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Synthesis and receptor binding assay of indolin-2-one derivatives as dopamine D4 receptor ligands

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Five indolin-2-one derivatives bearing piperazinylbutyl side chains attached to the amide nitrogen were synthesized from 2-indolinone. 1-(4-Bromobutyl)-indolin-2-one was reacted with 1-piperazinecarboxaldehyde to form 1-(4-(4-formyl-1-piperazinyl)butyl)indolin-2-one (**2**). In the presence of H_2SO_4, the aldehyde moiety was removed from 1-(4-(4-formyl-1-piperazinyl)butyl)indolin-2-one and then 1-(4-(1-piperazinyl)butyl)indolin-2-one (**3**) was obtained, this compound was reacted with benzaldehyde derivatives to give the target compounds **4 a-e** by *N*-alkylation reaction. The structures of the intermediates and the target compounds were characterized by ^{1}H NMR, ESI-MS spectra and elemental analyses. *In vitro* receptor binding assays at D_2, D_3, D_4 receptor subtypes of the target compounds were performed and the five compounds showed selectivity towards D_2-like receptors. Among them, 1-(4-(4-(4-hydroxybenzyl)-1-piperazinyl)butyl) indolin-2-one (**4c**) exhibited a remarkable affinity and selectivity to D_4 receptor with K_i value of 0.5 nM. The results indicated that 1-(4-(4-(4-hydroxybenzyl)-1- piperazinyl)butyl)indolin-2-one might be a potential dopamine D_4 receptor ligand.

1. Introduction

Disturbances of the dopaminergic system account for many neurological and neuropsychiatric diseases, including schizophrenia, Parkinson's disease, depression, tardive dyskinesia, attention deficit hyperactivity disorder (ADHD) and Tourette's syndrome (Michealraj et al. 2014). Five subtypes of dopamine receptors have been identified: D_1, D_2, D_3, D_4 and D_5 receptor. These subtypes are generally classified into two groups, D_1 like (D_1 and D_5) and D_2 like (D_2, D_3 and D_4) receptors, based on their pharmacologic properties and primary structure homology (Benes et al. 2012). The dopamine D_4 receptor is considered to be responsible for the antipsychotic effects of neuroleptics. The side effects of neuroleptic drugs, which primarily exert their effect *via* antagonism of D_2 receptors, are known to be due to D_2 receptor antagonism in the striatal regions of the brain. However, dopamine D_4 receptors are primarily located in areas of the brain other than striatum, suggesting that antagonists of the dopamine D_4 receptor will be devoid of extrapyramidal side effects. Consequently, D_4 receptor ligands, which are partial D_4 receptor agonists or antagonists, may have beneficial effects against psychoses (El-Mallakh et al. 2013; Cocker et al. 2014).

Most classical antipsychotic drugs bind to all D_2 like receptor subtypes while the atypical antipsychotic clozapine displays high affinity for D_4 receptor. Those ligands are far from ideal as some of them display affinity for other brain receptors, such as D_2, D_3, σ receptors, 5-HT receptors and this makes understanding of the experimental results using those radioligands as tracers complicated and difficult (Banerjee et al. 2013; Sampson et al. 2014; Tomlinson et al. 2015). Thus, ongoing efforts have been made to find selective ligands and radioligands for the D_4 receptor to facilitate the research into the function of the dopamine D_4 receptor and its correlations with various disorders (Abdelfattah et al. 2013; Moreland et al. 2004).

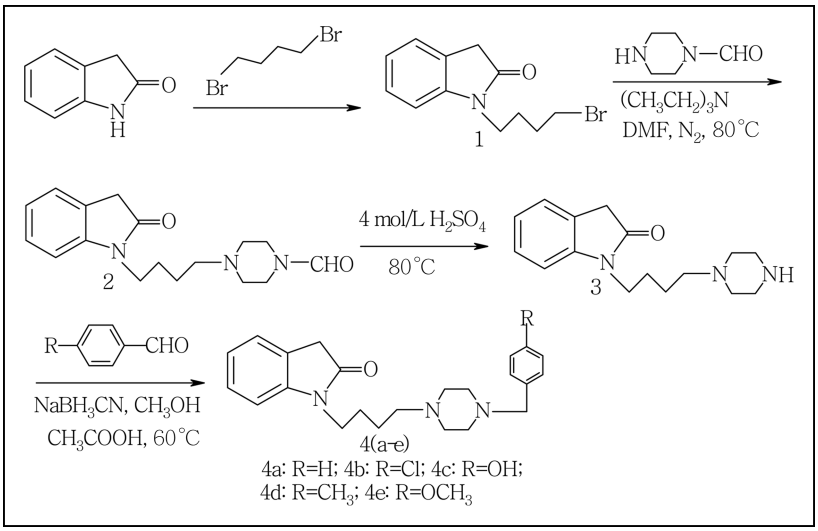
Some reports disclosed molecules with a butylamide linking chain, an extended aromatic terminal on one end and an arylpiperazine on the other end are selective dopamine D_3 receptor ligands, such as NGB 2904 (Banasikowski et al. 2012), GR 103691 (Cruz-Trujillo et al. 2013), BP 897 (Shinichiro et al. 2013). Structurally similar molecules made up of cyclic benzamides linked through an alkyl spacer to piperazines were evaluated as D_2/5-HT_2 receptor antagonists and non-classical antipsychotic agents (Résimont et al. 2010; Bhosale et al. 2014). The cyclic benzamide building blocks were linked to piperazines through a four-carbon spacer and that has been proven related with activity. The butyl spacer enabled the compound to adopt a folded conformation stabilized by an intramolecular hydrogen bond between the piperazine and the amide carbonyl. In turn, the flexible butyl chain allowed an optimal conformation and distance between the terminal pharmacophores resulting in optimal binding to the dopamine receptors.

In this study, five indolin-2-one derivatives as potential D_4 receptor ligands were synthesized and *in vitro* evaluated through receptor binding assay based on our previous work (Li et al. 2010).

2. Investigations, results and discussion

2.1. Synthesis of the indolin-2-one derivatives

Five indolin-2-one derivatives bearing piperazinylbutyl side chains attached to the amide nitrogen were synthesized from



Scheme 1: Synthesis of indolin-2-one derivatives **4a-e**.

Table 1: Receptor binding assay data of compounds 4a-e

Comp	K_i *(nM)*				
	D_2	D_3	$D_{4.2}$	$K_i\ D_2/K_i\ D_{4.2}$	$K_i\ D_3/K_iD_{4.2}$
4a	803	650	4.2	191.2	154.8
4b	820	692	8.1	101.2	85.4
4c	1815	1225	0.5	3630	2450
4d	915	756	5.5	166.4	137.5
4e	1020	935	12.0	85	77.9

2-indolinone. The approach for the synthesis of compounds **4a-e** included alkylation of 1-piperazinearboxaldehyde with 1-(4-bromobutyl)-indolin-2-one, amide hydrolysis and *N*-alkylation of 1-(4-(1-piperazinyl)butyl)indolin-2-one (**3**) with benzaldehyde derivatives. In the amination of the carbonyl compound, sodium cyanoborohydride was used as a selective reducing agent and acetic acid was used to adjust pH (Scheme).

2.2. Dopamine receptor ligands binding activity

The affinity of compounds **4a-e** for dopamine D_2, D_3, D_4 receptors were determined *in vitro* by measuring the ability to displace the dopamine receptor ligand [^{3}H]spiperone from dopamine receptors. All points were sampled in triplicate for each experiment and averaged values were reported. The affinities are given as K_i values in Table 1. In the receptor binding assay, compounds **4a-e** inhibited the [^{3}H]spiperone binding competitively to recombinant $D_{4.2}$ receptor with K_i(4.2, 8.1, 0.5, 5.5 and 12.0 nM), respectively. The differences in dopamine D_2, D_3 and $D_{4.2}$ receptor binding may be attributed to the difference of the side chain benzyl substituents. The dopamine D_{2long}, D_3 and $D_{4.2}$ receptor binding profiles of compounds **4a-e** indicate clear affinity for the $D_{4.2}$ receptor and weaker affinity for D_{2long} and D_3 subtypes. Thus, compounds **4a-e** could be potential ligands for $D_{4.2}$ receptor and compound **4c** is the most potent derivative, with $K_i = 0.5$ nM for the $D_{4.2}$ receptor, thus it is 3,630–fold selectivity over the D_2 receptor, 2,450-fold selectivity over the D_3 receptor, respectively.

The five indolin-2-one compounds showed affinity for the dopamine D_4 receptor, and when the R group at the benzyl side chain was changed from 4-H, 4-Cl to 4-hydroxyl, 4-methyl and 4-methoxyl, their affinities and selectivity for D_4 receptor changed significantly.

2.3. Conclusion

Five indolin-2-one derivatives bearing piperazinylbutyl side chains attached to the amide nitrogen were synthesized from 2-indolinone using a four-step procedure. Receptor binding assay disclosed affinity and selectivity of compounds **4a-e** towards the D_2-like receptors. Among them, 1-(4-(4-(4-hydroxybenzyl)-1-piperazinyl)butyl)indolin-2-one (**4c**) was the most potent one with $K_i = 0.5$ nM for the D_4 receptor and 3,630–fold selectivity over the D_2 receptor, 2,450-fold selectivity over the D_3 receptor. 1-(4-(4-(4-Hydroxybenzyl)-1-piperazinyl) butyl)indolin-2-one displayed higher affinity and selectivity towards dopamine D_4 receptor. The results indicated that 1-(4-(4-(4-hydroxybenzyl)-1-piperazinyl)butyl) indolin-2-one might be a potential dopamine D_4 receptor ligand.

3. Experimental

3.1. Materials and equipment

2-Indolinone was purchased from Fluka Chemie GmbH (Switzerland). 1-Piperazinecarboxaldehyde and 1,4-dibromobutane were obtained from Lancaster Synthesis Ltd (England). Benzaldehyde, 4-chlorobenzaldehyde, 4-hydroxybenzaldehyde, 4-methylbenzaldehyde 4-methoxybenzaldehyde and sodium cyanoborohydride were ordered from Acros Chemical Co., Ltd (Belgium). D_{2long}, D_3 receptors were purchased from Sigma Company and $D_{4.2}$ receptor was purchased from PerkinElmer Company (USA). Other chemicals were obtained from Shanghai Chemical Company (China) and used without further purification.

Thin layer chromatography (TLC) was carried out on glass palte precoated with GF_{254} silica gel (Sijia Biochemical Plastics Factory, Zhejiang, China) and was detected by a UV lamp (Anting Electronic Instrument Factory, Shanghai, China) at 254 nm. ^{1}H NMR assays were noted on an AVANCE 500 NMR spectrometer (BRUKER). Mass Spectra were recorded on a Micro-Mass GCT CA 055 mass spectrometer or a quadrupole mass spectrometer (Agilent LCMSD-SL, Agilent Technologies, Palo Alto, CA). The elemental analyses were performed on Elementar Vario EL III (Germany).

3.2. General procedure for the preparation of the indolin-2-one derivatives 4a-e

Indolin-2-one derivatives **4a-e** were synthesized according to the following procedure (Scheme).

Compound **1** (1-(4-bromobutyl)indolin-2-one) was prepared according to published procedures by the reaction of 1,4-dibromobutane with 2-indolinone.

Table 2: Receptor competition binding assay protocol

	TB* (μL)	NSB * (μL)	SB * (μL)	Control (μL)
Buffer solution	100	80	80	80
[^{3}H]Spiperone	20	20	20	20
Sample or Reference	/	(+)-Butaclamol 20	20	(DMF)20
Receptor	80	80	80	80

* TB: Total Binding; NSB: Nonspecific Binding; SB: Specific Binding.

A mixture of 1-(4-bromobutyl)indolin-2-one (0.536 g, 2.0 mmol), 1-piperazinecarboxaldehyde (0.342 g, 3.0 mmol) and triethylamine (3 mL) in absolute DMF (7 mL) was stirred and heated at 80 °C under nitrogen. The reaction course was monitored by TLC. At the end of the reaction, the mixture was poured into ice-water and extracted with chloroform. The extracts were passed through an anhydrous sodium sulfate column, filtered and evaporated under reduced pressure. The residue was then purified by flash column chromatography to give a light yellow oil (**2**).

A mixture of 1-(4-(4-formyl-1-piperazinyl)butyl)indolin-2-one (**2**) (0.300 g, 0.995 mmol) and 4 mol/L H_2SO_4 (10 mL) was heated at 80 °C for 9 h. The solution was cooled to room temperature and poured into 4 mol/L NaOH. The resulting basic suspension was extracted with dichloromethane and the organic layer was dried over anhydrous $MgSO_4$. After filtration, evaporation, the crude compound was purified by flash column chromatography and compound **3** was obtained.

To a solution of 1-(4-(1-piperazinyl)butyl)indolin-2-one (**3**) (0.120 g, 0.439 mmol) in methanol (10 mL), benzaldehyde (70 μL, 0.689 mmol) (or 4-chlorobenzaldehye 60 μL, 0.568 mmol; 4-hydroxybenzaldehye 70 μL, 0.647 mmol; 4-methylbenzaldehye 70 μL, 0.591 mmol; 4-methoxybenzaldehye 100 μL, 0.807 mmol), $NaBH_3CN$ (0.034 g, 0.550 mmol) and CH_3COOH (80 μL) were added. The mixture was stirred at room temperature for 3 h. After extraction the mixture with dichloromethane and water, the organic layer was passed through an anhydrous sodium sulfate column. Next, the residue was concentrated under reduced pressure and then purified by flash column chromatography to give **4a-e**, respectively.

*3.2.1. 1-(4-(4-Formyl-1-piperazinyl)butyl)indolin-2-one (**2**)*

Yield 65.1%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.66 (m, 2H, *J* = 7.5 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.75 (m, 2H, *J* = 7.5 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.45 (t, 2H, *J* = 7.5 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.62 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.18 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.53 (s, 2H, CH$_2$CON indolin), 3.75 (t, 2H, *J* = 7.7 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 6.80 (d, 1H, *J* = 2.5 Hz, 4-H), 7.02(dd, 1H, *J* = 2.2, 2.2 Hz, 5-H), 7.23 (dd, 1H, *J* = 2.2, 2.2 Hz, 6-H), 7.51 (d, 1H, *J* = 2.7 Hz, 7-H), 7.80 (s, 1H, CHO). ESI MS m/z (%):301.4 (M$^+$, 19), 302.4 (M + 1$^+$, 100). Anal. calcd for $C_{17}H_{23}N_3O_2$: C 67.75, H 7.69, N 13.94; found C 67.09, H 7.95, N 13.26.

*3.2.2. 1-(4-(1-Piperazinyl)butyl)indolin-2-one (**3**)*

Yield 76.2%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.65 (m, 2H, *J* = 7.1 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.77 (m, 2H, *J* = 7.6 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.44 (t, 2H, *J* = 7.6 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.65 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.23 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.55 (s, 2H, CH$_2$CON indolin), 3.77 (t, 2H, *J* = 7.5 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 6.80 (d, 1H, *J* = 2.4 Hz, 4-H), 7.07(dd, 1H, *J* = 2.3, 2.2 Hz, 5-H), 7.27 (dd, 1H, *J* = 2.2, 2.2 Hz, 6-H), 7.50 (d, 1H, *J* = 2.7 Hz, 7-H). ESI MS m/z (%): 274.4 (M + 1$^+$, 100). Anal. calcd for $C_{16}H_{23}N_3O$: C 70.29, H 8.48, N 15.37; found C 71.15, H 7.96, N 15.91.

*3.2.3. 1-(4-(4-Benzyl)-1-piperazinyl)butyl)indolin-2-one (**4a**)*

Yield 45.3%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.68 (m, 2H, *J* = 7.2 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.75 (m, 2H, *J* = 7.6 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.50 (t, 2H, *J* = 7.7 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.62 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.19 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.25(s, 2H, -N*CH$_2$*Ar-), 3.57 (s, 2H, CH$_2$CON indolin), 3.78 (t, 2H, *J* = 7.5 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 6.82 (d, 1H, *J* = 2.0 Hz, 4-H), 7.05(dd, 1H, *J* = 2.2, 2.2 Hz, 5-H), 7.26 (dd, 1H, *J* = 2.4, 2.4 Hz, 6-H), 7.28∼7.30 (m, 1H, Ar-H), 7.34∼7.36 (m, 4H, Ar-H), 7.50 (d, 1H, *J* = 2.8 Hz, 7-H). ESI MS m/z (%): 364.5 (M + 1$^+$, 100). Anal. calcd for $C_{23}H_{29}N_3O$: C 75.99, H 8.04, N 11.56; found C 75.06, H 8.55, N 11.93.

*3.2.4. 1-(4-(4-(4-Chlorobenzyl)-1-piperazinyl)butyl)indolin-2-one (**4b**)*

Yield 35.6%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.65 (m, 2H, *J* = 7.0 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.76 (m, 2H, *J* = 7.2 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.50 (t, 2H, *J* = 7.3 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.62 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.22 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.24(s, 2H, -N*CH$_2$*Ar-), 3.58 (s, 2H, CH$_2$CON indolin), 3.77 (t, 2H, *J* = 7.1 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 6.80 (d, 1H, *J* = 2.9 Hz, 4-H), 7.02(dd, 1H, *J* = 3.2, 2.2 Hz, 5-H), 7.19(t, 2H, *J* = 8.0 Hz, Ar-H), 7.27 (dd, 1H, *J* = 2.4, 3.5 Hz, 6-H), 7.37 (q, 2H, J = 6.0 Hz, Ar-H), 7.53 (d, 1H, *J* = 3.9 Hz, 7-H). ESI MS m/z (%): 398.9 (M + 1$^+$, 100). Anal. calcd for $C_{23}H_{28}N_3OCl$: C 69.42, H 7.09, N 10.56; found C 69.25, H 7.42, N 11.07.

*3.2.5. 1-(4-(4-(4-Hydroxybenzyl)-1-piperazinyl)butyl)indolin-2-one (**4c**)*

Yield 39.3%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.69 (m, 2H, *J* = 7.3 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.77 (m, 2H, *J* = 7.0 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.56 (t, 2H, *J* = 7.5 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.64 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.20 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.27(s, 2H, -N*CH$_2$*Ar-), 3.55 (s, 2H, CH$_2$CON indolin), 3.75 (t, 2H, *J* = 7.4 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 6.84 (d, 1H, *J* = 3.6 Hz, 4-H), 7.06(dd, 1H, *J* = 3.5, 2.9 Hz, 5-H), 7.22(t, 2H, *J* = 7.0 Hz, Ar-H), 7.25 (dd, 1H, *J* = 5.2, 3.9 Hz, 6-H), 7.38 (q, 2H, J = 7.0 Hz, Ar-H), 7.58 (d, 1H, *J* = 6.1 Hz, 7-H), 9.36 (s, 1H, OH). ESI MS m/z (%): 380.5 (M + 1$^+$, 100). Anal. calcd for $C_{23}H_{29}N_3O_2$: C 72.79, H 7.70, N 11.07; found C 72.92, H 7.86, N 11.32.

*3.2.6. 1-(4-(4-(4-Methylbenzyl)-1-piperazinyl)butyl)indolin-2-one (**4d**)*

Yield 40.1%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.63 (m, 2H, *J* = 7.6 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.73 (m, 2H, *J* = 7.2 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.49 (t, 2H, *J* = 7.0 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.55(s, 3H, -CH$_3$), 2.61 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.23 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.25 (s, 2H, -N*CH$_2$*Ar-), 3.59 (s, 2H, CH$_2$CON indolin), 3.81 (t, 2H, *J* = 7.0 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 6.82 (d, 1H, *J* = 2.7 Hz, 4-H), 7.06(dd, 1H, *J* = 2.5, 2.5 Hz, 5-H), 7.19(t, 2H, *J* = 7.0 Hz, Ar-H), 7.28 (dd, 1H, *J* = 2.7, 2.5 Hz, 6-H), 7.38 (t, 2H, J = 6.4 Hz, Ar-H), 7.56 (d, 1H, *J* = 3.6 Hz, 7-H). ESI MS m/z (%): 378.5 (M + 1$^+$, 70). Anal. calcd for $C_{24}H_{31}N_3O$: C 76.35, H 8.28, N 11.13; found C 76.91, H 8.82, N 10.55.

*3.2.7. 1-(4-(4-(4-Methoxybenzyl)-1-piperazinyl)butyl)indolin-2-one (**4e**)*

Yield 46.2%; Oil; ^{1}H NMR ($CDCl_3$, 500 MHz) *δ*: 1.62(m, 2H, *J* = 7.0 Hz, -CONCH$_2$CH$_2$*CH$_2$*CH$_2$N-), 1.72(m, 2H, *J* = 7.3 Hz, -CONCH$_2$*CH$_2$*CH$_2$CH$_2$N-), 2.50 (t, 2H, *J* = 7.2 Hz, -CONCH$_2$CH$_2$CH$_2$*CH$_2$*N), 2.62(br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.22 (br s, 4H, -(CH$_2$)$_2$N piperazinyl), 3.25 (s, 2H, -N*CH$_2$*Ar-), 3.57(s, 2H, CH$_2$CON indolin), 3.80(t, 2H, *J* = 7.2 Hz, -CON*CH$_2$*CH$_2$CH$_2$CH$_2$N), 3.85(s, 3H, -OCH$_3$), 6.83 (d, 1H, *J* = 2.9 Hz, 4-H), 7.02 (dd, 1H, *J* = 3.0, 3.0 Hz, 5-H), 7.22(t, 2H, *J* = 7.2 Hz, Ar-H), 7.27(dd, 1H, *J* = 2.7, 2.5 Hz, 6-H), 7.39 (t, 2H, J = 6.4 Hz, Ar-H), 7.60 (d, 1H, *J* = 3.6 Hz, 7-H). ESI MS m/z (%): 394.4 (M + 1$^+$, 70). Anal. calcd for C $C_{24}H_{31}N_3O_2$: C 73.25, H 7.94, N 10.68; found C 73.02, H 8.31, N 10.36.

3.3. In vitro receptor binding assays with D_{2long}, D_3, $D_{4.2}$ dopamine receptors

The *in vitro* receptor binding assay of compounds **4a-e** was performed by a modification of the procedure described by Hübner et al. (2000) and the protocol is given in Table 2. Briefly, the mixtures of protein, [^{3}H]spiperone and binding buffer (Tris-HCl 50 mmol/L, NaCl 120 mmol/L, KCl 5 mmol/L, $MgCl_2$ 5 mmol/L, $CaCl_2$ 1.5 mmol/L, EDTA 5 mmol/L, pH 7.4) were incu-

bated with different concentrations (1.0×10^{-11} mol/L~1.0×10^{-3} mol/L) of compounds **4a-e** in a total volume of 200 μL. Incubation was carried out at 30 °C for 1 h and was terminated by rapid filtration through Whatman GF/C glass fiber filters which were presoaked in 0.1% polyethyleneimine solution for 1 h, and filters were rinsed three times with 2 mL of ice-cold Tris-HCl buffer. Receptor-bound radioactivity was determined in a Beckman LS6500 liquid scintillation counter (USA). The specific binding of compounds **4a-e** were determined experimentally from the difference counts in the absence or presence of (+)-Butaclamol hydrochloride (Sigma-RBI Company), respectively. All assays were performed in triplicate.
Data were analyzed using Graphpad Prism programs.

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References

Abdelfattah MAO, Lehmann J, Abadi AH (2013) Discovery of highly potent and selective D4 ligands by interactive SAR study. Bioorg Med Chem Lett 23: 5077–5081.

Banasikowski TJ, Beninger RJ (2012) Reduced expression of haloperidol conditioned catalepsy in rats by the dopamine D3 receptor antagonists nafadotride and NGB 2904. Eur Neuropsychopharm 22: 761–768.

Benes J, Mravec B, Krizanova O, Kvetnansky R, Myslivecek J (2012) The restructuring of dopamine receptor subtype gene transcripts in c-fos KO mice. Brain Res Bull 87: 511–520.

Bhosale SH, Kanhed AM, Dash RC, Suryawanshi MR, Mahadik KR (2014) Design, synthesis, pharmacological evaluation and computational studies of 1-(biphenyl-4-yl)-2-[4-(substituted phenyl)-piperazin-1-yl]ethanones as potential antipsychotics. Eur J Med Chem 74: 358–365.

Cocker PJ, Foll BL, Rogers RD, Winstanley CA (2014) A selective role for dopamine D_4 receptors in modulating reward expectancy in a rodent slot machine task. Biol Psychiat 75: 817–824.

Cruz-Trujillo R, Avalos-Fuentes A, Rangel-Barajas C, Paz-Bermúdez F, Sierra A, Escartín-Perez E, Aceves J, Erlij D, Florán B (2013) D3 dopamine receptors interact with dopamine D1 but not D4 receptors in the GABAergic terminals of the SNr of the rat. Neuropharmacology 67: 370–378.

El-Mallakh RS, McKenzie C (2013) The dopamine D4/D2 receptor antagonist affinity ratio as a predictor of anti-aggression medication efficacy. Med Hypotheses 80: 530–533.

Hübner H, Haubmann C, Utz W, Gmeiner P (2000) Conjugated enynes as nonaromatic catechol bioisosteres: synthesis, binding experiments, and computational studies of novel dopamine receptor agonists recognizing preferentially the D_3 subtype. J Med Chem 43: 756–762.

Li GC, Wei WT, Yuan LH, Gao TJ (2010) Synthesis of benzopyrano[3,4-*c*]pyridin-5-ones as dopamine D_4 receptor ligands. Pharmazie 65: 791–793.

Michealraj KA, Jatana N, Jafurulla M, Narayanan L, Chattopadhyay A, Thelma BK (2014) Functional characterization of rare variants in human dopamine receptor D4 gene by genotype–phenotype correlations. Neuroscience 262: 176–189.

Moreland RB, Terranova MA, Chang R, Uchic ME, Matulenko MA, Surber BW, Stewart AO, Brioni JD (2004) [^{3}H] A-369508 ([2-[4-(2-cyanophenyl)-1-piperazinyl]-N- (3-methylphenyl) acetamide): an agonist radioligand selective for the dopamine D_4 receptor. Eur J Pharmacol 47: 147–154.

Résimont M, Liégeois JF (2010) Synthesis and in vitro binding studies of piperazine-alkyl-naphthamides: Impact of homology and sulphonamide/carboxamide bioisosteric replacement on the affinity for 5-HT_{1A}, α_{2A}, D4.2, D3 and D2L receptors. Bioorg Med Chem Lett 20(17): 5199–5202.

Sampson D, Zhu XY, Suresh VKE, Jagan RE, Edward O, Barbara B, Nazarius SL, Vincent S, Bryan LR, Seth Y (2014) Identification of a new selective dopamine D_4 receptor ligand. Bioorg Med Chem 22: 3105–3114.

Shinichiro N, Philip G, Hiroyoshi T, Fernando C, Tiffany C, Bernard LF, Benoit M, Bruce P, Ariel GG (2013) The potential role of dopamine D3 receptor neurotransmission in cognition. Eur Neuropsychopharm 23: 799–813.

Tomlinson A, Grayson B, Hayward A, Marshall KM, Neill NC (2015) Putative therapeutic targets for symptom subtypes of adult ADHD: D4 receptor agonism and COMT inhibition improve attention and response inhibition in a novel translational animal model. Eur Neuropsychopharm http://dx.doi.org/10.1016/j.euroneuro.2014.11.016.