Bioorganic & Medicinal Chemistry 17 (2009) 1849–1856

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and cytotoxic activity of new azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-ones

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ARTICLE INFO

Article history:
Received 31 October 2008
Revised 21 January 2009
Accepted 24 January 2009
Available online 31 January 2009

Keywords:
DLP
Radical oxidative
Azepinopyrroloisoquinolinones
Cytotoxic activity

ABSTRACT

A series of azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-ones (**3a–f**), that were conformationally restricted analogs of lead compound **2,** were designed as potential cytotoxic compounds and synthesized using a radical oxidative aromatic substitution reaction as the key step. Compounds **3a–f** were tested on five tumor cell lines to determine the conformational requirements for biological activity of compound **2**. The results show that conformational restrictions on compound **2**, generating the derivatives **3a–f**, do not appreciably reduce the cytotoxic activity of **2**, although compound **3d** (R = Br) showed good activity against U-251 cells. Preliminary structure–activity relationship studies with these compounds revealed the importance of halogens bonded to the isoquinoline moiety. Additionally, derivatives **3f** (R = NO_2) and **3b** (R = F) were cytotoxic to PC-3 and K-562 cells. However, none of the azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolinones inhibited the enzymatic activity of CDK1/cyclin B, CDK5/p25, or GSK-3.

1. Introduction

Cancer is a leading cause of death worldwide, and the total number of cancer deaths globally is projected to increase by 45% from 2007 to 2030 (from 7.9 to 11.5 million), partly because the world population is increasing and aging. During the same period, newly diagnosed cases of cancer are also estimated to increase by 37%, from 11.3 million in 2007 to 15.5 million in 2030. In most developed countries, cancer is the second leading cause of death after cardiovascular disease, and epidemiological evidence points to emergence of this trend in the less-developed world.[1] Despite questions on the future of synthetic small molecules as drugs, and predictions of their downfall, the worldwide drug market is still dominated by small molecules. At present, more than 80% of clinical drug candidate development by the top 20 pharmaceutical firms is based on small molecules,[2] and an analysis of developmental trends through 2007 showed that about half are chemically synthesized.[3] Pharmacophore-based methods have shown promising results in efforts to find pharmacologically active compounds relevant to a broad range of therapeutic areas. Drug design begins with comparisons of active and inactive compounds. This suggests how structural variations can change the biological activity of a molecule and permits development of a hypothesis concerning interactions between the molecule and its receptor. This approach is known as molecular mimicry and is based on determining the structural elements necessary for activity of the lead compound.[4]

1.1. Design

As part of our ongoing interest in the development of novel heterocyclic compounds inhibiting the growth of cancer cells, we have employed a structure–activity relationship analysis to generate new active leads from compound **1**.[5] Our previous studies suggested that a pyrroloazepine group and two aromatic groups, as found in compound **2**, are required for cytotoxic activity (Fig. 1).[6] As the mechanism underlying the antiproliferative activity of compound **2** is still unknown, further exploration of the pyrroloazepinone pharmacophore seemed advisable. Introduction of rigidity into bioactive compounds has proven very useful in studying conformational requirements for biological activity. One of the most efficient strategies for limiting the flexibility of compounds is the incorporation of conformationally restricted rings.[7] We therefore utilized this approach in the development of new cytotoxic derivatives from compound **2**. The conformation represented by structure **2** could be constrained by elimination of the 1-phenyl moiety and by attachment of the pyrrole nitrogen to the 2-phenyl ring of compound **2** through an ethylene linker, yielding the azepinopyrroloisoquinolinones **3**. These compounds have part of the pharmacophore found in the 7,12-dihydroindolo[3,2-*d*]benzazepin-6(5*H*)-ones (paullones) (**4**), which have been shown to restrict cellular proliferation by competitive inhibition of ATP binding.[8]

Here, we report the synthesis and cytotoxic activity of the 3-*R*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-one derivatives **3a**–**f**, which lack the 1-phenyl substituent and in which the 2-phenyl group is part of a bicyclic moiety that binds to the pyrroleazepine scaffold. Because compounds **3a-f** are closer analogs of

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doi:10.1016/j.bmc.2009.01.056

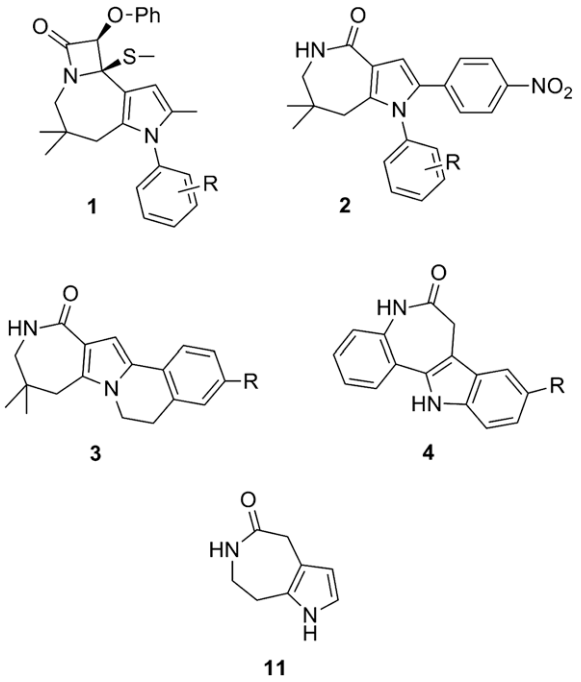


Figure 1.

paullones than are the compound **2** derivatives, we hypothesized that they would exhibit more potent antiproliferative activity. We also report on the CDK1/cyclin B, CDK5/p25, and GSK-3 inhibitory activities of these compounds. As intermediate compounds **9a–f** are chemically connected to the final products, suggesting that they may have pharmacological properties in common, the cytotoxic activities of the intermediates were also evaluated.

1.2. Chemistry

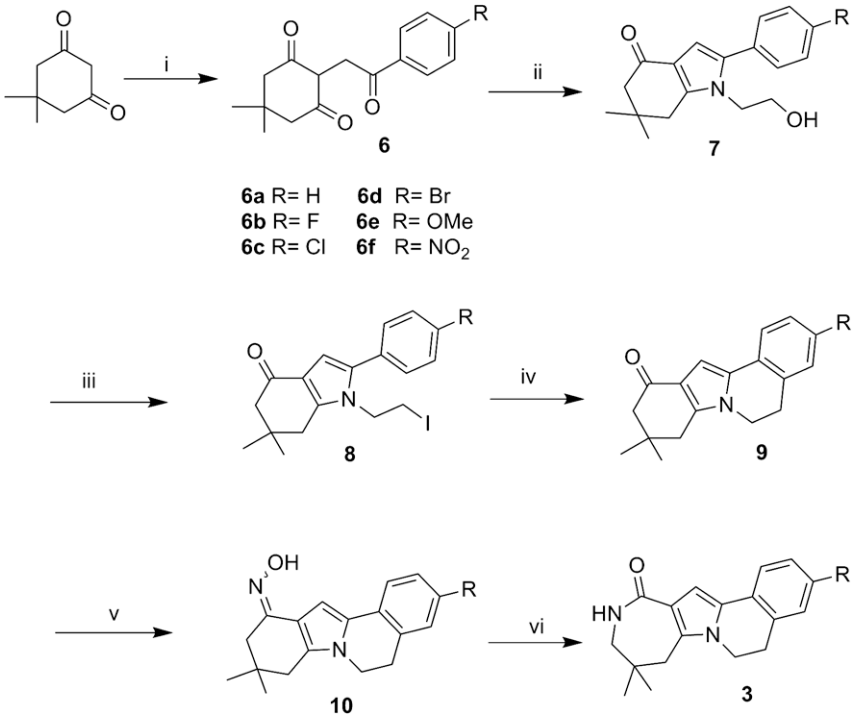
Construction of intermediates **9a–f** was the key step in obtaining compounds **3a–f**. The bond between the C-2-phenyl group and the *N*-ethyliodide moiety can be formed via C-2 radical oxidative aromatic substitution using lauroyl peroxide (DLP) as initiator and oxidant. The organic peroxides have been shown to be excellent reactants in radical aromatic substitution because they can act as both initiators and oxidants. Using peroxides avoids the toxicity of heavy metals present as organotin derivatives, and prevents premature reduction of the intermediate radical.[9] We started with synthesis of the 1,4-dicarbonyl compounds **6a–f**, as described previously[10] (Scheme 1). These compounds were condensed with 2-aminoethanol to generate the pyrrole derivatives **7a**–**f**. These derivatives were reacted with triphenylphosphine and iodine to obtain the desired iodo compounds **8a–f** in moderate overall yields. Cyclization of these iodides under the conditions described above gave excellent yields of the tetracyclic compounds **9a–f** with the *p*-fluoro compound **9b** being generated most efficiently.[11] Compounds **9a–f** were quantitatively oximated with hydroxylamine hydrochloride in ethanol, in the presence of sodium carbonate, to yield the oximes **10a–f**. Finally, regioselective Beckmann rearrangement of the *syn*- and *anti*-oximes **10a–f** yielded the pyrroloazepinoisoquinolines **3a–f** as the sole products. The group that migrates in the Beckmann rearrangement is thought to be that *anti* to the hydroxyl group. However, under the reaction conditions used, we have verified that the *anti* oximes of indolones undergo isomerization to the *syn* oximes prior to migration.[6,12]

1.3. Biological activity

We evaluated the abilities of compounds **3a–f** and **9a–f** to inhibit the growth of PC-3 prostate cancer, U-251 central nervous system cancer, K-562 leukemia, HCT-15 colon cancer, and MFC-7 breast cancer cells (Tables 1 and 2). Compound **3d** (R = Br) showed good inhibition of growth of all five cancer cell lines, with highest activity against U-251 cells (IC_{50} = 18.14 ± 1.02 μM). The chloro derivative **3c** showed moderate cytotoxicity against HCT-15 colon cancer cells, with an IC_{50} value in the micromolar range (IC_{50} = 39.65 ± 1.0 μM). Compound **3b** (R = F) was cytotoxic to K-562 (IC_{50} = 33.74 ± 3.8 μM) and MCF-7 (IC_{50} = 54.51 ± 3.4 μM) cancer cells, suggesting that derivatization of the 3-*R*-isoquinoline group with halogens yielded the best anticancer compounds. Interestingly, the *N*-(4-halogen)-pyrroloazepinone derivatives **2a** (R = Cl) and **2b** (R = Br) showed similar cytotoxic tendencies against the five cancer cell lines.[6] In addition, the nitro-derivative **3f** ($R = NO_2$) moderately inhibited the growth of three cancer cell lines, PC-3 (IC_{50} = 25.79 ± 3.2 μM), HCT-15 (IC_{50} = 29.50 ± 3.2 μM), and MCF-7 (IC_{50} = 43.14 ± 4.4 μM). By contrast, compounds **3a** (R = H) and **3e** (R = OMe) did not inhibit proliferation of any of the five cancer cell lines tested.

The activities of kenpaullone **4a** and alsterpaullone **4b** were also tested against the five cancer cell lines, and the cytotoxic activities of these compounds were compared with those of compounds **3a**–**f**. The most active inhibitors of proliferation of PC-3 prostate cells were compounds **3d** (R = Br; IC_{50} = 33.56 ± 2.8 μM) and **3f** ($R = NO_2$; IC_{50} = 25.79 ± 3.2 μM). Interestingly, kenpaullone **4a** had no activity against the PC-3 line, whereas alsterpaullone **4b** was over 20-fold more potent than were compounds **3d** and **3f**. Similarly, the bromine derivative compound **3d** was cytotoxic to U-251 central nervous system cancer cells and **4a** kenpaullone was inactive, whereas alsterpaullone **4b** was over 25-fold more potent than was compound **3d** (IC_{50} = 0.68 ± 0.02 μM vs IC_{50} = 18.14 ± 1.02 μM). Compounds **3b** (IC_{50} = 33.74 ± 3.8 μM) and **3d** (IC_{50} = 36.12 ± 3.6 μM) were more active than was compound **4a** against the K-562 leukemia cell line, whereas alsterpaullone **4b** was over 20-fold more potent than were either compounds **3b** or **3d** (IC_{50} = 1.58 ± 0.3 μM). The antiproliferative activities of compounds **3c**, **3d,** and **3f** against the HCT-15 colon cancer cell line (IC_{50} values in the low micromolar range) were similar to that of compound **4a** (IC_{50} = 23.86 ± 2.9 μM), whereas compound **4b** was over 50-fold more potent (IC_{50} = 0.62 ± 0.05 μM). The halogen derivatives compounds **3b** (IC_{50} = 54.51 ± 3.4 μM) and **3d** (IC_{50} = 43.15 ± 3.6 μM), and the nitro derivative compound **3f** (IC_{50} = 43.14 ± 4.4), were effective in killing breast cancer cells (MFC-7) with moderate cytotoxicity (IC_{50} values in the micromolar range), and compound **4a** was once again inactive, whereas compound **4b** was over 35-fold more potent than were compounds **3b**, **3d,** and **3f** (IC_{50} = 1.24 ± 0.1 μM). Unfortunately, compounds **3b**, **3d,** and **3f** did not inhibit the protein kinase activities of CDK1/cyclin B, CDK5/p25, or GSK-3 CDK1 (IC_{50} > 10 μM),[13] suggesting that another molecular mechanism is involved in the cytotoxic behavior of compounds **3a–f**.

Of the group of tetrahydroindolo[2,1-*a*]isoquinolin-11(8*H*)-ones **9a–f**, the most active were compound **9c** (R = Cl) against K-562 cells (IC_{50} = 10.21 ± 0.1 μM), followed by compound **9f** ($R = NO_2$) against HCT-15 cells (IC_{50} = 16.69 ± 1.4 μM). Although these activities are moderate, the potencies of these compounds were high compared to that of kenpaullone **4a**, which had an IC_{50} against K-562 cells of over 100 μM and an IC_{50} against HTC-15 cells of 23.86 ± 2.9 μM. However, the potencies of compounds **9c** and **9f** were lower than that of alsterpaullone **4b**. By contrast, compound **9d** (R = Br) showed moderate activity against all five cancer cell lines, particularly K-562 (IC_{50} = 23.86 ± 1.9 μM) and MCF-7 (IC_{50} = 34.96 ± 2.8 μM). Likewise, compound



Scheme 1. Synthesis of pyrroloazepinoisoquinoline derivatives **3a–f**. Reagents and conditions: (i) K_2CO_3, 4-$RC_6H_4COCH_2Br$, $CHCl_3$, rt, 48 h; (ii) 2-aminoethanol, AcOH, reflux, 12 h; (iii) Ph_3P, I_2, imidazole, CH_2Cl_2; (iv) dicumyl peroxide, chlorobenzene, reflux; (v) NH_2OH·HCl, AcONa, EtOH, reflux, 3 h; (vi) PPA, 80 °C, 3 h.

Table 1
The IC_{50} values (μM) of compounds **3a–f** to the five cancer cell lines[a]

Compd	R	PC-3 (prostate)	U-251 (CNS)	K-562 (leukemia)	HCT-15 (colon)	MCF-7 (breast)
3a	H	>100	>100	>100	>100	>100
3b	F	>100	>100	33.74 ± 3.8	>100	54.51 ± 3.4
3c	Cl	>100	>100	>100	39.65 ± 1.0	>100
3d	Br	33.56 ± 2.8	18.14 ± 1.02	36.12 ± 3.6	25.58 ± 2.4	43.15 ± 3.6
3e	OMe	>100	>100	>100	>100	>100
3f	NO_2	25.79 ± 3.2	>100	>100	29.50 ± 3.2	43.14 ± 4.4
2a	Cl	17.44 ± 1.87	28.82 ± 3.7	15.10 ± 3.9	20.04 ± 4.7	51.66 ± 3.6
2b	Br	21.1 ± 2.1	25.6 ± 4.5	>100	>100	>100
4a	Br	>100	>100	>100	23.86 ± 2.9	>100
4b	NO_2	1.12 ± 0.01	0.68 ± 0.02	1.58 ± 0.3	0.62 ± 0.05	1.24 ± 0.1

[a] Values are means of three experiments, (>100, not active).

Table 2
The IC_{50} values (μM) of compounds **9a–f** to the five cancer cell lines[a]

Compd	R	PC-3 (prostate)	U-251 (CNS)	K-562 (leukemia)	HCT-15 (colon)	MCF-7 (breast)
9a	H	>100	>100	36.83 ± 2.7	>100	>100
9b	F	>100	>100	27.22 ± 0.3	65.53 ± 0.9	>100
9c	Cl	>100	>100	10.21 ± 0.1	24.81 ± 10	41.82 ± 0.03
9d	Br	50.57 ± 4.3	53.33 ± 3.7	23.86 ± 1.9	35.99 ± 3.7	34.96 ± 2.8
9e	OMe	>100	>100	57.50 ± 1.3	>100	>100
9f	NO_2	>100	>100	73.68 ± 7.8	16.69 ± 1.4	>100
4a	Br	>100	>100	>100	23.86 ± 2.9	>100
4b	NO_2	1.12 ± 0.01	0.68 ± 0.02	1.58 ± 0.3	0.62 ± 0.05	1.24 ± 0.1

[a] Values are means of three experiments, (>100, not active).

9a (R = H) was active against K-562 cancer cells (IC_{50} = 36.83 ± 2.7 μM). All of derivatives **9a–f** were effective in killing leukemia cells (K-562) with moderate cytotoxicity in the micromolar range; compound **4a** was inactive and alsterpaullone **4b** was only about 7-fold more potent than was the most active azepinopyrroloisoquinoline **9c** (R= Cl) (IC_{50} = 1.58 ± 0.1 μM vs. IC_{50} = 10.21 ± 2.7 μM).

1.4. Conclusion

The results presented here indicate that conformational restrictions on compound **2**, generating the derivative compounds **3a–j**, do not appreciably reduce the cytotoxic activity of **2**, although one compound (**3d,** R = Br) is active mainly against U-251 cells. In addition, derivatives **3f** (R = NO_2) and **3b** (R = F) were cyto-

toxic to PC-3 and K-562 cells. Comparison of results using compounds **3a–f** and **9a–f** clearly shows that the action mechanisms of these groups are different, although a preliminary structure–activity relationship analysis for these compounds also revealed the importance of halogens in the benzene ring of the isoquinoline moiety. Likewise, the position of the amide moiety in compounds **3a–g** may also play an important role in determining relative activities. To test this hypothesis, compounds such as compound **11** will be synthesized and evaluated. Our results indicate that these new cytotoxic compounds may be useful as lead for the development of novel anticancer agents. Therefore, future studies will focus on determining the mechanism by which these new compounds inhibit tumor growth and on testing of combinations of appropriately positioned substituents.

2. Experimental

2.1. Chemistry

Melting points were determined using a Melt-Temp II melting point apparatus and are uncorrected. IR spectra were recorded using a Nicolet FT Magna-IR 750 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded using Varian Gemini 200 and UNITY-300 spectrometers, respectively, in deuterated chloroform solution containing tetramethylsilane as the internal standard with chemical shifts (δ) expressed downfield from TMS. Mass spectra were obtained using AX505-HA and SX-100 Jeol mass spectrometers. Reaction mixtures and chromatography fractions were concentrated using a rotary evaporator (ca. 20 °C/20 Torr). For column chromatography, the Merck Silica Gel 60 F254 was employed. Commercial grade reagents were used without further purification except where indicated.

2.2. Synthetic procedure

2.2.1. General procedure for the synthesis of 5,5-dimethyl-2-(2-oxo-2-phenylethyl)cyclohexane-1,3-diones, 6a–f

A slurry of dimedone (0.01 equiv), chloroketone (0.01 equiv), and anhydrous potassium carbonate (0.01 equiv) in chloroform was stirred at room temperature for 48 h. The mixture was then filtered; the insoluble salts were dissolved in water and the filtered solution was made acidic with concentrated HCl. The precipitate was filtered off, washed with water, and crystallized from aqueous alcohol.

2.2.1.1. 5,5-Dimethyl-2-(2-oxo-2-phenylethyl)cyclohexane-1,3-dione (6a). Yield 40% as a white solid; mp 171–173 °C; IR (KBr, cm^{-1}) ν_{max} 2961, 2659, 2577, 1685, 1569, 1383, 1252, 1039, 753; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.04 (s, 6H), 2.32 (s, 4H), 4.03 (s, 2H), 7.45–7.55 (m, 3H), 7.55–7.67 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 31.4, 31.6, 53.9, 106.6, 128.9, 129.8, 134.6, 135.2, 197.4, 203.4; MS(EI) m/z (rel intensity) 258(M$^+$, 38), 105(100).

2.2.1.2. 2-[2-(4-Fluorophenyl)-2-oxoethyl]-5,5-dimethylcyclohexane-1,3-dione (6b). Yield 67% as a white solid; mp 144–146 °C; IR (KBr, cm^{-1}) ν_{max} 2961, 2908, 2557, 1691, 1598, 1555, 1350, 1256, 1040, 832; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (s, 6H), 2.32 (s, 4H), 3.99 (s, 2H), 7.10–7.20 (m, 2H), 8.22–8.30 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 31.3, 31.6, 53.9, 106.4, 116.4, 131.0, 132.9, 169.4, 195.9, 202.1, 203.4; MS (EI) m/z (rel intensity) 276 (M$^+$, 36), 123 (100).

2.2.1.3. 2-[2-(4-Chlorophenyl)-2-oxoethyl]-5,5-dimethylcyclohexane-1,3-dione (6c). Yield 60% as a white solid; mp 163–164; IR (KBr, cm^{-1}) ν_{max} 3160, 2958, 2876, 1688, 1599, 1569, 1381, 1321, 1252, 1040, 814; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (s, 6H), 2.32 (s, 4H), 3.99 (s, 2H), 7.46 (dd, J = 1.7, 8.6 Hz, 2H), 8.15 (dd, J = 1.9, 8.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 31.2, 31.7, 53.9, 106.3, 129.2, 131.2, 133.4, 141.3, 196.1, 202.3, 203.1; MS (EI) m/z (rel intensity) 292 (M$^+$, 18), 139 (100).

2.2.1.4. 2-[2-(4-Bromophenyl)-2-oxoethyl]-5,5-dimethylcyclohexane-1,3-dione (6d). Yield 75% as a white solid; mp 154–155 °C; IR (KBr, cm^{-1}) ν_{max} 2960, 2925, 2653, 1694, 1581, 1556, 1384, 1234, 1151, 1042, 809; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05 (s, 6H), 2.32 (s, 4H), 3.98 (s, 2H), 7.60–7.65 (m, 2H), 8.07 (dd, J = 1.9, 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 31.6, 31.7, 48.7, 53.9, 106.3, 129.2, 131.2, 133.4, 141.3, 196.1, 202.3, 203.1; MS (EI) m/z (rel intensity) 336 (M$^+$, 38), 183 (100).

2.2.1.5. 2-[2-(4-Methoxyphenyl)-2-oxoethyl]-5,5-dimethylcyclohexane-1,3-dione (6e). Yield 50% as a white solid; mp 151–153 °C; IR (KBr, cm^{-1}) ν_{max} 3155, 2961, 2912, 2835, 1677, 1603, 1379, 1251, 1168, 1037, 822; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.04 (s, 6H), 2.31 (s, 4H), 3.88 (s, 3H), 3.97 (s, 2H), 6.95 (dd, J = 2.0, 8.8 Hz, 2H), 8.19 (dd, J = 2.0, 8.8 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2, 31.1, 31.3, 53.9, 55.6, 106.7, 132.5, 132.6, 164.9, 202.1, 203.4; MS (EI) m/z (rel intensity) 288 (M$^+$, 24), 135 (100).

2.2.1.6. 5,5-Dimethyl-2-[2-(4-nitrophenyl)-2-oxoethyl]cyclohexane-1,3-dione (6f). Yield 81% as a yellow-orange solid; mp 180–182 °C; IR (KBr, cm^{-1}) ν_{max} 3077, 2956, 2907, 2662, 1696, 1565, 1530, 1385, 1350, 1042, 846, 746, ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO) δ 1.07 (s, 6H), 2.32 (s, 4H), 3.95 (s, 2H), 8.13 (d, J = 8.9 Hz, 2H), 8.28 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.8, 31.6, 32.9, 46.1, 53.2, 107.6, 123.0, 128.6, 141.6, 149.3, 196.7; MS (EI) m/z (rel intensity) 303 (M$^+$, 94), 151 (100).

2.2.2. General procedure for the synthesis of 1-(2-hydroxyethyl)-6,6-dimethyl-2-phenyl-1,5,6,7-tetrahydro-4*H*-indol-4-ones (7a–f)

To a vigorously stirred suspension of triketone **6** (1 g, 3.88 mmol) in acetic acid (5 mL) was added ethanolamine (0.36 mL, 5.82 mmol). The resulting slurry was heated at 60 °C under a N_2 atmosphere until no more starting material was observed by TLC, and then the reaction mixture was allowed to warm to room temperature and poured into ice-water (10 mL). The solid was filtered and washed with cooled water. The crude mixture was purified by column chromatography on neutral aluminum oxide using a gradient of 30–50% AcOEt in hexane as eluent. Evaporation of the collected fractions gave 1-(2-hydroxyethyl)indolones **7a–f**.

2.2.2.1. 1-(2-Hydroxyethyl)-6,6-dimethyl-2-phenyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (7a). Yield 64% as a white solid; mp 172–174 °C; IR (KBr, cm^{-1}) ν_{max} 3325, 2955, 2883, 1624, 1475, 1061, 779, 703; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.16 (s, 6H), 2.32 (s, 2H), 2.79 (s, 2H), 3.54 (t, J = 6.2 Hz, 2H), 4.02 (t, J = 6.1 Hz, 3H), 6.42 (s, 1H), 7.41 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 28.2, 34.9, 35.9, 46.1, 51.5, 60.2, 104.6, 118.3, 127.2, 128.2, 128.8, 132.1, 135.4, 144.1, 192.4; MS (EI) m/z (rel intensity) 283 (M$^+$, 67), 183 (100); HRMS (FAB$^+$): calcd for $C_{18}H_{22}O_2N$: 284.1651, found: 284.1642.

2.2.2.2. 2-(4-Fluorophenyl)-1-(2-hydroxyethyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (7b). Yield 60% as a white solid; mp 161–163 °C; IR (KBr, cm^{-1}) ν_{max} 3340, 2954, 2879, 1629,1481, 1058, 847; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.16 (s,6H), 2.34 (s, 2H), 2.77 (s, 2H), 3.56 (t, J = 6.3 Hz, 2H), 3.98 (t, J = 6.3 Hz, 2H), 4.36 (s, 1H), 6.43 (s, 1H), 7.11 (dd, J = 5.4, 8.7 Hz, 2H), 7.41 (dd, J = 5.4, 9.0 Hz, 2H); ^{13}C NMR (75 MHz,

$CDCl_3$ + DMSO) δ 28.2, 34.8, 35.9, 45.9, 51.4, 60.3, 104.7, 114.7, 115.0, 118.3, 128.1, 130.7, 130.8, 134.3, 143.9, 160.1, 163.3, 192.8; MS (EI) *m*/*z* (rel intensity) 301 (M^+, 100), 201 (96); HRMS (FAB^+): calcd for $C_{18}H_{21}FO_2N$: 302.1556, found: 302.1554.

2.2.2.3. 2-(4-Chlorophenyl)-1-(2-hydroxyethyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (7c). Yield 55% as a white solid; mp 165–66 °C; IR (KBr, cm^{-1}) ν_{max} 3255, 2958, 2870, 1627, 1477, 1062, 820; ^1H NMR (300 MHz, $CDCl_3$) δ 1.10 (s, 6H), 2.23 (s, 2H), 2.70 (s, 2H), 2.77 (s,1H), 3.69 (d, J = 6.3 Hz, 2H), 4.02 (t, J = 6.2 Hz, 2H), 6.50 (s, 1H), 7.38 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.7, 35.4, 36.7, 46.5, 51.5, 61.4, 106.1, 119.2, 128.9, 130.7, 130.8, 134.0, 135.1, 144.9, 194.1; MS (EI) *m*/*z* (rel intensity) 317 (M^+, 98), 217 (100); HRMS (FAB^+): calcd for $C_{18}H_{21}ClO_2N$: 318.1261, found: 318.1251.

2.2.2.4. 2-(4-Bromophenyl)-1-(2-hydroxyethyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (7d). Yield 40% as a white solid; mp 159–160 °C; IR (KBr, cm^{-1}) ν_{max} 3258, 2953, 2872, 1628, 1475, 1063, 819; ^1H NMR (300 MHz, $CDCl_3$) δ 1.09 (s, 6H), 2.21 (s, 2H), 2.69 (s, 2H), 2.88 (s,1H), 3.71 (t, J = 5.5 Hz, 2H), 4.02 (t, J = 5.5 Hz, 2H), 6.49 (s, 1H), 7.32 (dd, J) 8.6, 1.9 Hz, 2H); 7.54 (dd, J = 8.6, 1.8 Hz, 2H,); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.7, 35.4, 36.6, 46.6, 51.3, 56.9, 61.3, 106.1, 119.0, 122.2, 131.0, 131.1, 131.8, 135.2, 145.4, 194.2; MS (EI) *m*/*z* (rel intensity) 361 (M^+, 100) 363 (M^++2, 99); HRMS (FAB^+): calcd for $C_{18}H_{21}BrO_2N$: 362.0756, found: 362.0742.

2.2.2.5. 1-(2-Hydroxyethyl)-2-(4-methoxyphenyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (7e). Yield 52% as a white solid; mp 155–56 °C; IR (KBr, cm^{-1}) ν_{max} 3331, 2953, 2897, 2835, 1635, 1483, 1250, 1180, 1030, 808; ^1H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 6H), 2.28 (s, 2H), 2.62 (br s, 1H), 2.72 (s, 2H), 3.68 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 4.01 (t, J = 5.7 Hz, 2H), 6.47 (s, 1H), 6.93 (dd, J = 9.0 Hz, 2H), 7.33 (dd,, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.7, 35.4, 36.7, 46.5, 51.2, 55.3, 61.6, 105.4, 114.0, 118.8, 124.6, 130.9, 136.2, 144.9, 159.4, 194.1; MS (EI) *m*/*z* (rel intensity) 313 (M^+, 100); HRMS (FAB^+): calcd for $C_{19}H_{24}O_3N$: 314.1758, found: 314.1756.

2.2.2.6. 1-(2-Hydroxyethyl)-6,6-dimethyl-2-(4-nitrophenyl)-1,5,6,7-tetrahydro-4*H*-indol-4-one (7f). Yield 60% as a yellow solid; mp 184–86 °C; IR (KBr, cm^{-1}) ν_{max} 3444, 2957, 2873, 1656, 1595, 1516, 1475, 1342, 859; ^1H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.13 (s, 6H), 1.95 (s, 1H), 2.30 (s, 2H), 2.74 (s, 2H), 3.75 (t, J = 5.7 Hz, 2H), 4.12 (t, J = 5.7 Hz, 2H), 6.66 (s, 1H), 7.64 (d, J = 9.0 Hz, 2H), 8.26 (d, J = 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.7, 35.4, 36.8, 46.7, 51.6, 61.5, 108.0, 119.8, 124.0, 129.5, 134.1, 138.9, 146.0, 146.9, 193.9; MS (EI) *m*/*z* (rel intensity) 328 (M^+, 80); 228 (100); HRMS (FAB^+): calcd for $C_{18}H_{21}O_4N_2$: 329.1501, found: 329.1508.

2.2.3. General procedure for the preparation of 1-(2-iodoethyl)-6,6-dimethyl-2-phenyl-1,5,6,7-tetrahydro-4*H*-indol-4-ones (8a–f)

Triphenylphosphine (1.5 equiv) and imidazole (1.5 equiv) were dissolved in dry CH_2Cl_2 (1.5 mL/mmol). The mixture was cooled in an ice bath, and iodine (1.5 equiv) was added with vigorous stirring over 10 min. The resulting slurry was warmed to room temperature, and a solution of hydroxyethyl tetrahydroindolone **7** (1.0 equiv) in CH_2Cl_2 was added dropwise over 15 min. The mixture was stirred for 1 h under a N_2 atmosphere. The solvent was removed, and the crude residue was purified by column chromatography on neutral aluminum oxide using hex/AcOEt as eluent.

2.2.3.1. 1-(2-Iodoethyl)-6,6-dimethyl-2-phenyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (8a). Yield 85% as a white solid; mp 143–45 °C; IR (KBr, cm^{-1}) ν_{max} 2952, 2923, 2867, 1656, 1471, 772, 705; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 6H), 2.40 (s, 2H), 2.71 (s, 2H), 3.03 (t, J = 8.1 Hz, 2H), 4.23 (t, J = 8.1 Hz, 2H), 6.56 (s, 1H), 7.34–7.47 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.7, 28.9, 35.6, 36.4, 46.4, 51.9, 106.2, 119.7, 128.2, 128.9, 129.0, 132.0,135.4, 143.0, 193.6; MS (EI) *m*/*z* (rel intensity) 393 (M^+, 100); HRMS (FAB^+): calcd for $C_{18}H_{21}ONI$: 394.0668, found: 394.0666.

2.2.3.2. 2-(4-Fluorophenyl)-1-(2-iodoethyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4H-indol-4-one (8b). yield 79% as a white solid; mp 140–141 °C; IR (KBr, cm^{-1}) ν_{max} 2952, 2925, 2861, 1658, 1469, 851; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 6H), 2.39 (s, 2H), 2.67 (s, 2H), 3.02 (t, J = 7.9 Hz, 2H), 4.20 (t, J = 7.9 Hz, 2H), 6.53 (s, 1H), 7.14 (dd, J = 8.8, 2.9 Hz, 2H), 7.33 (dd, J = 8.7, 2.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.5, 28.8, 35.6, 36.4, 46.3, 51.9, 106.4, 115.8, 116.1, 119.6, 130.8, 130.9, 134.2, 142.9, 193.5; MS (EI) *m*/*z* (rel intensity) 411 (M^+, 100); HRMS (FAB^+): calcd for $C_{18}H_{20}ONFI$: 412.0582, found: 412.0582.

2.2.3.3. 2-(4-Chlorophenyl)-1-(2-iodoethyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (8c). Yield 82% as a white solid; mp 125–126 °C; IR (KBr, cm^{-1}) ν_{max} 2957, 2929, 2872, 1651, 1472, 803; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 6H), 2.39 (s, 2H), 2.70 (s, 2H), 3.03 (t, J = 7.9 Hz, 2H), 4.22 (t, J = 7.9 Hz, 2H), 6.56 (s, 1H), 7.28 (d, J = 8.7 Hz, 2H), 7.41 (d, J = 8.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.5, 28.8, 35.6, 36.4, 46.4, 51.9, 106.6, 119.8, 129.1, 130.1, 130.5, 134.1, 134.3, 143.2, 193.5; MS (EI) *m*/*z* (rel intensity) 427 (M^+, 100); HRMS (FAB^+): calcd for $C_{18}H_{21}ONClI$: 428.0278, found: 428.0285.

2.2.3.4. 2-(4-Bromophenyl)-1-(2-iodoethyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (8d). Yield 71% as a white solid; mp 119–120 °C; IR (KBr, cm^{-1}) ν_{max} 2955, 2871, 1654, 1466, 805; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 6H), 2.39 (s, 2H), 2.70 (s, 2H), 3.03 (t, J = 7.9 Hz, 2H), 4.22 (t, J = 7.9 Hz, 2H), 6.56 (s, 1H), 7.23 (dd, J = 9.0, 1.8 Hz, 2H), 7.57 (dd, J = 8.8, 2.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.5, 28.8, 35.6, 36.4, 46.4, 51.9, 106.7, 119.8, 122.5, 130.4, 131.0, 132.1, 134.1, 143.3, 193.5; MS (EI) *m*/*z* (rel intensity) 471 (M^+, 06), 473 (M^++2, 07), 83 (100); HRMS (FAB^+): calcd for $C_{18}H_{21}ONBrI$: 471.9773, found: 471.9782.

2.2.3.5. 1-(2-Iodoethyl)-2-(4-methoxyphenyl)-6,6-dimethyl-1,5,6,7-tetrahydro-4*H*-indol-4-one (8e). Yield 72% as a white solid; mp 134–135 °C; IR (KBr, cm^{-1}) ν_{max}: 2955, 2969, 2840, 1652, 1469, 1414, 1248, 1171, 839; ^1H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 6H), 2.39 (s, 2H), 2.69 (s, 2H), 3.03 (t, J = 7.4 Hz, 2H), 3.86 (s, 3H), 4.20 (t, J = 7.6 Hz, 2H), 6.50 (s, 1H), 6.96 (dd, J = 8.8, 2.0 Hz, 2H), 7.28 (dd, J = 8.8, 2.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.8, 28.9, 35.5, 36.4, 46.4, 51.9, 55.3, 105.7, 114.2, 119.5, 124.3, 130.4, 135.2, 142.6, 159.6, 193.6; MS (EI) *m*/*z* (rel intensity) 423 (M^+, 100), HRMS (FAB^+): calcd for $C_{19}H_{23}O_2NI$: 424.0773, found: 424.0774.

2.2.3.6. 1-(2-Iodoethyl)-6,6-dimethyl-2-(4-nitrophenyl)-1,5,6,7-tetrahydro-4*H*-indol-4-one (8f). Yield 69% as a yellow solid; mp 150–151 °C; IR (KBr, cm^{-1}) ν_{max} 2954, 2926, 2867, 1656, 1599, 1518, 1470, 1342, 856; ^1H NMR (300 MHz, $CDCl_3$) δ 1.20 (s, 6H), 2.42 (s, 2H), 2.73 (s, 2H), 3.06 (t, J = 7.5 Hz, 2H), 4.31 (t, J = 7.5 Hz, 2H), 6.72 (s, 1H), 7.53 (d, J = 9.0 Hz, 2H), 8.31 (d, J = 9.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.5, 28.8, 35.6, 36.5, 46.6, 51.9, 108.6, 120.4, 124.3, 128.9, 133.1, 138.5, 144.6, 147.1, 193.4; MS (EI) *m*/*z* (rel intensity) 438 (M^+, 100); HRMS (FAB^+): calcd for $C_{18}H_{20}O_3N_2I$: 439.0519, found: 439.0521.

2.2.4. General procedure for the synthesis of 9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-ones (9a–f)

To a degassed solution of the corresponding iodo derivative (1.0 equiv) in refluxing chlorobenzene (7 mL/mmol) was added dicumyl peroxide (1.5 equiv) portionwise (0.3 equiv/1.5 h). The reaction was carried out under a N_2 atmosphere for 7.5 h. The mixture was then allowed to warm to room temperature and evaporated to dryness. The crude residue was purified by column chromatography on silica gel (AcOEt/hex).

2.2.4.1. 9,9-Dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (9a). Yield 82% as a white crystalline solid; mp 148–150 °C; IR (KBr, cm^{-1}) ν_{max} 2957, 2871, 1647, 1486, 762; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 6H), 2.38 (s, 2H), 2.65 (s, 2H), 3.08 (t, J = 6.6, 2H), 3.97 (t, J = 6.6, 2H), 6.87 (s, 1H), 7.12–7.28 (m, 3H), 7.53 (d, J = 7.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.8, 35.3, 35.6, 41.0, 52.1, 99.9, 119.8, 122.9, 126.6, 127.5, 127.9, 128.7, 130.3, 131.2, 142.1, 193.3; MS (EI) m/z (rel intensity 265 (M^+, 81), 181 (100); HRMS (FAB^+): calcd for $C_{18}H_{20}ON$: 266.1545, found: 266.15435.

2.2.4.2. 3-Fluoro-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (9b). Yield 91% as a white crystalline solid; mp 179–180 °C IR (KBr, cm^{-1}) ν_{max} 2955, 2869, 1646, 1482, 826; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 6H), 2.39 (s, 2H), 2.65 (s, 2H), 3.08 (t, J = 6.8, 2H), 3.98 (t, J = 6.8, 2H), 6.81 (s, 1H), 6.89–7.01 (m, 2H), 7.49 (dd, J = 8.5, 2.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.8, 28.9, 35.4, 35.6, 40.7, 52.1, 99.6, 114.7, 119.8, 124.6, 125.0, 130.6, 132.5, 142.1, 159.7, 163.0, 193.5; MS (EI) m/z (rel intensity) 283 (M^+, 100); HRMS (FAB^+): calcd for $C_{18}H_{20}ONF$: 284.1451, found: 284.1452.

2.2.4.3. 3-Chloro-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (9c). yield 85% as a white crystalline solid; mp 199–200 °C; IR (KBr, cm^{-1}) ν_{max} 2955, 2868, 1647, 1481, 829; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 6H), 2.39 (s, 2H), 2.65 (s, 2H), 3.07 (t, J = 6.7, 2H), 3.97 (t, J = 6.7, 2H), 6.85 (s, 1H), 7.18 (d, J = 1.99 Hz, 1H), 7.22 (dd, J = 2.16, 8.28 Hz, 1H), 7.44 (d, J = 8.28 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.7, 28.8, 28.8, 35.3, 35.6, 40.7, 52.1, 100.4, 119.9, 124.2, 127.2, 127.7, 127.9, 130.3, 131.8, 131.9, 142.4, 193.4; MS (EI) m/z (rel intensity) 299 (M^+, 100); 301(M^+ + 2, 31); HRMS (FAB^+): calcd for $C_{18}H_{20}ONCl$: 300.1155, found: 300.1149.

2.2.4.4. 3-Bromo-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (9d). Yield 53% as a white crystalline solid; mp 190–191 °C; IR (KBr, cm^{-1}) ν_{max} 2953, 2871, 1649, 1470, 825; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 6H), 2.38 (s, 2H), 2.65 (s, 2H), 3.07 (t, J = 6.8 Hz, 2H), 3.97 (t, J = 6.7 Hz, 2H), 6.87 (s, 1H), 7.34 (s, 1H), 7.38 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 28.8, 35.3, 35.6, 40.7, 52.1, 100.5, 119.8, 120.0, 124.5, 127.7, 130.4, 130.6, 130.8, 132.2, 142.4, 193.4; MS (EI) m/z (rel intensity) 343 (M^+, 76), 344 (M^+ + 2, 75), 259 (100); HRMS (FAB^+): calcd for $C_{18}H_{20}ONBr$: 344.0650, found: 344.0661.

2.2.4.5. 3-Methoxy-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (9e). Yield 53% as a white crystalline solid; mp 201–203 °C; IR (KBr, cm^{-1}) ν_{max}: 2951, 2872, 2835, 1654, 1481, 1426, 1161, 1035, 826; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 6H), 2.38 (s, 2H), 2.65 (s, 2H), 3.06 (t, J = 6.4 Hz, 2H), 3.82 (s, 3H), 3.96 (t, J = 6.8 Hz, 2H), 6.75 (s, 1H), 6.83 (dd, J = 2.8, 8.4 Hz, 2H), 7.46 (d, J = 8.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.9, 29.2, 35.3, 35.6, 40.9, 52.1, 55.3, 98.4, 113.1, 113.3, 119.7, 121.8, 124.4, 131.4, 132.0, 141.8, 158.4, 193.4; MS (EI) m/z (rel intensity) 295 (M^+, 85), 211 (100). HRMS (FAB^+): calc for $C_{19}H_{22}O_2N$: 296.1657, found: 296.1651.

2.2.4.6. 9,9-Dimethyl-3-nitro-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (9f). Yield 75% as a yellow crystalline solid; mp 230–231 °C; IR (KBr, cm^{-1}) ν_{max} 2954, 2879, 1653, 1575,1514, 1330, 807; ^1H NMR (300 MHz, $CDCl_3$) δ 1.17 (s, 6H), 2.41 (s, 2H), 2.69 (s, 2H), 3.21 (t, J = 6.6 Hz, 2H), 4.06 (t, J = 6.6 Hz, 2H), 7.07 (s, 1H), 7.61 (d, J = 8.4 Hz, 1H), 8.10–8.15 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.8, 28.8, 35.3, 35.7, 40.9, 52.1, 103.9, 120.8, 123.2, 123.6, 129.5, 130.8, 134.8, 143.7, 145.6, 193.2; MS (EI) m/z (rel intensity) 310 (M^+, 100); HRMS (FAB^+): calcd for $C_{18}H_{19}O_3N_2$: 311.1396, found: 311.1395.

2.2.5. General procedure for the synthesis of 9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (*syn*/*anti*) oximes (10a–f)

To a vigorously stirred solution of tetrahydroindolo[2,1-*a*]isoquinoline **9** (0.5 g, 1.88 mmol) in ethanol (10 mL) was added hydroxylamine hydrochloride (0.458 g, 6.59 mmol) and sodium acetate (0.85 g, 10.36 mmol) dissolved in a minimal quantity of water. The resulting mixture was refluxed for 3 h, after which the mixture was allowed to cool to room temperature. The crude product was filtered, washed with cold water and purified by column chromatography on silica gel using hexane–ethyl acetate 1:1 as eluent.

2.2.5.1. 9,9-Dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (syn/anti) oximes (10a). Yield 86% as a white solid; mp 218–220 °C; IR (KBr, cm^{-1}) ν_{max} 3251, 3155, 1651, ^1H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.10–1.20 (H-13,1$\acute{3}$), 2.30–2.31 (H-10), 2.55–257(H-8), 3.06 (H-5), 3.96 (H-6), 7.05–7.26 (Ar-H), 7.31 (H-12), 7.46–7.59 (Ar-H). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO) δ 7.6, 28.1, 31.7, 32.1, 34.5, 35.3, 42.4, 97.1, 104.6, 110.9, 121.5, 124.9, 126.4, 127.2, 128.5, 129.3, 132.7, 134.1, 148.2; MS (EI) m/z (rel intensity) 280(M^+, 100).

2.2.5.2. 3-Fluoro-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (*syn*/*anti*) oximes (10b). Yield 61% as a white solid; mp 236–237 °C; IR (KBr, cm^{-1}) ν_{max} 3245, 3154, 1647, ^1H NMR (300 MHz, $CDCl_3$+DMSO) δ 1.10 (H-13,1$\acute{3}$), 2.32–234 (H-10), 2.54–256 (H-8), 3.06 (H-5), 3.96 (H-6), 6.89–6.95(Ar-H), 7.27 (H-12), 7.41–7.52 (Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 27.7, 28.4, 31.9, 32.3, 34.7, 35.5, 42.5, 97.8, 104.5, 111.1, 123.4, 123.5, 125.1, 125.2, 128.1, 131.7, 131.8, 134.3, 148.5; MS (EI) m/z (rel intensity) 298 (M^+, 100).

2.2.5.3. 3-Chloro-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (*syn*/*anti*) oximes (10c). Yield 83% as a white solid; mp 250–254 °C; IR (KBr, cm^{-1}) ν_{max} 3242, 3152, 1653, ^1H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.08 (H-13,1$\acute{3}$), 2.24–2.26 (H-10), 2.55–2.56 (H-8), 3.05 (H-5), 3.96 (H-6), 7.18 (Ar-H), 7.26 (H-12), 7.45 (Ar-H), 10.13 (br s); ^{13}C NMR (75 MHz,$CDCl_3$ + DMSO) δ 27.8, 28.1, 28.4, 32.1, 35.3, 42.7, 97.8, 105.4, 111.5, 123.0, 126.6, 127.4, 127.5, 127.5, 129.7, 131.6, 134.3, 147.6; MS (EI) m/z (rel intensity) 314 (M^+, 100).

2.2.5.4. 3-Bromo-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (*syn*/*anti*) oximes (10d). Yield 88% as a white solid; mp 246–248 °C; IR (KBr, cm^{-1}) ν_{max} 3238, 3114, 1649, ^1H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.11–1.12 (H-13,1$\acute{3}$), 2.37–239 (H-10), 2.57–259 (H-8), 3.06 (H-5), 3.97 (H-6), 7.33–7.39 (Ar-H), 7.56 (H-12); ^{13}C NMR (50 MHz, $CDCl_3$+DMSO) δ 27.5, 27.8, 28.2, 32.3, 35.2, 41.9, 97.9, 105.2, 110.7, 118.1, 123.3, 127.5, 128.2, 129.5, 130.1, 131.6, 135.7, 149.0; MS (EI) m/z (rel intensity) 358 (M^+, 100).

2.2.5.5. 3-Methoxy-9,9-dimethyl-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (*syn*/*anti*) oximes (10e). Yield 93% as a white solid; mp 249–250 °C; IR (KBr, cm^{-1}) ν_{max} 3246, 3155, 1646,

^{1}H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.12–1.14 (H-13,1$\acute{3}$), 2.41–2.43 (H-10), 2.61–2.63 (H-8), 3.06 (H-5), 3.99 (H-6), 6.77–6.84 (Ar-H), 7.14 (H-12); 7.46 (Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0, 27.8, 32.2, 34.6, 40.8, 102.4, 109.1, 112.1, 120.6, 122.8, 129.3, 131.0, 136.3, 150.3, 157.1; MS (EI) 310.

2.2.5.6. 9,9-Dimethyl-3-nitro-5,8,9,10-tetrahydroindolo[2,1-*a*]isoquinolin-11(6*H*)-one (*syn*/*anti*) oximes (10f). Yield 80% as a yellow solid, mp 239–240 °C; IR (KBr, cm^{-1}) ν_{max} 3240, 3152, 1652, ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO) δ 1.12–1.14 (H-13,1$\acute{3}$), 2.43–2.45 (H-10)), 2.62–263 (H-8), 3.17–3.24 (H-5), 4.00–4.09 (H-6), 7.55 (H-12), 7.57–7.66 (Ar-H), 8.04–8.13 (Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO) δ 27.4, 27.8, 28.1, 31.8, 32.4, 34.5, 35.2, 35.6, 41.5, 101.5, 108.7, 111.6, 122.8, 127.5, 127.8, 130.0, 134.5, 135.8, 138.0, 143.9, 144.2, 148.9, 150.9; MS (EI) *m*/*z* (rel intensity) 325 (M^+, 100).

2.2.6. General procedure for the synthesis of azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-ones (3a–f)

To a mixture of phosphorus pentoxide (9 g) and phosphoric acid (5 mL) was added the oxime (0.5 g). The resulting mixture was stirred and heated at 80 °C for 3 h. The mixture was allowed to warm to room temperature and then poured into ice-water, after which it was neutralized with sodium carbonate and extracted with methylene chloride (3 × 10 mL). The organic layer was dried over sodium sulfate. The solvent was removed in vacuo, and the crude residue was purified by column chromatography on silica gel using a gradient of 50–100% hexane–ethyl acetate as eluent.

2.2.6.1. 9,9-Dimethyl-5,6,8,9,10,11-hexahydro-12*H*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-one (3a). Yield 28% as a white solid; mp 236–237 °C; IR (KBr, cm^{-1}) ν_{max} 3275, 3188, 3049, 2963, 1639, 1472, 1314, 1147, 764; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 6H), 2.72 (s, 2H), 3.09 (m, 4H), 3.96 (t, J = 6.6, 2H), 6.99 (s, 1H), 7.10–7.29 (m, 3H), 7.49–7.54 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.2, 28.2, 33.1, 40.3, 51.8, 104.6, 113.2, 114.6, 122.1, 125.8, 126.8, 127.3, 128.1, 129.1, 129.5, 168.4; MS (EI) *m*/*z* (rel intensity) 280 (M^+, 100). Anal. Calc. for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.12; H, 7.19; N, 9.96.

2.2.6.2. 3-Fluoro-9,9-dimethyl-5,6,8,9,10,11-hexahydro-12*H*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-one (3b). Yield 49% as a white solid, mp 216–218 °C; IR (KBr, cm^{-1} ν_{max} 3473, 3275, 3187, 3045, 2906, 2876, 1638, 1478, 1398, 1316, 1157, 818; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 6H), 2.68 (s, 2H), 3.06 (m, J = 6.8, 4H), 3.93 (t, J = 6.8 Hz, 2H), 6.4 (br s, 1H), 6.80–7.02 (m, 2H), 6.97 (s, 1H), 7.5 (dd, J = 5.6, 8.6 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 29.0, 34.1, 40.3, 40.6, 52.6, 105.0, 114.3, 114.8, 124.4, 124.6, 133.0, 127.3, 128.1, 129.1, 129.5, 169.2; MS (EI) *m*/*z* (rel intensity) 298 (M^+, 100), Anal. Calc. for $C_{18}H_{19}FN_2O$: C, 72.46; H, 6.42; F, 6.37; N, 9.39. Found: C, 72.48; H, 6.42; N, 9.35.

2.2.6.3. 3-Chloro-9,9-dimethyl-5,6,8,9,10,11-hexahydro-12*H*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-one (3c). Yield 65% as a white solid, mp 242–244 °C; IR (KBr, cm^{-1}) ν_{max} 3491, 3413, 3273, 3183, 3041, 2961, 2901, 1638, 1474, 1314, 1152, 820; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.13 (s, 6H), 2.69 (s, 2H), 3.06 (t, J = 6.6 Hz, 2H), 3.10 (d, J = 5.4 Hz, 2H), 3.94 (t, J = 6.6 Hz, 2H), 6.59 (br s, 1H), 7.01 (s, 1H), 7.18 (d, J = 1.5 Hz, 1H), 7.22 (dd, J = 2.1, 8.1 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 28.8, 33.9, 40.3, 40.7, 52.7, 105.7, 115.2, 124.2, 127.2, 127.7, 127.8, 131.5, 131.6, 133.9, 169.2; MS (EI) *m*/*z* (rel intensity) 314(M^+, 100), 316. Anal. Calc. for $C_{18}H_{19}ClN_2O$: C, 68.67; H, 6.08; N, 8.90. Found: C, 68.65; H, 6.08; N, 8.92.

2.2.6.4. 3-Bromo-9,9-dimethyl-5,6,8,9,10,11-hexahydro-12*H*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-one (3d). Yield 47% as a white solid, mp 230–232 °C; IR (KBr, cm^{-1}) ν_{max} 3402, 3274, 3187, 3044, 2957, 1638, 1472, 1314, 1150, 819; ^{1}H NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ 1.13 (s, 6H), 2.71 (s, 2H), 3.08 (t, J = 6.6 Hz, 2H), 3.13 (d, 2H), 3.95 (t, J = 6.6 Hz, 2H), 6.70 (s, 1H), 7.07 (br s, 1H), 7.34–7.41 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.3, 28.1, 28.3, 33.1, 40.4, 40.9, 51.8, 105.3, 114.8, 118.9, 122.3, 131.6, 133.7, 168.3; MS (EI) *m*/*z* (rel intensity) 358 (M^+, 100). Anal. Calc. for $C_{18}H_{19}BrN_2O$: C, 60.18; H, 5.33; N, 7.80. Found: C, 60.20; H, 5.33; N, 7.82.

2.2.6.5. 3-Methoxy-9,9-dimethyl-5,6,8,9,10,11-hexahydro-12*H*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]iso quinolin-12-one (3e). Yield 34% as a beige solid, mp 259–261 °C; IR (KBr, cm^{-1}) ν_{max} 3477, 3272, 3179, 3035, 2960, 1637, 1484, 1392, 1300, 1252, 1166, 816; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 6H), 2.69 (s, 2H), 3.03–3.10 (m, J = 6.6 Hz, 4H), 3.82 (s, 3H), 3.92 (t, J = 6.6 Hz, 2H), 6.50 (br s, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.82 (dd, J = 2.7, 8.4 Hz, 1H), 6.91 (s, 1H),7.48 (d, J = 8.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 29.2, 33.9, 40.4, 40.8, 55.3, 52.7, 103.7, 113.1, 114.9, 121.8, 124.3, 129.9, 131.5, 132.4, 133.2, 158.3, 169.4; MS (EI) *m*/*z* (rel intensity) 310 (M^+, 100), Anal. Calc. for $C_{19}H_{22}N_2O$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.53; H, 7.14; N, 9.04.

2.2.6.6. 9,9-Dimethyl-3-nitro-5,6,8,9,10,11-hexahydro-12*H*-azepino[3′,4′:4,5]pyrrolo[2,1-*a*]isoquinolin-12-one (3f). Yield 25% as a pale yellow solid, mp 263–265 °C; IR (KBr, cm^{-1}) ν_{max} 3405, 3268, 3182, 3041, 2963, 2927, 2878, 1616, 1513, 1470, 1324, 1089, 1025, 800; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (s, 6H), 2.73 (s, 2H), 3.10 (d, 2H), 3.20 (t, J = 6.3 Hz, 2H), 4.02 (t, J = 6.6 Hz, 2H), 6.48 (br s, 1H), 7.23 (s, 1H), 7.60 (d, J = 8.4 Hz, 1H), 8.11 (d, J = 2.1 Hz, 1H), 8.14 (dd, J = 2.4, 8.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.9, 28.8, 34.2, 40.2, 40.6, 52.6, 109.4, 116.9, 123.0, 123.2, 123.5, 128.0, 130.3, 134.8, 135.4, 145.4, 168.7; MS (EI) *m*/*z* (rel intensity) 325(M^+, 100), Anal. Calc. for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.9. Found: C, 66.46; H, 5.89; N, 12.93.

2.3. Cytotoxic activity

Cancer lines were obtained from the National Cancer Institute of the United States. The lines were those of colon cancer (HTC-15), breast cancer (MCF-7), leukemia (K-562), central nervous system cancer (U-251), and prostate cancer (PC-3). Cytotoxicity data were determined using the protein-bound-sulforhodamine (SRB) assay in microculture; the assay measures cell growth as described by Monks[14] method. Cell lines were cultivated in RPMI-1640 medium with 10% (v/v) fetal bovine serum, 2 μM L-glutamine, 100 IU/mL penicillin G, 100 mg/mL streptomycin sulfate, and 0.25 μg/mL of amphotericin B (Gibco Grand Island, NY) Incubation was at 37 °C in a 5%, CO_2 atmosphere and 95% relative humidity. After growth, cells were detached with a 0.1% (w/v) trypsin-EDTA solution, counted in a hemocytometer, and diluted with complete medium to 5×10^4 cells/mL (K-562, MCF-7), 7.5×10^4 cells/mL (U-251, PC-3), or 10×10^4 cells/mL (HTC-15). Microtiter plate wells were filled with 100 μL aliquots of cell suspension and incubated. After 24 h cells were treated with logarithmically-diluted concentrations of sample compounds. Test samples were dissolved in DMSO to provide stock solutions (40 μM) and were then diluted to yield solutions of 100, 31, 10, 3.1 and 1 μM, for use in treatment of cell suspensions. After 48 h, cultures were fixed in situ by addition of 50 μL 50% (w/v) trichloroacetic acid and incubated for 60 min at 4 °C. Cells were harvested by centrifugation, washed three times, and dried. Cell pellets were suspended in 100 μL 0.4% (w/v) SRB solution in 1% (v/v) acetic acid for 30 min. Unlinked SRB was removed by washing with 1% (v/v) acetic acid and protein-linked dye was extracted with 10 μM tris(hydroxymethyl)methaneamine solution, and quantitated by spectrometry at 515 nm. The IC_{50}

values were calculated according to Monks.[14] Experiments were conducted in triplicate, and means and standard errors calculated. The compounds 9-bromo-7,12-dihydroindolo-[3,2-*d*][1]benzazepin-6(5*H*)-one (kenpaullone **4a)** and 9-nitro-7,12-dihydroindolo-[3,2-*d*][1]benzazepin-6(5*H*)-one (alsterpaullone **4b)** were acquired from A.G. Scientific, Inc. San Diego,CA.[15]

Enzyme inhibition assays were conducted in the laboratory of Professor L. Meijer following the protocol described in Ref. 13.

Acknowledgments

We thank Professor L. Meijer and Oliver Lozach, CNRS, Station Biologique, Roscoff, France, for performing he kinase assays that were supported by the Ministèrie de la Recherche/INSERM/CNRS 'Molècules et Cibles Thèrapeutiques' Programme. Financial support from the DGAPA, UNAM (Project PAPIIT-IN213407), is gratefully acknowledged. M.M.A thanks CONACYT for a scholarship and we also thank R. Patiño, H. Rios, A. Peña, N. Zavala, L. Velasco, and J. Pérez for technical assistance. Contribution No. 2648 from Instituto de Química, UNAM.

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