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Original article

New 1-arylindoles based serotonin 5-HT$_7$ antagonists. Synthesis and binding evaluation studies

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ABSTRACT

Based on 5-HT$_{1A}$ and 5-HT$_7$ ligand MR25003 scaffold, a new series of 1-aryl indole analogues were prepared and evaluated against 5-HT$_7$ receptors. Modulations of aryl moieties provided a large number of new indolic derivatives. Most of compounds tested have displayed 5-HT$_7$ affinity in the nanomolar range. Among them, 1-(naphthyl)indole derivative **3p** (*Ki* (5-HT$_7$) = 4.5 nM) showed also a good selectivity over 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_6$ receptors. This compound was pharmacology characterized as an antagonist.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that mediates its effects on the central and peripheral nervous system via interaction with receptors. At least seven serotonin receptor families have been identified on the basis of their sequence homology, pharmacology, and signal transduction [1]. The human 5-HT$_7$ receptor subtype is the most recent 5-HT receptor and was first identified by Bard in 1993 [2]. The seven-transmembrane domain G-protein-coupled receptor 5-HT$_7$ was found to be positively coupled to an adenylate cyclase second messenger system. Its widespread distribution in CNS (thalamus, hypothalamus, limbic and cortical regions) is correlated with important functional roles in thermoregulation, circadian rhythm, endocrine regulation, sleeping, schizophrenia, depression and memory [3]. The 5-HT$_7$ receptor subtype was also found in the periphery (spleen, kidney, heart, coronary artery, smooth muscle and gastrointestinal tract). Currently, numerous nanomolar agonists and antagonists of 5-HT$_7$ receptors have been reported [4]. A remarkable structural diversity of 5-HT$_7$ ligands is observed indicating the continued interest in the development of 5-HT$_7$ ligands with suitable drug-like properties for pre-clinical validation. Moreover, new selective 5-HT$_7$ receptor ligands may be an important and indispensable research tool for precise determination of functional and physiopathological role of 5-HT$_7$ receptor subtype.

The *N*-phenylpyrrole moiety emerged as a 5-HT$_7$ pharmacophore from a virtual screening performed on a chemolibrary and realized by Rault et al., in 2005 [5]. Pharmacomodulation studies led to the preparation of MR25003 as a potent 5-HT$_{1A}$/5-HT$_7$ ligand (*Ki* 5-HT$_{1A}$ = 41 nM, *Ki* 5-HT$_7$ = 21 nM).

In continuation of our research work on the design of 5-HT$_7$ ligands [6,7] and based on 5-HT$_7$ pharmacophore model for 5-HTR antagonism (one basic centre, two hydrophobic groups, a one hydrogen bond acceptor) [8], we have developed a new series of 5-HT$_7$ 1-arylindole ligands. In this project, we have modulated the nature of the central heterocycles of MR25003 by substituting the pyrrole ring by an indole to evaluate the impact in the 5-HT$_7$ binding affinity and the 5-HT$_7$/5-HT$_{1A}$ selectivity (Fig. 1). The structural modifications comprised also modulation of aryl substituent at the N1 position of indole and piperazine.

2. Results and discussion

2.1. Chemistry

Target compounds of general structure **I** were obtained in two steps from commercially available indole-3-carbaldehyde **1**

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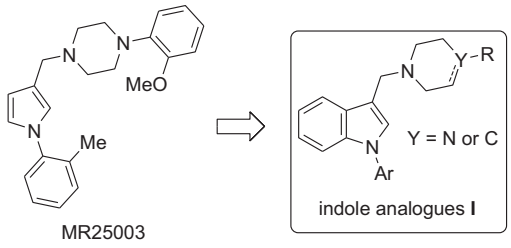


Fig. 1. Structure of MR25003 and the proposed indole analogues.

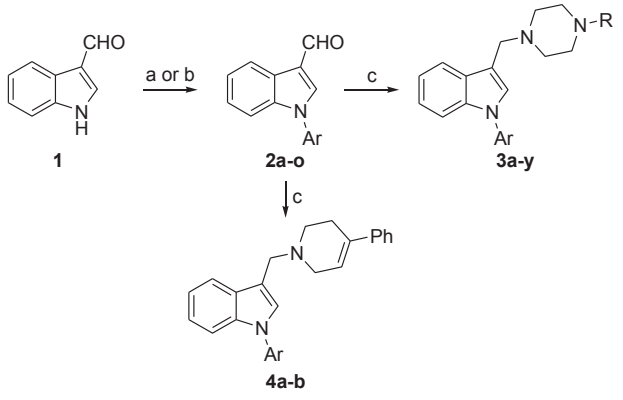
(Scheme 1). *N*-Arylation of **1** was mediated by copper(I) oxide in the presence of aryl halide in basic medium. After optimization, we found that the use of 0.3 equivalents of copper(I) oxide, 2 equivalents of aryl bromide or aryl iodide and 2 equivalents of potassium carbonate in dimethylformamide at reflux led to the total consumption of **1** and gave 1-arylindole-3-carbaldehydes **2a**–**n** in moderate-to-good yields (Table 1).

The use of *ortho*-substitued aryl halide decreases dramatically the reaction yield (entry 2, 5, 7, 8, 9, 11 and 13). In the case of 1-iodo-2,5-dimethylbenzene, the formation of the coupling compound was not observed. The preparation of **2o** was also problematic. Copper mediated *N*-arylation of **1** with 2-iodopyridine led to the 1,2-bis(pyridin-2-yl)indole-3-carbaldehyde in 41% yield [9]. An alternative method was carried out to get the desired compound **2o**. This compound was obtained from the sodium salt of **1** through a nucleophilic substitution reaction with 2-fluoropyridine to give **2o** in 84% yield [9].

Compounds **2a**–**o** were submitted to a reductive amination with *N*-substituted piperazines or 4-phenyl-1,2,3,6-tetrahydropyridine and sodium cyanoborohydride (1 equivalent) to provide final compounds **3** and **4** in fair yields (46–92%).

2.2. Biological studies

All prepared compounds **3** and **4** were tested as fumarate salts (except **3j**, **3n** and **3v**: free bases) in competition binding experiments for native serotonin 5-HT_{1A} (rat hippocampus) and cloned human 5-HT_7 (stably expressed in HEK-293 cells) receptors, according to previously published procedures [10]. Binding results of **3** and **4** are reported in Table 2.



Scheme 1. Synthesis of compounds **2**, **3** and **4**. Reagents and conditions: (a) ArX (2 equiv), Cu_2O (0.3 equiv), K_2CO_3 (2 equiv), DMF, rflx, 3 days (**2a**–**n**); (b) NaH (1.5 equiv), 2-fluoropyridine (1.7 equiv), DMF, 100 °C, 16 h (**2o**); (c) *N*-substituted piperazine or 4-phenyl-1,2,3,6-tetrahydropyridine (2 equiv), $NaBH_3CN$ (1 equiv), AcOH (1 equiv), MeOH, 70 °C, 18 h (**3**–**4**).

Table 1
Copper-mediated arylation yield.

Entry	Ar	**2** – Yield (%)
1[a]	Ph	2a – 89
2[a]	*o*-MePh	2b – 23
3[a]	*m*-MePh	2c – 75
4[a]	*p*-MePh	2d – 67
5[a]	*o*-EtPh	2e – 25
6[b]	*p*-(4-Ph)Ph	2f – 72
7[a]	*o*-iPrPh	2g – 21
8[a]	*o*,*m*-diMePh	2h – 20
9[a]	naphth-1-yl	2i – 23
10[b]	naphth-2-yl	2j – 92
11[a]	*o*-F_3CPh	2k – 8
12[a]	*o*-MeOPh	2l – 68
13[a]	*o*-NCPh	2m – 25
14[a]	pyridin-3-yl	2n – 70
15[c]	pyridin-2-yl	2o – 86

[a] Aryl iodide.
[b] Aryl bromide.
[c] SN_{Ar}.

The affinities (*Ki*) for the most potent 1-arylindole derivatives **3** for the 5-HT_7 receptor were determined to be between 4.5 and 20 nM. For compounds **4a** and **4b**, about a 2-fold decrease in 5-HT_7 affinity was observed (*Ki* = 33–92 nM) was observed when compared to their arylpiperazine analogues **3a** and **3d**, respectively. Compound with a phenyl group at the N1 position of indole shows nanomolar 5-HT_7 affinities (**3a**, *Ki* = 54 nM). To improve the affinity, the introduction of *ortho*-substituent such as an alkyl group (Me, iPr) on phenyl ring is primordial. In these cases, high 5-HT_7R affinity was measured (**3d**, *Ki* = 18 nM; **3k**, *Ki* = 15 nM, **3m**, *Ki* = 18 nM). The shift of the same substituent from *ortho*-position to *meta*- or *para*-position led to decrease in 5-HT_7 affinity (**3g**, *Ki* = 65 nM; **3h**, *Ki* = 94 nM). A bulky substituent such as biphenyl or naphth-2-yl at

Table 2
Receptor binding results for *N*-arylindoles **3** and **4**.

			Ki ± SEM (nM)[a]	
3,**4**[b]	Ar	R	5-HT1A	5-HT7
3a	Ph	Ph	216 ± 8	54 ± 3
3b	Ph	*o*-MeOPh	10 ± 1	10.8 ± 0.3
3c	Ph	*o*-HOPh	17 ± 1	27 ± 1
3d	*o*-MePh	Ph	95 ± 3	18 ± 2
3e	*o*-MePh	*o*-MeOPh	15 ± 1	8.7 ± 0.4
3f	*o*-MePh	Me	2912 ± 180	3907 ± 430
3g	*m*-MePh	Ph	90 ± 11	65 ± 8
3h	*p*-MePh	Ph	332 ± 21	94 ± 7
3i	*o*-EtPh	Ph	134 ± 9	48 ± 6
3j	*p*-(4-Ph)Ph	Ph	49,000	1920 ± 120
3k	*o*-iPrPh	Ph	118 ± 9	15 ± 2
3l	*o*-iPrPh	*o*-MeOPh	58 ± 5	17 ± 2
3m	*o*,*m*-diMePh	Ph	324 ± 40	18 ± 3
3n	*o*,*m*-diMePh	*o*-MeOPh	17 ± 2	6.9 ± 1.1
3o	naphth-1-yl	Ph	545 ± 38	34 ± 4
3p	naphth-1-yl	*o*-MeOPh	70 ± 12	4.5 ± 1
3q	naphth-1-yl	*o*-HOPh	57 ± 6	20 ± 3
3r	naphth-2-yl	Ph	1950 ± 130	4440 ± 470
3s	*o*-F_3CPh	Ph	81 ± 11	20 ± 3
3t	*o*-MeOPh	Ph	86 ± 3	70 ± 3
3u	*o*-NCPh	Ph	36 ± 2	41 ± 4
3v	pyridin-2-yl	Ph	95 ± 13	189 ± 16
3w	pyridin-2-yl	*o*-MeOPh	55 ± 8	62 ± 4
3x	pyridin-3-yl	Ph	190 ± 18	60 ± 8
4a	Ph	Ph	89 ± 4	92 ± 6
4b	*o*-MePh	Ph	178 ± 3	33 ± 4

[a] Inhibition constants (*Ki*) were determined from three separate experiments, run in triplicate.
[b] Compounds tested as fumarate salts except for **3j**, **3n** and **3v** (free bases).

the N1 position of indole dramatically reduced the affinity for the 5-HT_7 receptor (**3j**, *Ki* = 1920 nM; **3r**, *Ki* = 4440 nM). Interestingly, the replacement of phenyl ring by a naphth-1-yl group retained the affinity (**3o**, *Ki* = 34 nM). Substitution of the phenyl by a pyridinyl moiety led to weaker 5-HT_7 ligands (**3v**, *Ki* = 189 nM; **3x**, *Ki* = 60 nM) and even for 2-pyridyl group, 5-HT_{1A} affinity was better than 5-HT_7.

As regards the *N*-substituent of the piperazine, the presence of a phenyl or a substituted phenyl substituent was crucial to retain the 5-HT_7 affinity (**3d**, *Ki* = 18 nM; **3e**, *Ki* = 8.7 nM). This observation was confirmed by the poor binding result obtained for **3f** (*Ki* = 3907 nM). The presence of *ortho*-methoxyphenylpiperazine (*o*-MPP) instead of phenyl or 2-hydroxyphenyl group led to the most potent 5-HT_7 ligands (**3b**, *Ki* = 10.8 nM; **3e**, *Ki* = 8.7 nM; **3n**, *Ki* = 6.9 nM; **3p**, *Ki* = 4.5 nM).

Finally, we examined the 5-HT_7/5-HT_{1A} receptor selectivity for the 1-arylindole series. The 5-HT_7 selectivity was improved when the phenyl group was not substituted (e.g. **3a**, **3d**, **3m**, **3o**). *o*-MPP is a well-known 5-HT_{1A} privileged structure, this data was confirmed by the binding results. Compound **3b** shows no selectivity and **3e**, **3l**, **3n** a low 2–3 fold 5-HT_7 receptors selectivity over 5-HT_{1A} receptors. An exception was observed for the compound **3p** where the selectivity over 5-HT_{1A} receptors was retained (15 fold) compared to **3o** (16 fold). Compounds **3n** and **3p** were selected as lead compounds, exhibiting low nanomolar 5-HT_7 affinity and moderate 5-HT_7 selectivity (especially **3p**).

Both compounds **3n** and **3p** displayed a good selectivity over 5-HT_{2A} and 5-HT_6 receptors (Table 3). The 5-HT_7 agonist and antagonist effects of ligands **3n** and **3p** were assessed by determining the adenylate cyclase activity [11]. The biological studies were performed by CEREP (Celle Levescaut, France). Compounds **3n** and **3p** did not display agonist activity but showed an antagonist activity (data not reported).

The feasibility of imaging the 5-HT_7 receptors in the brain by positron emission tomography (PET) studies represents an interesting field to provide significant advances in the understanding of the neurobiology and eventual dysfunctions of the 5-HT_7 receptor [12]. Considering the low nanomolar affinity of **3p** for the 5-HT7 receptors and in spite of a moderate 5-HT_7/5-HT_{1A} receptor selectivity, the compound **3q** can be considered as a first potential ^{11}C radiolabelling precursor of 1-arylindole series.

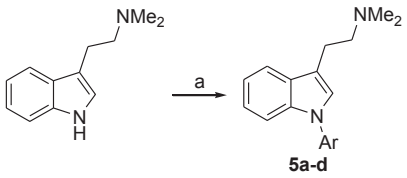
1-Aryltryptamines **5** were also prepared to evaluate their 5-HT_7 and 5-HT_{1A} binding affinities (Scheme 2). Following the same *N*-arylation reaction to get **2**, compounds **5** were prepared from commercially available *N*,*N*-dimethyltryptamine in good yields (85–92%). The 5-HT_7 binding results were disappointing compared to those of corresponding **3** (Table 4).

Thus, a *N*,*N*-dimethylaminoethyl chain turned out to be favourable for 5-HT_{1A} and selectivity over 5-HT_7 receptors. Compounds **5** were nanomolar 5-HT_{1A} receptor ligands and the derivative **5a** showed the highest 5-HT_{1A} selectivity versus 5-HT_7 receptors (19 fold).

Table 3
5-HT_{2A} and 5-HT_6 receptor binding affinities for **3n** and **3p**.

	Inhibition (%)[a]			
3[b]	5-HT_{2A} at 1 μM	5-HT_{2A} at 10 nM	5-HT_6 at 1 μM	5-HT_6 at 10 nM
3n	0	0	86	0
3p	10	6	50	3

[a] Experiments performed by CEREP.
[b] **3n**: free base; **3p**: fumarate salt.



Scheme 2. Synthesis of compounds **5**. Reagents and conditions: (a) ArX (1.3 equiv), Cu_2O (0.3 equiv), K_2CO_3 (1.5 equiv), DMF, MW, 120 min 240 °C.

3. Conclusion

In conclusion, we analysed the impact of the substitution of the pyrrole ring of MR25003 by indole nucleus on 5-HT_7/5-HT_{1A} binding affinities. 1-Arylindoles **3** were prepared in two steps and showed nanomolar 5-HT_7 receptor affinity. Among the compounds tested, the 1-naphthyl derivative **3p** was found to be a high affinity 5-HT_7R antagonist with acceptable selectivity profile versus 5-HT receptors.

4. Experimental section

4.1. Chemistry

General methods: Commercial reagents (Fluka, Aldrich) were used without purification. Solvents were distillated prior to use. Melting points were determined using a Büchi capillary instrument and are uncorrected. IR spectra were recorded on a Perkin–Elmer 681 infrared spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane as an internal standard. The following abbreviations were used to designate the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. Mass spectra were recorded with a Perkin–Elmer SCIEX API spectrometer. Elemental analyses were performed on a Thermoquest Flash 1112 series EA analyser. Elemental analyses were found to be within ±0.4 of the theoretical values. Purity of tested compounds was >95%. Thin Layer Chromatography (TLC) analyses were conducted on aluminium sheets silica gel Merck $60F_{254}$. The spots were visualized using an ultraviolet light. Flash chromatography was carried out on silica gel 60 (40–63 μm, Merck) using the indicated solvents. The light petroleum ether refers to the fraction boiling at 40–60 °C.

4.1.1. General procedure for preparation of compounds *2a–2n*

A suspension of indole-3-carbaldehyde **1** (0.411 g, 2.8 mmol), Cu_2O (0.3 equiv), K_2CO_3 (2.0 equiv) and aryl halide (2.0 equiv) in anhydrous DMF (5.6 mL) was refluxed for 72 h. After cooling to RT, the reaction mixture was filtrated over a celite pad eluting with EtOAc. Solvents were removed and the residue dissolved in EtOAc (20 mL) washed successively by 2.5% aqueous NH_4OH, 1 M HCl and saturated aqueous NaCl. The organic phase was dried over Na_2SO_4,

Table 4
Receptor binding results for 1-aryltryptamines **5**.

		Ki ± SEM (nM)[a]	
5[b]	Ar	5-HT_{1A}	5-HT_7
5a	Ph	62 ± 8	1200 ± 109
5b	*o*-MePh	59 ± 4	283 ± 21
5c	*o*,*m*-diMePh	76 ± 10	317 ± 28
5d	naphth-1-yl	144 ± 16	649 ± 47

[a] Inhibition constants (*Ki*) were determined from three separate experiments, run in triplicate.
[b] Compounds tested as fumarate salts.

filtered and concentrated. The residue was purified by flash column chromatography on silica gel (PE/EtOAc 9:1 to 7:3) to furnish the desired compound.

*4.1.1.1. 1-Phenyl-1H-indole-3-carbaldehyde (**2a**) (CAS: 32542-59-9).* Starting aryl halide = iodobenzene. Yield: 89% (557 mg); mp 70–72 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.40 (m, 2H, H_{Ar}), 7.46–7.62 (m, 6H, H_{Ar}), 7.93 (s, 1H, H_{Ar}), 8.39 (dd, 1H, J = 2.3, 6.1 Hz, H_{Ar}), 10.12 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.2 (CH), 119.8 (Cq), 122.3 (CH), 123.6 (CH), 124.7 (CH), 124.9 (2 × CH), 125.6 (Cq), 128.4 (CH), 130.1 (2 × CH), 137.6 (Cq), 138.2 (Cq), 138.3 (CH), 185.0 (CHO); IR (KBr, cm^{-1}): 3100, 3040, 1655, 735, 690; MS (ESI): m/z 222 $[M+H]^+$.

*4.1.1.2. 1-(2-Methylphenyl)-1H-indole-3-carbaldehyde (**2b**) (CAS: 196496-26-1).* Starting aryl halide = 1-iodo-2-methylbenzene. Yield: 23% (153 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H, CH_3), 7.03 (d, 1H, J = 7.9 Hz, H_{Ar}), 7.29–7.38 (m, 4H, H_{Ar}), 7.40–7.47 (m, 2H, H_{Ar}), 7.78 (s, 1H, H_{Ar}), 8.38 (dd, 1H, J = 1.1, 6.0 Hz, H_{Ar}), 10.11 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.6 (CH_3), 111.2 (CH), 119.4 (Cq), 122.2 (CH), 123.3 (CH), 124.6 (CH), 124.9 (Cq), 127.3 (CH), 127.9 (CH), 129.6 (CH), 131.6 (CH), 135.6 (Cq), 136.7 (Cq), 138.5 (Cq), 138.9 (CH), 185.1 (CHO); IR (KBr, cm^{-1}): 3100, 3040, 1660, 745; MS (ESI): m/z 236 $[M+H]^+$.

*4.1.1.3. 1-(3-Methylphenyl)-1H-indole-3-carbaldehyde (**2c**).* Starting aryl halide = 1-iodo-3-methylbenzene. Yield: 75% (153 mg); mp 109–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.46 (s, 3H, CH_3), 7.24–7.37 (m, 5H, H_{Ar}), 7.41–7.48 (m, 2H, H_{Ar}), 7.81 (s, 1H, H_{Ar}), 8.40 (d, 1H, J = 7.1 Hz, H_{Ar}), 10.05 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.3 (CH_3), 111.1 (CH), 119.4 (Cq), 121.6 (CH), 122.1 (CH), 123.2 (CH), 124.4 (CH), 125.2 (CH), 125.4 (Cq), 128.9 (CH), 129.6 (CH), 137.3 (Cq), 137.8 (Cq), 138.3 (Cq), 140.0 (CH), 184.8 (CHO); IR (KBr, cm^{-1}): 3047, 2810, 1716, 1668, 1607, 1497, 1461, 1177, 1081; MS (ESI): m/z 236 $[M+H]^+$.

*4.1.1.4. 1-(4-Methylphenyl)-1H-indole-3-carbaldehyde (**2d**).* Starting aryl halide = 1-iodo-4-methylbenzene. Yield: 67% (415 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (s, 3H, CH_3), 7.29–7.42 (m, 6H, H_{Ar}), 7.46 (dd, 1H, J = 1.9, 6.8 Hz, H_{Ar}), 7.89 (s, 1H, H_{Ar}), 8.38 (dd, 1H, J = 1.9, 6.8 Hz, H_{Ar}), 10.07 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.2 (CH_3), 111.1 (CH), 119.4 (Cq), 122.2 (CH), 123.3 (CH), 124.5 (CH), 124.7 (2 × CH), 125.5 (Cq), 130.5 (2 × CH), 135.5 (Cq), 137.6 (Cq), 138.4 (CH + Cq), 184.9 (CHO); IR (KBr, cm^{-1}): 3045, 2919, 2733, 1675, 1386, 1080, 814, 754; MS (ESI): m/z 236 $[M+H]^+$.

*4.1.1.5. 1-(2-Ethylphenyl)-1H-indole-3-carbaldehyde (**2e**).* Starting aryl halide = 1-iodo-2-ethylbenzene. Yield: 25% (175 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (*t*, 3H, J = 7.5 Hz, CH_3), 2.39 (*q*, 2H, J = 7.5 Hz, CH_2), 7.04 (d, 1H, J = 7.9 Hz, H_{Ar}), 7.22–7.39 (m, 4H, H_{Ar}), 7.50–7.54 (m, 2H, H_{Ar}), 7.81 (s, 1 H, H_{Ar}), 8.47 (d, 1H, J = 7.5 Hz, H_{Ar}), 10.07 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4 (CH_3), 23.5 (CH_2), 110.6 (CH), 118.7 (Cq), 121.5 (CH), 122.6 (CH), 124.0 (CH), 124.1 (Cq), 126.7 (CH), 127.5 (CH), 129.3 (CH), 129.4 (CH), 135.4 (Cq), 138.3 (Cq), 139.1 (CH), 140.9 (Cq), 184.4 (CHO), IR (neat, cm^{-1}): 3055, 2972, 2722, 1682, 1379, 1077, 746; MS (ESI): m/z 250 $[M+H]^+$.

*4.1.1.6. 1-(Biphenyl-4-yl)-1H-indole-3-carbaldehyde (**2f**).* Starting aryl halide = 1-bromo-4-phenylbenzene. Yield: 72% (599 mg); mp 152–153 °C (EtOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36–7.40 (m, 2H, H_{Ar}), 7.42–7.44 (m, 1H, H_{Ar}), 7.49 (d, 2H, J = 7.7 Hz, H_{Ar}), 7.53–7.57 (m, 1H, H_{Ar}), 7.60 (d, 2H, J = 8.5 Hz, H_{Ar}), 7.66 (d, 2H, J = 7.1 Hz, H_{Ar}), 7.80 (d, 2H, J = 8.5 Hz, H_{Ar}), 7.96 (s, 1H, H_{Ar}), 8.39–8.42 (m, 1H, H_{Ar}), 10.14 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.3 (CH), 119.9 (Cq), 122.4 (CH), 123.6 (CH), 124.8 (CH), 125.2 (2 × CH), 125.7 (Cq), 127.3 (2 × CH), 128.1 (CH), 128.7 (2 × CH), 129.2 (2 × CH), 137.3 (Cq), 137.6 (Cq), 138.2 (CH), 139.8 (Cq), 141.3 (Cq), 185.1 (CHO); IR (KBr, cm^{-1}): 3027, 2943, 2821, 1597, 1489, 1456, 1251, 1003, 761, 744; MS (ESI): m/z 298 $[M+H]^+$.

*4.1.1.7. 1-[2-(1-Methylethyl)phenyl]-1H-indole-3-carbaldehyde (**2g**).* Starting aryl halide = 1-iodo-2-(1-methylethyl)benzene. Yield: 21% (155 mg); mp 99–101 °C (EtOAc/PE); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.11 (d, 3H, J = 6.8 Hz, CH_3), 1.18 (d, 3H, J = 6.8 Hz, CH_3), 2.59–2.64 (m, 1H, CH), 7.04 (d, 1H, J = 7.9 Hz, H_{Ar}), 7.28–7.42 (m, 4H, H_{Ar}), 7.57 (m, 2H, H_{Ar}), 7.79 (s, 1H, H_{Ar}), 8.41 (d, 1H, J = 7.5 Hz, H_{Ar}), 10.13 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.5 (CH_3), 24.6 (CH_3), 27.9 (CH), 111.1 (CH), 119.2 (Cq), 122.0 (CH), 123.2 (CH), 124.5 (CH), 124.6 (Cq), 127.0 (CH), 127.1 (CH), 128.2 (CH), 130.1 (Cq), 135.1 (Cq), 139.3 (CH), 139.4 (CH), 146.5 (Cq), 185.0 (CHO); IR (KBr, cm^{-1}): 3056, 2720, 1654, 1575, 1460, 1456, 792, 748; MS (ESI): m/z 264 $[M+H]^+$.

*4.1.1.8. 1-(2,3-Dimethylphenyl)-1H-indole-3-carbaldehyde (**2h**).* Starting aryl halide = 1-iodo-2,3-dimethylbenzene. Yield: 20% (140 mg); mp 90–91 °C (EtOAc/PE); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.93 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 7.02 (d, 1H, J = 7.9 Hz, H_{Ar}), 7.17 (d, 1H, J = 7.5 Hz, H_{Ar}), 7.24–7.38 (m, 4H, H_{Ar}), 7.76 (s, 1H, H_{Ar}), 8.38 (d, 1H, J = 7.5 Hz, H_{Ar}), 10.10 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1 (CH_3), 20.3 (CH_3), 111.2 (CH), 119.0 (Cq), 121.9 (CH), 123.0 (CH), 124.3 (CH), 124.6 (Cq), 125.2 (CH), 126.4 (CH), 130.7 (CH), 134.0 (Cq), 136.5 (Cq), 138.5 (Cq), 138.9 (Cq), 139.2 (CH), 184.8 (CHO); IR (KBr, cm^{-1}): 3111, 2918, 2813, 1664, 1609, 1578, 1476, 1456, 1381, 1084, 758; MS (ESI): m/z 250 $[M+H]^+$.

*4.1.1.9. 1-(Naphth-1-yl)-1H-indole-3-carbaldehyde (**2i**).* Starting aryl halide = 1-iodonaphthalene. Yield: 23% (175 mg); mp 110–112 °C (CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.01 (d, 1H, J = 8.3 Hz, H_{Ar}), 7.22–7.27 (m, 1H, H_{Ar}), 7.35–7.40 (m, 2H, H_{Ar}), 7.46 (*t*, 1H, J = 7.7 Hz, H_{Ar}), 7.56–7.66 (m, 3H, H_{Ar}), 7.95 (s, 1H, H_{Ar}), 8.01 (d, 1H, J = 8.8 Hz, H_{Ar}), 8.06 (d, 1H, J = 8.6 Hz, H_{Ar}), 8.44 (d, 1H, J = 7.7 Hz, H_{Ar}), 10.15 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.5 (CH), 119.7 (Cq), 122.2 (CH), 122.6 (CH), 123.4 (CH), 124.6 (CH), 124.9 (Cq), 125.3 (CH), 125.5 (CH), 127.2 (CH), 127.7 (CH), 128.6 (CH), 129.9 (CH), 130.0 (Cq), 134.3 (Cq), 134.5 (Cq), 139.4 (Cq), 139.9 (CH), 185.1 (CHO); IR (KBr, cm^{-1}): 3115, 3051, 2836, 1649, 1530, 1181, 771, 748; MS (ESI): m/z 272 $[M+H]^+$.

*4.1.1.10. 1-(Naphth-2-yl)-1H-indole-3-carbaldehyde (**2j**).* Starting aryl halide = 2-bromonaphthalene. Yield: 92% (699 mg); mp 121–122 °C (EtOAc/PE); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31–7.41 (m, 2H, H_{Ar}), 7.53 (d, 1H, J = 7.7 Hz, H_{Ar}), 7.57–7.62 (m, 3H, H_{Ar}), 7.88–7.97 (m, 4H, H_{Ar}), 8.02 (d, 1H, J = 8.7 Hz, H_{Ar}), 8.44 (d, 1H, J = 8.1 Hz, H_{Ar}), 10.11 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.2 (CH), 119.8 (Cq), 122.3 (CH), 122.8 (CH), 123.1 (CH), 123.6 (CH), 124.7 (CH), 125.6 (Cq), 127.0 (CH), 127.5 (CH), 127.9 (CH), 128.0 (CH), 130.1 (CH), 132.6 (Cq), 133.5 (Cq), 135.5 (Cq), 137.6 (Cq), 138.5 (CH), 185.0 (CHO); IR (KBr, cm^{-1}): 3092, 3054, 2815, 1656, 1530, 1474, 1454, 1296, 1134, 733; MS (ESI): m/z 272 $[M+H]^+$.

*4.1.1.11. 1-[2-(Trifluoromethyl)phenyl]-1H-indole-3-carbaldehyde (**2k**).* Starting aryl halide = 1-iodo-2-(trifluoromethyl)benzene. Yield: 8% (65 mg); mp 93–95 °C (EtOAc/PE); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.99 (d, 1H, J = 8.1 Hz, H_{Ar}), 7.29–7.39 (m, 2H, H_{Ar}), 7.50 (d, 1H, J = 7.3 Hz, H_{Ar}), 7.69–7.78 (m, 2H, H_{Ar}), 7.80 (s, 1H, H_{Ar}), 7.93 (dd, 1H, J = 1.5, 7.5 Hz, H_{Ar}), 8.37 (d, 1H, J = 7.5 Hz, H_{Ar}), 10.11 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 111.3 (CH), 120.2 (Cq), 122.5 (CH), 123.2 (Cq, J_{C-F} = 271.7 Hz), 123.8 (CH), 124.9 (Cq), 125.1 (CH), 128.2

(CH, J_{C-F} = 4.9 Hz), 128.8 (Cq, J_{C-F} = 31.1 Hz), 130.4 (CH), 131.1 (CH), 133.8 (CH), 135.9 (Cq, J_{C-F} = 2.3 Hz), 140.1 (Cq), 140.3 (CH), 185.6 (CHO); IR (KBr, cm^{-1}): 3045, 2734, 1662, 1605, 1578, 1504, 1464, 1312, 1057, 776, 754; MS (ESI): *m/z* 290 $[M+H]^+$.

*4.1.1.12. 1-(2-Methoxyphenyl)-1H-indole-3-carbaldehyde (**2l**).* Starting aryl halide = 1-iodo-2-methoxybenzene. Yield: 68% (478 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 3.63 (s, 3H, OCH_3), 7.10–7.15 (m, 2H, H_{Ar}), 7.20 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 7.29–7.37 (m, 2H, H_{Ar}), 7.41–7.51 (m, 2H, H_{Ar}), 7.87 (s, 1H, H_{Ar}), 8.38 (d, 1H, *J* = 7.1 Hz, H_{Ar}), 10.07 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.3 (CH_3), 111.1 (CH), 112.2 (CH), 118.7 (Cq), 120.6 (CH), 121.5 (CH), 122.7 (CH), 123.9 (CH), 124.5 (Cq), 125.7 (Cq), 127.4 (CH), 129.8 (CH), 137.9 (Cq), 140.2 (CH), 153.8 (Cq), 184.7 (CHO); IR (neat, cm^{-1}): 3053, 1657, 1597, 741; MS (ESI): *m/z* 252 $[M+H]^+$.

*4.1.1.13. 2-(3-Formyl-1H-indol-1-yl)benzonitrile (**2m**) (CAS: 260553-34-2).* Starting aryl halide = 2-iodobenzonitrile. Yield: 25% (175 mg); mp 110–111 °C ($EtOH/Et_2O$); ^1H NMR (300 MHz, $CDCl_3$): δ 7.28–7.31 (m, 1H, H_{Ar}), 7.35–7.46 (m, 2H, H_{Ar}), 7.66 (td, 2H, *J* = 1.3, 7.7 Hz, H_{Ar}), 7.85 (td, 1H, *J* = 1.5, 8.3 Hz, H_{Ar}), 7.94 (d, 1H, *J* = 7.7 Hz, H_{Ar}), 8.00 (s, 1H, H_{Ar}), 8.40 (dd, 1H, *J* = 1.8, 5.8 Hz, H_{Ar}), 10.16 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 110.1 (Cq), 110.7 (CH), 115.6 (Cq), 120.3 (Cq), 122.3 (CH), 123.8 (CH), 124.9 (CH), 125.0 (Cq), 127.7 (CH), 129.2 (CH), 134.3 (CH), 134.5 (CH), 137.5 (Cq), 138.5 (CH), 139.8 (CH), 185.1 (CHO); IR (KBr, cm^{-1}): 3021, 2928, 1672, 1185, 1139, 723, 704; MS (ESI): *m/z* 247 $[M+H]^+$.

*4.1.1.14. 1-(Pyridin-3-yl)-1H-indole-3-carbaldehyde (**2n**) (CAS: 95504-82-8).* Starting aryl halide = 3-iodopyridine. Yield: 70% (440 mg); mp 138–140 °C (MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 7.39–7.48 (m, 3H, H_{Ar}), 7.65 (br s, 1H, H_{Ar}), 7.94 (s, 1H, H_{Ar}), 8.00 (d, 1H, *J* = 8.3 Hz, H_{Ar}), 8.41 (d, 1H, *J* = 8.3 Hz, H_{Ar}), 8.78 (br s, 1H, H_{Ar}), 8.92 (br s, 1H, H_{Ar}), 10.16 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 110.6 (CH), 120.4 (Cq), 122.5 (CH), 122.9 (CH), 124.4 (CH), 125.1 (CH), 125.5 (Cq), 132.3 (CH), 137.3 (Cq), 137.7 (CH), 146.0 (CH), 149.3 (CH), 149.8 (Cq), 185.0 (CHO); IR (KBr, cm^{-1}): 3073, 2738, 1673, 1654, 1616, 1587, 1485, 1463, 1083, 788, 739; MS (ESI): *m/z* 223 $[M+H]^+$.

*4.1.1.15. 1-(Pyridin-2-yl)-1H-indole-3-carbaldehyde (**2o**) (CAS: 890095-85-9).* The compound was prepared from sodium salt of indole-3-carbaldehyde and 2-fluoropyridine as described in reference [9]. Yield: 86% (541 mg); mp: 114–115 °C (MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 7.33–7.45 (m, 3H, H_{Ar}), 7.64 (d, 1H, *J* = 8.3 Hz, H_{Ar}), 7.95 (td, 1H, *J* = 1.1, 7.7 Hz, H_{Ar}), 8.02–8.05 (m, 1H, H_{Ar}), 8.38 (s, 1H, H_{Ar}), 8.40 (dd, 1H, *J* = 1.3, 5.3 Hz, H_{Ar}), 8.64 (dd, 1H, *J* = 1.3, 4.7 Hz, H_{Ar}), 10.16 (s, 1H, CHO); ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.8 (CH), 115.7 (CH), 120.5 (Cq), 122.2 (CH), 122.3 (CH), 123.8 (CH), 125.1 (CH), 126.3 (Cq), 136.2 (Cq), 137.0 (CH), 139.0 (CH), 149.4 (CH), 150.9 (Cq), 185.4 (CHO); IR (KBr, cm^{-1}): 3101, 3050, 2821, 1649, 1224, 1083, 739; MS (ESI): *m/z* 223 $[M+H]^+$.

*4.1.2. General procedure for preparation of compounds **3** and **4***

The derivative **2** (0.5 mmol) was added to a solution of the amine (1.0 mmol, 2.0 equiv) and acetic acid (1.0 equiv) in MeOH (2.0 mL). The sodium cyanoborohydride (1.0 equiv) was then added at room temperature in one portion and reaction mixture was refluxed overnight. After cooling to room temperature, 12 N HCl was added carefully under fume hood until pH 2. Stirring was continued for 15 min and 50% aqueous NaOH was added to obtain pH 10–12. Methanol was then eliminated under vacuum and the residue diluted in water and extracted with dichloromethane (3×). Resulting organic layers were washed with saturated NaCl solution and dried over Na_2SO_4. After removal of the solvent, the crude product was purified by flash chromatography on silica gel using system solvent indicated below.

*4.1.2.1. 1-Phenyl-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3a**).* System solvent: CH_2Cl_2/MeOH 97:3; yield: 61% (112 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.78 (br s, 4H, CH_2N), 3.27 (br s, 4H, CH_2N), 3.90 (s, 2H, CH_2Ar), 6.85 (*t*, 1H, *J* = 7.5 Hz, H_{Ar}), 6.92 (d, 2H, *J* = 7.9 Hz, H_{Ar}), 7.18–7.28 (m, 4H, H_{Ar}), 7.33–7.40 (m, 2H, H_{Ar}), 7.52 (d, 4H, *J* = 4.4 Hz, H_{Ar}), 7.57 (d, 1H, *J* = 8.7 Hz, H_{Ar}), 7.81 (d, 1H, *J* = 7.4 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.3 (2 CH_2), 53.2 (2 × CH_2), 53.7 (CH_2), 110.6 (CH), 113.6 (Cq), 116.1 (2 × CH), 119.6 (CH), 120.1 (CH), 120.3 (CH), 122.6 (CH), 124.3 (2 × CH), 126.4 (CH), 127.5 (CH), 129.2 (2 × CH), 129.4 (Cq), 129.7 (2 × CH), 136.2 (Cq), 139.8 (Cq), 151.5 (Cq); IR (neat, cm^{-1}): 3080, 3040, 3020, 1595, 740, 690; MS (ESI): *m/z* 368 $[M+H]^+$; Anal. calcd for $C_{25}H_{25}N_3$ $C_4H_4O_4$: C 72.03, H 6.04, N 8.70, found: C 72.14, H 6.10, N 8.91; mp (fumarate); 174–176 °C dec. (EtOH).

*4.1.2.2. 3-{[4-(2-Methoxyphenyl)piperazinyl]methyl}-1-phenyl-1H-indole (**3b**).* System solvent: CH_2Cl_2/MeOH 98:2; yield: 73% (145 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.70 (br s, 4H, CH_2N), 3.03 (br s, 4H, CH_2N), 3.76 (s, 3H, OCH_3), 3.79 (s, 2H, CH_2Ar), 6.74–6.92 (m, 4H, H_{Ar}), 7.08–7.16 (m, 3H, H_{Ar}), 7.24–7.28 (m, 1H, H_{Ar}), 7.42 (d, 4H, *J* = 4.2 Hz, H_{Ar}), 7.47 (d, 1H, *J* = 7.9 Hz, H_{Ar}), 7.74 (d, 1H, *J* = 7.2 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.8 (2 × CH_2), 53.4 (2 × CH_2), 53.5 (CH_2), 55.4 (CH_3), 110.5 (CH), 111.1 (CH), 113.6 (Cq), 118.3 (CH), 120.0 (CH), 120.2 (CH), 121.0 (CH), 122.5 (CH), 122.8 (CH), 124.2 (2 × CH), 126.3 (CH), 127.5 (CH), 129.6 (Cq), 129.6 (2 × CH), 136.2 (Cq), 139.8 (Cq), 141.5 (Cq), 152.3 (Cq); IR (neat, cm^{-1}): 3080, 3040, 3020, 1595, 745, 695; MS (ESI): *m/z* 398 $[M+H]^+$; Anal. calcd for $C_{26}H_{27}N_3O$ $C_4H_4O_4$: C 70.16, H 6.08, N 8.18, found: C 70.30, H 6.18, N 8.45; mp (fumarate): 187–188 °C dec. ($EtOH/Et_2O$).

*4.1.2.3. 2-{4-[(1-Phenyl-1H-indol-3-yl)methyl]piperazinyl}phenol (**3c**).* System solvent: CH_2Cl_2/MeOH 99:1 to 98:2; yield: 72% (139 mg); foam; ^1H NMR (300 MHz, $CDCl_3$): δ 2.60–2.85 (m, 4H, CH_2N), 2.92 (br s, 4H, CH_2N), 3.87 (s, 2H, CH_2Ar), 6.85 (dt, 1H, *J* = 1.5, 7.5 Hz, H_{Ar}), 6.94 (dt, 1H, *J* = 1.3, 7.8 Hz, H_{Ar}), 7.07 (dt, 1H, *J* = 1.3, 7.8 Hz, H_{Ar}), 7.16–7.27 (m, 2H, H_{Ar}), 7.32–7.39 (m, 2H, H_{Ar}), 7.51–7.60 (m, 6H, H_{Ar}), 7.83–7.85 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.6 (2 × CH_2), 53.5 (CH_2Ar), 53.8 (2 × CH_2), 110.6 (CH), 113.3 (Cq), 114.1 (CH), 119.97 (CH), 120.03 (CH), 120.3 (CH), 121.5 (CH), 122.6 (CH), 124.2 (2 × CH), 126.34 (CH), 126.36 (CH), 127.5 (CH), 129.4 (Cq), 129.7 (2 × CH), 136.1 (Cq), 139.2 (Cq), 139.7 (Cq), 151.6 (Cq); IR (neat, cm^{-1}): 3320, 3050, 1664, 1596, 740, 696; Anal. calcd for $C_{25}H_{25}N_3O$ $1/2C_4H_4O_4$: C 73.45, H 6.16, N 9.52, found: C 73.38, H 6.09, N 9.48; mp (fumarate): 161–163 °C ($EtOH/Et_2O$).

*4.1.2.4. 3-[(4-Phenylpiperazinyl)methyl]-1-(2-methylphenyl)-1H-indole (**3d**).* System solvent: CH_2Cl_2/MeOH 95:5; yield: 78% (149 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.75 (*t*, 4H, *J* = 4.5 Hz, CH_2N), 3.24 (*t*, 4H, *J* = 4.9 Hz, CH_2N), 3.88 (s, 2H, CH_2Ar), 6.85 (*t*, 1H, *J* = 7.4 Hz, H_{Ar}), 6.93 (d, 2H, *J* = 8.3 Hz, H_{Ar}), 7.02–7.05 (m, 1H, H_{Ar}), 7.17–7.19 (m, 3H, H_{Ar}), 7.23–7.28 (m, 2H, H_{Ar}), 7.32–7.34 (m, 2H, H_{Ar}), 7.35–7.39 (m, 2H, H_{Ar}), 7.81–7.84 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.9 (CH_3), 49.3 (2 × CH_2), 53.2 (2 × CH_2), 53.7 (CH_2), 110.7 (CH), 112.4 (Cq), 116.1 (2 × CH), 119.6 (CH), 119.7 (CH), 119 (CH), 122.3 (CH), 126.9 (2 × CH), 128.2 (CH), 128.3 (CH), 128.4 (Cq), 129.2 (2 × CH), 131.3 (CH), 135.8 (Cq), 137.5 (Cq), 138.2 (Cq), 151.5 (Cq); IR (neat, cm^{-1}): 3080, 3040, 3020, 1595, 740, 690; MS (ESI): *m/z* 382 $[M+H]^+$; Anal. calcd for $C_{26}H_{27}N_3$ $C_4H_4O_4$: C 72.71, H 6.28, N 8.44, found: C 72.71, H 6.32, N 8.60; mp (fumarate): 185 °C dec. ($EtOH/Et_2O$).

*4.1.2.5. 3-{[4-(2-Methoxyphenyl)piperazinyl]methyl}-1-(2-methylphenyl)-1H-indole (**3e**).* System solvent: CH_2Cl_2/MeOH 97:3; yield 83% (170 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (s, 3H, CH_3), 2.80 (br s, 4H, CH_2N), 3.13 (br s, 4H, CH_2N), 3.86 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2Ar), 6.84–7.04 (m, 5H, H_{Ar}), 7.16–7.19 (m, 3H, H_{Ar}), 7.32–7.33 (m, 2H, H_{Ar}), 7.36–7.38 (m, 2H, H_{Ar}), 7.82–7.85 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.9 (CH_3), 50.9 (2 × CH_2), 53.4 (2 × CH_2), 53.6 (CH_2), 55.4 (OCH_3), 110.6 (CH), 111.2 (CH), 112.5 (Cq), 118.3 (CH), 119.7 (CH), 119.8 (CH), 121.0 (CH), 122.2 (CH), 122.9 (CH), 126.8 (CH), 128.2 (2 × CH), 128.3 (CH), 128.5 (Cq), 131.2 (CH), 135.8 (Cq), 137.4 (Cq), 138.3 (Cq), 141.6 (Cq), 152.4 (Cq); IR (neat, cm^{-1}): 3040, 3020, 2805, 1590, 740; MS (ESI): *m/z* 412 $[M+H]^+$; Anal. calcd for $C_{27}H_{29}N_3O$ $C_4H_4O_4$: C 70.57, H 6.30, N 7.96, found: C 70.76, H 6.35, N 8.05; mp (fumarate): 180–181 °C (EtOH/Et_2O).

*4.1.2.6. 1-(2-Methylphenyl)-3-[(4-methylpiperazinyl)methyl]-1H-indole (**3f**).* System solvent: EtOAc/EtOH 2:8 + 1% Et_3N; yield 73% (116 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.06 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.44–2.66 (m, 8H, CH_2N), 3.83 (s, 2H, CH_2Ar), 6.99–7.02 (m, 1H, H_{Ar}), 7.14–7.19 (m, 3H, H_{Ar}), 7.29–7.31 (m, 2H, H_{Ar}), 7.35–7.37 (m, 2H, H_{Ar}), 7.77–7.80 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.6 (CH_3), 45.9 (CH_3), 52.8 (2 × CH_2), 53.3 (CH_2), 55.1 (2 × CH_2), 110.3 (CH), 112.3 (Cq), 119.4 (CH), 119.6 (CH), 121.9 (CH), 126.6 (CH), 127.8 (2 × CH), 127.9 (CH), 128.1 (Cq), 130.9 (CH), 135.4 (Cq), 137.1 (Cq), 137.9 (Cq); IR (neat, cm^{-1}): 3043, 2934, 2796, 1497, 1459, 1262, 1163, 1009, 735; MS (ESI): *m/z* 320 $[M+H]^+$; Anal. calcd for $C_{21}H_{25}N_3$ $C_4H_4O_4$: C 68.95, H 6.71, N 9.65, found: C 70.01, H 6.59, N 9.47; mp (fumarate): 199–200 °C (EtOH/Et_2O).

*4.1.2.7. 1-(3-Methylphenyl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3g**).* System solvent: EtOAc/PE 5:95 to 10:90; yield: 67% (128 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.64 (s, 3H, CH_3), 2.94 (br s, 4H, CH_2N), 3.43 (br s, 4H, CH_2N), 4.05 (s, 2H, CH_2Ar), 7.12–7.15 (m, 3H, H_{Ar}), 7.36–7.56 (m, 9H, H_{Ar}), 7.85 (d, 1H, *J* = 7.0 Hz, H_{Ar}), 8.14 (d, 1H, *J* = 6.4 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 21.3 (CH_3), 48.9 (2 × CH_2), 53.0 (2 × CH_2), 53.5 (CH_2), 110.5 (CH), 113.3 (Cq), 115.8 (2 × CH), 119.3 (CH), 119.9 (CH), 120.0 (CH), 120.9 (CH), 122.4 (CH), 124.6 (CH), 126.9 (CH), 127.2 (CH), 128.9 (2 × CH + Cq), 129.3 (CH), 136.0 (Cq), 139.4 (Cq), 139.5 (Cq), 151.3 (Cq); IR (neat, cm^{-1}): 3054, 2825, 1600, 1496, 1460, 1006; MS (ESI): *m/z* 382 $[M+H]^+$; Anal. calcd for $C_{26}H_{27}N_3$ $C_4H_4O_4$: C 72.41, H 6.28, N 8.44, found: C 72.19, H 6.20, N 8.27; mp (fumarate): 192 °C dec. (EtOH/Et_2O).

*4.1.2.8. 1-(4-Methylphenyl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3h**).* System solvent: EtOAc/PE 2:8; yield: 83% (158 mg); mp: 80–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (s, 3H, CH_3), 2.74 (br s, 4H, CH_2N), 3.23 (br s, 4H, CH_2N), 3.84 (s, 2H, CH_2Ar), 6.84 (*t*, 1H, *J* = 7.1 Hz, H_{Ar}), 6.92 (d, 2H, *J* = 8.3 Hz, H_{Ar}), 7.16–7.38 (m, 9H, H_{Ar}), 7.53 (d, 1H, *J* = 7.9 Hz, H_{Ar}), 7.82 (d, 1H, *J* = 7.1 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9 (CH_3), 48.9 (2 × CH_2), 52.9 (2 × CH_2), 53.4 (CH_2), 110.4 (CH), 113.0 (Cq), 115.8 (2 × CH), 119.3 (CH), 119.8 (CH), 119.9 (CH), 122.3 (CH), 123.9 (2 × CH), 127.3 (CH), 128.9 (2 × CH), 129.1 (Cq), 130.0 (2 × CH), 135.8 (Cq), 136.1 (Cq), 137.0 (Cq), 151.2 (Cq); IR (KBr, cm^{-1}): 3036, 2810, 1601, 1502, 1457, 1055; MS (ESI): *m/z* 382 $[M+H]^+$; Anal. calcd for $C_{26}H_{27}N_3$ $C_4H_4O_4$ 1/4H_2O: C 71.76, H 6.32, N 8.37, found: C 71.74, H 6.12, N 8.19; mp (fumarate): 192 °C dec. (EtOH/Et_2O).

*4.1.2.9. 1-(2-Ethylphenyl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3i**).* System solvent: EtOAc/PE 5:95 to 10:90; yield: 58% (114 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (*t*, 3H, *J* = 7.5 Hz, CH_3), 2.57 (m, 2H, CH_2), 2.89 (br s, 4H, CH_2N), 3.38 (br s, 4H, CH_2N), 4.02 (s, 2H, CH_2Ar), 6.98 (*t*, 1H, *J* = 7.5 Hz, H_{Ar}), 7.07 (d, 2H, *J* = 8.1 Hz, H_{Ar}), 7.17–7.20 (m, 1H, H_{Ar}), 7.31–7.44 (m, 7H, H_{Ar}), 7.53–7.55 (m, 2H, H_{Ar}), 7.95–8.05 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.9 (CH_3), 24.2 (CH_2), 49.1 (2 × CH_2), 53.0 (2 × CH_2), 53.6 (CH_2), 110.5 (CH), 112.1 (Cq), 116.0 (2 × CH), 119.5 (CH), 119.6 (CH), 119.7 (CH), 122.2 (CH), 126.7 (CH), 128.2 (Cq), 128.4 (CH), 128.5 (2 × CH), 129.0 (2 × CH), 129.5 (CH), 137.5 (Cq), 137.8 (Cq), 141.8 (Cq), 151.4 (Cq); IR (neat, cm^{-1}): 3056, 2819, 1600, 1496, 1462, 1378, 742, 693; MS (ESI) *m/z* 396 $[M+H]^+$; Anal. calcd for $C_{27}H_{29}N_3$ $C_4H_4O_4$ 1/2H_2O: C 71.52, H 6.58, N 8.07, found: C 71.44, H 6.36, N 7.97. mp (fumarate): 169–171 °C (EtOH/Et_2O).

*4.1.2.10. 1-(Biphenyl-4-yl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3j**).* The near white solid product was isolated from reaction mixture by filtration; yield: 70% (155 mg); mp 131–132 °C (EtOAc/PE); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.76 (br s, 4H, CH_2N), 3.24 (br s, 4H, CH_2N), 3.88 (s, 2H, CH_2Ar), 6.85 (*t*, 1H, *J* = 7.2 Hz, H_{Ar}), 6.93 (d, 2H, *J* = 7.9 Hz, H_{Ar}), 7.19–7.29 (m, 4H, H_{Ar}), 7.38–7.41 (m, 2H, H_{Ar}), 7.49 (*t*, 2H, *J* = 7.2 Hz, H_{Ar}), 7.58–7.67 (m, 5H, H_{Ar}), 7.74 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 7.84 (d, 1H, *J* = 7.0 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.3 (2 × CH_2), 53.3 (2 × CH_2), 53.7 (CH_2), 110.7 (CH), 113.8 (Cq), 116.1 (2 × CH), 119.6 (CH), 120.2 (CH), 120.4 (CH), 122.7 (CH), 124.4 (2 × CH), 127.1 (2 × CH), 127.4 (CH), 127.6 (CH), 128.3 (2 × CH), 129.0 (2 × CH), 129.2 (2 × CH), 129.5 (Cq), 136.3 (Cq), 139.0 (Cq), 139.3 (Cq), 140.3 (Cq), 151.5 (Cq); IR (KBr, cm^{-1}): 3027, 1596, 1522, 1489, 1456, 1231, 760, 743; MS (ESI): *m/z* 444 $[M+H]^+$. Anal. calcd for $C_{31}H_{29}N_3$: C 83.94, H 6.59, N 9.47, found: C 83.32, H 6.76, N 9.41.

*4.1.2.11. 1-[2-(1-Methylethyl)phenyl]-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3k**).* System solvent: EtOAc/PE 1:9 to 3:7; yield: 50% (102 mg); oil; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.18 (d, 3H, *J* = 6.8 Hz, CH_3), 1.22 (d, 3H, *J* = 6.8 Hz, CH_3), 2.73–2.83 (m, 5H, CH_2N + CH), 3.32 (br s, 4H, CH_2N), 3.97 (s, 2H, CH_2Ar), 6.92 (*t*, 1H, *J* = 7.2 Hz, H_{Ar}), 7.01 (d, 2H, *J* = 7.9 Hz, H_{Ar}), 7.09–7.12 (m, 1H, H_{Ar}), 7.24–7.35 (m, 7H, H_{Ar}), 7.50–7.59 (m, 2H, H_{Ar}), 7.91–7.93 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.6 (CH_3), 24.7 (CH_3), 27.8 (CH), 49.2 (2 × CH_2), 53.0 (2 × CH_2), 53.6 (CH_2), 110.5 (CH), 112.0 (Cq), 116.1 (2 × CH), 119.6 (CH), 119.7 (2 × CH), 122.3 (CH), 126.6 (CH), 126.8 (CH), 128.2 (Cq), 128.7 (CH), 128.9 (CH), 129.0 (CH), 129.1 (2 × CH), 136.8 (Cq), 138.4 (Cq), 146.9 (Cq), 151.5 (Cq); IR (neat, cm^{-1}): $\tilde{\nu}$ 3057, 2963, 2818, 1600, 1496, 1461, 1380, 1006, 742; MS (ESI): *m/z* 410 $[M+H]^+$; Anal. calcd for $C_{28}H_{31}N_3$ $C_4H_4O_4$ 1/3H_2O: C 72.29, H 6.76, N 7.90, found: C 72.19, H 6.69, N 8.01; mp (fumarate): 160–161 °C (EtOH/Et_2O).

*4.1.2.12. 3-{[4-(2-Methoxyphenyl)piperazinyl]methyl}-1-[2-(1-methylethyl)phenyl]-1H-indole (**3l**).* System solvent: EtOAc/PE 4:6 to 9:1; yield: 60% (132 mg); foam; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (d, 3H, *J* = 6.8 Hz, CH_3), 1.13 (d, 3H, *J* = 6.8 Hz, CH_3), 2.17 (m, 1H, CH), 2.79 (br s, 4H, CH_2N), 3.12 (br s, 4H, CH_2N), 3.85 (s, 3H, OCH_3), 3.89 (s, 2H, CH_2Ar), 6.84–7.01 (m, 5H, H_{Ar}), 7.15–7.18 (m, 3H, H_{Ar}), 7.24–7.33 (m, 2H, H_{Ar}), 7.42–7.50 (m, 2H, H_{Ar}), 7.81–7.84 (m, 2H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.6 (CH_3), 24.7 (CH_3), 27.9 (CH), 50.9 (2 × CH_2), 53.4 (2 × CH_2), 53.6 (CH_2), 55.4 (CH_3), 110.6 (CH), 111.2 (CH), 112.2 (Cq), 118.4 (CH), 119.6 (CH), 119.7 (CH), 121.1 (CH), 122.2 (CH), 122.9 (CH), 126.6 (CH), 126.8 (CH), 128.4 (Cq), 128.8 (CH), 128.9 (CH), 129.0 (Cq), 136.9 (Cq), 138.4 (Cq), 141.6 (Cq), 147.1 (Cq), 152.4 (Cq); IR (KBr, cm^{-1}): 3043, 2956, 1495, 1459, 1237, 1027, 738; MS (ESI): *m/z* 440 $[M+H]^+$; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{29}H_{34}N_3O$: 440.27019, found: 440.27021.

*4.1.2.13. 1-(2,3-Dimethylphenyl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3m**).* System solvent: EtOAc/PE 2:8 to 3:7; yield: 80% (159 mg); mp 151–152 °C (EtOAc/PE); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.94 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 2.75 (br s, 4H, CH_2N), 3.24 (br s, 4H, CH_2N), 3.87 (s, 2H, CH_2Ar), 6.82 (*t*, 1H, *J* = 7.2 Hz, H_{Ar}), 6.93 (d, 2H, *J* = 8.5 Hz, H_{Ar}), 6.99–7.03 (m, 1H, H_{Ar}), 7.15–7.29 (m, 8H, H_{Ar}), 7.82–7.85 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5 (CH_3), 20.5

(CH_3), 49.3 (2 × CH_2), 53.1 (2 × CH_2), 53.7 (CH_2), 110.7 (CH), 112.2 (Cq), 116.0 (2 × CH), 119.5 (CH), 119.6 (CH), 119.8 (CH), 122.2 (CH), 125.8 (CH), 126.1 (CH), 128.3 (Cq), 128.5 (CH), 129.1 (2 × CH), 129.7 (CH), 134.5 (Cq), 137.7 (Cq), 138.2 (Cq), 138.5 (Cq), 151.5 (Cq); IR (KBr, cm^{-1}): 2931, 2812, 1601, 1578, 1480, 1387, 1080, 739; SM (ESI): *m/z* 396 $[M+H]^+$; Anal. calcd for $C_{27}H_{29}N_3$ $C_4H_4O_4$: C 72.78, H 6.50, N 8.21, found: C 72.45, H 6.64, N 8.16; mp (fumarate): >200 °C (EtOH/Et_2O).

*4.1.2.14. 3-{[4-(2-Methoxyphenyl)piperazinyl]methyl}-1-(2,3-dimethylphenyl)-1H-indole (**3n**).* System solvent: EtOAc/PE 4:6; yield: 60% (128 mg); mp 135–136 °C (EtOAc/PE); ^1H NMR (300 MHz, $CDCl_3$): δ 1.94 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.82 (br s, 4H, CH_2N), 3.14 (br s, 4H, CH_2N), 3.86 (s, 3H, OCH_3), 3.91 (s, 2H, CH_2Ar), 6.84–7.04 (m, 5H, H_{Ar}), 7.16–7.28 (m, 6H, H_{Ar}), 7.82–7.85 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.5 (CH_3), 20.5 (CH_3), 50.8 (2 × CH_2), 53.3 (2 × CH_2), 53.6 (CH_2), 55.4 (CH_3), 110.7 (CH), 111.1 (CH), 112.1 (Cq), 118.3 (CH), 119.6 (CH), 119.7 (CH), 121.0 (CH), 122.1 (CH), 122.8 (CH), 125.8 (CH), 126.1 (CH), 128.4 (Cq), 128.6 (CH), 129.6 (CH), 134.5 (Cq), 137.6 (Cq), 138.2 (Cq), 138.5 (Cq), 141.5 (Cq), 152.3 (Cq); IR (KBr, cm^{-1}): 3048, 2997, 1501, 1478, 1448, 1238, 1004, 733; MS (ESI): *m/z* 426 $[M+H]^+$; Anal. calcd for $C_{28}H_{31}N_3O$: C 79.02, H 7.34, N 9.87, found: C 78.88, H 7.33, N 10.00.

*4.1.2.15. 1-(Naphth-1-yl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3o**).* System solvent: EtOAc/PE 1:9; yield: 46% after recristallization from MeOH (96 mg); mp 140–141 °C (MeOH); ^1H NMR (300 MHz, $CDCl_3$): δ 2.94 (br s, 4H, CH_2N), 3.42 (br s, 4H, CH_2N), 4.08 (s, 2H, CH_2Ar), 7.02 (*t*, 1H, *J* = 7.5 Hz, H_{Ar}), 7.10 (d, 2H, *J* = 7.5 Hz, H_{Ar}), 7.20 (d, 1H, *J* = 7.9 Hz, H_{Ar}), 7.31 (dd, 1H, *J* = 7.4 Hz, H_{Ar}), 7.35–7.37 (m, 1H, H_{Ar}), 7.43 (dd, 2H, *J* = 7.5 Hz, H_{Ar}), 7.50 (s, 1H, H_{Ar}), 7.55–7.58 (m, 1H, H_{Ar}), 7.64–7.77 (m, 4H, H_{Ar}), 8.09–8.11 (m, 3H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.2 (2 × CH_2), 53.1 (2 × CH_2), 53.6 (CH_2), 110.9 (CH), 112.8 (Cq), 116.0 (2 × CH), 119.5 (CH), 119.9 (CH), 120.0 (CH), 122.4 (CH), 123.4 (CH), 125.1 (CH), 125.5 (CH), 126.6 (CH), 126.9 (CH), 128.3 (CH), 128.4 (CH), 128.5 (Cq), 129.1 (2 × CH), 129.2 (CH), 130.4 (Cq), 134.5 (Cq), 135.9 (Cq), 138.4 (Cq), 151.4 (Cq); IR (KBr, cm^{-1}): 3054, 2904, 2799, 1598, 1575, 1499, 1450, 1407, 1001, 738, 690; Anal. calcd for $C_{29}H_{27}N_3$ $C_4H_4O_4$.1/4H_2O: C 73.66, H 5.90, N 7.81, found: C 73.81, H 5.68, N 7.64; mp (fumarate): 202–203 °C dec. (EtOH/Et_2O).

*4.1.2.16. 3-{[4-(2-Methoxyphenyl)piperazinyl]methyl}-1-(naphth-1-yl)-1H-indole (**3p**).* System solvent: EtOAc/PE 2:8 to 1:1; yield: 70% (157 mg); foam; ^1H NMR (300 MHz, $CDCl_3$): δ 2.87 (br s, 4H, CH_2N), 3.18 (br s, 4H, CH_2N), 3.88 (s, 3H, OCH_3), 3.97 (s, 2H, CH_2Ar), 6.87–7.06 (m, 5H, H_{Ar}), 7.14–7.26 (m, 2H, H_{Ar}), 7.37 (s, 1H, H_{Ar}), 7.41–7.46 (m, 1H, H_{Ar}), 7.52–7.63 (m, 4H, H_{Ar}), 7.93 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 7.94–8.00 (m, 2H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.9 (2 × CH_2), 53.4 (2 × CH_2), 53.7 (CH_2), 55.4 (CH_3), 110.9 (CH), 111.2 (CH), 113.0 (Cq), 118.3 (CH), 119.9 (2 × CH), 121.0 (CH), 122.3 (CH), 122.8 (CH), 123.6 (CH), 125.2 (CH), 125.6 (CH), 126.7 (CH), 126.9 (CH), 128.3 (CH), 128.4 (CH), 128.7 (Cq), 129.4 (CH), 130.5 (Cq), 134.5 (Cq), 136.1 (Cq), 138.4 (Cq), 141.6 (Cq), 152.4 (Cq); IR (KBr, cm^{-1}): 3045, 2935, 2811, 1594, 1499, 1452, 1238, 1005, 742; MS (ESI): *m/z* 448 $[M+H]^+$; Anal. calcd for $C_{30}H_{29}N_3O$ $C_4H_4O_4$: C 72.45, H 5.90, N 7.46, found: C 72.68, H 6.11, N 7.51.

*4.1.2.17. 2-{4-[(Naphthyl-1H-indol-3-yl)methyl]piperazinyl}phenol (**3q**).* System solvent: EtOAc/PE 3:7 to 1:1; yield: 60% (130 mg); foam; ^1H NMR (300 MHz, $CDCl_3$): δ 2.85 (br s, 4H, CH_2N), 3.01 (br s, 4H, CH_2N), 3.99 (s, 2H, CH_2Ar), 6.92 (*t*, 1H, *J* = 7.7 Hz, H_{Ar}), 7.03 (d, 1H, *J* = 7.9 Hz, H_{Ar}), 7.09–7.16 (m, 2H, H_{Ar}), 7.20–7.32 (m, 3H, H_{Ar}), 7.42 (s, 1H, H_{Ar}), 7.45–7.50 (m, 1H, H_{Ar}), 7.55–7.67 (m, 4H, H_{Ar}), 7.97–8.04 (m, 3H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 52.7 (2 × CH_2), 53.7 (2 × CH_2), 53.8 (CH_2), 111.0 (CH), 112.6 (Cq), 114.1 (CH), 119.9 (CH), 120.1 (2 × CH), 121.6 (CH), 122.4 (CH), 123.4 (CH), 125.2 (CH), 125.6 (CH), 126.4 (CH), 126.7 (CH), 127.0 (CH), 128.4 (CH), 128.5 (CH), 128.6 (Cq), 129.4 (CH), 130.5 (Cq), 134.5 (Cq), 135.9 (Cq), 138.5 (Cq), 139.2 (Cq), 151.6 (Cq); IR (KBr, cm^{-1}): 3049, 2936, 1595, 1491, 1453, 1247, 1003, 741; HRMS-ESI: *m/z* $[M+H]^+$ calcd for $C_{29}H_{28}N_3O$: 434.22324, found: 434.22329.

*4.1.2.18. 1-(Naphth-2-yl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3r**).* System solvent: EtOAc/PE 3:7 to 1:1; yield: 78% (162 mg); mp 128–129 °C (EtOAc/PE); ^1H NMR (300 MHz, $CDCl_3$): δ 2.62 (br s, 4H, CH_2N), 3.14 (br s, 4H, CH_2N), 3.79 (s, 2H, CH_2Ar), 6.75 (*t*, 1H, *J* = 7.2 Hz, CH_2), 6.91 (d, 2H, *J* = 8.1 Hz, H_{Ar}), 7.15–7.27 (m, 4H, H_{Ar}), 7.54–7.62 (m, 2H, H_{Ar}), 7.67 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 7.72 (s, 1H, H_{Ar}), 7.79 (dd, 1H, *J* = 2.1, 8.8 Hz, H_{Ar}), 7.83 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 8.01–8.05 (m, 2H, H_{Ar}), 8.11–8.14 (m, 2H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.3 (2 × CH_2), 53.2 (2 × CH_2), 53.6 (CH_2), 110.7 (CH), 113.8 (Cq), 116.1 (2 × CH), 119.6 (CH), 120.2 (CH), 120.4 (CH), 121.7 (CH), 122.7 (CH), 123.2 (CH), 126.1 (CH), 127.0 (CH), 127.6 (CH), 127.8 (CH), 127.9 (CH), 129.2 (2 × CH), 129.5 (Cq), 129.7 (CH), 131.8 (Cq), 133.9 (Cq), 136.4 (Cq), 137.2 (Cq), 151.5 (Cq); IR (KBr, cm^{-1}): 3058, 2935, 1595, 1507, 1469, 1452, 1236, 1132, 1002, 744; MS (ESI): *m/z* 418 $[M+H]^+$; Anal. calcd for $C_{29}H_{27}N_3$: C 83.42, H 6.52, N 10.06, found: C 83.60, H 6.49, N 9.56; mp (fumarate): 128–129 °C (EtOH/Et_2O).

*4.1.2.19. 3-[(4-Phenylpiperazinyl)methyl]-1-[2-(trifluoromethyl)phenyl]-1H-indole (**3s**).* System solvent: EtOAc/PE 2:8; yield: 49% (93 mg); mp 101–103 °C (EtOAc/PE); ^1H NMR (300 MHz, $CDCl_3$): δ 2.78 (br s, 4H, CH_2N), 3.36 (br s, 4H, CH_2N), 3.92 (s, 2H, CH_2Ar), 6.89 (*t*, 1H, *J* = 7.3 Hz, H_{Ar}), 6.98 (d, 2H, *J* = 8.1 Hz, H_{Ar}), 7.03–7.09 (m, 1H, H_{Ar}), 7.22–7.28 (m, 3H, H_{Ar}), 7.31 (dd, 2H, *J* = 7.7 Hz, H_{Ar}), 7.50 (d, 1H, *J* = 7.7 Hz, H_{Ar}), 7.62 (*t*, 1H, *J* = 7.4 Hz, H_{Ar}), 7.71 (*t*, 1H, *J* = 7.4 Hz, H_{Ar}), 7.87–7.92 (m, 2H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.2 (2 × CH_2), 52.9 (2 × CH_2), 53.4 (CH_2), 110.4 (CH), 112.7 (Cq), 116.1 (2 × CH), 119.6 (CH), 119.8 (CH), 120.2 (CH), 122.6 (CH), 125.0 (Cq, J_{C-F} = 271.0 Hz), 127.5 (CH, J_{C-F} = 5.0 Hz), 128.4 (Cq), 128.6 (Cq, J_{C-F} = 30.5 Hz), 128.7 (CH), 129.1 (2 × CH), 129.3 (CH), 131.0 (CH), 132.9 (CH), 137.5 (Cq), 138.9 (Cq), 151.4 (Cq); IR (KBr, cm^{-1}): 3039, 2928, 2821, 1601, 1503, 1465, 1451, 1316, 1055, 1004, 748; HRMS (CI): *m/z* $[M+H]^+$ calcd for $C_{26}H_{25}F_3N_3$: 436.2001, found: 436.2003; mp (fumarate): 165–167 °C (EtOH/Et_2O).

*4.1.2.20. 1-(2-Methoxyphenyl)-3-[(4-phenylpiperazinyl)methyl]-1H-indole (**3t**).* System solvent: CH_2Cl_2/MeOH 99:1 to 98:2; yield: 73% (145 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.74 (br s, 4H, CH_2N), 3.24 (br s, 4H, CH_2N), 3.79 (s, OCH_3), 3.85 (s, 2H, CH_2Ar), 6.84 (d, 1H, *J* = 7.3 Hz, H_{Ar}), 6.91–6.94 (m, 2H, H_{Ar}), 7.05–7.28 (m, 8H, H_{Ar}), 7.35–7.41 (m, 2H, H_{Ar}), 7.79–7.82 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.2 (2 × CH_2), 53.1 (2 × CH_2), 53.7 (CH_2Ar), 55.7 (OCH_3), 111.0 (CH), 112.3 (Cq), 112.5 (CH), 116.0 (2 × CH), 119.5 (CH), 119.69 (CH), 119.73 (CH), 120.9 (CH), 122.1 (CH), 128.0 (CH), 128.1 (Cq), 128.5 (CH), 128.6 (Cq), 128.9 (CH), 129.1 (2 × CH), 137.2 (Cq), 151.5 (Cq), 154.3 (Cq); IR (neat, cm^{-1}): 3060, 1666, 1597, 745, 691; MS (ESI): *m/z* 398 $[M+H]^+$; Anal. calcd for $C_{26}H_{27}N_3O$ $C_4H_4O_4$: C 70.16, H 6.08, N 8.18, found: C 70.26, H 6.00, N 8.18; mp (fumarate): 179 °C dec. (EtOH/Et_2O).

*4.1.2.21. 2-{[3-(4-Phenylpiperazinyl)methyl]indol-1-yl}benzonitrile (**3u**).* System solvent: CH_2Cl_2/MeOH 99:1; yield: 71% (139 mg); ^1H NMR (300 MHz, $CDCl_3$): δ 2.74 (br s, 4H, CH_2N), 3.22 (br s, 4H, CH_2N), 3.84 (s, 2H, CH_2Ar), 6.83 (*t*, 1H, *J* = 7.3 Hz, H_{Ar}), 6.92–6.94 (m, 2H, H_{Ar}), 7.20–7.29 (m, 4H, H_{Ar}), 7.33–7.36 (m, 1H, H_{Ar}), 7.40 (s, 1H, H_{Ar}), 7.49 (dt, 1H, *J* = 1.1, 7.5 Hz, H_{Ar}), 7.63–7.65 (m, 1H, H_{Ar}), 7.75 (dt, 1H, *J* = 1.5, 7.5 Hz, H_{Ar}), 7.85 (d, 1H, *J* = 7.9 Hz, H_{Ar}), 7.86 (d, 1H, *J* = 7.3 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.2 (2 × CH_2), 53.2

(2 × CH_2), 53.5 (CH_2Ar), 109.6 (Cq), 110.3 (CH), 115.0 (Cq), 116.0 (2 × CH), 116.6 (Cq),119.5 (CH), 120.4 (CH), 121.0 (CH), 123.1 (CH), 127.2 (CH), 127.3 (CH), 127.5 (CH), 129.1 (2 × CH), 129.3 (Cq), 133.9 (CH), 134.6 (CH), 136.7 (Cq), 141.9 (Cq), 151.4 (Cq); IR (neat, cm^{-1}): 3060, 2227, 1666, 1596, 747, 694; MS (ESI): *m/z* 393 $[M+H]^+$; Anal. calcd for $C_{26}H_{24}N_4$ $C_4H_4O_4$: C 70.85, H 5.55, N 11.02, found: C 70.68, H 5.52, N 10.79; mp (fumarate): 173–175 °C dec. (EtOH/Et_2O).

*4.1.2.22. 3-[(4-Phenylpiperazinyl)methyl]-1-(pyridin-2-yl)-1H-indole (**3v**).* System solvent EtOAc/PE 1:1 to 7:3; yield: 92% (170 mg); mp 113–114 °C (EtOAc/PE); ^1H NMR (300 MHz, $CDCl_3$): δ 2.74 (br s, 4H, CH_2N), 3.23 (br s, 4H, CH_2N), 3.84 (s, 2H, CH_2Ar), 6.84 (*t*, 1H, *J* = 7.2 Hz, H_{Ar}), 6.92 (d, 2H, *J* = 8.1 Hz, H_{Ar}), 7.16 (dd, 1H, *J* = 5.1, 7.0 Hz, H_{Ar}), 7.24–7.35 (m, 4H, H_{Ar}), 7.51 (d, 1H, *J* = 8.3 Hz, H_{Ar}), 7.74 (s, 1H, H_{Ar}), 7.79–7.84 (m, 2H, H_{Ar}), 8.21 (d, 1H, *J* = 8.3 Hz, H_{Ar}), 8.57 (d, 1H, *J* = 3.8 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.3 (2 × CH_2), 53.2 (2 × CH_2), 53.7 (CH_2), 113.1 (CH), 114.4 (CH), 115.5 (Cq), 116.1 (2 × CH), 119.6 (CH), 119.9 (CH), 120.1 (CH), 121.2 (CH), 123.4 (CH), 125.3 (CH), 129.1 (2 × CH), 130.5 (Cq), 135.6 (Cq), 138.4 (CH), 149.0 (CH), 151.5 (Cq), 152.5 (Cq); IR (KBr, cm^{-1}): 3058, 2951, 1595, 1484, 1450, 1220, 1128, 1002, 744; MS (ESI): *m/z* 369 $[M+H]^+$; Anal. calcd for $C_{24}H_{24}N_4$: C 78.23, H 6.56, N 15.21, found: C 77.80, H 6.34, N 15.59.

*4.1.2.23. 3-{[4-(2-Methoxyphenyl)piperazinyl]methyl}-1-(pyridin-2-yl)-1H-indole (**3w**).* System solvent: EtOAc/PE 1:1; yield: 65% (130 mg); mp 131–132 °C (EtOAc/PE); ^1H NMR (300 MHz, $CDCl_3$): δ 2.93 (br s, 4H, CH_2N), 3.19 (br s, 4H, CH_2N), 3.83 (s, 3H, OCH_3), 4.01 (s, 2H, CH_2Ar), 6.82–7.02 (m, 4H, H_{Ar}), 7.13–7.17 (m, 1H, H_{Ar}), 7.22–7.34 (m, 2H, H_{Ar}), 7.54 (d, 1H, *J* = 8.3 Hz, H_{Ar}), 7.74–7.91 (m, 3H, H_{Ar}), 8.25 (d, 1H, *J* = 8.1 Hz, H_{Ar}), 8.55 (d, 1H, *J* = 3.6 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.9 (2 × CH_2), 52.8 (3 × CH_2), 55.3 (CH_3), 111.1 (CH), 112.9 (CH), 113.4 (CH), 114.5 (CH), 118.3 (CH), 119.5 (CH), 120.0 (CH), 120.9 (CH), 121.3 (CH), 123.4 (CH), 126.5 (CH), 130.3 (Cq), 135.4 (CH), 138.5 (CH), 140.9 (CH), 148.8 (Cq), 148.9 (Cq), 152.1 (Cq), 152.3 (Cq); IR (KBr, cm^{-1}): 3053, 2992, 1471, 1448, 1434, 1240, 1124, 747; MS (ESI): *m/z* 399 $[M+H]^+$; Anal. calcd for $C_{25}H_{26}N_4O$ $C_4H_4O_4$: C 67.69, H 5.88, N 10.89, found: C 67.89, H 6.10, N 10.90; mp (fumarate): 140–141 °C (EtOH/Et_2O).

*4.1.2.24. 3-[(4-Phenyl-1-piperazinyl)methyl]-1-(pyridin-3-yl)-1H-indole (**3x**).* System solvent: EtOAc/PE 6:4 + 1% Et_3N; yield: 70% (129 mg); mp 85–86 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 2.78 (br s, 4H, CH_2N), 3.28 (br s, 4H, CH_2N), 3.89 (s, 2H, CH_2Ar), 6.90 (*t*, 1H, *J* = 7.2 Hz, H_{Ar}), 6.98 (d, 2H, *J* = 8.3 Hz, H_{Ar}), 7.27–7.34 (m, 4H, H_{Ar}), 7.37 (s, 1H, H_{Ar}), 7.49 (dd, 1H, *J* = 4.7, 8.1 Hz, H_{Ar}), 7.59 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 7.88 (d, 1H, *J* = 7.9 Hz, H_{Ar}), 7.93 (d, 1H, *J* = 7.1 Hz, H_{Ar}), 8.65 (d, 1H, *J* = 3.8 Hz, H_{Ar}), 8.91 (d, 1H, *J* = 2.3 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 49.2 (2 × CH_2), 53.2 (2 × CH_2), 53.5 (CH_2), 110.0 (CH), 114.8 (Cq), 116.0 (2 × CH), 119.6 (CH), 120.3 (CH), 120.7 (CH), 123.1 (CH), 124.1 (CH), 126.7 (CH), 129.1 (2 × CH), 129.5 (Cq), 131.1 (CH), 136.1 (Cq), 136.3 (Cq), 145.5 (CH), 147.4 (CH), 151.4 (Cq); IR (KBr, cm^{-1}): 3036, 2941, 2824, 1599, 1557, 1488, 1457, 1002, 743; MS (ESI): *m/z* 369 $[M+H]^+$; Anal. calcd for $C_{24}H_{24}N_4$ $C_4H_4O_4$: C 69.41, H 5.82, N 11.56, found: C 69.25, H 5.92, N 11.40; mp (fumarate): 150–151 °C (EtOH/Et_2O).

*4.1.2.25. 1-Phenyl-3-[(3,6-dihydro-4-phenyl-1(2H)-pyridinyl)methyl]-1H-indole (**4a**).* System solvent: CH_2Cl_2/MeOH 98:2; yield: 66% (121 mg); foam; ^1H NMR (300 MHz, $CDCl_3$): δ 2.60 (s, 2H, CH_2N), 2.85 (*t*, 2H, *J* = 5.7 Hz, CH_2N), 3.31 (br s, 2H, CH_2N), 3.93 (s, 2H, CH_2Ar), 6.10 (s, 1H, =CH), 7.18–7.44 (m, 9H, H_{Ar}), 7.48–7.56 (m, 4H, H_{Ar}), 7.59 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 7.83 (d, 1H, *J* = 5.8 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.7 (CH_2), 50.5 (CH_2), 53.4 (CH_2), 53.9 (CH_2), 111.1 (CH), 114.2 (Cq), 120.4 (CH), 120.7 (CH), 122.6 (CH), 123.0 (CH), 124.6 (2 × CH), 125.4 (2 × CH), 127.8 (CH), 127.4 (CH), 127.9 (CH), 128.8 (2 × CH), 130.0 (Cq), 130.1 (2 × CH), 135.4 (Cq), 136.5 (Cq), 140.2 (Cq), 141.5 (Cq); IR (neat, cm^{-1}): 3046, 1636, 1594, 1498, 742, 694; MS (ESI): *m/z* 365 $[M+H]^+$; Anal. calcd for $C_{26}H_{24}N_2$ $C_4H_4O_4$: C 74.98, H 5.87, N 5.83, found: C 75.30, H 5.84, N 5.96; mp (fumarate): 165–167 °C dec. (EtOH).

*4.1.2.26. 3-[(3,6-Dihydro-4-phenyl-1(2H)-pyridinyl)methyl]-1-(2-methylphenyl)-1H-indole (**4b**).* System solvent: CH_2Cl_2/MeOH 98:2; yield: 50% (95 mg); foam; ^1H NMR (300 MHz, $CDCl_3$): δ 2.09 (s, 3H, CH_3), 2.60 (s, 2H, CH_2N), 2.85 (*t*, 2H, *J* = 5.8 Hz, CH_2N), 3.31 (br s, 2H, CH_2N), 3.95 (s, 2H, CH_2Ar), 6.09 (s, 1H, =CH), 7.01–7.06 (m, 1H, H_{Ar}), 7.15–7.41 (m, 12H, H_{Ar}), 7.80–7.84 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8 (CH_3), 28.2 (CH_2), 50.0 (CH_2), 52.9 (CH_2), 53.2 (CH_2), 110.6 (CH), 112.4 (Cq), 119.6 (CH), 119.7 (CH), 122.2 (2 × CH), 124.9 (2 × CH), 126.8 (CH), 126.9 (CH), 128.1 (CH), 128.2 (CH), 128.3 (CH), 128.4 (2 × CH), 128.5 (Cq), 131.2 (CH), 134.9 (Cq), 135.7 (Cq), 137.3 (Cq), 138.2 (Cq), 141.0 (Cq); IR (neat, cm^{-1}): 3052, 1660, 1598, 1498, 742, 694; MS (ESI): *m/z* 379 $[M+H]^+$; Anal. calcd for $C_{27}H_{26}N_2$ $C_4H_4O_4$: C 75.28, H 6.11, N 5.66, found: C 75.37, H 6.12, N 5.79; mp (fumarate): 155–157 °C dec. (EtOH).

*4.1.3. General procedure for preparation of **5***

N-arylations were performed under microwave heating using a Biotage Initiator at 2.45 GHz in a seal processed vial (0.5–2.0 mL). The specified reaction temperature was fixed by continuous power delivery adjustment. *N,N*-dimethyltryptamine (0.55 mmol), aryl iodide (1.3 equiv), Cu_2O (0.3 equiv) and K_2CO_3 (1.5 equiv) in anhydrous DMF (1.2 mL) were heated 120 min at 240 °C under argon. After cooling to room temperature, the reaction mixture was filtrated on Celite eluting with EtOAc. Filtrate was then concentrated under vacuum and chromatographied on silica gel using the system solvent CH_2Cl_2/MeOH 95:5 + εNH_4OH.

*4.1.3.1. N,N-Dimethyl-1-phenyl-1H-indol-3-ethanamine (**5a**) (CAS: 109692-21-9).* Yield: 90% (131 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.40 (s, 6H, $N(CH_3)_2$), 2.71–2.77 (m, 2H, CH_2), 3.01–3.07 (m, 2H, CH_2), 7.13–7.23 (m, 3H, H_{Ar}), 7.30–7.37 (m, 1H, H_{Ar}), 7.49–7.58 (m, 5H, H_{Ar}), 7.66 (d, 1H, *J* = 7.4 Hz, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.6 (CH_2), 45.5 (2 × CH_3), 60.2 (CH_2), 110.5 (CH), 115.5 (Cq), 119.1 (CH), 119.9 (CH), 122.4 (CH), 124.0 (2 × CH), 125.2 (CH), 126.0 (CH), 129.0 (Cq), 129.5 (2 × CH), 135.9 (Cq), 139.8 (Cq); IR (neat, cm^{-1}): 3043, 1596, 1500, 737, 695; MS (ESI): *m/z* 265 $[M+H]^+$; Anal. calcd for $C_{18}H_{20}N_2$ $C_4H_4O_4$: C 69.46, H 6.39, N 7.36, found: C 69.31, H 6.44, N 6.99; mp (fumarate): 120–122 °C (EtOH/Et_2O).

*4.1.3.2. N,N-Dimethyl-1-(2-methylphenyl)-1H-indol-3-ethanamine (**5b**).* Yield: 92% (141 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.07 (s, 3H, CH_3), 2.40 (s, 6H, $N(CH_3)_2$), 2.73–2.76 (m, 2H, CH_2), 3.02–3.07 (m, 2H, CH_2), 7.00–7.03 (m, 2H, H_{Ar}), 7.14–7.19 (m, 2H, H_{Ar}), 7.27–7.38 (m, 4H, H_{Ar}), 7.66–7.69 (m, 1H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8 (CH_3), 23.8 (CH_2), 45.6 (2 × CH_3), 60.5 (CH_2), 110.6 (CH), 114.4 (Cq), 119.0 (CH), 119.4 (CH), 122.1 (CH), 126.1 (CH), 126.8 (CH), 127.9 (Cq), 128.0 (CH), 128.1 (CH), 131.2 (CH), 135.8 (Cq), 137.3 (Cq), 138.4 (Cq); IR (neat, cm^{-1}): 3048, 2925, 1498, 1460, 738; MS (ESI): *m/z* 279 $[M+H]^+$; Anal. calcd for $C_{19}H_{22}N_2$ $C_4H_4O_4$: C 70.03, H 6.64, N 7.10, found: C 70.31, H 6.72, N 7.35; mp (fumarate): 110–111 °C (EtOH/Et_2O).

*4.1.3.3. N,N-Dimethyl-1-(2,3-dimethylphenyl)-1H-indol-3-ethanamine (**5c**).* Yield: 92% (148 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 1.92 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 2.42 (s, 6H, $N(CH_3)_2$), 2.74–2.79 (m, 2H, CH_2), 3.04–3.09 (m, 2H, CH_2), 6.98–7.01 (m, 2H, H_{Ar}), 7.12–7.24 (m, 5H, H_{Ar}), 7.65–7.69 (m, 1H, H_{Ar}); ^{13}C NMR

(75 MHz, $CDCl_3$): δ 14.3 (CH_3), 20.3 (CH_3), 23.4 (CH_2), 45.3 (2 × CH_3), 60.5 (CH_2), 110.6 (CH), 113.8 (Cq), 118.8 (CH), 119.2 (CH), 121.9 (CH), 125.6 (CH), 125.9 (CH), 126.3 (CH), 127.7 (Cq), 129.3 (CH), 134.3 (Cq), 137.4 (Cq), 138.2 (Cq), 138.3 (Cq); IR (neat, cm^{-1}): 3048, 2940, 1479, 1455, 737; MS (ESI): *m/z* 293 $[M+H]^+$; Anal. calcd for $C_{20}H_{24}N_2$ $C_4H_4O_4$: C 70.57, H 6.91, N 6.86, found: C 70.74, H 6.75, N 6.50; mp (fumarate): 145–146 °C (EtOH/Et_2O).

*4.1.3.4. N,N-Dimethyl-1-(naphth-1-yl)-1H-indol-3-ethanamine (**5d**).* Yield: 85% (147 mg); oil; ^1H NMR (300 MHz, $CDCl_3$): δ 2.50 (s, 6H, $N(CH_3)_2$), 2.90 (br s, 2H, CH_2), 3.16 (br s, 2H, CH_2), 7.03 (d, 1H, *J* = 7.5 Hz, H_{Ar}), 7.11–7.22 (m, 3H, H_{Ar}), 7.41–7.60 (m, 5H, H_{Ar}), 7.73 (d, 1H, *J* = 7.2 Hz, H_{Ar}), 7.94–7.98 (m, 2H, H_{Ar}); ^{13}C NMR (75 MHz, $CDCl_3$): δ 23.9 (CH_2), 45.6 (2 × CH_3), 60.5 (CH_2), 110.9 (CH), 114.9 (Cq), 119.1 (Cq), 119.7 (CH), 122.3 (CH), 123.6 (CH), 125.1 (CH), 125.6 (CH), 126.6 (CH), 126.9 (CH), 127.3 (CH), 128.2 (CH), 128.3 (2 × CH), 130.6 (Cq), 134.6 (Cq), 136.2 (Cq), 138.3 (Cq); IR (neat, cm^{-1}): 3043, 2931, 1469, 1454, 737; MS (ESI): *m/z* 315 $[M+H]^+$; Anal. calcd for $C_{22}H_{22}N_2$ $C_4H_4O_4$: C 72.54, H 6.09, N 6.51, found: C 72.21, H 5.93, N 6.28; mp (fumarate): 168–170 °C (EtOH/Et_2O).

4.2. Biological studies

4.2.1. Cell culture and preparation of cell membranes

HEK293 cells with stable expression of human serotonin 5-HT_{7b}R (prepared with the use of Lipofectamine 2000) were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were grown in Dulbeco's Modifier Eagle Medium containing 10% dialysed foetal bovine serum and 500 mg/ml G418 sulphate. For membranes preparations, cells were subcultured in 10 cm diameter dishes, grown to 90% confluence, washed twice with pre-warmed to 37 °C phosphate buffered saline (PBS) and were pelleted by centrifugation (200 g) in PBS containing 0.1 mM EDTA and 1 mM dithiothreitol, and stored at −80 °C. Cell pellets were thawed and homogenized in 20 volumes of assay buffer using an Ultra Turrax tissue homogenizer, centrifuged twice at 35,000 *g* for 20 min at 4 °C, with incubation for 15 min at 37 °C in between and final pellets were frozen at −80 °C until used in experiments. For 5-HT_{1A}R binding assay frozen rat hippocampi were homogenized in 20 volumes of ice-cold Tris–HCl buffer (50 mM, pH = 7.4 at 25 °C), and were centrifuged at 35,000 *g* for 15 min. The pellet was resuspended in the same volume of buffer, incubated for 10 min at 37 °C and centrifuged once again. The final pellet was frozen at −80 °C until used for binding assays.

4.2.2. Radioligand binding assays for 5-HT_{1A} and 5-HT_7 receptors

To determine the affinity of the synthesized compounds for serotonin 5-HT_{1A} and 5-HT_7 receptors were employed according to previously published procedures [10]. This was accomplished by displacement of [^{3}H]-8-OH-DPAT from rat hippocampus homogenate for 5-HT_{1A}R, and [^{3}H]-5-CT from cloned human 5-HT_{7b}R stably expressed in HEK293 cells.

On the day of experiment, frozen pellets were thawed and suspended in 50 mM Tris–HCl supplemented with: 4 mM $MgCl_2$, 10 mM pargyline and 0.1% ascorbate. Assay samples were incubated in a total volume of 1 mL at 37 °C for 15 min for 5-HT_{1A}R and in 0.2 mL in 96-well microtitre plates for 1 h at 37 °C for 5-HT_{7b}R. The process of equilibration was terminated by rapid filtration through Whatman GF/B filters using the Brandell cell harvester for 5-HT_{1A}R or through unifilter plates with a 96-well cell harvester (PerkinElmer) in the case of 5-HT_{7b}R. Radioactivity retained on the filters was quantified on a Beckman LS6500 scintillation counter or on a MicroBeta plate reader (Perkin Elmer), for 5-HT_{1A}R and 5-HT_7R, respectively.

For displacement studies the assay samples contained 1 nM [^{3}H]-8-OH-DPAT (170 Ci/mmol, PerkinElmer) for 5-HT_{1A}R and 0.6 nM [^{3}H]-5-CT (93.0 Ci/mmol, Hartmann Analytic GmbH) for 5-HT_7R. Both assays used 10 μM of 5-HT for non-specific binding determination. Each compound was tested in triplicate at 7–8 concentrations (10^{-11}–10^{-4} M). The inhibition constants (*Ki*) were calculated from the Cheng–Prusoff equation [13]. Results were expressed as means of at least three separate experiments.

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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.2014.01.055.

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