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Original article

Carboline- and phenothiazine-derivated heterocycles as potent SIGMA-1 protein ligands

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ABSTRACT

Sigma 1 receptors are associated with neurodegenerative and psychiatric disorders. These receptors, *via* their chaperoning functions that counteract endoplasmic reticulum stress and block neurodegeneration, may serve as a target for a new generation of antidepressants or neuroprotective agents. The involvement of these receptors has also been observed in neuropathic pain and cancer. Only a few ligands, such as Igmesine and Anavex 2-73, have been involved in clinical trials. Thus the development of sigma 1 ligands is of interest to a new generation of drugs. Previous work in our lab underlined the potency of benzannulated bicyclic compounds as interesting ligands. Herein the work was extended to a series of novel tricyclic compounds. Carboline- and phenothiazine-derivated compounds were designed and synthesized. *In vitro* competition binding assays for sigma 1 and 2 receptors showed that most of them have high affinity for sigma 1 receptor ($K_i = 2.5–18$ nM), and selectivity toward sigma 2 receptor, without cytotoxic effects on SY5Y cells.

1. Introduction

Sigma 1 receptors (S1R) are unique non-opioid receptors located at the endoplasmic reticulum (ER)–mitochondrion interface known as the MAM (mitochondrion associated endoplasmic reticulum membrane) [1,2]. They are ubiquitously expressed in the central nervous system, but are also found in peripheral organs including lung, liver, kidney, eye and heart [1]. One S1R subtype was cloned in 1996 [3]. It is an integral membrane protein of 223 amino acids with three hydrophobic domains [1]. In contrast, sigma 2 receptors (S2R) have not been cloned yet.

S1R, as an ER chaperone protein, is regulated by its association with another chaperone protein, BiP (or GRP 78). S1R regulates intra-mitochondrial calcium homoeostasis, ER stress and signal transduction. Indeed, when ER Ca^{2+} is depleted, S1R chaperone IP_3 receptors localize at the MAM to ensure Ca^{2+} transmission between the ER and mitochondria [4]. S1R are chaperones that correct misfolded proteins, stabilize other ER proteins and regulate ER-associated degradation (ERAD) [5]. Moreover, S1R localization is dynamic, and S1R are able to translocate from the MAM to the plasma membrane, where they may regulate a variety of functional proteins, including ion channels, receptors and kinases. Thus these receptors are involved in the regulation of numerous neurotransmitter systems [4].

The involvement of S1R in depression [6,7], drug abuse [8], neurodegenerative diseases [9,10], but also in pain [11,12] and cancer proliferation [13], has been reviewed.

Several compounds have undergone clinical trials, but no selective S1R ligands have so far been marketed, although many drugs on the market show affinity for S1R. The selective S1R ligand Igmesine has been studied in phase 3 clinical trials for depression [14]. A phase 2 study is underway to evaluate the mixed muscarinic-S1R agonist Anavex 2-73 in patients with Alzheimer's disease [15,16].

In previous work in our laboratory, a series of tetrahydroisoquinoline-hydantoin (tic-hydantoin)–derived

Abbreviations: ER, endoplasmic reticulum; MAM, mitochondrion associated endoplasmic reticulum membrane; S1R, sigma 1 receptors; S2R, sigma 2 receptors; BiP, immunoglobulin heavy-chain binding protein; GRP78, glucose-regulated protein 78.

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compounds were described, with potent affinity for S1R in the nanomolar range, high selectivity toward S2R and low cytotoxicity (IC_{50} > 100 μM), underlining the interest of compounds **1** and **2** (Fig. 1) [17–20]. These ligands were evaluated in different pharmacological models, and compound **1** showed high efficiency in a cocaine addiction model [21], ischaemia and experimental autoimmune encephalomyelitis [22].

Tic-hydantoin derivatives **1** showed all of the properties compatible with development except metabolic stability. The majority of metabolites resulted from tic-hydantoin instability. However, demethylated and debenzylated compounds have been identified among all the metabolites of compound **1** (unpublished data). An isoindoline moiety was therefore introduced to avoid this metabolic instability (compound **2**).

To solve this problem of instability, we looked for Tic-hydantoin substitutes. Considering the hydrophobic moiety of the S1R pharmacophore [23], the interest of heterocycles such as benzomorphanes, benzofurans [24], benzothiazolinones [25] and benzoxazolinones [26] has been underlined. We recently described benzannulated derivatives as potent and selective S1R ligands [27]. These compounds included benzoxazolinone, benzoxazinone, benzothiazinone, benzimidazolone and benzimidazole as heterocycles with propylbenzylmethylamine and propylisoindoline.

In this paper, we report our efforts to replace the tic-hydantoin core with different tricyclic heterocycles. We decided to focus our attention on the evaluation of fused cyclopentabenzoxazole as tricyclic analogues of our previously described bicyclic ligands, carboline and phenothiazine moieties. In the light of our previous studies, we selected 3-(N-benzyl-N-methylamino)propyle and 3-(isoindolin-2-yl)propyle moieties, already introduced on derivatives **1**, **2** [18,20]. 3-(N-phenylethyl-N-methylamino)propyle and 3-(pyrrolidin-1-yl)propyle were also selected for this study [27].

2. Chemistry

A series of tricyclic derivatives was synthesized by nucleophilic substitution of amino heterocycles with chloroalkylamino side chains (Scheme 1). The heterocycles (**a**–**h**) were either commercially available, such as 2,3,4,9-tetrahydro-*1H*-pyrido[3,4-b] indole (beta carboline, **a**), phenoxazine (**e**) and phenothiazine (**f**), or synthesized (Scheme 1). 1-Phenyl-2,3,4,9-tetrahydro-*1H*-pyrido[3,4-b] indole (**b**) was synthesized, starting from tryptamine, benzaldehyde and trifluoroacetic acid in dichloromethane, by Pictet–Spengler reaction [28]. 2,3,4,5-Tetrahydro-*1H*-pyrido [4,3-b]indole (gamma-carboline, **c**) was prepared in one step, starting from phenyl hydrazine and 4-piperidinone in ethanol [29]. 1,2,3,4-Tetrahydrobenzofuro[3,2-c]pyridine (**d**) was synthesized in two steps from the corresponding O-phenylhydroxylamine and ethyl-4-oxopiperidine-1-carboxylate in ethanol, followed by deprotection of the carbamate to generate the tricyclic derivative [30]. The tricyclic cyclopentabenzoxazolone derivatives (**g**–**h**) were prepared in three steps starting from benzoxazolone in a Friedel–Crafts reaction with a mixture of aluminium trichloride in DMF and acetic acid chloride. Crotonization of the resulting derivatives with potassium hydroxide and the corresponding benzaldehyde in ethanol, followed by cyclization in polyphosphoric acid, yielded the tricyclic benzoxazolone derivatives (**g**–**h**) [31].

According to the general procedure shown in Scheme 2, a library of 22 novel tricyclic derivatives was synthesized.

Starting from 3-bromo-1-chloropropane, reaction with appropriate amines yielded amino side chains **4a**–**c** and **5**. These intermediates were prepared in CH_3CN or DMF in the presence of K_2CO_3 with the appropriate substituted amine such as *N*-methylbenzylamine, *N*-methyl-*N*-phenylethylamine, pyrrolidine or isoindoline (yields: 30–95%). The preparation of compounds **6**–**9** involved direct nucleophilic substitution using eight tricyclic scaffolds (**a**–**h**) under various conditions (procedure A, B, C) with different bases (K_2CO_3, NaOH, NaH), in CH_3CN or DMF. Tricyclic moieties were all introduced on disubstituted amine (**6a**–**f**, **7a**–**f**, **9a**–**f**), except for pyrrolidine (**8a**–**b**) and tricyclic benzoxazolone (**6g**–**h**), to give final compounds.

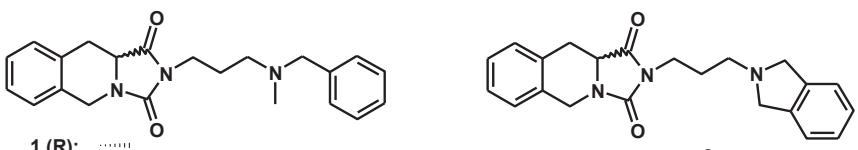
Procedure A (K_2CO_3, CH_3CN) was adopted with tricyclic compounds **a**–**d**, **g**–**h** and gave acceptable yields. This procedure A was also applied to tricyclic scaffold **c** to afford compound **6c** with very low yield (3%), indeed we observed dramatic degradation in this reaction. For the synthesis of compounds **7c** and **9c**, procedure B was applied (NaOH, H_2O, CH_3CN), which gave better yields (respectively 15% and 26%) with fewer degradation. For tricyclic compounds **e** and **f**, a stronger base was necessary, procedure C (NaH, DMF) was thus applied.

The structures of all final compounds were determined by ^1H NMR, ^{13}C NMR and LC-MS analyses. Purity was evaluated under two different HPLC conditions, and exceeded 96%. Results are presented in the experimental section (SI). For the pharmacological evaluation, all final products were converted into their water soluble hydrochloride salts.

3. Results and discussion

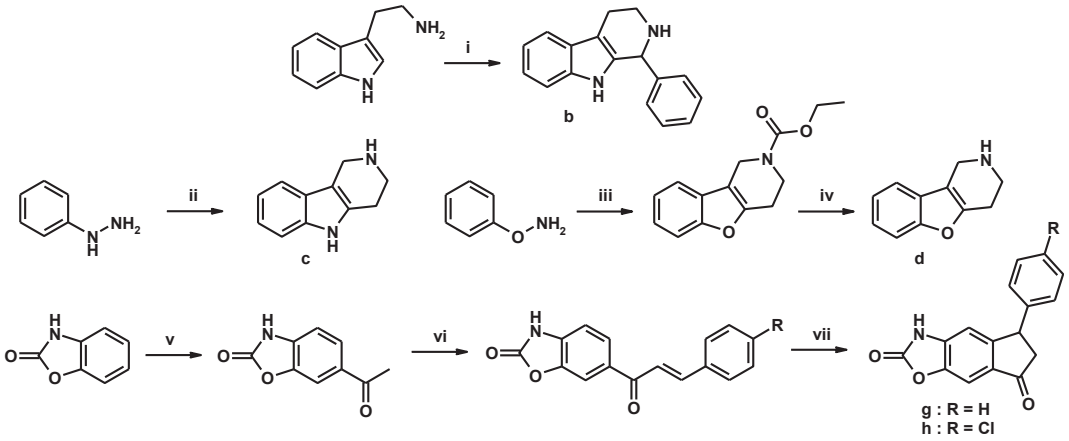
3.1. Biological evaluations and SAR

S1R and S2R affinities were investigated in competition experiments with radioligands according to the methods described by Ganapathy et al. [32]. In the S1R assay, the selective ligand [^3H](+)-pentazocine was employed as the radioligand. Since a selective S2R radioligand was not commercially available, the non-selective ligand [^3H]-DTG was employed in the S2R assay, in the presence of an excess of non-tritiated (+)-pentazocine, which selectively occupies S1R. In both assays, Jurkat cell membranes were used as a source of receptors.



Ki (nM)	S1R	S2R	S2R/S1R
1 (R)	7.1	1000	141
1 (S)	4.5	496	110
2	5.3	545	103

Fig. 1. Structure of Tic-hydantoin-derivated compounds **1** and **2**.

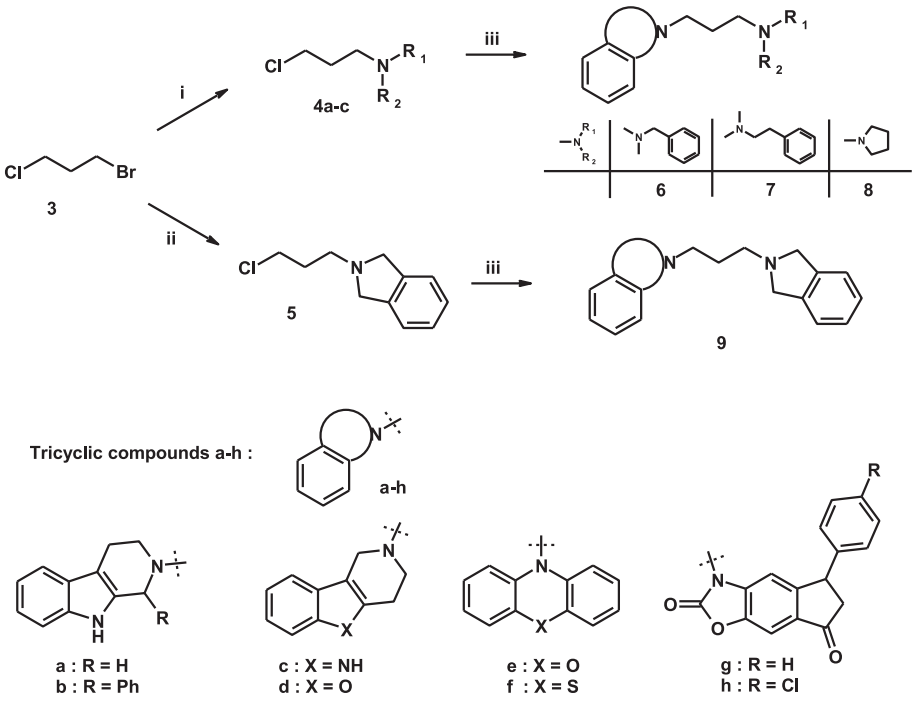


Reagents and conditions: *i*: benzaldehyde, trifluoroacetic acid, CH_2Cl_2, rt, 16h (70%); *ii*: 4-piperidonone, absolute ethanol, rt, 2h (64%); *iii*: ethyl-4-oxopiperidin-1-yl carboxylate, HCl, absolute ethanol, reflux, 2h (84%); *iv:* potassium hydroxide, water, ethanol, reflux, 24h (94%); *v:* CH_3COCl, $AlCl_3$, DMF, 80°C, 4h (75%); vi: potassium hydroxide, water, ethanol, rt, 16h (R= H, Cl: 85%); vii: polyphosphoric acid, 120°C, 1h (R= H: 70% and R= Cl: 30%).

Scheme 1. Preparation of tricyclic compounds **b**, **d**, **g**, **h.**

The K_i values for S1R were determined from the corresponding IC_{50} values for each compound **6**–**9**. For compounds showing high affinity for S1R, the K_i values for S2R and the S2R/S1R selectivity ratios were also calculated (Table 1).

The tic-hydantoin moiety was replaced with various tricyclic scaffolds such as beta- or gamma-carboline, tetrahydrobenzofuropyridine, phenoxazine, phenothiazine and cyclopentabenzoxazolone. From the results obtained, it appears that the replacement of the tic-hydantoin core with different tricyclic derivatives can strongly affect the affinity for S1R and S2R. Indeed, although a variety of modulations leads to a dramatic loss of S2R/S1R selectivity compared to the lead compounds **1** and **2**, high affinity for S1R is often maintained and sometimes improved.



Reagents and conditions: *i*: *N*-methyl-*N*-benzylamine, or *N*-methyl-*N*-phenylethylamine, or pyrrolidine (0.34 eq), K_2CO_3 (0.66 eq), DMF, 70°C to rt, 24h (95%); *ii*: isoindoline (0.18 eq), K_2CO_3 (0.36 eq), CH_3CN, 70°C to rt, 24 h (77%); *iii*: 3-chloropropan-1-amine derivative **4a-c** or **5** (1.2 eq), various heterocycles **a-h** (1 eq), K_2CO_3, NaOH or NaH, DMF or CH_3CN, 70°C.

Scheme 2. Synthesis of final compounds **6a**–**h**, **7a**–**f**, **8a**–**b**, **9a**–**f**.

Table 1
Affinity for S1R and S2R, and cytotoxicity evaluation of target compounds **6**–**9**.

Compound	K_i (nM)		K_i (σ_2)/K_i (σ_1)	IC_{50} (μM) SY5Y	IC_{50}/K_i (σ_1)
	σ_1	σ_2			
Haloperidol	11.9	175	15	–	–
1(S)	4.5	496[a]	110	>100	>20,000
1(R)	7.1	764[a]	108	19.5	2746
2	5.3	416[a]	78	nd	nd
6a	6.2	15	2.4	19.7	3177
6b	4.3	100	23	9.3	2163
6c	18	130	7	16.9	939
6d	2.0	<10	<5	68.9	34,450
6e	>200	180	<1	71.9	<350
6f	28	280	10	17.1	611
6g	72	180	2.5	>100	>1389
6h	160	50	0.3	5.0	31
7a	7.2	91	13	11.6	1611
7b	11	93	8	11.8	1073
7c	11	200	18	33.2	3018
7d	2.5	78	31	25.4	10,160
7e	18	80	4	46.9	2606
7f	11	310	28	8.6	782
8a	>200	>400	–	32.9	<160
8b	36	310	9	47.8	1328
9a	8.0	110	14	24.9	3112
9b	8.4	110	13	18.3	2179
9c	72	180	2.5	50.0	694
9d	11	9.9	0.9	78.9	7173
9e	>200	140	<1	11.0	<55
9f	12	94	8	83.3	6942

Mean IC_{50} values for 2–3 independent experiments are shown with less than 10% deviation.
[a] Rat cerebral cortex membranes were used as a source of σ_2 receptors. nd: Not determined.

The nature of the tricyclic moieties influences affinity for S1R and S2R, and therefore the S2R/S1R selectivity. In the beta-carboline series, with or without a phenyl ring at the 1 position, compounds **6a**–**b**, **7a**–**b**, and **9a**–**b** displayed good affinity for S1R (K_i = 4.3–11 nM) and moderate affinity for S2R (K_i = 91–110 nM), except for derivative **6a** (K_i = 15 nM). The S2R/S1R selectivity of these compounds (from 2.4 to 23) was lower than the tic-hydantoin derivatives (greater than 103). For beta-carboline derivatives with pyrrolidine, the affinity for S1R was low for **8a** (K_i > 200 nM) and moderate for **8b** (K_i = 36 nM). Despite a moderate affinity, this last compound displayed S2R/S1R selectivity (9) due to a low affinity for S2R (K_i = 310 nM). Therefore, the pyrrolidine side chain was not introduced on the other heterocycles. Substitution with gamma-carboline as heterocycle gave compounds **6c**, **7c**, and **9c**. While derivatives **6c** and **7c** showed good affinity for S1R, with K_i values of 18 and 11 nM respectively, compound **9c** showed only moderate affinity (K_i = 72 nM). Compared to the beta-carboline series, modulation of the position of the nitrogen atom with the gamma-carboline derivatives induced lower affinity for S1R and S2R of compounds **6c**, **7c**, and **9c** (K_i = 130–200 nM), but similar S2R/S1R selectivity (values from 2.5 to 18). Replacement of the NH group in the gamma-carboline by an oxygen atom resulted in the tetrahydrobenzofuropyridine series. This series showed high affinity for S1R, with K_i values between 2.0 and 11 nM for compounds **6d**, **7d** and **9d**. Compared to the gamma carboline series, replacement of the NH group by an oxygen atom in the tetrahydrobenzofuropyridine compounds induced a significant increase in affinity for S1R, but mainly for S2R, with a K_i value lower than 10 nM and a K_i value of 9.9 for **6d** and **9d** respectively, leading to a loss of S2R/S1R selectivity (<5 and 0.9). Therefore, among the 22 evaluated tricyclic compounds, it is worth emphasizing that the best selectivity (S2R/S1R = 31) was obtained for compound **7d** (K_i (S1R) = 2.5 nM and K_i (S2R) = 78 nM). It has to be noted that better selectivity was obtained in our previously described Tic-hydantoin series (S2R/S1R = 141 for compound **1(*R*)**) [17–19], or even in spiro[2]benzopyran-1,4′-piperidine series (S2R/S1R superior to 1000) [33].

Using phenoxazine as the heterocyclic moiety gave disappointing results, with low affinity for S1R (K_i > 200 nM), and without selectivity for compounds **6e** and **9e**. Derivative **7e** showed good affinity for S1R (K_i = 18 nM), and moderate affinity for S2R (K_i = 80 nM); however it showed very low S2R/S1R selectivity (4). Replacement of the oxygen atom by a sulphur atom resulted in compounds **6f**, **7f** and **9f**. Compared to the phenoxazine series, phenothiazine compounds **6f**, **7f** and **9f** showed better affinity for S1R (K_i = 11–28 nM) and S2R/S1R selectivity (8–28). Compounds **6g**–**h**, with cyclopentabenzoxazole moieties, displayed disappointing affinity for S1R (K_i = 72–160 nM) and no S2R/S1R selectivity (0.3–2.5). We observed that introduction of a chlorine atom on compound **6h**, compared to **6g**, induced more affinity for S2R (K_i = 50 nM) than for S1R (K_i = 160 nM). Consequently, the other disubstituted amines (**7**–**9**) were not introduced on tricyclic benzoxazolone moieties **g** and **h**. The addition of a cyclopentyle ring to afford the cyclopentabenzoxazole scaffold induced a dramatic loss of affinity for S1R and selectivity for S2R compared to our previously reported benzoxazolinone compounds [27].

Among the *N*-methylbenzylamine, *N*-methyl-*N*-phenylethylamine and isoindoline moieties, *N*-methyl-*N*-phenylethylamine gave the best S2R/S1R selectivity (31 for **7d** and 28 for **7f**) in the tricyclic series (**7a**–**f**), with good affinity for S1R (K_i = 2.5–18 nM) and moderate affinity for S2R (K_i = 78–310 nM).

3.2. Evaluation of cytotoxic effects

To determine the potential cytotoxic effects of our synthetic derivatives, the human neuroblastoma cell line SY5Y was treated with the whole compounds at different concentrations up to 100 μM. Cell viability was calculated using a colorimetric MTT assay. All tricyclic derivatives exhibited moderate to low cytotoxicity (Table 1). Compound **6h** showed the highest cytotoxicity, 5.0 μM, with a low selectivity index, $IC_{50(SY5Y)}/K_{i\ (S1R)}$ ratio of 31. Compound **6b** showed high cytotoxicity, 9.3 μM, but a very good selectivity index, $IC_{50(SY5Y)}/K_{i\ (S1R)}$ ratio of 2163. Excellent selectivity indexes were obtained with compounds **6d**, **7d** and **9d**, with ratios of 7173 to 34,450. All these derivatives contained tetrahydrobenzofuropyridine as the tricyclic moiety. The gamma-carboline series, with compounds **6c** and **9c**, showed low selectivity index, 939 and 694 respectively. Derivatives **6e**, **6h**, **8a** and **9e** showed both low affinities for S1R (superior to 160 nM) and low selectivity index ($IC_{50(SY5Y)}/K_{i\ (S1R)}$ ratio: 31–350). Finally, compounds (**6a**–**b**, **7a**–**b**, **8a**–**b** and **9a**–**b**), with a beta-carboline moiety, have intermediate but high selectivity index, with values from 1073 to 3177.

4. Conclusion

S1R is an ER chaperone protein that has been implicated in many neurodegenerative and neuropsychiatric disorders, and in the response to cellular oxidative stress. Thus, its ligands may be of interest as therapeutic agents. We synthesized compounds with tricyclic scaffolds and various disubstituted amines, and evaluated their affinity for sigma receptors and their cytotoxic effect on neuronal cells. This study completed our previously work on benzannulated bicyclic compounds as interesting S1R ligands. Most of these tricyclic compounds showed high affinity for S1R and very low cytotoxicity. Their selectivity indexes against S2R, ranging from 1 to 31, provide non-selective and selective sigma-1 ligands respectively. This study identified compound **7d** as a very efficient and non-cytotoxic derivative. Though it has moderate selectivity

for S2R, this compound **7d** was selected for further *in vivo* evaluations. Work is in progress.

5. Experimental section

5.1. Chemistry

5.1.1. General

Chemicals and solvents were obtained from commercial sources, and used without further purification unless otherwise noted. Reactions were monitored by TLC performed on Macherey–Nagel Alugram® Sil 60/UV_{254} sheets (thickness 0.2 mm). Purification of products was carried out by either column chromatography or thick layer chromatography. Column chromatography was carried out on using Macherey–Nagel silica gel (230–400 mesh). Thick layer chromatography was performed on glass plates coated with Macherey–Nagel Sil/UV_{254} (thickness 2 mm), from which the pure compounds were extracted with the following solvent system: DCM/MeOH(NH_3), 90:10. NMR spectra were recorded on a Bruker DRX 300 spectrometer (operating at 300 MHz for ^{1}Hand 75 MHz for ^{13}C). Chemical shifts are expressed in ppm relative to either tetramethylsilane (TMS) or to residual proton signal in deuterated solvents. Chemical shifts are reported as position (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = double doublet, br = broad and m = multiplet), coupling constant (J in Hz), relative integral and assignment. The attributions of protons and carbons were achieved by analysis of 2D experiments (COSY, HSQC and HMBC). Mass spectra were recorded on a Varian triple quadrupole 1200 W mass spectrometer equipped with a non-polar C18 TSK-gel Super ODS (4.6 × 50 mm) column, using electrospray ionisation and a UV detector (diode array). The purity of final compounds was verified by two types of high pressure liquid chromatography (HPLC) columns: C18 Interchrom UPTISPHERE and C4 Interchrom UPTISPHERE. Analytical HPLC was performed on a Shimadzu LC-2010AHT system equipped with a UV detector set at 254 nm and 215 nm. Compounds were dissolved in 50 μL methanol and 950 μL buffer A, and injected into the system. The following eluent systems were used: buffer A (H_2O/TFA, 100:0.1) and buffer B (CH_3CN/H_2O/TFA, 80:20:0.1). HPLC retention times (HPLC t_R) were obtained at a flow rate of 0.2 mL/min for 35 min using the following conditions: a gradient run from 100% of buffer A over1 min, then to 100% of buffer B over the next 30 min.

5.1.2. General procedure for synthesis of compounds **4a–c**

A 1.00 g (16.9 mmol) amount of *N*-methylbenzylamine, *N*-methyl-*N*-phenylethylamine, or pyrrolidine was dissolved in 25 mL of DMF. After addition of potassium carbonate (4.6 g, 33.0 mmol), the resulting mixture was heated at 70 °C. After 30 min, the solution was allowed to cool to room temperature. A 4.9 mL (49.5 mmol) amount of 1-bromo-3-chloropropane was added and the reacting mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and 60 mL water added to the residue. The product was extracted with 3 × 30 mL of dichloromethane. The combined organic fractions were washed with water and dried over sodium sulphate. The solvent was evaporated under reduced pressure. Purification by column chromatography (DCM:MeOH(NH_3), 99:1 (v/v)) was performed and enabled to collect the product as an oil.

5.1.2.1. N-Benzyl-3-chloro-N-methylpropan-1-amine **4** [34]. Purification by column chromatography (DCM) was performed and enabled collection of the product as a colourless oil (3.2 g, 95%). TLC: R_f 0.4 (DCM:MeOH(NH_3), 99:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40–7.22 (m, 5H, H_{aro}); 3.65 (t, J = 7.1 Hz, 2H, CH_2); 3.52 (s, 2H, CH_2); 2.55 (t, J = 7.0 Hz, 2H, CH_2); 2.21 (s, 3H, CH_3); 1.98 (p, J = 7.1 Hz, 2H, CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 198.09; 200.09. Found: 198.01; 199.96. HPLC (C_4, 35 min): t_R 8.01 min, P_{HPLC} 96%; HPLC (C_{18}, 35 min): t_R 14.69 min, P_{HPLC} 96%.

5.1.2.2. 3-Chloro-N-methyl-N-phenylethylpropan-1-amine **4b** [27]. Purification by column chromatography (DCM:MeOH(NH_3), 97:3 (v/v)) was performed and enabled to collect the product as a yellowish liquid (2.28 g, 65%). TLC: R_f 0.7 (DCM:MeOH(NH_3), 90:10, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33–7.27 (m, 2H, H_{aro}); 7.42–7.16 (m, 3H, H_{aro}); 3.57 (t, J = 7.2 Hz, 2H, CH_2); 2.78 (m, 2H, CH_2); 2.63 (m, 2H, CH_2); 2.56 (t, J = 7.1 Hz, 2H, CH_2); 2.31 (s, 3H, CH_3); 1.93 (p, J = 7.0 Hz, 2H, CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 212.1; 214.1; Found: 212.1; 214.1.

5.1.2.3. 1-(3-Chloropropyl)pyrrolidine **4c** [27,35]. Purification by column chromatography (DCM:MeOH(NH_3), 99:1 (v/v)) was performed and enabled to collect the product as a brown oil (703 mg, 30%). TLC: R_f 0.5 (DCM:MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 3.60 (t, J = 6.9 Hz, 2H, CH_2); 2.85 (t, J = 7.0 Hz, 2H, CH_2); 2.49 (m, 4H, 2 CH_2); 1.98 (p, J = 7.1 Hz, 2H, CH_2); 1.78 (m, 4H, 2 CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 148.1; 150.1; Found: 148.0; 150.0.

5.1.3. N-(3-Chloropropyl)isoindoline **5** [36]

A 1.0 g (8.4 mmol) amount of isoindoline was dissolved in 25 mL of acetonitrile. After addition of potassium carbonate (2.3 g, 16.8 mmol), the resulting mixture was heated at 70 °C. After 30 min, the solution was allowed to cool to room temperature. A 4.2 mL (47.2 mmol) amount of 1-bromo-3-chloropropane was added and the reaction mixture stirred at room temperature for 24 h. The solvent was removed under reduced pressure and 60 mL water added to the residue. The product was extracted with 3 × 30 mL dichloromethane. The combined organic fractions were washed with water and dried over sodium sulphate. The solvent was evaporated under reduced pressure. Purification by column chromatography (DCM:MeOH(NH_3), 99:1 (v/v)) was performed and enabled collection of the product as a brown liquid (1.27 g, 77%). TLC: R_f 0.7 (DCM:MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.21 (s, 4H, H_{aro}); 3.95 (s, 4H, 2 CH_2); 3.69 (t, J = 6.8 Hz, 2H, CH_2); 2.90 (t, J = 7.1 Hz, 2H, CH_2); 2.07 (p, J = 7.0 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.0 (2 C_{aro}); 126.7 (2 C_{aro}); 122.3 (2 C_{aro}); 59.1 (2 CH_2); 53.0 (CH_2); 43.1 (CH_2); 31.9 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 196.05; 198.07. Found: 195.95; 197.91.

5.1.4. General procedure for final compounds

5.1.4.1. Procedure A. One equivalent of appropriate heterocyclic derivative was dissolved in acetonitrile. Three equivalents of potassium carbonate and 1.2 equivalents of appropriate 3-chloropropan-1-amine derivative were added. The resulting mixture was heated at 70 °C until the disappearance of starting material. The reaction was monitored by TLC. After 24–96 h, the solvent was removed under reduced pressure and a 60 mL amount of water added to the residue. The crude product was extracted with dichloromethane. The combined organic fractions were washed with water and dried over magnesium sulphate. Purification by thick layer chromatography or column chromatography was performed.

5.1.4.2. Procedure B. One equivalent of appropriate heterocyclic derivative and 1.2 equivalents of appropriate 3-chloropropan-1-amine derivative were dissolved in a mixture of water:-acetonitrile (2:1, v/v). Three equivalents of sodium hydroxide were added. The resulting mixture was heated at 50 °C until the disappearance of starting material. The reaction was monitored by TLC. After 24–48 h, the product was extracted with ethyl acetate. The combined organic fractions were washed with water and dried over

magnesium sulphate. Purification by thick layer chromatography or column chromatography was performed.

5.1.4.3. Procedure C. The reaction was carried out under a nitrogen atmosphere. One equivalent of appropriate heterocyclic derivative was dissolved in anhydrous DMF. Four equivalents of sodium hydride were added carefully and the resulting mixture was stirred at room temperature for 30 min. Then 1.2 equivalents of appropriate 3-chloropropan-1-amine derivative were added. The reacting mixture was stirred at room temperature until the disappearance of starting material. The reaction was monitored by TLC. The reaction was quenched with 50 mL of water and the crude product extracted with ethyl acetate. The combined organic fractions were washed with water and dried over magnesium sulphate. Purification by column chromatography or thick layer chromatography was performed.

5.1.4.3.1. 2-[3-(N-benzyl-N-methylamino)propyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***6a***. Procedure A. The compound was purified by column chromatography (DCM: MeOH(NH_3), 9:1, v/v) and obtained as orange solid (202 mg, 35%). Melting point: 112 °C. TLC: R_f 0.5 (DCM:MeOH(NH_3), 8:2, v/v). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 10.71 (br s, 1H, NH); 7.37–7.19 (m, 7H, H_{aro}); 7.04–6.90 (m, 2H, H_{aro}); 3.60 (br s, 2H, CH_2); 3.48 (s, 2H, CH_2); 2.74 (m, 2H, CH_2); 2.66 (m, 2H, CH_2); 2.52 (t, J = 7 Hz, 2H, CH_2); 2.42 (t, J = 7 Hz, 2H, CH_2); 2.14 (s, 3H, CH_3); 1.73 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 139.6 (C_{aro}); 136.4 (C_{aro}); 133.3 (C_{aro}); 129.2 (2 C_{aro}); 128.6 (2 C_{aro}); 127.3 (C_{aro}); 127.1 (C_{aro}); 120.7 (C_{aro}); 118.7 (C_{aro}); 117.8 (C_{aro}); 111.4 (C_{aro}); 106.9 (C_{aro}); 62.0 (CH_2); 55.8 (CH_2); 55.3 (CH_2); 51.3 (CH_2); 50.6 (CH_2); 42.3 (CH_3); 25.1 (CH_2); 21.7 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 334.2; Found: 334.0. HPLC (C_4, 35 min): t_R 10.2 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 15.6 min, P_{HPLC} 97%.

5.1.4.3.2. 2-[3-(N-Benzyl-N-methylamino)propyl]-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***6b***. Procedure A. The compound was purified by thick layer chromatography (cyclohexane:ethyl acetate, 6:4 (v/v) + 0.1% MeOH(NH_3)) and obtained as yellow solid (160 mg, 47%). TLC: R_f 0.5 (CycloHex:EtOAc, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.54 (br s, 1H, NH); 7.42–7.23 (m, 11H, H_{aro}); 7.22–7.05 (m, 3H, H_{aro}); 4.60 (br t, J = 2 Hz, 1H, CH); 3.46 (s, 2H, CH_2); 3.35 (m, 1H, CH_2); 2.93 (m, 2H, CH_2); 2.79–2.62 (m, 2H, CH_2); 2.50–2.35 (m, 2H, CH_2); 2.27 (m, 1H, CH_2); 2.16 (s, 3H, CH_3); 1.79 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.4 (C_{aro}); 139.1 (C_{aro}); 136.2 (C_{aro}); 135.0 (C_{aro}); 129.1 (2 C_{aro}); 129.0 (2 C_{aro}); 128.6 (2 C_{aro}); 128.3 (2 C_{aro}); 127.2 (C_{aro}); 126.9 (C_{aro}); 121.5 (C_{aro}); 119.3 (C_{aro}); 118.3 (C_{aro}); 110.8 (C_{aro}); 109.1 (C_{aro}); 109.0 (C_{aro}); 64.7 (CH_2); 62.2 (CH_2); 55.4 (CH_2); 52.1 (CH_2); 48.4 (CH_2); 42.2 (CH_3); 25.0 (CH_2); 21.3 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 410.2; Found: 410.0. HPLC (C_4, 35 min): t_R 19.4 min, P_{HPLC} 96%; HPLC (C_{18}, 35 min): t_R 18.0 min, P_{HPLC} 97%.

5.1.4.3.3. 2-[3-(N-Benzyl-N-methylamino)propyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole ***6c***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 95:5 to 94:6 (v/v)) and obtained as a yellow oil (15 mg, 3%). TLC: R_f 0.4 (DCM: MeOH(NH_3), 8:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.16 (br s, 1H, NH); 7.40 (m, 1H, H_{aro}); 7.37–7.22 (m, 6H, H_{aro}); 7.17–7.03 (m, 2H, H_{aro}); 3.78 (br s, 2H, CH_2); 3.55 (s, 2H, CH_2); 2.97–2.81 (m, 4H, 2 CH_2); 2.73 (t, J = 7 Hz, 2H, CH_2); 2.52 (t, J = 7 Hz, 2H, CH_2); 2.26 (s, 3H, CH_3); 1.93 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 138.7 (C_{aro}); 136.1 (C_{aro}); 131.9 (C_{aro}); 129.2 (2 C_{aro}); 128.3 (2 C_{aro}); 127.2 (C_{aro}); 126.0 (C_{aro}); 121.3 (C_{aro}); 119.3 (C_{aro}); 117.5 (C_{aro}); 110.7 (C_{aro}); 108.0 (C_{aro}); 62.3 (CH_2); 55.9 (CH_2); 55.4 (CH_2); 50.7 (CH_2); 49.6 (CH_2); 42.2 (CH_3); 25.0 (CH_2); 23.5 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 334.2; Found: 334.0. HPLC (C_4, 35 min): t_R 5.3 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 14.9 min, P_{HPLC} 97%.

5.1.4.3.4. 2-[3-(N-Benzyl-N-methylamino)propyl]-1,2,3,4-tetrahydrobenzofuro[3,2-c]pyridine ***6d***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 99:1 (v/v)) and obtained as a yellowish oil (65 mg, 57%). TLC: R_f 0.4 (DCM:MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46–7.24 (m, 7H, H_{aro}); 7.23–7.18 (m, 2H, H_{aro}); 3.64 (br t, J = 2 Hz, 2H, H_1); 3.54 (s, 2H, CH_2); 2.94–2.85 (m, 4H, 2 CH_2); 2.68 (t, J = 7 Hz, 2H, CH_2); 2.50 (t, J = 7 Hz, 2H, CH_2); 2.25 (s, 3H, CH_3); 1.87 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.7 (C_{aro}); 152.1 (C_{aro}); 129.3 (2 C_{aro}); 128.4 (2 C_{aro}); 127.4 (C_{aro}); 127.1 (C_{aro}); 123.2 (C_{aro}); 122.4 (C_{aro}); 118.2 (2 C_{aro}); 111.6 (C_{aro}); 111.0 (C_{aro}); 62.0 (CH_2); 55.3 (CH_2); 55.2 (CH_2); 50.3 (CH_2); 48.7 (CH_2); 41.9 (CH_3); 25.1 (CH_2); 24.2 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 335.2; Found: 335.1. HPLC (C_4, 35 min): t_R 17.0 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 15.0 min, P_{HPLC} > 99%.

5.1.4.3.5. 10-[3-(N-Benzyl-N-methylamino)propyl]phenoxazine ***6e***. Procedure C. The compound was purified by column chromatography (CycloHex:EtOAc, 3:2 (v/v) + 0.1% MeOH(NH_3)) and obtained as a purple oil (219 mg, 58%). TLC: R_f 0.5 (CycloHex:EtOAc, 3:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.39–7.29 (m, 5H, H_{aro}); 6.81–6.50 (m, 8H, H_{aro}); 3.58 (t, J = 7 Hz, 2H, CH_2); 3.54 (s, 2H, CH_2); 2.50 (t, J = 7 Hz, 2H, CH_2); 2.26 (s, 3H, CH_3); 1.85 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.0 (2 C_{aro}); 133.2 (2 C_{aro}); 129.7 (C_{aro}); 128.7 (2 C_{aro}); 123.7 (3 C_{aro}); 121.0 (3 C_{aro}); 115.5 (3 C_{aro}); 111.4 (2 C_{aro}); 61.4 (CH_2); 53.8 (CH_2); 41.5 (CH_2); 41.4 (CH_3); 22.1 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 345.2; Found: 345.2. HPLC (C_4, 35 min): t_R 14.0 min, P_{HPLC} 98%; HPLC (C_{18}, 35 min): t_R 21.3 min, P_{HPLC} 98%.

5.1.4.3.6. 10-[3-(N-Benzyl-N-methylamino)propyl]phenothiazine ***6f***. Procedure C. The compound was purified by column chromatography (CycloHex:EtOAc, 3:2 (v/v) + 0.1% MeOH(NH_3)) and obtained as purple oil (238 mg, 66%). TLC: R_f 0.4 (CycloHex:EtOAc, 3:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.30–7.13 (m, 9H, H_{aro}); 6.97–6.88 (m, 4H, H_{aro}); 3.98 (t, J = 7 Hz, 2H, CH_2); 3.62 (s, 2H, CH_2); 2.67 (t, J = 7 Hz, 2H, CH_2); 2.26 (s, 3H, CH_3); 2.11 (p, ^3J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.1 (2 C_{aro}); 129.5 (2 C_{aro}); 129.2 (C_{aro}); 128.5 (2 C_{aro}); 127.7 (C_{aro}); 127.5 (2 C_{aro}); 127.3 (2 C_{aro}); 125.4 (2 C_{aro}); 122.6 (2 C_{aro}); 115.7 (2 C_{aro}); 61.6 (CH_2); 54.1 (CH_2); 44.8 (CH_2); 41.5 (CH_3); 23.9 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 361.1; Found: 361.2. HPLC (C_4, 35 min): t_R 14.6 min, P_{HPLC} 96%; HPLC (C_{18}, 35 min): t_R 24.7 min, P_{HPLC} 97%.

5.1.4.3.7. 3-[3-(N-Benzyl-N-methylamino)propyl]-2,3-dihydro-2,5-dioxo-7-phenylcyclopenta-benzoxazole ***6g***. Procedure A with DMF. The compound was purified by column chromatography (petroleum ether:ethyl acetate, 7:3 (v/v) + 0.1% MeOH(NH_3)) and obtained as a beige solid (190 mg, 39%). Melting point: 93 °C.TLC: R_f 0.2 (PE:EtOAc 7:3, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.56 (s, 1H, H_{aro}); 7.39–7.14 (m, 8H, H_{aro}); 7.08 (dd, J = 8 Hz, J = 2 Hz, 2H, H_{aro}); 6.85 (s, 1H, H_{aro}); 4.54 (dd, J = 8 Hz, J = 3 Hz, 1H, CH_2); 2.88 (m, 2H, CH_2); 3.38 (d, J = 5 Hz, 2H, CH_2); 3.26 (dd, J = 19 Hz, J = 4 Hz, 1H, CH_2); 2.71 (dd, J = 19 Hz, J = 4 Hz, 1H, CH_2); 2.38 (t, J = 7 Hz, 2H, CH_2); 2.02 (s, 3H, CH_3); 1.91 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 204.0 (CO); 155.4 (C_{aro}); 154.3 (CO); 143.4 (C_{aro}); 142.8 (C_{aro}); 138.7 (C_{aro}); 138.2 (C_{aro}); 131.5 (C_{aro}); 129.1 (2 C_{aro}); 128.8 (2 C_{aro}); 128.3 (2 C_{aro}); 127.5 (2 C_{aro}); 127.2 (C_{aro}); 127.1 (C_{aro}); 105.6 (C_{aro}); 104.1 (C_{aro}); 62.0 (CH_2); 53.9 (CH_2); 47.0 (CH_2); 44.5 (CH_2); 41.9 (CH_3); 40.6 (CH_2); 25.4 (CH_2). LCMS (ESI$^+$): Calc. for [M+H]: 427.19; Found: 427.01. HPLC (C_4, 35 min): t_R 21.8 min, P_{HPLC} 99%; HPLC (C_{18}, 35 min): t_R 20.5 min, P_{HPLC} 99%.

5.1.4.3.8. 3-[3-(N-Benzyl-N-methylamino)propyl]-2,3-dihydro-2,5-dioxo-7-(4-chlorophenyl) cyclopentabenzoxazole ***6h***. Procedure A with DMF. The compound was purified by column chromatography (DCM:MeOH(NH_3), 98:2, v/v) and obtained as a yellow solid (150 mg, 27%). Melting point: 146 °C.TLC: R_f 0.5 (DCM:MeOH(NH_3), 98:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.57 (s,

1H, H_{aro}); 7.34–7.24 (m, 4H, H_{aro}); 7.25–7.15 (m, 3H, H_{aro}); 6.99 (d, $J = 8$ Hz, 2H, H_{aro}); 6.82 (s, 1H, H_{aro}); 4.50 (dd, $J = 8$ Hz, $J = 4$ Hz, 1H, CH_2); 3.88 (m, 2H, CH_2); 3.38 (d, $J = 4$ Hz, 2H, CH_2); 3.25 (dd, $J = 19$ Hz, $J = 8$ Hz, 1H, CH_2); 2.65 (dd, $J = 19$ Hz, $J = 4$ Hz, 1H, CH_2); 2.39 (t, $J = 7$ Hz, 2H, CH_2); 2.02 (s, 3H, CH_3); 1.91 (p, $J = 7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 203.5 (CO); 154.8 (C_{aro}); 154.3 (CO); 142.9 (C_{aro}); 141.9 (C_{aro}); 138.7 (C_{aro}); 138.5 (C_{aro}); 133.0 (C_{aro}); 131.5 (C_{aro}); 129.2 (2 C_{aro}); 128.9 (2 C_{aro}); 128.8 (C_{aro}); 128.3 (2 C_{aro}); 127.1 (2 C_{aro}); 105.5 (C_{aro}); 104.2 (C_{aro}); 62.0 (CH_2); 53.9 (CH_2); 46.8 (CH_2); 43.9 (CH_2); 41.8 (CH_3); 40.7 (CH_2); 25.5 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 461.16; 463.16; Found: 461.01; 463.04. HPLC (C_4, 35 min): t_R 23.7 min, P_{HPLC} 96%; HPLC (C_{18}, 35 min): t_R 17.2 min, P_{HPLC} 96%.

5.1.4.3.9. 2-[3-(N-Methyl-N-phenylethylamino)propyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***7a***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 99:1 to 97:3 (v/v)) and obtained as an orange oil (300 mg, 51%). TLC: R_f 0.6 (DCM:MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.11 (br s, 1H, NH); 7.48 (m, 1H, H_{aro}); 7.34–7.27 (m, 3H, H_{aro}); 7.25–7.18 (m, 3H, H_{aro}); 7.17–7.08 (m, 2H, H_{aro}); 3.63 (br s, 2H, CH_2); 2.91–2.77 (m, 6H, 3 CH_2); 2.73 (m, 2H, CH_2); 2.62 (t, $J = 7$ Hz, 2H, CH_2); 2.55 (t, $^3J = 7$ Hz, 2H, CH_2); 2.36 (s, 3H, CH_3); 1.82 (p, $J = 7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.5 (C_{aro}); 136.2 (C_{aro}); 131.9 (C_{aro}); 128.7 (2 C_{aro}); 128.4 (2 C_{aro}); 127.2 (C_{aro}); 126.1 (C_{aro}); 121.3 (C_{aro}); 119.3 (C_{aro}); 118.0 (C_{aro}); 110.7 (C_{aro}); 108.3 (C_{aro}); 59.9 (CH_2); 55.9 (CH_2); 55.6 (CH_2); 51.3 (CH_2); 50.4 (CH_2); 42.2 (CH_3); 33.7 (CH_2); 25.3 (CH_2); 21.3 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 348.2; Found: 348.0. HPLC (C_4, 35 min): t_R 9.3 min, $P_{HPLC} > 99\%$; HPLC (C_{18}, 35 min): t_R 16.4 min, $P_{HPLC} > 99\%$.

5.1.4.3.10. 2-[3-(N-Methyl-N-phenylethylamino)propyl]-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***7b***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 98:2 to 96:4 (v/v)) and obtained as a yellow oil (135 mg, 38%). TLC: R_f 0.6 (DCM:MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.62 (m, 1H, H_{aro}); 7.40–7.28 (m, 9H, H_{aro} and NH); 7.26–7.08 (m, 6H, H_{aro}); 4.59 (br t, $J = 2$ Hz, 1H, CH); 3.35 (m, 1H, CH_2); 2.95 (m, 2H, CH_2); 2.82–2.58 (m, 7H, CH and 3 CH_2); 2.57–2.38 (m, 2H, CH_2); 2.32 (s, 3H, CH_3); 1.76 (p, $J = 7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.4 (C_{aro}); 140.3 (C_{aro}); 136.3 (C_{aro}); 134.9 (C_{aro}); 129.1 (2 C_{aro}); 128.7 (2 C_{aro}); 128.6 (2 C_{aro}); 128.4 (2 C_{aro}); 128.0 (C_{aro}); 127.1 (C_{aro}); 126.1 (C_{aro}); 121.5 (C_{aro}); 119.3 (C_{aro}); 118.3 (C_{aro}); 110.8 (C_{aro}); 109.0 (C_{aro}); 64.7 (CH_2); 59.4 (CH_2); 55.5 (CH_2); 52.0 (CH_2); 48.4 (CH_2); 42.0 (CH_3); 33.5 (CH_2); 24.8 (CH_2); 21.3 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 424.2; Found: 424.1. HPLC (C_4, 35 min): t_R 11.0 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 18.6 min, P_{HPLC} 96%.

5.1.4.3.11. 2-[3-(N-Methyl-N-phenylethylamino)propyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole ***7c***. Procedure B. The compound was purified by thick layer chromatography (DCM:MeOH(NH_3), 9:1 (v/v)) and obtained as a yellow oil (25 mg, 15%). TLC: R_f 0.3 (DCM:MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.96 (br s, 1H, NH); 7.41 (m, 1H, H_{aro}); 7.33–7.18 (m, 6H, H_{aro}); 7.17–7.05 (m, 2H, H_{aro}); 3.74 (s, br, 2H, CH_2); 2.95–2.79 (m, 6H, 3 CH_2); 2.73–2.65 (m, 4H, 2 CH_2); 2.58 (t, $J = 7.1$ Hz, 2H, CH_2); 2.39 (s, 3H, CH_3); 1.89 (p, $J = 7.3$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.2 (C_{aro}); 136.1 (C_{aro}); 131.9 (C_{aro}); 128.7 (2 C_{aro}); 128.4 (2 C_{aro}); 126.1 (C_{aro}); 126.1 (C_{aro}); 121.2 (C_{aro}); 119.3 (C_{aro}); 117.5 (C_{aro}); 110.6 (C_{aro}); 108.5 (C_{aro}); 59.4 (CH_2); 55.9 (CH_2); 55.6 (CH_2); 50.7 (CH_2); 49.6 (CH_2); 42.1 (CH_3); 33.5 (CH_2); 25.0 (CH_2); 23.6 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 348.2; Found: 348.0. HPLC (C_4, 35 min): t_R 8.3 min, P_{HPLC} 98%; HPLC (C_{18}, 35 min): t_R 16.1 min, P_{HPLC} 98%.

5.1.4.3.12. 2-[3-(N-Methyl-N-phenylethylamino)propyl]-1,2,3,4-tetrahydrobenzofuro[3,2-c]pyridine ***7d***. Procedure A. The compound was purified by thick layer chromatography (DCM:MeOH(NH_3), 9:1 (v/v)) and obtained as a colourless oil (71 mg, 60%). TLC: R_f 0.5 (DCM: MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49–7.14 (m, 9H, H_{aro}); 3.64 (br t, $J = 2$ Hz, 2H, CH_2); 3.00–2.73 (3, 8H, 4 CH_2); 2.71–2.67 (m, 4H, 2 CH_2); 2.51 (s, 3H, CH_3); 1.96 (p, $J = 7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.7 (C_{aro}); 152.1 (C_{aro}); 138.8 (C_{aro}); 128.7 (2 C_{aro}); 128.6 (2 C_{aro}); 127.1 (C_{aro}); 126.5 (C_{aro}); 123.3 (C_{aro}); 122.4 (C_{aro}); 118.2 (C_{aro}); 111.5 (C_{aro}); 111.0 (C_{aro}); 58.8 (CH_2); 55.2 (CH_2); 55.0 (CH_2); 50.4 (CH_2); 48.7 (CH_2); 41.6 (CH_3); 32.6 (CH_2); 24.3 (CH_2); 24.1 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 349.2; Found: 349.1. HPLC (C_4, 35 min): t_R 10.9 min, $P_{HPLC} > 99\%$; HPLC (C_{18}, 35 min): t_R 15.8 min, $P_{HPLC} > 99\%$.

5.1.4.3.13. 10-[3-(N-Methyl-N-phenylethylamino)propyl]phenoxazine ***7e***. Procedure C. The compound was purified by column chromatography (CycloHex:EtOAc, 3:2 (v/v) + 0.1% MeOH(NH_3)) and obtained as a yellow oil (200 mg, 51%). TLC: R_f 0.2 (CycloHex:EtOAc, 3:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.33–7.18 (m, 5H, H_{aro}); 6.82–6.74 (m, 2H, H_{aro}); 6.68–6.61 (m, 4H, H_{aro}); 6.54–6.50 (m, 2H, H_{aro}); 3.53 (t, $J = 7$ Hz, 2H, CH_2); 2.83 (m, 2H, CH_2); 2.65 (m, 2H, CH_2); 2.54 (t, $J = 7$ Hz, 2H, CH_2); 2.35 (s, 3H, CH_3); 1.81 (p, $J = 7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.0 (2 C_{aro}); 140.0 (C_{aro}); 133.3 (2 C_{aro}); 128.7 (2 C_{aro}); 128.5 (2 C_{aro}); 126.2 (2 C_{aro}); 123.7 (C_{aro}); 120.8 (2 C_{aro}); 115.3 (2 C_{aro}); 111.42 (2 C_{aro}); 59.3 (CH_2); 54.7 (CH_2); 42.0 (CH_2); 41.7 (CH_3); 33.6 (CH_2); 22.7 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 359.2; Found: 359.1. HPLC (C_4, 35 min): t_R 13.63 min, P_{HPLC} 96%; HPLC (C_{18}, 35 min): t_R 24.0 min, P_{HPLC} 96%.

5.1.4.3.14. 10-[3-(N-Methyl-N-phenylethylamino)propyl]phenothiazine ***7f***. Procedure C. The compound was purified by column chromatography (CycloHex:EtOAc, 3:2 (v/v) + 0.1% MeOH(NH_3)) and obtained as a brown oil (150 mg, 40%). TLC: R_f 0.2 (CycloHex:EtOAc, 3:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 77.32–7.10 (m, 9H, H_{aro}); 6.97–6.84 (m, 4H, H_{aro}); 3.94 (t, $J = 7$ Hz, 2H, CH_2); 2.78–2.62 (m, 2H, CH_2); 2.62–2.51 (m, 4H, 2 CH_2); 2.35 (s, 3H, CH_3); 2.04 (p, $J = 7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.2 (2 C_{aro}); 139.6 (C_{aro}); 128.7 (2 C_{aro}); 128.5 (2 C_{aro}); 127.6 (C_{aro}); 127.3 (2 C_{aro}); 126.2 (2 C_{aro}); 125.4 (2 C_{aro}); 122.6 (2 C_{aro}); 115.8 (2 C_{aro}); 59.1 (CH_2); 54.6 (CH_2); 44.9 (CH_2); 42.0 (CH_3); 32.8(CH_2); 26.3 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 375.1; Found: 374.9. HPLC (C_4, 35 min): t_R 14.4 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 25.4 min, P_{HPLC} 98%.

5.1.4.3.15. 2-[3-(Pyrrolidin-1-yl)propyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4,-b]indole ***8a***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 96:4 (v/v)) and obtained as a yellow oil (100 mg, 20%). TLC: R_f 0.6 (DCM: MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.35 (br s, 1H, NH); 7.49 (d, $J = 8$ Hz, 1H, H_8); 7.34 (d, $^3J_{5\text{-}6} = 8$ Hz, 1H, H_5); 7.17 (td, $J = 8.2$ Hz, $J = 1.2$ Hz, 1H, H_6); 7.11 (td, $J = 8.1$ Hz, $J = 1.1$ Hz, 1H, H_7); 4.70 (br s, 2H, CH_2); 4.25 (m, 2H, CH_2); 3.85 (m, 2H, CH_2); 2.85 (m, 2H, CH_2); 2.77 (m, 6H, 3 CH_2); 2.04 (p, $J = 7$ Hz, 2H, CH_2); 1.91–1.84 (m, 4H, 2 CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 136.2 (C_{aro}); 130.4 (C_{aro}); 127.0 (C_{aro}); 121.8 (C_{aro}); 119.6 (C_{aro}); 118.0 (C_{aro}); 111.0 (C_{aro}); 63.7 (CH_2); 54.1 (CH_2); 53.4 (2 CH_2); 50.8 (CH_2); 42.2 (CH_2); 27.8 (CH_2); 23.5 (2 CH_2); 21.5 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 284.2; Found: 284.1. HPLC (C_4, 35 min): t_R 10.6 min, P_{HPLC} 96%; HPLC (C_{18}, 35 min): t_R 15.7 min, P_{HPLC} 96%.

5.1.4.3.16. 1-Phenyl-2-[3-(pyrrolidin-1-yl)propyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***8b***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 99:1 to 95:5 (v/v)) and obtained as a yellow oil (35 mg, 12%). TLC: R_f 0.6 (DCM: MeOH(NH_3), 8:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.55 (m, 1H, H_{aro}); 7.39–7.31 (m, 5H, H_{aro}); 7.22–7.06 (m, 3H, H_{aro}); 4.59 (br t, $J = 2$ Hz, 1H, CH_2); 3.33 (m, 1H, CH_2); 2.99 (m, 2H, CH_2); 2.82–2.77 (m, 2H, CH_2); 2.62–2.51 (m, 5H, CH and 2 CH_2); 2.45 (m, 1H, CH_2); 2.33 (m, 1H, CH_2); 1.90–1.67 (m, 6H, 3 CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.4 (C_{aro}); 136.3 (C_{aro}); 134.9 (C_{aro}); 129.1 (2 C_{aro}); 128.6 (2 C_{aro}); 128.0 (C_{aro}); 127.1 (C_{aro}); 121.5 (C_{aro}); 119.3 (C_{aro}); 118.3 (C_{aro}); 110.8 (C_{aro}); 108.9 (C_{aro}); 64.7 (CH_2); 54.3 (CH_2); 54.1 (2 CH_2);

51.9 (CH_2); 48.3 (CH_2); 26.2 (CH_2); 23.4 (2 CH_2); 21.2 (CH_2). LCMS (ESI^+): Calc. for [M+H]:360.2; Found: 360.0. HPLC (C_4, 35 min): t_R 9.9 min, P_{HPLC} 99%; HPLC (C_{18}, 35 min): t_R 15.4 min, P_{HPLC} 98%.

5.1.4.3.17. 2-[3-(Isoindolin-2-yl)propyl]-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***9a***. Procedure A. The compound was purified by column chromatography (DCM:MeOH(NH_3), 99:1 to 96:4 (v/v)) and obtained as an orange solid (100 mg, 36%). Melting point: 197 °C. TLC: R_f 0.5 (DCM: MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.85 (br s, 1H, NH); 7.49 (m, 1H, H_{aro}); 7.31 (m, 1H, H_{aro}); 7.21 (s, 4H, H_{aro}); 7.17–7.06 (m, 2H, H_6 and H_7); 3.97 (s, 4H, 2 CH_2); 3.73 (br t, J = 2 Hz, 1H, CH); 2.95–2.81 (m, 6H, 3 CH_2); 2.75 (t, J = 7 Hz, 2H, CH_2); 1.94 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 140.0 (2 C_{aro}); 136.1 (C_{aro}); 131.8 (C_{aro}); 126.8 (2 C_{aro}); 122.3 (2 C_{aro}); 121.3 (C_{aro}); 119.3 (C_{aro}); 118.0 (C_{aro}); 110.6 (C_{aro}); 108.0 (C_{aro}); 59.1 (2 CH_2); 55.8 (CH_2); 54.2 (CH_2); 51.2 (CH_2); 50.6 (CH_2); 26.9 (CH_2); 21.4 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 332.2; Found: 332.0. HPLC (C_4, 35 min): t_R 9.0 min, P_{HPLC} > 99%; HPLC (C_{18}, 35 min): t_R 15.2 min, P_{HPLC} > 99%.

5.1.4.3.18. 2-[3-(Isoindolin-2-yl)propyl]-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole ***9b***. Procedure A. The compound was purified by thick layer chromatography (cyclohexane: ethyl acetate, 1:1 (v/v) + 0.1% MeOH(NH_3)) and obtained as a yellow oil (200 mg, 59%). TLC: R_f 0.5 (DCM: MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.55 (m, 1H, H_{aro}); 7.41–7.28 (m, 5H, H_{aro}); 7.25–7.07 (m, 7H, H_{aro}); 4.61 (br t, J = 2 Hz, 1H, CH); 3.89 (s, 4H, 2 CH_2); 3.39 (m, 1H, CH_2); 2.95 (m, 2H, CH_2); 2.82–2.67 (m, 3H, CH_2); 2.65–2.46 (m, 2H, CH_2); 1.84 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 141.5 (C_{aro}); 139.9 (2 C_{aro}); 136.3 (C_{aro}); 135.0 (C_{aro}); 129.2 (2 C_{aro}); 128.7 (2 C_{aro}); 128.0 (C_{aro}); 127.1 (C_{aro}); 126.7 (2 C_{aro}); 122.2 (2 C_{aro}); 121.5 (C_{aro}); 119.3 (C_{aro}); 118.3 (C_{aro}); 110.8 (C_{aro}); 109.0 (C_{aro}); 64.7 (CH_2); 59.0 (2 CH_2); 54.1 (CH_2); 51.9 (CH_2); 48.4 (CH_2); 26.7 (CH_2); 21.3 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 408.2; Found: 408.0. HPLC (C_4, 35 min): t_R 11.9 min, P_{HPLC} 98%; HPLC (C_{18}, 35 min): t_R 17.0 min, P_{HPLC} 96%.

5.1.4.3.19. 2-[3-(Isoindolin-2-yl)propyl]-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole ***9c***. Procedure B. The compound was purified by thick layer chromatography (cyclohexane:ethyl acetate, 1:1 (v/v) + 0.1% MeOH(NH_3)) and obtained as a yellow oil (41 mg, 26%). TLC: R_f 0.4 (CycloHex:EtOAc, 1:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.98 (br s, 1H, NH); 7.41 (m, 1H, H_{aro}); 7.30 (m, 1H, H_{aro}); 7.21 (s, 4H, H_{aro}); 7.17–7.04 (m, 2H, H_{aro}); 4.00 (s, 4H, H_{aro}); 3.82 (br t, J = 2 Hz, 2H, CH_2); 3.02–2.77 (m, 8H, 4 CH_2); 2.01 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 139.7 (2 C_{aro}); 136.1 (C_{aro}); 131.8 (C_{aro}); 126.8 (2 C_{aro}); 126.1 (C_{aro}); 122.3 (2 C_{aro}); 121.3 (C_{aro}); 119.4 (C_{aro}); 117.5 (C_{aro}); 110.7 (C_{aro}); 108.0 (C_{aro}); 59.1 (2 CH_2); 55.7 (CH_2); 54.2 (CH_2); 50.6 (CH_2); 49.6 (CH_2); 26.6 (CH_2); 23.5 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 332.2; Found: 332.0.

5.1.4.3.20. 2-[3-(Isoindolin-2-yl)propyl]-1,2,3,4-tetrahydrobenzofuro[3,2-c]pyridine ***9d***. Procedure A. The compound was purified by thick layer chromatography (DCM:MeOH(NH_3), 9:1 (v/v)) and obtained as a brown oil (67 mg, 58%). TLC: R_f 0.4 (DCM: MeOH(NH_3), 9:1, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.43–7.39 (m, 2H, H_{aro}); 7.24–7.19 (m, 6H, H_{aro}); 4.01 (s, 4H, H_{aro}); 3.68 (br t, J = 2 Hz, 2H, CH_2); 2.97–2.84 (m, 6H, 3 CH_2); 2.78 (t, J = 7 Hz, 2H, CH_2); 1.97 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 154.7 (C_{aro}); 152.1 (C_{aro}); 139.6 (2 C_{aro}); 127.2 (C_{aro}); 126.9 (2 C_{aro}); 123.2 (C_{aro}); 122.4 (2 C_{aro}); 122.3 (C_{aro}); 118.2 (C_{aro}); 111.6 (C_{aro}); 111.0 (C_{aro}); 59.1 (2 CH_2); 55.4 (CH_2); 54.2 (CH_2); 50.4 (CH_2); 48.7 (CH_2); 26.9 (CH_2); 24.3 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 333.2; Found: 333.2. HPLC (C_4, 35 min): t_R 16.7 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 14.7 min, P_{HPLC} 96%.

5.1.4.3.21. 10-[3-(Isoindolin-2-yl)propyl]phenoxazine ***9e***. Procedure C. The compound was purified by column chromatography (CycloHex:EtOAc, 3:2 (v/v) + 0.1% MeOH(NH_3)) and obtained as a brown oil (314 mg, 83%). TLC: R_f 0.5 (CycloHex:EtOAc, 3:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.27 (s, 4H, H_{aro}); 6.83–6.76 (m, 2H, H_{aro}); 6.69–6.60 (m, 6H, H_{aro}); 3.98 (s, 4H, 2 CH_2); 3.68 (t, J = 7 Hz, 2H, CH_2); 2.85 (t, J = 7 Hz, 2H, CH_2); 1.94 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 144.9 (2 C_{aro}); 139.1 (2 C_{aro}); 133.3 (2 C_{aro}); 127.1 (2 C_{aro}); 123.7 (2 C_{aro}); 122.4 (2 C_{aro}); 120.9 (2 C_{aro}); 115.4 (2 C_{aro}); 111.4 (2 C_{aro}); 59.2 (2 CH_2); 53.8 (CH_2); 41.6 (CH_2); 24.2 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 343.1; Found: 343.0. HPLC (C_4, 35 min): t_R 13.2 min, P_{HPLC} 99%; HPLC (C_{18}, 35 min): t_R 23.9 min, P_{HPLC} > 99%.

5.1.4.3.22. 10-[3-(Isoindolin-2-yl)propyl]phenothiazine ***9f***. Procedure C. The compound was purified by column chromatography (CycloHex:EtOAc, 3:2 (v/v) + 0.1% MeOH(NH_3)) and obtained as a brown oil (199 mg, 56%). TLC: R_f 0.2 (CycloHex:EtOAc, 3:2, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.23–7.10 (m, 8H, H_{aro}); 6.98–6.88 (m, 4H, H_{aro}); 4.02 (t, J = 7 Hz, 2H, CH_2); 3.93 (s, 4H, 2 CH_2); 2.89 (t, J = 7 Hz, 2H, CH_2); 2.09 (p, J = 7 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 145.3 (2 C_{aro}); 139.6 (2 C_{aro}); 127.5 (2 C_{aro}); 127.3 (2 C_{aro}); 126.9 (2 C_{aro}); 125.3 (2 C_{aro}); 122.5 (2 C_{aro}); 122.3 (2 C_{aro}); 115.7 (2 C_{aro}); 59.2 (2 CH_2); 53.6 (CH_2); 45.2 (CH_2); 26.3 (CH_2). LCMS (ESI^+): Calc. for [M+H]: 359.1; Found: 358.9. HPLC (C_4, 35 min): t_R 13.6 min, P_{HPLC} 97%; HPLC (C_{18}, 35 min): t_R 24.0 min, P_{HPLC} 96%.

5.2. In vitro testing

5.2.1. Assay for binding to σ receptors

The σ binding assays were performed by CEREP (Poitiers, France), according to Ganapathy et al. [32]. The σ_1 binding assay was carried out by incubating Jurkat cell membranes (10–20 mg protein per tube) with [^{3}H](+)-pentazocine (15 nM) and a range of concentrations of test compounds, at 37 °C for 2 h, in 5 mM Tris/HCl buffer (pH = 7.4). The σ_2 binding assay was performed by incubating Jurkat cell membranes (10–20 mg protein per tube) with [^{3}H]-DTG (25 nM) in the presence of (+)-pentazocine (1 μM) to saturate σ_1 receptors, and a range of concentrations of test compounds, at room temperature for 1 h in 5 mM TrisHCl buffer (pH = 7.4). The final assay volume was 0.5 mL. Binding was terminated by rapid filtration through Whatman GF/B filters, which were then washed with 5 × 1 mL ice-cold NaCl solution and allowed to dry before bound radioactivity was measured using liquid scintillation counting. Nonspecific binding was determined, in both assays, under similar conditions, but in the presence of 10 μM unlabelled haloperidol. Inhibition constants (K_i) were calculated from the IC_{50} values according to the method of Cheng and Prusoff (1973).

5.2.2. Cell culture and cytotoxicity assay

The human neuroblastoma cell line (SY5Y) was cultured in DMEM (Dulbecco's Modified Eagle Medium) (Gibco) supplemented with 2 mM L-glutamine, 100 μg/ml streptomycin, 100 IU/mL penicillin, 1 mM non-essential amino acids and 10% (v/v) heat-inactivated foetal bovine serum (Sigma Aldrich), and grown at 37 °C in a humidified incubator with 5% CO_2.

Cells were seeded at 2000 cells per well onto 96-well plates in DMEM medium. Cells were starved for 24 h to obtain synchronous cultures, and were then incubated in culture medium that contained various concentrations of test compounds, each dissolved in less than 0.1% DMSO. After 72 h of incubation, cell growth was estimated by the colorimetric MTT (thiazolyl blue tetrazolium bromide) assay.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.10.053.

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