

A solid phase parallel synthesis of diverse amides as dopamine D₃ receptor ligands

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Abstract

A solid phase parallel synthesis using SynPhase™ technology was used to couple a series of 21 carboxylic with three different 4-(4-arylpiperazinyl)butanamines. The resulting library was evaluated as dopamine D₃ receptor ligands giving rise to several compounds with affinities in the low nanomolar concentration range (9e and 9n with binding affinities at D₃ receptors of 0.10 and 0.35 nM respectively).

Keywords: D₃ receptors, ligand, dopamine, amide

Introduction

Since its discovery in 1990 [1], the dopamine D₃ receptor has been widely studied [2]. Its localisation in the limbic area of the brain [3] as well as the early investigations with agonists [4] or antagonists [5] led to the current situation that the therapeutic use of such compounds is mainly directed towards drug abuse. More recent studies have shown that potential therapeutic uses could be in other neurological and neuropsychiatric disorders [6]. The aromatic amide **BP 897**⁵ (Figure 1) is the first representative of potent selective antagonists or partial agonists at this receptor. It has been used as prototype for further analogues by many groups.

Modification of the aromatic amide (Figure 2) has mainly focused on its replacement with other polyaromatics such as fluorene NGB2904 [7] and biphenyl GR103691, [8] or heteroaromatics like benzothiophene FAUC346 [9] or benzofurane A [10]. More recently, isosteric replacement of the

aromatic amide with pyrimidone [11] or incorporation into a benzodiazepinedione [12] has been reported.

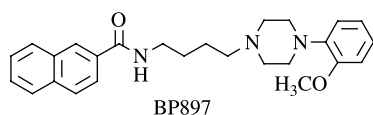
In the continuation of the previously reported modulations of **BP897** [13], we have performed some variations of both amide and aryl piperazine moieties using a combinatorial solid phase approach. Thus, three sub-series (**8–10**) of compounds were synthesized corresponding to variation of R¹ substituent (Figure 3). Concerning the amide part, three kinds of R² substituents have been introduced: aryl (**a–g**), heteroaryl (**h–j**) and aryl connected through variable alkyl linkers (**k–u**).

Materials and methods

General

Syntheses were performed on SynPhase™ Lanterns (Code SPPS, D series, Linker BAL, 35 μmoles). ¹H NMR Spectra were recorded on a Jeol GSX spectrometer at 270 MHz. High resolution mass

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Figure 1. Chemical structure of **BP897**.

spectra (HRMS) were recorded at the “Centre Régional de Mesures Physiques de l’Ouest” on a ZabSpec ToF Micromass spectrometer using a LSIMS (Cs⁺) or electrospray ionisation mode. Reagent-grade solvents were purchased from chemical suppliers and used directly without further purification unless otherwise specified. Thin-layer chromatography using CH₂Cl₂/CH₃OH (95/5) as an eluent was performed on Merck silica gel 60 F254 (layer thickness: 0.22 mm) and assessed the purity of the compounds (>95%). The compounds were visualised using UV light, ninhydrin or iodine. The structure of compounds was checked using ¹H NMR and HRMS.

Synthesis

Anchorage of amines to BAL lanterns. To a solution of a primary amine (1 M), NaBH₃CN (0.063 g; 0.1 M) and AcOH (0.1 mL) in *N,N*-dimethylformamide (DMF) (10 mL) were added 21 SynPhase Lanterns **1**. The solution was heated to 60°C for 16 h. The lanterns **2–4** were then washed with 3 × DMF and 3 × CH₂Cl₂ (general procedure) and dried under vacuum. One lantern of each amine pool was taken to

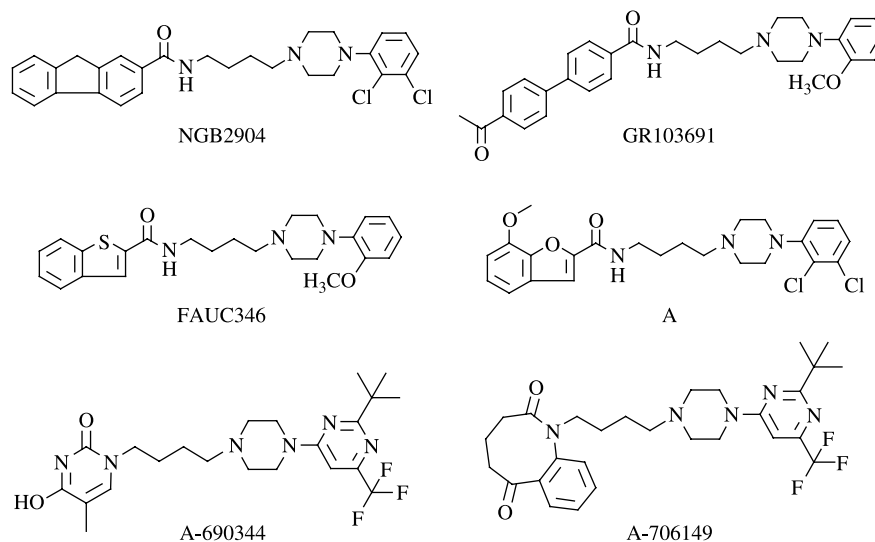
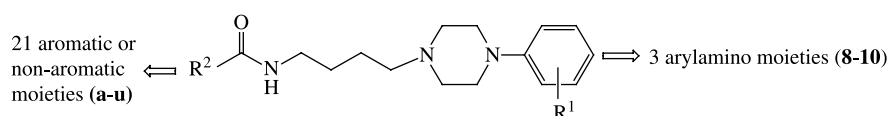
form 21 identical pools that will react with acyl chlorides or carboxylic acids.

Formation of amide bond from acyl chlorides. To a pool of 3 lanterns **2–4** was added a solution of *N*-methylmorpholine (0.25 M) and R²-COCl (0.25 M) in dichloromethane (2 mL) for 1 h at room temperature. The lanterns **5a–7b** were then washed according to the general procedure and dried under vacuum.

Formation of amide bond from carboxylic acids. To a pool of 3 lanterns **2–4** was added a solution of diisopropyl carbodiimide (DIC, 0.2 M), hydroxybenzotriazole (HOBt, 0.2 M) and R²-COOH (0.2 M) in DMF (2 mL) for 16 h at room temperature. The lanterns **5c–7u** were then washed according to the general procedure and dried under vacuum.

Cleavage. The lanterns **5a–7u** were then separated in a 96-well plate and 0.5 mL of trifluoroacetic acid in CH₂Cl₂ (20:80) was added. After 1 h at room temperature, the lanterns were removed and the cleavage solutions were evaporated under vacuum to yield the compounds **8a–10u** (average yield: 48%).

N-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}-benzamide, trifluoroacetate salt (**8a**). ¹H NMR

Figure 2. Aromatic analogs of **BP897**.Figure 3. Exploration of R¹ and R² parts of **BP897**.

(CD₃OD) δ (ppm): 1.77 (qt, $J = 7.0$ Hz, $J = 6.7$ Hz, 2H), 1.90 (m, 2H), 3.16 (t, $J = 12.0$ Hz, 2H), 3.34 (m, 6H), 3.49 (t, $J = 6.9$ Hz, 2H), 3.53 (d, $J = 13.0$ Hz, 2H), 3.71 (d, $J = 12.0$ Hz, 2H), 7.17 (dd, $J = 2.2$, $J = 7.3$, 1H), 7.32 (m, 2H), 7.48 (t, $J = 7.5$ Hz, 2H), 7.56 (t, $J = 7.5$ Hz, 1H), 7.85 (m, 2H). R_f (CH₂Cl₂/CH₃OH: 95/5) = 0.30.

N-{4-[4-(3-(Trifluoromethyl)phenyl)piperazin-1-yl]butyl}-5-phenylpentanamide, trifluoroacetate salt (**9r**). ¹H NMR (DMSO-*d*₆) δ (ppm): 1.61 (m, 2H), 1.66 (q, $J = 3.5$ Hz, 4H), 1.80 (m, $J = 3.6$ Hz, $J = 7.31$ Hz, 2H), 2.25 (t, $J = 6.8$ Hz, 2H), 2.64 (t, $J = 6.8$ Hz, 2H), 3.13 (m, 2H), 3.24 (m, 6H), 3.33 (t, $J = 1.6$ Hz, 2H), 3.66 (m, 2H), 3.91 (m, 2H), 7.21 (m, 8H), 7.49 (t, $J = 8.3$ Hz, 1H). R_f (CH₂Cl₂/CH₃OH: 95/5) = 0.25.

N-{4-[4-(2-Fluorophenyl)-piperazin-1-yl]-butyl}-5,6,7,8-tetrahydronaphthyl-2-amide, trifluoroacetate salt (**10d**). ¹H NMR (DMSO-*d*₆) δ (ppm): 1.73 (qt, $J = 7.0$ Hz, $J = 7.3$ Hz, 2H), 1.86 (m, 6H), 2.82 (d, $J = 3.2$ Hz, 4H), 3.14 (t, $J = 12.2$ Hz, 2H), 3.31 (m, 6H), 3.47 (t, $J = 6.7$ Hz, 2H), 3.62 (d, $J = 13.3$ Hz, 2H), 3.69 (d, $J = 12.1$ Hz, 2H), 7.12 (m, 5H), 7.54 (m, 2H). R_f (CH₂Cl₂/CH₃OH: 95/5) = 0.38.

9b. HRMS [M + H]⁺ calcd for C₂₂H₂₅F₄N₃O: 424.2012; found: 424.2012. **8h**: HRMS [M + H]⁺ calcd for C₂₀H₂₃BrCl₂N₄O: 485.0510; found: 485.0510. **9l**: HRMS [M + H]⁺ calcd for C₃₆H₃₈F₃N₃O₃: 618.2943; found: 618.2941. **8c**: HRMS [M + H]⁺ calcd for C₂₂H₂₇Cl₂N₃O₃S: 484.1228; found: 484.1234. **10g**: HRMS [M + H]⁺ calcd for C₂₈H₃₂FN₃O₂: 462.2556; found: 462.2550. **9r**: HRMS [M + H]⁺ calcd for C₂₅H₃₄FN₃O: 412.2764; found: 412.2762.

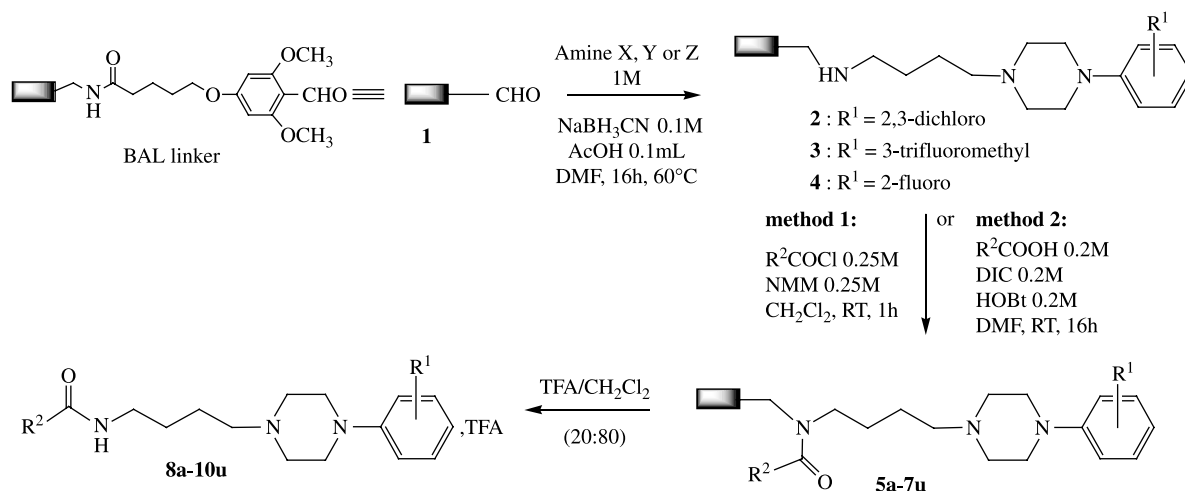
Pharmacological characterisation

Affinity of compounds for the human D₃ receptor was determined by [³H] spiperone binding CHO cells that had been transfected by the cDNA coding for the human D₃ receptor (hD₃). [³H]Spiperone (0.5 at 2 nM) binding was performed in the presence of 2.5 to 5 μ g of membrane proteins in a medium containing 120 mM of NaCl, 5 mM of KCl, and 50 mM of Tris HCl at pH 7.4; incubation for 60 min at room temperature was performed. Non-specific binding was estimated in the presence of 10 μ M haloperidol. Non-transfected cells are devoid of any specific binding. The products were tested in duplicate experiments at five different concentrations: 0.1, 1, 10, 100 and 1 000 nM.

Results and discussion

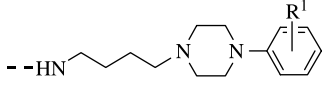
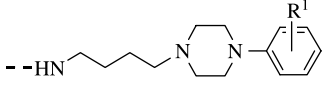

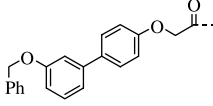

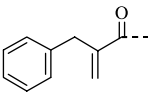
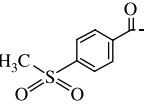
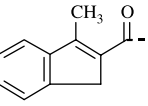
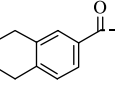
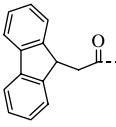
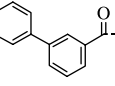
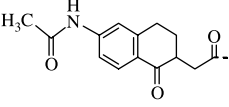
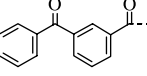
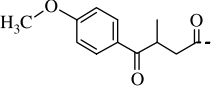
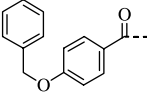
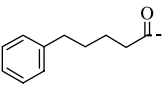
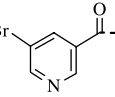
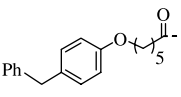
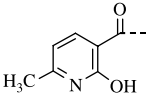
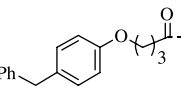

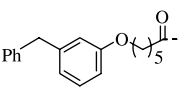
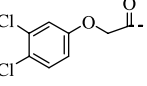
To minimize operations encountered in solution phase synthesis, we selected SynPhase™ Lanterns [14] with BAL linker (Mimotopes Pty Ltd) that offer synthesis characteristics and functionality comparable to classical resins. Their easy and fast handling facilitates parallel synthesis of milligram scale individualised compounds (35 μ mol per support) using the “split-pool” procedure [15]. BAL linker allows fixation of primary amines through reductive amination then formation of amides that can easily be cleaved from the support.

The first step of our work on solid support (Scheme 1) according to the split-pool technique consisted in a reductive amination to the BAL linker. Thus we formed three pools of 21 BAL lanterns **1** and put each one in a solution of the desired amine [16,17] (1 M) in DMF in the presence of NaBH₃CN and a catalytic amount of acetic acid. The temperature was maintained at 60°C for 16 h in the separate solutions (10 mL) affording three pools (2–4).



Scheme 1. Synthetic scheme for compounds **8a-10u**.

Table I. Binding affinities K_i [nM] of **8a-10u** at D₃ receptors using a stable transfected CHO cell line.

R ²				R ²			
	8:R ¹ =2,3-dichloro	9:R ¹ =3-trifluoromethyl	10:R ¹ =2-fluoro		8:R ¹ =2,3-dichloro	9:R ¹ =3-trifluoromethyl	10:R ¹ =2-fluoro
a 	1.1	0.40	1.7	l 	4.2	7.7	25
b 	0.40	1.4	1.8	m 	3.5	9.9	22
c 	1.2	1.4	2.0	n 	0.85	0.35	0.4- 1
d 	4.0	1.2	2.6	o 	6.0	2.25	16
e 	0.70	0.10	1.0	p 	3.0	9.2	73
f 	1.4	1.2	1.5	q 	90	31	109
g 	0.50	2.0	1.5	r 	2.0	12	19
h 	2.8	100	41	s 	3.5	2.0	8.6
i 	2.6	2.5	15	t 	3.7	3.8	34
j 	3.3	30	40	u 	7.0	22	18
k 	6.6	21	60				

The next step aimed at forming the amide bond from acyl chlorides or carboxylic acids. Thus 21 identical pools were formed with one lantern of each series 2–4. Two pools (**a** and **b**) were reacted for 1 h with acyl chlorides in the presence of *N*-methylmorpholine in dichloromethane. The other ones (**c** to **u**) were placed in DMF for 16 h in the presence of carboxylic acids and an equimolar mixture of DIC and

HOBt, as usually performed in peptidic bond formation. In our synthesis, DIC that generates soluble diisopropylurea was preferred to dicyclohexylcarbodiimide (DCC).

For the final cleavage step, lanterns **5a-7u** were distributed in a 96 well plate and a mixture of trifluoroacetic acid in dichloromethane was added, leading to 5 to 20 mg of the separate amides **8a-10u** as

their trifluoroacetate salts (average yield of 48%) after evaporation of the solvents. It is noteworthy that only amides could be separated from the support leading to very good purity according to TLC controls. ¹H NMR Spectra of several compounds showed satisfactory results.

Binding affinities of **8a-10u** at D₃ receptors were then evaluated. Within the (2,3-dichlorophenyl)piperazine series (compounds **8a-u**, Table I), all compounds except **8q** display affinities in low nanomolar concentration range. This substitution generally gives better affinities than 3-trifluoromethyl (series **9**) or 2-fluoro (series **10**). The substitution has only little influence on the ranking order of potencies except for **8q**, **9h**, **10q**.

Benzamide substitution has been discussed previously [13] with 2-methoxyphenylpiperazine derivatives: 2-substitution with methyl and benzoyl chains was found detrimental. In these series we found that fluoro substitution is well tolerated (**8-10b**) probably due to its small size or an internal hydrogen bond involving the amidic NH keeping the aromatic in a good orientation. Substitution in 3- or 4-position is well tolerated even with bulky substituents such as phenyl, benzoyl or benzyloxy **8e-10g**. Heteroaromatics (pyridine **8h-10i**, pyrazine **8-10j**) show a slight decrease in affinity, probably due to their hydrophilic nature located too close to the amide. Note that 2-substitution with an hydroxyl (**8-10i**) is also well tolerated, probably due to an intermolecular hydrogen bond.

The presence of a spacer between aromatic and amide part of the molecules **8k-10u** always seems unfavourable except for **8-10n** whose structure is related to one of the previously described **FAUC346** or **A-706149**.

In summary, we consider that solid phase parallel chemistry using SynPhase™ Lanterns was beneficial to our objective especially in terms of ease and purity of the final compounds. The obtaining of diverse compounds with nanomolar *K_i* values at human dopamine D₃ receptors will allow selectivity and pharmacokinetic studies to find the best potential candidates for further development.

Acknowledgements

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