.

Having the reaction conditions in hands, different functional groups were included into the final isoindolo[2,1-a]quinolin-11(5*H*)-one skeleton 6a–l, employing various anilines in combination with phthalic aldehyde and isoprene as the alkene (Table 2). The cycloaddition reaction occurred with complete regio and stereoselectivity to give only one isomer in each reaction in good to high yields (68–90%).

The mechanistic elucidation of such cycloaddition<sup>30</sup> processes has been studied and is still under discussion, debating between a concerted or a step-wise mechanisms.<sup>31,32</sup> However, numerous practical studies and theoretical

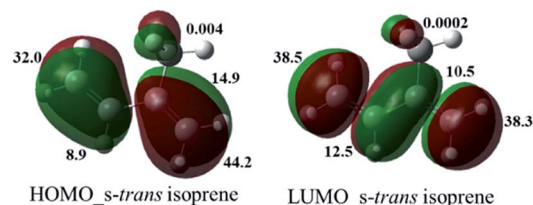
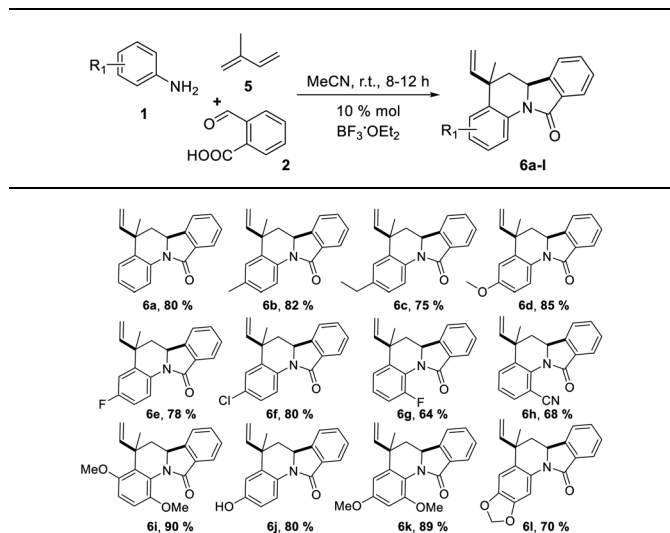


Fig. 3 HOMO and LUMO electronic density of isoprene.



Table 2 Reaction scope<sup>a</sup>

<sup>a</sup> Reaction run for 0.5 mmol of substrate, aniline (0.5 mmol), phthalic aldehyde (0.6 mmol) and isoprene to room temperature; catalyst load was 10% mol using 5 mL of solvent in a 20 mL flask under N<sub>2</sub> atmosphere.

investigations support a non-concerted mechanism for the Lewis acid catalysed aza DA reaction.<sup>33-36</sup>

In our previous work on AIIQ products **4a-m** we mentioned that amide formation ( $\gamma$ -lactamization) may occur directly after aldimine formation and prior to [4 + 2] cycloaddition of the acyliminium intermediate.<sup>15</sup> In this present study, in order to elucidate this mechanistic route, we achieved isolation of *N*-phenyl-3-hydroxy-isoindolinone (**A**) under the same reaction conditions (see ESI<sup>†</sup>). Noteworthy that **A** is known as an excellent starting material for isoindolo[2,1-*a*]quinolin-11-ones synthesis.<sup>13</sup> Thus, the reasonable mechanism can be envisioned as initial de-hydroxylation of isoindolinones type **A** by a Lewis acid, BF<sub>3</sub>·OEt<sub>2</sub>, to generate *N*-acyliminium cation **B** followed by electrophilic attack of cation **B** to isoprene **5**, leading to a new cation **C** (Scheme 2). Then, its intramolecular Friedel-Crafts reaction can afford intermediate **D** which gives isoindolo[2,1-*a*]quinolinone ring **6a** through 1,3-H shift step.<sup>13</sup>

Looking for an evidence of the reaction mechanism, we used the trapping control strategy.<sup>33,34</sup> Thus, BF<sub>3</sub>·OEt<sub>2</sub>-catalysed reaction of

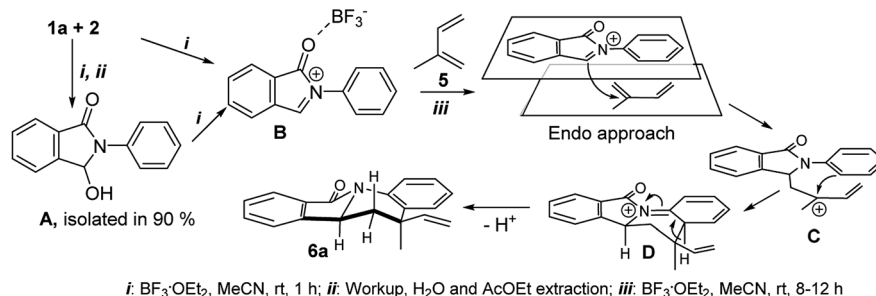
isoindolinone (**A**) and isoprene (**5**) in methanol was carried out (see ESI<sup>†</sup>). Unfortunately, the obtained results were insufficient to draw definite conclusions. It was observed that methanol affected drastically on over the course of the reaction, decreasing the yield of the final product **6a** to 20% and generating an unseparated mixture of poly-methoxylated adducts of **B** in which isoprene moiety was not incorporated. This indicates that there was an interaction between the molecules of methanol and intermediate ionic species like **B-C** during the cycloaddition process. With this outcome, we believed that formation of final isoindolo[2,1-*a*]quinolin-11-ones **6** using the three-component reaction of **1**, **2** and **5** occurs *via* generation of *N*-acyliminium cations which react with isoprene following a step-wise addition-cyclisation mechanism as indicated in Scheme 2.

Regarding to the obtention of two possible diastereomers *cis/trans*, associated to the vinyl and methyl position, we performed a structural elucidation using monocystal X-ray analysis (Fig. 4). Thus, we observed that the reference proton 6a-H is oriented in *axial* position as same as methyl group and vinyl group is *pseudo-equatorial* oriented. As it is known, DA reaction, normal and inverse version, are favoured though of an endo approach because it maximizes the secondary orbital overlap.<sup>37</sup> Therefore, explanation of this behaviour could be associated with no covalent interaction between aryl moiety and vinyl group in the endo approach (Scheme 2).

Having a complete structural description of IIQ molecules, and following our medicinal chemistry program, we evaluated their *in silico* and *in vitro*, molecular properties and activity as antitumoral compounds.

## Biology

***In silico* oral bioavailability and toxicity risk profile of the synthesized compounds.** In addition to the new synthesis of isoindolo[2,1-*a*]quinoline-11(5*H*)-ones, the main idea is to identify a lead biologically active substance with potential and selective activity against cancer strains. In this sense, we addressed to perform an *in silico* screening of the final products based on molecular descriptors. Therefore, using on-line Molinspiration platform<sup>38</sup> and DataWarrior software,<sup>39</sup> both series of compounds **4a-m** and **6a-l**, were subjected to the Lipinski's rule of five analysis (drug-likeness), which postulates if a chemical substance can be orally active in humans. Each structural fragment of the obtained molecules **4a-m**, **6a-l** (Table 3), is analysed for the Molinspiration program within a collection compounds created by shredding 3300 traded drugs, as



Scheme 2 Mechanistic proposal of the isoindolo[2,1-*a*]quinolin-11(5*H*)-one skeleton formation.



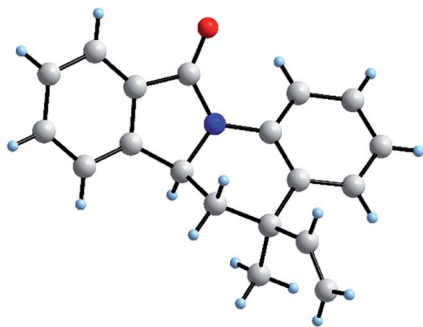


Fig. 4 Stick-ball model of **6a** asymmetric unit from monocystal X-ray diffraction (see ESI†).

well as 15 000 commercially available chemicals, yielding a complete list of all available orally fragments.<sup>38</sup>

Conversely to the Adriamycin (Adr) as reference, the obtained calculations demonstrated that all synthesized compounds contain high bioavailability properties, and they are between almost all parameters established by this rule of five (molecular weight = 275.35–416.43 g mol<sup>-1</sup>,  $c \log P = 3.870$ – $6.183$ ,  $nNO = 2$ – $7$ , and  $nOHNH = 0$ – $1$ )<sup>40</sup> (Table 3). Compounds with  $c \log P$ , higher than five units (**4h**–**4j**, **4m**, **6c** and **6k**), showed just one violation, maintaining their potential bioavailability to permeate the lipidic membrane. In this case,  $c \log P$  property is especially affected by the inclusion of apolar groups as methyl (**4i**) and ethyl (**4j**), compounds with highest  $c \log P$ .

In the AIIQ series, 4-hydroxy-3-methoxyphenyl moiety (**4a**–**4g**) provides a better solubility than the 4-methoxyphenyl (**4h**–**4m**) in the IIQ core.

The TPSA values minor to 140 Å<sup>2</sup> are associated to good cell membrane permeability. Values below to 60 Å<sup>2</sup> are accepted for those compounds able to cross the hematoencephalic barrier. Considering this, the TPSA values obtained for the synthesized compounds (20.309–95.595 Å<sup>2</sup>) allow us to confirm their drug-relevant properties regarding to cell membrane permeation. The TPSA value has been shown to be a very good descriptor characterizing drug absorption, including intestinal absorption, bioavailability, Caco-2 permeability and blood–brain barrier penetration.<sup>41</sup> Chemotherapy cancer treatment showed several side effects in drug treatment and reveals a diminution in the life quality of patient, showing hair fall out, vomit, not remember things, among others.<sup>42</sup>

These side effects are produced because anticancer drugs are generally toxic and kill normal cells due to their low selectivity.

Therefore, in order to assess preliminary to these kind of possible pharmacological properties of compounds **4a**–**m** and **6a**–**l**, their toxicity profile evaluation was performed employing the DataWarrior software.<sup>39</sup> It may point to the presence of certain fragments generally responsible for the irritant, mutagenic, tumorigenic, or reproductive effects in these molecules.<sup>43</sup> As shown in Table 4, any of the structural frames included in the final products afford a high toxic property.

A moderate risk was observed for some compounds in the irritant subject, mainly the vinyl isoindoloquinoline series. On

Table 3 Molecular properties of the obtained isoindoloquinolines **4a**–**m**, **6a**–**l**

| IIQ       | Molecular formula   | Molecular properties |        |                         |       |         |       |       |
|-----------|---|----------------------|--------|-------------------------|-------|---------|-------|-------|
|           |   | $c \log P$           | TPSA   | MW, g mol <sup>-1</sup> | $nNO$ | $nOHNH$ | $nRB$ | Viol. |
| <b>4a</b> | C <sub>24</sub> H <sub>21</sub> NO <sub>3</sub>               | 4.307                | 49.771 | 371.44                  | 4     | 1       | 2     | 0     |
| <b>4b</b> | C <sub>24</sub> H <sub>20</sub> FNO <sub>3</sub>              | 4.446                | 49.771 | 389.43                  | 4     | 1       | 2     | 0     |
| <b>4c</b> | C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> | 4.241                | 95.595 | 416.43                  | 7     | 1       | 3     | 0     |
| <b>4d</b> | C <sub>25</sub> H <sub>20</sub> N <sub>2</sub> O <sub>3</sub> | 4.013                | 73.563 | 396.45                  | 5     | 1       | 2     | 0     |
| <b>4e</b> | C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O <sub>5</sub> | 4.217                | 95.595 | 416.43                  | 7     | 1       | 3     | 0     |
| <b>4f</b> | C <sub>24</sub> H <sub>20</sub> FNO <sub>3</sub>              | 4.422                | 49.771 | 389.43                  | 4     | 1       | 2     | 0     |
| <b>4g</b> | C <sub>25</sub> H <sub>21</sub> NO <sub>5</sub>               | 4.173                | 68.239 | 415.44                  | 6     | 1       | 2     | 0     |
| <b>4h</b> | C <sub>24</sub> H <sub>21</sub> NO <sub>2</sub>               | 5.024                | 29.543 | 355.44                  | 3     | 0       | 2     | 1     |
| <b>4i</b> | C <sub>25</sub> H <sub>23</sub> NO <sub>2</sub>               | 5.449                | 29.543 | 369.46                  | 3     | 0       | 2     | 1     |
| <b>4j</b> | C <sub>26</sub> H <sub>25</sub> NO <sub>2</sub>               | 5.916                | 29.543 | 383.49                  | 3     | 0       | 3     | 1     |
| <b>4k</b> | C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O <sub>4</sub> | 4.959                | 75.367 | 400.43                  | 6     | 0       | 3     | 0     |
| <b>4l</b> | C <sub>25</sub> H <sub>21</sub> NO <sub>4</sub>               | 4.890                | 48.011 | 399.45                  | 5     | 0       | 2     | 0     |
| <b>4m</b> | C <sub>28</sub> H <sub>23</sub> NO <sub>2</sub>               | 6.183                | 29.543 | 405.50                  | 3     | 0       | 2     | 1     |
| <b>6a</b> | C <sub>19</sub> H <sub>17</sub> NO                            | 4.167                | 20.309 | 275.35                  | 2     | 0       | 1     | 0     |
| <b>6b</b> | C <sub>20</sub> H <sub>19</sub> NO                            | 4.592                | 20.309 | 289.38                  | 2     | 0       | 1     | 0     |
| <b>6c</b> | C <sub>21</sub> H <sub>21</sub> NO                            | 5.06                 | 20.31  | 303.40                  | 2     | 0       | 2     | 1     |
| <b>6d</b> | C <sub>20</sub> H <sub>19</sub> NO <sub>2</sub>               | 4.200                | 29.543 | 305.38                  | 3     | 0       | 2     | 0     |
| <b>6e</b> | C <sub>19</sub> H <sub>16</sub> FNO                           | 4.307                | 20.309 | 293.34                  | 2     | 0       | 1     | 0     |
| <b>6f</b> | C <sub>19</sub> H <sub>16</sub> ClNO                          | 4.821                | 20.309 | 309.80                  | 2     | 0       | 1     | 0     |
| <b>6g</b> | C <sub>24</sub> H <sub>16</sub> FNO                           | 4.283                | 20.309 | 293.34                  | 2     | 0       | 1     | 0     |
| <b>6h</b> | C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O              | 3.870                | 44.10  | 300.36                  | 3     | 0       | 1     | 0     |
| <b>6i</b> | C <sub>21</sub> H <sub>21</sub> NO <sub>3</sub>               | 4.185                | 38.777 | 335.40                  | 4     | 0       | 3     | 0     |
| <b>6j</b> | C <sub>19</sub> H <sub>17</sub> NO <sub>2</sub>               | 4.18                 | 29.54  | 305.38                  | 4     | 0       | 2     | 0     |
| <b>6k</b> | C <sub>21</sub> H <sub>21</sub> NO <sub>3</sub>               | 5.18                 | 38.78  | 335.40                  | 4     | 0       | 3     | 0     |
| <b>6l</b> | C <sub>20</sub> H <sub>17</sub> NO <sub>3</sub>               | 4.034                | 38.777 | 319.36                  | 4     | 0       | 1     | 0     |
| Adr       | C <sub>27</sub> H <sub>29</sub> NO <sub>11</sub>              | 0.567                | 206.08 | 543.52                  | 12    | 7       | 5     | 3     |



Table 4 Toxicity risk, drug-likeness and drug-score of compounds 4a–m, 6a–l

| IIQ | Potential risk <sup>a</sup> |      |      |           | Drug-likeness | Drug-score |
|-----|-----------------------------|------|------|-----------|---------------|------------|
|     | Mut.                        | Tum. | Irr. | Rep. eff. |               |            |
| 4a  | ■                           | ■    | ■    | ■         | 0.13          | 0.47       |
| 4b  | ■                           | ■    | ■    | ■         | -1.73         | 0.33       |
| 4c  | ■                           | ■    | ■    | ■         | -6.85         | 0.27       |
| 4d  | ■                           | ■    | ■    | ■         | -4.28         | 0.27       |
| 4e  | ■                           | ■    | ■    | ■         | -7.05         | 0.22       |
| 4f  | ■                           | ■    | ■    | ■         | -1.34         | 0.34       |
| 4g  | ■                           | ■    | ■    | ■         | -0.33         | 0.36       |
| 4h  | ■                           | ■    | ■    | ■         | 0.01          | 0.43       |
| 4i  | ■                           | ■    | ■    | ■         | -1.51         | 0.30       |
| 4j  | ■                           | ■    | ■    | ■         | -0.44         | 0.32       |
| 4k  | ■                           | ■    | ■    | ■         | -6.92         | 0.26       |
| 4l  | ■                           | ■    | ■    | ■         | -0.47         | 0.33       |
| 4m  | ■                           | ■    | ■    | ■         | -2.47         | 0.15       |
| 6a  | ■                           | ■    | ■    | ■         | -4.77         | 0.28       |
| 6b  | ■                           | ■    | ■    | ■         | -3.35         | 0.26       |
| 6c  | ■                           | ■    | ■    | ■         | -2.08         | 0.11       |
| 6d  | ■                           | ■    | ■    | ■         | -4.97         | 0.28       |
| 6e  | ■                           | ■    | ■    | ■         | -6.62         | 0.26       |
| 6f  | ■                           | ■    | ■    | ■         | -4.79         | 0.23       |
| 6g  | ■                           | ■    | ■    | ■         | -6.25         | 0.26       |
| 6h  | ■                           | ■    | ■    | ■         | -6.16         | 0.03       |
| 6i  | ■                           | ■    | ■    | ■         | -6.68         | 0.27       |
| 6j  | ■                           | ■    | ■    | ■         | -6.56         | 0.23       |
| 6k  | ■                           | ■    | ■    | ■         | -5.85         | 0.26       |
| 6l  | ■                           | ■    | ■    | ■         | -5.32         | 0.24       |
| Adr | ■                           | ■    | ■    | ■         | 7.19          | 0.55       |

<sup>a</sup> ■, drug-like properties; ■, moderate risk; ■, high risk.

the other hand, a moderate mutagenic risk was observed for compounds **4e**, **4m** and **6a**. Ciano group at the C-1 position afford a potential high risk regarding to reproductive effects.

Apparently, obtained compounds have not functional groups that could be attributed to them potential toxicity and drug score values are closer to the Adriamycin value. In the same sense, there is remarkable that mostly AIIQ showed better drug score values than VIIQ molecules. Adriamycin evaluation in DataWarrior platform afford us a high drug likeness value, in agreement with its widely recognized properties and thus to have a good molecular properties theoretical approach. On the other hand, as complement of this preliminary high throughput screening, *in silico* analysis, we performed the evaluation of the growth inhibitory activity against four cancer cell lines, for the synthetic compounds **4a–m**, **6a–l**.

A moderate risk was observed for some compounds in the irritant subject, mainly the vinyl isoindoloquinoline series. On the other hand, a moderate mutagenic risk was observed for compounds **4e**, **4m** and **6a**. Ciano group at the C-1 position afford a potential high risk regarding to reproductive effects. Apparently, obtained compounds have not functional groups that could be attributed to them potential toxicity and drug score values are closer to the Adriamycin value. In the same sense, there is remarkable that mostly AIIQ showed better drug score values than VIIQ molecules. On the other hand, as complement of this preliminary high throughput screening, *in silico* analysis, we performed the evaluation of the growth inhibitory activity against four cancer cell lines, for the synthetic compounds **4a–m**, **6a–l**.

**Cytotoxic activity.** Motivated for the recent results on anti-cancer activity of quinoline and tetrahydroquinoline molecules,<sup>44,45</sup> we tested our library of compounds **4a–m**, **6a–l** using a well-known biological assay, the MTT test, in order to discovery a new potential lead compound in anticancer therapy (Table 5). After evaluation, it was noted that C-5 aryl substituted molecules AIIQ **4a–m** displayed better anticancer inhibition properties, in contract, the C-5 vinyl group of molecules VIIQ **6a–l** decrease the cytotoxicity activity.

We also observed that both series, AIIQ and VIIQ showed positive response in the assay where five AIIQ (**4a**, **4b**, **4e**, **4g** and **4h**) and four VIIQ (**6g**, **6i**, **6k** and **6l**) have activity at least against PC3, pancreas cancer cell line and SKBR3 (breast carcinoma, overexpresses the HER2/*c-erb-2* gene). Noteworthy, that any tested compounds are toxic for normal human dermis fibroblasts (Table 5).

According with our previous report, phenolics, *i.e.*, 4-methoxyphenyl and 4-hydroxy-3-methoxyphenyl groups in the tetrahydroquinoline derivatives are responsible for anticancer properties.<sup>20</sup> Similarly, these functionalized aromatic fragments in the IIQ core provide biological activity against malignant tumour. As results, there have been discovered two AIIQ molecules, **4a** and **4h**, which showed potent cytotoxicity against four cancer cell lines, being non-toxic for normal cells. Moreover, comp. **4a** with 4-hydroxy-3-methoxyphenyl moiety derived from starting eugenol molecule, resulted in a less active anticancer agent than its analogue **4h** with 4-methoxyphenyl fragment that is resulting from anethole. This analogue displays the best activity of all series, reaching a 79 nM IC<sub>50</sub> value against HeLa cancer cell line. In addition, its great antitumor action was obtained for the other cell lines MCF-7, 310 nM; SKBR3, 1434 nM and PC3, 210 nM (Table 5). Interesting to note, one, an additional hydroxy group in C-5 aryl moiety of comp. **4a** worthens anticancer activity highlighting its MCF-7, PC3 and HeLa cells inhibition that is more effective that Adriamycin, a reference compound.

According to the *in silico* calculations (Table 3), comp. **4a** is less lipophilic molecule and possesses more TPSA values, *i.e.*, less capacity of cell membrane permeability than comp. **4h**. Moreover, both molecules showed the best drug score values of the series for these two compounds and their toxicity values for fibroblasts using MTT assay were chord with the risk properties



Table 5 Cytotoxic activity of the isoindoloquinolines 4a–m, 6a–l<sup>a</sup>

| IIQ | Cancer cells lines, cytotoxicity, IC <sub>50</sub> (μM) |                     |                     |                     |             |
|-----|---|---------------------|---------------------|---------------------|-------------|
|     | MCF-7   | SKBR3               | PC3                 | HeLa                | Fibroblasts |
| 4a  | 41.65 ± 1.02  | 76.67 ± 1.02        | <b>21.48 ± 1.05</b> | <b>24.90 ± 1.02</b> | >100        |
| 4b  | >100  | 92.24 ± 1.04        | 35.85 ± 1.03        | >100                | >100        |
| 4c  | >100  | >100                | >100                | >100                | >100        |
| 4d  | >100  | >100                | >100                | >100                | >100        |
| 4e  | >100  | >100                | 65.77 ± 1.25        | >100                | >100        |
| 4f  | >100  | >100                | >100                | >100                | >100        |
| 4g  | >100  | >100                | 47.47 ± 1.00        | <b>23.16 ± 1.02</b> | >100        |
| 4h  | <b>0.31 ± 1.03</b>                                      | <b>14.34 ± 1.03</b> | <b>0.21 ± 1.00</b>  | <b>0.079 ± 1.18</b> | >100        |
| 4i  | >100  | >100                | >100                | >100                | >100        |
| 4j  | >100  | >100                | >100                | >100                | >100        |
| 4k  | >100  | >100                | >100                | >100                | >100        |
| 4l  | >100  | >100                | >100                | >100                | >100        |
| 4m  | >100  | >100                | >100                | >100                | >100        |
| 6a  | >100  | >100                | >100                | >100                | >100        |
| 6b  | >100  | >100                | >100                | >100                | >100        |
| 6c  | >100  | >100                | >100                | >100                | >100        |
| 6d  | >100  | >100                | >100                | >100                | >100        |
| 6e  | >100  | >100                | >100                | >100                | >100        |
| 6f  | >100  | >100                | >100                | >100                | >100        |
| 6g  | >100  | >100                | 83.48 ± 1.00        | >100                | >100        |
| 6h  | >100  | >100                | >100                | >100                | >100        |
| 6i  | >100  | 96.48 ± 1.05        | 64.61 ± 1.00        | >100                | >100        |
| 6j  | >100  | 79.23 ± 1.00        | >100                | >100                | >100        |
| 6k  | >100  | 40.14 ± 1.00        | <b>23.89 ± 1.05</b> | >100                | >100        |
| 6l  | >100  | >100                | 63.47 ± 1.06        | >100                | >100        |
| Adr | 0.74 ± 0.05   | 1.65 ± 0.08         | 2.35 ± 0.4          | 3.62 ± 0.12         | 2.45 ± 0.37 |

<sup>a</sup> Marked in bold parameters indicated notable interesting anticancer activity.

obtained from DataWarrior platform (Table 4). Due to the importance of this new potential anticancer compounds, more studies are performed in advance to demonstrate more biological properties based on cancer diseases.

## Conclusion

In this research, we developed mild, efficient synthesis of novel vinyl isoindoloquinoline derivatives. In addition, we prepared and tested analogous aryl isoindoloquinoline molecules. Bio inspection of new molecules of both series against cancer cell lines in different studies confirmed that they are promising anticancer agents.

Among them, 5-(4-methoxyphenyl)isoindoloquinoline **4h** was shown to be more positioned as a new potential compound, which is under ongoing researches in our laboratories, displaying an excellent cytotoxic activity (79–1434 nM), even more in three cancer cell lines (MCF-7, PC3 and HeLa) than the reference compound, the Adriamycin.

## Experimental

### Chemistry

General information and NMR spectra are reported in ESI.†

Aryl isoindolo[2,1-*a*]quinolines **4a–m** were easily prepared via three-component reaction of respective anilines, isoeugenol/*trans*-anethole and *ortho*-phthalaldehydic acid following

described procedure.<sup>15</sup> Characterization data and spectra information for these series are reported in ESI.† The new vinyl derivatives are reported here and in the ESI.† All compounds were purified and characterized before biological tests.

**General procedure for the synthesis of the 5-vinyl-5-methyl-isoindolo[2,1-*a*]quinolin-11(5*H*)-ones 6a–l.** In a 100 mL Schlenk reactor 0.17 g (1.8 mmol) of aniline, 0.3 g (2.0 mmol) *o*-phthalaldehydic acid were dissolved in 20 mL of dry MeCN and N<sub>2</sub> atmosphere. After 5 min 0.026 g (0.18 mmol) of BF<sub>3</sub>·OEt<sub>2</sub> were added. Then, after 20 min 0.37 g (5.4 mmol) of isoprene were added and the reaction mixture was stirred at room temperature for over 8–12 h according to TLC. Afterwards, the reaction mass was treated with a Na<sub>2</sub>CO<sub>3</sub> saturated solution and extracted with ethyl acetate (2 × 20 mL). The organic layer was separated and dried with Na<sub>2</sub>SO<sub>4</sub>. The organic solvent was removed in vacuum to afford the respective 5-vinyl-5-methyl-isoindolo[2,1-*a*]quinolines **6a–l**, which were purified by column chromatography (silica gel, petroleum ether/EtOAc) to afford pure substances.

*Trans*-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-*a*]quinolin-11(5*H*)-one (**6a**). Were obtained 400 mg (1.45 mmol, 80%), white solid; mp: 182–183 °C; IR (KBr): 2947, 1682 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ (ppm) 1.43 (3H, d, *J* = 1.0 Hz, CH<sub>3</sub>), 2.24 (1H, dd, *J* = 12.4, 7.1 Hz, CH<sub>2</sub>), 2.36 (1H, dd, *J* = 12.4, 7.0 Hz, CH<sub>2</sub>), 4.86–4.66 (1H, m, CH), 4.97 (1H, dd, *J* = 10.0, 2.5 Hz, =CH<sub>2</sub>), 5.03 (1H, d, *J* = 2.6 Hz, =CH<sub>2</sub>), 5.97 (1H, ddd, *J* = 16.7, 9.9, 0.9 Hz, =CH), 7.07 (1H, td, *J* = 7.2, 1.7 Hz, 3-H), 7.21–7.12 (3H, m, 1-H and 2-H), 7.34 (1H,



td,  $J = 7.4, 1.6$  Hz, 8-H), 7.38 (1H, dd,  $J = 7.4, 1.8$  Hz, 9-H), 7.44–7.39 (1H, m, 7-H), 7.65 (1H, dd,  $J = 7.3, 1.6$  Hz, 10-H).  $^{13}\text{C}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 24.4, 40.6, 42.9, 54.1, 112.1, 122.2, 124.0, 124.3, 125.0, 125.7, 125.8, 129.2, 130.2, 132.2, 133.9, 137.7, 142.8, 147.4, 164.9; GC/MS (70 eV),  $t_{\text{R}} = 24.117$  min,  $m/z$  (%) 275 ( $\text{M}^+$ , 70), 260 (100), 232 (10); anal. calc. for  $\text{C}_{19}\text{H}_{17}\text{NO}$ : C, 82.88; H, 6.22; N, 5.09. Found: C, 82.80; H, 6.12; N, 5.19.

*Trans-3,5-dimethyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6b)*. Were obtained 410 mg (1.41 mmol, 82%), white solid; mp: 193–195 °C; IR (KBr): 2940, 1660  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.63–1.50 (4H, m,  $\text{CH}_3$  and  $\text{CH}_2$ ), 2.26 (3H, s, 3- $\text{CH}_3$ ), 2.38 (1H, d,  $J = 12.3$  Hz,  $\text{CH}_2$ ), 5.08 (1H, d,  $J = 12.2$  Hz, 6a-H), 5.16 (1H, d,  $J = 10.9$  Hz,  $=\text{CH}_2$ ), 5.30 (1H, d,  $J = 17.3$  Hz,  $=\text{CH}_2$ ), 5.93 (1H, dd,  $J = 17.0, 10.5$  Hz,  $=\text{CH}$ ), 7.03 (1H, s, 4-H), 7.09 (1H, d,  $J = 8.1$  Hz, 2-H), 7.57 (1H, d,  $J = 7.3$  Hz, 9-H), 7.69 (1H, d,  $J = 7.4$  Hz, 8-H), 7.75 (1H, d,  $J = 7.4$  Hz, 7-H), 7.80 (1H, d,  $J = 7.8$  Hz, 10-H), 8.27 (1H, d,  $J = 8.0$  Hz, 1-H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 20.7, 22.1, 27.2, 40.0, 40.5, 54.9, 112.5, 119.7, 122.8, 123.4, 127.4, 128.6, 128.9, 131.9, 132.3, 132.5, 132.8, 145.0, 146.7, 165.1; GC/MS (70 eV):  $t_{\text{R}} = 25.042$  min,  $m/z$  (%) 289 ( $\text{M}^+$ , 80), 274 (100), 246 (5); anal. calc. for  $\text{C}_{20}\text{H}_{19}\text{NO}$ : C, 83.01; H, 6.62; N, 4.84. Found: C, 82.95; H, 6.59; N, 4.75.

*Trans-3-ethyl-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6c)*. Were obtained 375 mg (1.23 mmol, 75%) white solid; mp: 187–188 °C; IR (KBr): 2809, 1666  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.56 (4H, br. s,  $\text{CH}_3$  and  $\text{CH}_2$ ), 1.78 (1H, s,  $\text{CH}_2$ ), 2.27 (3H, s,  $\text{CH}_3$ ), 2.43–2.28 (2H, m,  $\text{CH}_2$ ), 5.1 (1H, d,  $J = 10.1$ ,  $=\text{CH}_2$ ), 5.17 (1H, d,  $J = 10.2$  Hz,  $=\text{CH}_2$ ), 5.31 (1H, d,  $J = 17.4$  Hz, 6a-H), 5.96 (1H, br. d,  $J = 17.1$  Hz,  $=\text{CH}$ ), 7.04 (1H, s, 4-H), 7.10 (1H, d,  $J = 8.2$  Hz, 2-H), 7.59 (1H, br. s, 9-H), 7.69 (1H, br. s, 8-H), 7.75 (1H, s, 7-H), 7.82 (1H, s, 1-H), 8.29 (1H, d,  $J = 8.2$  Hz, 10-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 20.7, 27.2, 29.4, 40.0, 40.5, 54.9, 68.2, 112.5, 119.7, 122.8, 123.4, 127.4, 128.6, 128.9, 131.9, 132.3, 132.5, 132.8, 145.0, 146.7, 165.1; GC/MS (70 eV):  $t_{\text{R}} = 25.014$  min,  $m/z$  (%) 303 ( $\text{M}^+$ , 70), 274 (100), 246 (5); anal. calc. for  $\text{C}_{21}\text{H}_{21}\text{NO}$ : C, 83.13; H, 6.98; N, 4.62. Found: C, 83.08; H, 7.06; N, 4.55.

*Trans-5-methyl-3-methoxy-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6d)*. Were obtained 425 mg (1.39 mmol, 85%), white solid; mp: 178–179 °C; IR (KBr): 2947, 1680  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.56 (4H, m, 5- $\text{CH}_3$  and  $\text{CH}_2$ ), 2.44–2.29 (1H, m,  $\text{CH}_2$ ), 3.72 (3H, s,  $\text{CH}_3\text{O}$ ), 5.05 (1H, d,  $J = 12.5, 2.6$  Hz, 6a-H), 5.17 (1H,  $J = 10.1$ ,  $=\text{CH}_2$ ), 5.31 (1H, d,  $J = 17.4$ ,  $=\text{CH}_2$ ), 5.96 (1H, dd,  $J = 17.4, 10.6$  Hz,  $=\text{CH}$ ), 6.76 (1H, d,  $J = 3.0, 4\text{-H}$ ), 6.90 (1H, dd,  $J = 9.0, 3.0$  Hz, 2-H), 7.55 (1H, 't',  $J = 7.4$  Hz, 9-H), 7.67 (1H, 't',  $J = 7.4$  Hz, 8-H), 7.74 (1H, br. d,  $J = 7.6$  Hz, 7-H), 7.79 (1H, br. d,  $J = 7.5$  Hz, 10-H), 8.33 (1H, d,  $J = 9.0$  Hz, 1-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 27.6, 40.7, 40.9, 55.3, 55.6, 112.6, 113.1, 114.2, 121.4, 123.2, 123.7, 128.8, 129.0, 132.4, 132.6, 134.9, 145.3, 147.0, 155.9, 165.2; GC/MS (70 eV),  $t_{\text{R}} = 26.638$  min,  $m/z$  (%) 305 ( $\text{M}^+$ , 90), 290 (100), 262 (10); anal. calc. for  $\text{C}_{20}\text{H}_{19}\text{NO}_2$ : C, 78.66; H, 6.27; N, 4.59. Found: C, 78.59; H, 6.19; N, 4.67.

*Trans-3-fluor-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6e)*. Were obtained 390 mg (1.33 mmol, 78%), white solid; mp: 163–165 °C; IR (KBr): 2979, 1660  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.72–1.50 (4H, m, 5- $\text{CH}_3$  and  $\text{CH}_2$ ), 2.41 (1H, d,  $J = 12.4$  Hz,  $\text{CH}_2$ ), 5.10 (1H, d,  $J = 12.5$  Hz, 6a-H),

5.19 (1H, d,  $J = 10.1$  Hz,  $=\text{CH}_2$ ), 5.33 (1H, d,  $J = 17.1$  Hz,  $=\text{CH}_2$ ), 6.01–5.98 (1H, m,  $=\text{CH}$ ), 7.04 (1H, d,  $J = 8.3$  Hz, 4-H), 7.16 (1H, m, 2-H), 7.71 (1H, br. s, 9-H), 7.77 (1H, s, 8-H), 7.82 (2H, br. s, 7-H and 10-H), 8.41 (1H, br. s, 1-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 27.1, 39.9, 40.4, 54.9, 113.2, 113.7, 113.9, 114.8, 121.6, 122.8, 123.5, 128.7, 131.4, 131.6, 132.5, 135.6, 144.8, 146.0, 165.2; GC/MS (70 eV),  $t_{\text{R}} = 24.055$  min,  $m/z$  (%) 293 ( $\text{M}^+$ , 80), 278 (100), 250 (5); anal. calc. for  $\text{C}_{19}\text{H}_{16}\text{FNO}$ : C, 77.80; H, 5.50; F, 6.48, N, 4.77. Found: C, 77.86; H, 5.42; N, 4.66.

*Trans-3-chloro-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6f)*. Were obtained 400 mg (1.29 mmol, 80%), white solid; mp: 225–226 °C; IR (KBr): 2948, 1682  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.44 (3H, d,  $J = 1.1$  Hz,  $\text{CH}_3$ ), 2.26 (1H, dd,  $J = 12.4, 7.1$  Hz,  $\text{CH}_2$ ), 2.37 (1H, dd,  $J = 12.5, 7.0$  Hz,  $\text{CH}_2$ ), 4.77–4.53 (1H, m, 6a-H), 4.96 (1H, dd,  $J = 10.0, 2.5$  Hz,  $=\text{CH}_2$ ), 5.03 (1H, d,  $J = 2.6$  Hz,  $=\text{CH}_2$ ), 5.99 (1H, ddq,  $J = 16.8, 10.1, 1.1$  Hz,  $=\text{CH}$ ), 7.16 (1H, d,  $J = 1.1$  Hz, 4-H), 7.20 (2H, d,  $J = 1.1$  Hz, 1-H and 2-H), 7.39–7.33 (2H, m, 8-H and 9-H), 7.41 (1H, ddd,  $J = 6.9, 2.1, 0.7$  Hz, 7-H), 7.65 (1H, dd,  $J = 7.1, 1.8$  Hz, 10-H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 24.1, 39.6, 40.1, 42.5, 53.6, 111.5, 124.8, 125.1, 125.3, 125.5, 129.5, 129.7, 129.8, 131.3, 136.1, 136.6, 142.0, 146.8, 163.7; GC/MS (70 eV),  $t_{\text{R}} = 25.855$  min,  $m/z$  (%) 309 ( $\text{M}^+$ , 80), 294 (100), 259 (20); anal. calc. for  $\text{C}_{19}\text{H}_{16}\text{ClNO}$ : C, 73.66; H, 5.21; Cl, 11.44; N, 4.52. Found: C, 73.71; H, 5.29; N, 4.59.

*Trans-1-fluor-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6g)*. Were obtained 320 mg (1.1 mmol, 64%); white solid; mp: 150–152 °C;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.33 (3H, d,  $J = 1.1$  Hz,  $\text{CH}_3$ ), 2.34 (1H, dd,  $J = 12.5, 7.0$  Hz,  $\text{CH}_2$ ), 2.43 (1H, dd,  $J = 12.5, 7.0$  Hz,  $\text{CH}_2$ ), 4.91 (1H, t,  $J = 6.9$  Hz, 6a-H), 4.96 (1H, dd,  $J = 10.0, 2.5$  Hz,  $=\text{CH}_2$ ), 5.03 (1H, dd,  $J = 16.9, 2.4$  Hz,  $=\text{CH}_2$ ), 6.05–5.93 (1H, m,  $=\text{CH}$ ), 6.94 (1H, td,  $J = 7.8, 1.5$  Hz, 2-H), 7.02 (1H, dd,  $J = 7.5, 1.6$  Hz, 4-H), 7.15 (1H, td,  $J = 7.5, 5.0$  Hz, 3H), 7.40–7.31 (2H, m, 8-H and 9-H), 7.45–7.40 (1H, m, 7-H), 7.70–7.60 (1H, m, 10-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 25.1, 40.0, 41.0, 42.4, 58.7, 112.0, 116.6, 122.1, 125.0, 125.7, 126.5, 130.2, 132.0, 134.8, 141.9, 146.1, 156.3, 158.3, 164.4; GC/MS (70 eV),  $t_{\text{R}} = 10.9$  min,  $m/z$  (%) 293 ( $\text{M}^+$ , 100), 278 (80), 250 (30); anal. calc. for  $\text{C}_{19}\text{H}_{16}\text{FNO}$ : C, 77.80; H, 5.50; F, 6.48, N, 4.77. Found: C, 77.85; H, 5.45; N, 4.85.

*Trans-1-ciano-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6h)*. Were obtained 340 mg (1.13 mmol, 68%), white solid; mp: 198–199 °C; IR (KBr): 2950, 1660  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.34 (3H, d,  $J = 1.1$  Hz,  $\text{CH}_3$ ), 2.34 (1H, dd,  $J = 12.5, 7.0$  Hz,  $\text{CH}_2$ ), 2.43 (1H, dd,  $J = 12.5, 7.0$  Hz,  $\text{CH}_2$ ), 4.94–4.90 (1H, m, CH), 4.97 (1H, dd,  $J = 10.0, 2.5$  Hz,  $=\text{CH}_2$ ), 5.04 (1H, dd,  $J = 16.9, 2.4$  Hz,  $=\text{CH}_2$ ), 5.99 (1H, ddd,  $J = 16.7, 9.9, 0.9$  Hz, CH), 7.35–7.27 (2H, m, 3-H and 4-H), 7.44–7.35 (3H, m, 7-H, 8-H and 9-H), 7.48 (1H, dd,  $J = 7.0, 2.0$  Hz, 2-H), 7.68–7.62 (1H, m, 10-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 25.0, 40.0, 42.1, 42.4, 57.7, 109.6, 112.2, 115.8, 125.2, 125.7, 125.9, 129.1, 130.2, 131.5, 134.0, 135.9, 138.6, 141.6, 145.2, 164.4; GC/MS (70 eV),  $t_{\text{R}} = 25.1$  min,  $m/z$  (%) 300 ( $\text{M}^+$ , 100), 285 (80), 254 (30); anal. calc. for  $\text{C}_{20}\text{H}_{16}\text{NO}$ : C, 79.98; H, 5.37; N, 9.33. Found: C, 80.01; H, 5.42; N, 9.29.

*Trans-1,4-dimethoxy-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6i)*. Were obtained 450 mg (1.34 mmol, 90%), white solid; mp: 180–181 °C; IR (KBr): 2948, 1660  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR





(400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.27 (1H, 't',  $J = 12.6$  Hz,  $\text{CH}_2$ ), 1.56 (3H, s,  $\text{CH}_3$ ), 2.28 (1H, dd,  $J = 13.2, 2.6$  Hz,  $\text{CH}_2$ ), 3.69 (3H, s,  $\text{OCH}_3$ ), 3.77 (3H, s,  $\text{OCH}_3$ ), 5.06–4.81 (3H, m,  $=\text{CH}_2$ , and 6a-H), 6.07 (1H, dd,  $J = 17.5, 10.6$  Hz,  $=\text{CH}$ ), 6.89 (1H, d,  $J = 9.1$  Hz, 3-H), 7.01 (1H, d,  $J = 9.1$  Hz, 2-H), 7.55 (1H, t,  $J = 7.0$  Hz, 9-H), 7.65 (1H, m, 8-H), 7.70 (1H, d,  $J = 7.5$  Hz, 7-H), 7.77 (1H, d,  $J = 7.5$  Hz, 10-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 25.2, 41.1, 47.3, 55.0, 55.9, 56.1, 110.1, 110.2, 111.8, 122.9, 123.6, 124.9, 125.3, 128.4, 131.8, 131.9, 146.4, 147.1, 147.3, 151.6, 163.4; GC/MS (70 eV),  $t_R = 26.678$  min,  $m/z$  (%) 335 ( $\text{M}^+$ , 100), 320 (30), 306 (10), 361 (25); anal. calc. for  $\text{C}_{21}\text{H}_{21}\text{NO}_3$ : C, 75.20; H, 6.31; N, 4.18. Found: C, 75.29; H, 6.40; N, 4.08.

*Trans-5-hydroxy-5-vinyl-6,6a-dihydro-[1,3]dioxolo[4,5-g]isoindolo[2,1-a]quinolin-11(5H)-one (6j)*. Were obtained 390 mg (1.34 mmol, 78%), white solid; mp: 150–152 °C; IR (KBr): 3435, 2890, 1682  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.54 (4H, d,  $J = 8.5$  Hz,  $\text{CH}_3$  and  $\text{CH}_2$ ), 2.41–2.23 (1H, m,  $\text{CH}_2$ ), 5.02 (1H, d,  $J = 11.9$  Hz, 6a-H), 5.15 (1H, d,  $J = 10.6$  Hz,  $=\text{CH}_2$ ), 5.29 (1H, d,  $J = 17.3$  Hz,  $=\text{CH}_2$ ), 5.91 (1H, dd,  $J = 17.3, 10.5$  Hz,  $=\text{CH}_2$ ), 6.65 (1H, d,  $J = 2.9$  Hz, 4-H), 6.71 (1H, dd,  $J = 8.8, 2.8$  Hz, 2-H), 7.54 (1H, d,  $J = 7.5$  Hz, 9-H), 7.65 (1H, d,  $J = 7.4$  Hz, 8-H), 7.71 (1H, d,  $J = 7.2$  Hz, 7-H), 7.77 (1H, d,  $J = 7.3$  Hz, 10-H), 8.20 (1H, d,  $J = 8.7$  Hz, 1-H), 9.28 (1H, s, OH);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 18.0, 27.6, 31.1, 40.6, 41.2, 55.3, 112.8, 115.2, 121.5, 123.6, 127.4, 128.9, 132.4, 132.6, 134.8, 145.2, 147.2, 154.1, 165.1 GC/MS (70 eV),  $t_R = 35.56$  min,  $m/z$  (%) 291 ( $\text{M}^+$ , 100), 276 (55), 246 (90); anal. calc. for  $\text{C}_{19}\text{H}_{17}\text{NO}_2$ : C, 78.33; H, 5.88; N, 4.81. Found: C, 78.44; H, 5.97; N, 4.75.

*Trans-1,3-dimethoxy-5-methyl-5-vinyl-6,6a-dihydroisoindolo[2,1-a]quinolin-11(5H)-one (6k)*. Were obtained 400 mg (1.19 mmol, 80%), white solid; mp: 160–161 °C; IR (KBr): 2948, 1660  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.35 (3H, d,  $J = 0.9$  Hz,  $\text{CH}_3$ ), 2.35 (1H, dd,  $J = 12.4, 7.1$  Hz,  $\text{CH}_2$ ), 2.43 (1H, dd,  $J = 12.4, 7.0$  Hz,  $\text{CH}_2$ ), 3.71 (3H, s,  $\text{OCH}_3$ ), 3.78 (3H, s,  $\text{OCH}_3$ ), 4.92 (1H, t,  $J = 6.9$  Hz, 6a-H), 4.97 (1H, dd,  $J = 10.1, 2.4$  Hz,  $=\text{CH}_2$ ), 5.04 (1H, dd,  $J = 16.9, 2.4$  Hz,  $=\text{CH}_2$ ), 6.00 (1H, dd,  $J = 16.8, 10.1, 1.0$  Hz,  $=\text{CH}$ ), 6.54 (1H, d,  $J = 1.4$  Hz, 4-H), 6.84 (1H, d,  $J = 1.4$  Hz, 2-H), 7.37 (2H, dd,  $J = 5.4, 3.6$  Hz, 8H and 9H), 7.43–7.39 (1H, m, 7-H), 7.75–7.63 (1H, m, 10H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 25.1, 39.4, 42.4, 42.7, 55.7, 57.3, 58.5, 100.1, 107.1, 111.9, 122.5, 125.6, 125.7, 130.2, 131.9, 135.8, 141.3, 145.0, 155.1, 157.0, 164.5; GC/MS (70 eV),  $t_R = 25.138$  min,  $m/z$  (%) 335 ( $\text{M}^+$ , 70), 320 (100), 288 (50), 256 (50); anal. calc. for  $\text{C}_{21}\text{H}_{21}\text{NO}_3$ : C, 75.20; H, 6.31; N, 4.18. Found: C, 75.27; H, 6.38; N, 4.27.

*Trans-5-methyl-5-vinyl-6,6a-dihydro-[1,3]dioxolo[4,5-g]isoindolo[2,1-a]quinolin-11(5H)-one (6l)*. Were obtained 350 mg (1.10 mmol, 70%), white solid; mp: 193–194 °C; IR (KBr): 2890, 1682  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 1.55–1.47 (4H, m,  $\text{CH}_3$  and  $\text{CH}_2$ ), 2.40–2.34 (1H, dd,  $J = 13.4, 2.28$  Hz,  $\text{CH}_2$ ), 5.02 (1H, d,  $J = 11.0$  Hz, 6a-H), 5.13 (1H, d,  $J = 10.6$  Hz,  $=\text{CH}_2$ ), 5.26 (1H, d,  $J = 17.5$  Hz,  $=\text{CH}_2$ ), 5.88 (2H, dd,  $J = 17.3, 10.5$  Hz,  $=\text{CH}$ ), 6.02–5.96 (2H, m,  $\text{CH}'_2$ ), 6.72 (1H, s, 4-H), 7.54 (1H, t,  $J = 7.2$  Hz, 9-H), 7.67 (1H, t,  $J = 7.1$  Hz, 8-H), 7.73 (1H, d,  $J = 7.3$  Hz, 7-H), 7.77 (1H, d,  $J = 7.3$  Hz, 10-H), 7.94 (1H, s, 1-H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ )  $\delta$  (ppm) 27.7, 40.3, 40.5, 55.1, 100.8, 101.2, 107.9, 112.6, 122.7, 123.3, 126.2, 128.6, 128.9, 131.8, 132.3, 143.6, 144.7, 145.6, 146.7, 165.0; GC/MS (70 eV),  $t_R = 28.88$  min,  $m/z$  (%) 319 ( $\text{M}^+$ , 100), 304 (55), 274

(55), 246 (90); anal. calc. for  $\text{C}_{20}\text{H}_{17}\text{NO}_3$ : C, 75.22; H, 5.37; N, 4.39. Found: C, 75.30; H, 5.43; N, 4.46.

## Biology

Human tumour cell lines and culture media: PC3 (prostate carcinoma), HeLa (cervical epithelial carcinoma) were grown in RPMI 1640 medium (Invitrogen) supplemented with 10% heat inactivated fetal bovine serum (FBS), 1% of L-glutamine, 1% streptomycin, 100 units per mL penicillin (all obtained from Sigma Aldrich USA). MCF-7 (breast carcinoma, no overexpresses the HER2/c-erb-2 gene), SK-BR-3 (breast carcinoma, overexpresses the HER2/c-erb-2 gene) and primary culture of normal human dermis fibroblast used as control cells were grown in DMEM medium (GIBCO). Cells were grown in a humidified incubator with 5%  $\text{CO}_2$  and 95% air at 37 °C until they reach the exponential growth phase. For treatments exponentially growing cells were collected, counted, re-suspended in fresh culture medium, and incubated in 96 sterile well plates.

**Cytotoxicity evaluation by MTT assay.** Cell viability was assessed using the MTT assay, which is based on the ability of viable cells to metabolically reduce a yellow tetrazolium salt (MTT; Sigma) to a purple formazan product. This reaction takes place when mitochondrial reductases are active. Cells were grown in 96-well plates (5 × 103 cells per well) for 24 hours. Cultures were carried out at 37 °C in a humidified atmosphere with 5%  $\text{CO}_2$  cells were incubated with the synthetic products or chemotherapeutic drugs in 100  $\mu\text{L}$  of complete culture medium containing 0, 1, 5, 10, 25, 100  $\mu\text{g mL}^{-1}$  concentrations each one compounds for 72 hours. After incubation, the medium was removed, and the cells were treated with 100  $\mu\text{L}$  0.4  $\text{mg mL}^{-1}$  MTT for 3 h at 37 °C. Subsequently, the MTT is discarded and the reaction is revealed with 100  $\mu\text{L}$  DMSO. The solubilized formazan product was quantified with the help of a microtiter plate reader TECAN-sunrise at 570 nm. Adriamycin was used as a positive control in the assay. In all cases the compounds were dissolved in DMSO, at the final concentration in the culture medium was lower than 1%, a concentration that had neither cytotoxic effect nor caused any interference with the colorimetric detection method.<sup>46</sup>

**Selectivity index (SI).** The selectivity index was calculated as the  $\text{IC}_{50}$  (control cells)/ $\text{IC}_{50}$  (tumoral cell line) ratio. A selectivity index >1 indicates that the cytotoxicity on tumoral cells surpassed that on healthy non-tumoral cells.<sup>47</sup>

**Statistical analysis.** All experiments were performed at least three times. The results are expressed as mean  $\pm$  SD. Anova test were performed. Only *post hoc* Dunnet test  $p < 0.01$  was considered to be statistically significant. The dose-response curves were plotted with the OriginPro ver.8.0 programs, and 50% growth inhibitory concentrations ( $\text{IC}_{50}$ ) of synthetic products or chemotherapeutic drugs were determined by a non-linear regression of individual experiments calculated through computation with GraphPad prism v.5.02 software program (Intuitive Software for Science, San Diego, CA, USA).



## Conflicts of interest

The authors declare that they have no competing interests.

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## Notes and references

- Z. Sui, J. Altom, V. Nguyen, J. Fernandez, J. Bernstein, J. J. Hiliard, J. F. Barrett, B. L. Podlogar and K. A. Ohemeng, *Bioorg. Med. Chem.*, 1998, **6**, 735–742.
- T. Lübbers, P. Angehrn, A. Gmünder and S. Herzig, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 4708–4714.
- M. Garavis, B. López-Méndez, A. Somoza, J. Oyarzabal, C. Dalvit, A. Villasante, R. Campos-Olivas and C. González, *ACS Chem. Biol.*, 2014, **9**, 1559–1566.
- M. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, *J. Am. Chem. Soc.*, 1966, **88**, 3888–3890.
- P. Pigeon and B. Decroix, *Synth. Commun.*, 1998, **28**(13), 2507–2516.
- P. Pigeon, M. Othman, P. Netchitaïlo and B. Decroix, *J. Heterocycl. Chem.*, 1999, **36**, 691–695.
- J. Epsztajn, A. Józwiak, P. Kołuda, I. Sadokierska and I. Wilkowska, *Tetrahedron*, 2000, **56**, 4837–4844.
- V. Mamane and Y. Fort, *Tetrahedron Lett.*, 2006, **47**, 2337–2340.
- Y. Zhou, L. Qian and W. Zhang, *Synlett*, 2009, **5**, 0843–0847.
- Ch. Reddy, S. Babu and R. Padmavathi, *ChemistrySelect*, 2016, **1**, 2952–2959.
- E. V. Boltukhina, F. I. Zubkov and A. V. Varlamov, *Chem. Heterocycl. Compd.*, 2006, **42**, 971–1001.
- F. I. Zubkov, E. V. Boltukhina, K. F. Turchin, R. S. Borisova and A. V. Varlamov, *Tetrahedron*, 2005, **61**, 4099–4113.
- (a) W. Zhang, A. Zheng, Z. Liu, L. Yang and Z. Liu, *Tetrahedron Lett.*, 2005, **46**, 5691–5694; (b) Z. Al-Jaroudi, P. P. Mohapatra, T. S. Cameron and A. Jha, *Synthesis*, 2016, **48**, 4477–4488; (c) M. O'Brien, R. Weagle, D. Corkum, M. Kuanar, P. Mohapatra and A. Jha, *Mol. Diversity*, 2017, **21**, 455–462.
- S. Khadem, K. Udachin, G. Enright, M. Prakesch and P. Arya, *Tetrahedron Lett.*, 2009, **50**, 6661–6664.
- D. R. Merchán-Arenas and V. V. Kouznetsov, *J. Org. Chem.*, 2014, **79**, 5327–5333.
- O. Ghashghaei, C. Masdeu, C. Alonso, F. Palacios and R. Lavilla, *Drug Discov. Today Technol.*, 2018, **29**, 71–79.
- U. Hsing-Janlgi, C. Ericn, A. Browne and E. Sewy, *Can. J. Chem.*, 1988, **66**, 2345–2347.
- P. J. Gregoire, J. M. Mellor and G. D. Merriman, *Tetrahedron*, 1995, **51**, 6133–6144.
- A. Katritzky and M. F. Gordeev, *J. Org. Chem.*, 1993, **58**, 4049–4053.
- A. Muñoz, F. Sojo, D. R. Merchán Arenas, V. V. Kouznetsov and F. Arvelo, *Chem.-Biol. Interact.*, 2011, **189**, 215–221.
- L. Chen, L. Feng, Y. He, M. Huang, Y. Liu, H. Yun and M. Zhuo, WO 2012/001020A1, 2012.
- V. V. Kouznetsov, *Tetrahedron*, 2009, **65**, 2721–2750.
- S. Kobayashi, H. Ishitani and S. Nagayama, *Synthesis*, 1995, **09**, 1195–1202.
- H.-Y. Noh, S.-W. Kim, S. I. Paek, H.-J. Ha, H. Yun and W. K. Lee, *Tetrahedron*, 2005, **61**, 9281–9290.
- M. E. Squillacote and F. Liang, *J. Org. Chem.*, 2005, **70**, 6564–6573.
- C.-M. Wang, Z.-H. Liu, Y.-K. Chen, J.-M. Han, Y.-L. Chen, M.-M. Miao and H. Cao, *Comput. Theor. Chem.*, 2013, **1017**, 174–181.
- J. Hernández Muñoz, *Synlett*, 2017, **23**, 1101–1102.
- E. Ohgaki, J. Motoyoshiya, S. Narita, T. Kakurai, S. Hayashi and K. Hirakawa, *J. Chem. Soc., Perkin Trans. 1*, 1990, **1**, 3109–3112.
- V. V. Kouznetsov, A. R. Romero Bohórquez, L. Astudillo Saavedra and R. Fierro Medina, *Mol. Divers.*, 2006, **10**, 29–37.
- L. S. Povarov, *Russ. Chem. Rev.*, 1967, **36**, 656.
- D. Bello, R. Ramon and R. Lavilla, *Curr. Org. Chem.*, 2010, **14**, 332–356.
- I. Muthukrishnan, V. Sridharan and J. C. Menéndez, *Chem. Rev.*, 2019, **119**(8), 5057–5191.
- S. Hermitage, J. A. K. Howard, D. Jay, R. G. Pritchard, M. R. Probert and A. Whiting, *Org. Biomol. Chem.*, 2004, **2**, 2451–2460.
- R. Marques, J. O. S. Varejão, A. Sousa, S. Castañeda and S. A. Fernandes, *Org. Biomol. Chem.*, 2019, **17**, 2913–2922.
- A. Jha, T.-Y. Chou, Z. Jaroudi, B. D. Ellis and T. S. Cameron, *Beilstein J. Org. Chem.*, 2014, **10**, 848–857.
- L. R. Domingo, M. J. Aurell, J. A. Sáez and S. M. Mekelleche, *RSC Adv.*, 2014, **4**, 25268–25278.
- J. I. Garcia, J. A. Mayoral and L. Salvatella, *Eur. J. Org. Chem.*, 2005, **1**, 85–90.
- <http://www.molinspiration.com/services>.
- <http://www.organic-chemistry.org/prog/peo/>.
- C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3–25.
- P. Ertl, B. Rohde and P. Selzer, *J. Med. Chem.*, 2000, **43**, 3714–3717.
- <https://www.cdc.gov/cancer/survivors/patients/side-effects-of-treatment.htm>, accessed 4.16.2020.
- T. Sander, J. Freyss, M. Von Korff and C. Rufener, *J. Chem. Inf. Model.*, 2015, **55**, 460–473.
- S. Jain, V. Chandra, P. Kumar Jain, K. Pathak, D. Pathak and A. Vaidya, *Arabian J. Chem.*, 2016, 4920–4946.
- R. Musiol, *Expert Opin. Drug Discovery*, 2017, **12**, 583–597.
- T. Mosmann, *J. Immunol. Methods*, 1983, **65**, 55–63.
- D. Callacondo, A. Quispe, S. Lindo and A. Vaisberg, *Rev. Peru. Med. Exp. Salud Publica*, 2008, **25**, 380–385.

