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# Synthesis of benzopyrano[3,4-*c*]pyridin-5-ones as dopamine $D_4$ receptor ligands

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The dopamine $D_4$ receptor is highly expressed in prefrontal cortex, hippocampus, amygdala, hypothalamus and is hypothesized to relate with the pathophysiology and pharmacotherapy of schizophrenia while its level in brain regions is much lower. To date, no specific ligand is available for the study of $D_4$ receptor *in vivo*. In this study, we report the synthesis and *in vitro* receptor binding assay of three benzopyrano[3,4-*c*]pyridin-5-ones as potential dopamine $D_4$ receptor ligands. These new compounds have higher affinity and selectivity toward dopamine $D_4$ receptor and their $K_i$ values for $D_4$ receptor are in the nanomolar (nM) range.

## 1. Introduction

The dopamine $D_4$ receptor was cloned in 1991 (Van Tol et al. 1991) and its potential clinical importance in neuropsychiatric diseases has inspired growing interest in its pathophysiological role. About 0.7% of the general population suffers from schizophrenia at some point in their life and it is one of the most common central nervous system disorders in the world while its aetiology is unclear (Tye et al. 2009).

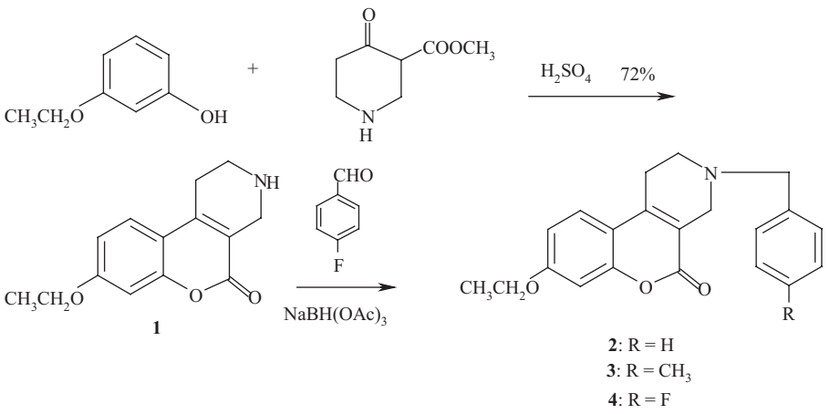
Since Arvid Carlsson and his colleagues first disclosed that antipsychotic agents upregulated dopamine turnover in rodent brain in 1963, the dopamine hypothesis of schizophrenia has been regarded as the primary theory for antipsychotic drug design (Lauzon et al. 2010; Jackson et al. 2009). Over the past decades, great efforts have been made to develop selective, high affinity ligands and radioligands for the dopamine $D_4$ receptor (Ehlicht et al. 2009; Rostom 2010). However, to present, most candidates are not ideal. The distribution of $D_4$ receptors is not very clear and its level in schizophrenia is currently controversial due to the lack of specific ligands and radioligands for the $D_4$ receptor subtype.

Benzopyrano[3,4-*c*]pyridin-5-ones (Unangst et al. 1997), as potential selective $D_4$ receptor antagonists, have chemical structures quite different from those of previously reported selective $D_4$ receptor antagonists. Recently, the synthesis and evaluation of $^{11}C$ (Zhang et al. 2002) and $^{123}I$ labeled benzopyrano[3,4-*c*]pyridine-5-ones were reported as dopamine $D_4$ receptor radioligands (Staelens et al. 2005). Based on the structure-activity analysis of benzopyrano[3,4-*c*]pyridin-5-ones as dopamine $D_4$ receptor ligands and our previous work (Li et al. 2005a, 2009b), putative $D_4$ receptor ligands with the structure of benzopyrano[3,4-*c*]pyridin-5-one were prepared and biologically evaluated.

## 2. Investigatious, results and discussion

8-Ethoxy-1,2,3,4-tetrahydrobenzopyrano[3,4-*c*]pyridin-5-one was synthesized from 3-ethoxyphenol and methyl-4-oxo-3-piperidinecarboxylate hydrochloride based on an intermolecular cycloaddition reaction under strongly acidic condition. An electron-donating function *meta* to the hydroxy group in the substituted phenol is required for the cyclization process employed in this reaction. 3-Benzyl-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano[3,4-*c*]pyridin-5-one (2), 3- (4-methylbenzyl)-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano [3, 4-*c*]pyridin5-one (3), 3-(4-fluorobenzyl)-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano[3,4-*c*]pyridin-5-one (4) were obtained through *N*-alkylation reaction of the intermediate with benzaldehyde, 4-methylbenzaldehyde and 4-fluorobenzaldehyde in the presence of sodium triacetoxyborohydride. In a second step, 1,3-dimethyl-2-imidazolidinone was used as a polar aprotic solvent and sodium triacetoxyborohydride as a selective reducing agent. Compared with other reductive amination procedures, it gives higher yields and fewer side-products using sodium triacetoxyborohydride as a selective reductive reagent.

The affinity of the target compounds 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 expressed in Chinese hamster ovary cells. All points were sampled in triplicate for each experiment. All experiments were repeated three times and averaged values were reported. The data were fitted using a sigmoid model to obtain $IC_{50}$ values, which represent the concentrations corresponding to 50% of maximal inhibition. The inhibition constant ($K_i$) values for compounds **2, 3, 4** were calculated from $IC_{50}$ values for a one-site model with the Cheng-Prusoff equation $K_i = \frac{IC_{50}}{1+[ligand]/K_D}$. The affinities are given as $K_i$ values in the Table. In the receptor binding assay, compounds 2, 3, 4 inhibited the [$^3$H]spiperone binding competitively to recombinant $D_{4.2}$ receptor with *K; values of* 2.2, 2.8 and 4.1 nM, respectively. Their affinities for dopamine $D_2$, $D_3$ receptors were negligible. The differences in dopamine $D_2$, $D_3$ and $D_4$ receptor binding may be attributed to the structure of benzopyrano[3,4-*c*]pyridin-5-ones' and the change of the side chain benzyl substituents. The dopamine $D_{2long}$, $D_3$ and $D_{4.2}$ receptor binding profiles of compounds **2, 3, 4** clearly indicate a good affinity for the $D_{4.2}$




**1**

**2**: R = H
**3**: R = $CH_3$
**4**: R = F

Scheme: Synthesis of benzopyrano[3,4-*c*]pyridin-5-ones

**Table: Receptor binding assay results of compounds 2, 3, 4**

| compd. | $K_i$ (nM) | | | |
|---|---|---|---|---|
| | $D_2$ | $D_3$ | $D_4$ | $D_2/D_4$ |
| **2** | 500 | 70 | 2.2 | 227 |
| **3** | 1200 | 420 | 2.8 | 429 |
| **4** | >5800 | >3000 | 4.1 | >1415 |

receptor and a weaker affinity for $D_{2long}$ and $D_3$ subtypes. Thus, compounds 2, 3, 4 could be potential ligands for the $D_{4.2}$ receptor. The three 8-ethoxy compounds showed higher affinity for the dopamine $D_4$ receptor, and when the R group at the benzyl side chain changed from 4-H to 4-methyl, 4-fluoro, their affinities for $D_4$ receptor were slightly lowered while their selectivity toward the $D_4$ receptor increased significantly.

In conclusion, three benzopyrano[3,4-*c*]pyridin-5-ones were prepared and their *in vitro* receptor binding activities were evaluated. These compounds have potent affinities for the dopamine $D_4$ receptor. Especially, 3-(4-fluorobenzyl)-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano[3,4-*c*]pyridin-5-one (**4**) has higher selectivity for the dopamine $D_4$ receptor and may be used to investigate the dopamine $D_4$ receptor in the central nervous system.

## 3. Experimental

### *3.1. Materials and chemistry*

3-Ethoxyphenol, sodium triacetoxyborohydride, benzaldehyde, 4-methylbenzaldehyde and 4-fluorobenzaldehyde were purchased from Acros Chemical Co., Ltd (Belgium). 1,3-Dimethylimidazolidinone was ordered from Lancaster Synthesis Ltd (England). 3-Methoxycarbonyl-4-piperidone hydrochloride was obtained from Fluka Chemie GmbH (Switzerland). [$^3$H]Spiperone was purchased from Amersham Pharmacia Biotech UK Ltd. (England). Other chemicals were obtained from Shanghai Chemical Company (China) and were analytically pure. $D_{4.2}$ receptor was purchased from PerkinElmer Company and $D_{2long}$, $D_3$ receptor were purchased from Sigma Company.

Melting points were determined on a WRS-1A digital melting point apparatus (Shanghai Jingke Physics Optics Apparatus Co. Ltd, China) and are uncorrected. All IR analyses were recorded as potassium bromide disks on an AVATAR 370 FT-IR spectrometer (Thermo Nicolet). $^1$H NMR, $^{19}$F NMR assays were noted on an AVANCE 500 NMR spectrometer (BRUKER) and chemical shifts were reported in ppm. Mass Spectra were recorded on a MicroMass GCT CA 055 mass spectrometer. The general procedure for the preparation of benzopyrano[3,4-*c*]pyridin-5-ones was shown in the Scheme.

#### *3.1.1. Synthesis of 8-ethoxy-1,2,3,4-tetrahydrobenzopyrano[3,4-c] pyridin-5-one(1)*

A mixture of 3-ethoxyphenol (0.138 g, 1 mmol) and methyl-4-oxo-3-piperidine carboxylate hydrochloride (0.194 g, 1 mmol) was cooled in an ice-water bath to 5 °C and 2.0 mL solution of 72% $H_2SO_4$ was added dropwise. Thereafter, the mixture was stirred at room temperature for 48 h and then 3 g ice-water was added slowly, followed by addition of $NH_4OH$ to adjust the pH of the resulting mixture to about 9. Stirring was kept on until a granular precipitate was formed. The solid was filtered and washed sequentially with 3% aqueous NaOH solution and 10% MeOH in $H_2O$. Recrystallization from acetonitrile gave 0.162 g of 1: yield 65.9%; yellow powder; m.p. 236–238 °C; $^1$H NMR (DMSO-$d_6$, 500 MHz)δ: 1.39 (t, 3H, $CH_3$), 2.50 (br s, 1H, NH), 2.68 (t, *J*=5.5 Hz, 2H, $CH_2$), 2.95 (t, *J*=5.6 Hz, 2H, $CH_2$), 3.52 (s, 2H, $CH_2$), 4.15 (t, 2H, $CH_2O$), 6.93 (m, 2H, 2×H), 7.59 (d, *J*=8.5 Hz, 1H); IR (KBr) *v*: 3440, 1720, 1590, 1400,1270, 1140, 856 cm$^{-1}$; ESI MS (m/z) (%) 246.2 (M+1$^+$).

#### *3.1.2. Synthesis of 3-benzyl-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano[3,4-c] pyridin-5-one(2)*

Compound **1** (0.123 g, 0.501 mmol), benzaldehyde (70 μL, 0.689 mmol), THF (2 mL), 1,3-dimethyl-2-imidazolidinone (0.5 mL) and acetic acid (50 μL) were mixed and stirred for 15 min, then sodium triacetoxyborohydride (0.165 g, 0.77 mmol) was added. The mixture was stirred for 20 h under nitrogen and then 30 mL ice-water was added to it to form sediment. The precipitated solid was filtered and washed with $H_2O$, then recrystallized form ethyl acetate. Finally, a yellow solid, compound **2** (0.059 g) was obtained. Yield 35.5%; m.p.126-129 °C; $^1$H NMR (DMSO-$d_6$, 500 MHz) *δ*: 1.32 (t, 3H, $CH_3$), 2.73 (t, *J*=5.6 Hz, 2H), 2.85 (t, *J*=5.6 Hz, 2H), 3.23 (s, 2H), 3.69 (s, 2H), 4.13 (t, 2H, $CH_2O$), 6.71 (d, *J*=2.0 Hz, 1H), 6.80 (dd, *J*=2.2, 2.2 Hz, 1H), 7.28∼7.30 (m, 1H), 7.34∼7.36 (m, 4H), 7.53 (d, *J*=8.6 Hz, 1H); IR (KBr) *v*: 3350, 2920, 1680, 1610, 1450, 1410, 1160, 1080, 847, 754, 702 cm$^{-1}$; ESI MS (*m/z*) (%): 336.4(M+1$^+$).

#### *3.1.3. Synthesis of 3-(4-methylbenzyl)-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano [3,4-c]pyridin-5-one (**3**)*

A solution of **1** (0.123 g, 0.501 mmol) and 4-methylbenzaldehyde (70 μL, 0.591 mmol) was reacted with sodium triacetoxyborohydride (0.160 g, 0.75 mmol) as described in section **3.1.2**. Then the mixture was added to 30 mL ice and $H_2O$. The precipitated product was filtered and washed with $H_2O$, recrystallized from EtOH to give 0.101 mg of compound 3: yellow solid, yield 57.9%; m.p.182-185 °C; $^1$H NMR (DMSO-$d_6$, 500 MHz) *δ*: 1.31 (t, 3H, $CH_3$), 2.30 (s, 3H, $CH_3$), 2.70 (t, *J*=5.4 Hz, 2H), 2.85 (t, *J*=5.4 Hz, 2H), 3.22 (s, 2H), 3.68 (s, 2H), 4.11 (t, 2H, $CH_2O$), 6.69 (d, *J*=2.2 Hz, 1H), 6.81 (dd, *J*=2.2, 2.2 Hz, 1H), 7.18 (t, *J*=2.2 Hz, 2H), 7.39 (q, *J*=5.6 Hz, 2H), 7.52 (d, *J*=8.6 Hz, 1H); IR (KBr) *v*: 3430, 2920, 1710, 1620, 1510, 1470, 1350, 1280, 1220, 1160, 1090, 847, 756 cm$^{-1}$; ESI MS (m/z) (%) 350.5 (M+1$^+$).

#### *3.1.4. Synthesis of 3-(4-fluorobenzyl)-8-ethoxy-1,2,3,4-tetrahydrobenzopyrano [3,4-c]pyridin-5-one (**4**)*

A solution of compound **1** (0.123 g, 0.501 mmol) and 4-fluorobenzaldehyde (60 μL, 0.568 mmol), was reacted with sodium triacetoxyborohydride (0.160 g, 0.75 mmol) as described in section **3.1.2**. Then the mixture was added to 30 mL ice and $H_2O$. The precipitated solid was filtered and washed with $H_2O$. Recrystallization from aqueous MeCN gave 0.120 mg of compound **4**: yellow solid, yield 67.8%; m.p.135-138 °C; $^1$H NMR (DMSO-$d_6$, 500 MHz) *δ*: 1.31 (t, 3H, $CH_3$), 2.79 (t, *J*=5.4 Hz, 2H), 2.89 (t, *J*=5.4 Hz, 2H), 3.49 (s, 2H), 3.73 (s, 2H), 4.11 (t, 2H, $CH_2O$), 6.82 (d, *J*=2.2 Hz, 1H), 6.87 (dd, *J*=2.2, 2.2 Hz, 1H), 7.02 (t, *J*=2.2 Hz, 2H), 7.36 (q, *J*=5.6 Hz, 2H), 7.43 (d, *J*=8.6 Hz, 1H); $^{19}$F NMR (DMSO-$d_6$, 470 MHz) *δ*: -116.83 (s, 1F); IR (KBr) *v*: 3430, 2940, 1710, 1610, 1510, 1480, 1350, 1250, 1220, 1160, 1020, 847, 812 cm$^{-1}$; ESI MS (m/z) (%) 354.4 (M+1$^+$).

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

The *in vitro* receptor binding assay of compounds **2, 3, 4** was performed by a modification of the procedure described by Thibous et al. (2000). 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 incubated with different concentrations ($1.0\times10^{-11}$mol/L$\sim1.0\times10^{-3}$mol/L) of compounds **2, 3, 4** 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. The specific binding of compounds **2, 3, 4** were determined experimentally from the difference counts in the absence or presence of (+)-butaclamol hydrochloride (Sigma-RBI Company). All assays were performed in triplicate. Data were analyzed using Graphpad Prism programs.

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