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Parallel synthesis and dopamine D_3/D_2 receptor screening of novel {4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}carboxamides

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Abstract—We have applied a fast and high-yielding method for the parallel amidation of 4-[4-(2-methoxyphenyl)piperazin-1-yl]-butylamine yielding analogs of the partial dopamine receptor agonist BP 897. Using this amino scaffold prepared in solution and polymer-bound carboxylic acid equivalents, we have synthesized a series of high affinity dopamine D_3 receptor ligands. The novel compounds were obtained in good to excellent yield and purity. Biological evaluation included determination of binding affinities at hD_{2S} and hD_3 receptor subtypes. From the 22 novel structures presented here, compound **4v** showed high affinity (K_i (hD_3) 1.6 nM) and a 136-fold preference for the D_3 receptor versus that for the D_2 receptor subtype. Our results suggest that this derivatization technique is a useful method to speed up structure–activity relationships studies on dopamine receptor subtype modulators.

1. Introduction

Despite considerable progress in computer based drug discovery techniques, deeper understanding of structure–activity relationships in part still relies heavily on trial and error. Therefore it is favorable, to be able to synthesize arrays of pure test candidates rapidly. In the ongoing search for high affinity and D_3 versus D_2 receptor-preferring ligands, the partial agonist BP 897 (**1**) was taken as a lead structure to synthesize novel dopamine receptor ligands (Fig. 1). The rational for this interest is the fact that the dopamine D_3 receptor is recognized as a potential therapeutic target for the treatment of various neurological and psychiatric disorders.[1–4] Hitherto performed modification of the *N*-butyl-carboxamide linker of **1** by constraining the alkyl chain in rings or by replacing the amide system turned out to be detrimental for D_3 receptor affinity.[5] Thus, further variation of the amide moiety of **1** as exclusive diversity site, was envisioned. Knowledge gained from previously reported molecular modeling studies supported the structural advantages of the amide

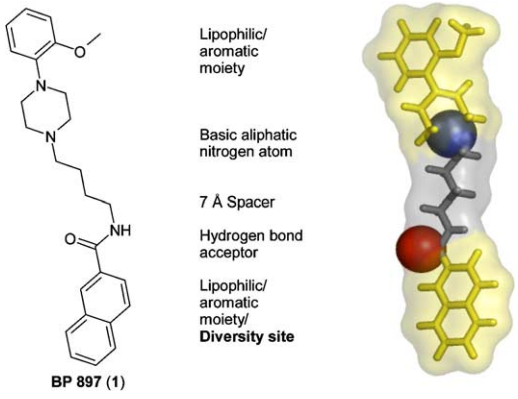

Figure 1. Pharmacophore for D_3 receptor binding of compound BP 897 (**1**).

bridge. While simple synthetic transformations such as amide bond formation can easily be performed rapidly, work-up procedures often have to be optimized for every individual new molecule.

This time-consuming step keeps the medicinal chemist from the productive part of a given project. Here we suggest to use polymer-bound active carboxylic acid

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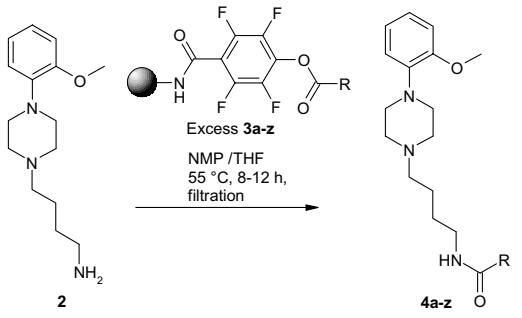
equivalents for the fast parallel synthesis of *N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}carboxamides (**4a**–**z**) fulfilling the requirements for the postulated pharmacophore model depicted above.

2. Results

Throughout the last years, the use of safety-catch linkers for the construction of chemo selective acylating agents in convergent polymer-assisted solution-phase (cPASP) synthesis of biologically active adenosine analogs using Kenner's safety-catch linker was demonstrated.[6,7] The quantitative *N*-selective acylation of amino groups, the straightforward product separation by filtration and the absence of impurities of residual coupling reagents being the main advantages of this approach. In continuation of our efforts to contribute to the identification of biologically active compounds via parallel synthesis, a set of *N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-carboxamides was envisioned. Because the synthesis of these derivatives is markedly less demanding than the preparation of multifunctional nucleoside derivatives in terms of regioselective modification of the nucleoside scaffold, the advantages of the chemo selective Kenner linker are overcome by the disadvantage of the additional activation step using toxic and expensive reagents, additional washing steps due to the activation procedure and the impossibility of being recycled.

Since a great number of phenolic linkers have been proposed as tools for the preparation of polymer-bound acylating species, recently, we decided to add such a simple couple and release linker to our PASP technique repertoire.[8–10] Our approach focused on the use of the 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid linker for this purpose. Carboxylic acids were immobilized on the linker attached to aminomethylated polystyrene yielding reagents **3a**–**z** and subsequently transferred to amine **2** in solution under very mild conditions (Scheme 1). Candidates **4a**–**z** for biological evaluation could thus be obtained in nearly quantitative yield, little loss of amine **2**, in purity up to 95% in parallel. For the preparation of a great number of derivatives, the issue of linker recycling has been studied, but this anticipated advantage over the Kenner linker could not be proven experimentally to date.



Scheme 1. PASP synthesis of compounds **4a**–**z**. R in compounds **4a**–**z** equals R in **3a**–**z** and can be deduced from Table 1.

Existing pharmacophore models for dopamine D_2 and D_3 receptor ligands suggest that an extended and more linear conformation in the aliphatic or aryl spacers should be crucial for dopamine D_3 receptor selectivity and careful replacement of the naphthyl moiety by other aromatic residues, namely heterocycles are well tolerated.[5,11,12] This information was used as a tool to select a set of 24 carboxylic acid residues for the construction of **4a**–**z**. For the resulting novel compounds presented here, structural diversity in the amide moiety (benzamides, alkyl amides, aryl–alkyl amides) had a major influence on down to single digit nanomolar D_3 receptor affinity.

Some compounds, namely **4v** (*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}-4-phenoxybenzamide) displayed a favorable pharmacological profile (K_i (hD_3) 1.6 nM; K_i (hD_2) 219.4 nM; selectivity ratio of 136).

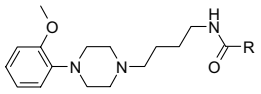
Initially, 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid was attached onto aminomethylated polystyrene using *N*,*N*′-diisopropylcarbodiimide as activating agent. Addition of bromophenol blue allowed a naked-eye control of the crucial linker anchorage. The initial deep blue suspension turned green, and slowly faded to yellow. The alteration of the color indicated the quantitative disappearance of free amino groups and assured a complete and exhaustive loading of the starting polymer. We prepared the polymer-bound acylation reagents by loading the commercially available carboxylic acids onto polymer-bound 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid applying *N*,*N*′-diisopropylcarbodiimide as described.[9] Due to the tetrafluoro substitution of the linker, a labile ester bond is formed and the applied amine **2** exhibits sufficient nucleophilic properties to be acylated without the need of further activation steps.

After repetitive washing to remove residual *N*,*N*′-diisopropylcarbodiimide, *N*,*N*′-diisopropylurea, and base impurities from the polymer beads, the active polymer-bound acids were used as acylation reagents **3a**–**z**.

The resulting reagents **3a**–**z** were subsequently incubated with a solution of amine **2**. The amine **2** was subjected to the acylation reaction as a free base resulting from ion exchange chromatography to remove hydrogen chloride from the storage form, the tri-HCl salt. In the course of the reaction, the limiting amounts of **2** were quenched, resulting in pure solutions of the target amides **4a**–**z**. After filtration and evaporation of the solvent under reduced pressure, compounds **4a**–**z** were obtained in excellent yield and fair to high relative purity (Fig. 1 and Table 1). For comparative purposes, the activity of BP 897 (**1**) was determined in the same experiment and is also reported (Table 1).

Exhaustive automated washing procedures (here an ACT Vanguard system was applied) and the use of volatile solvents for the acylation reaction (in this case freshly distilled THF) led to test samples of high quality and purity (in the range of 71–95% with an acceptable average of 83%) without the need of further time-consuming purification processes. Downstream HPLC

Table 1. Dopamine receptor subtype affinities K_i [nM], selectivity ratio and purity of derivatives **4a**–**z** produced via route outlined in Scheme 1



Entry	R	Receptor subtype		Ratio[a]	Yield [%]	Purity[b] [%]	From entry
		D_2	D_3	D_2:D_3			
1[c]	2-Naphthyl	186	0.67	277			
4a	2-Methylpropyl	>250	123		97	73.9	**3a**
4b	3-Oxobutyl	>250	889		93	85.0	**3b**
4c	Pentyl	>250	87		95	73.8	**3c**
4d	1-Cyclohexylmethyl	321.6	2.0	160.8	95	71.2	**3d**
4e	3-Cyclohexylpropyl	>250	23.7		89	71.7	**3e**
4f	3-Phenoxypropyl	>250	13.9		99	83.3	**3f**
4g	4-Phenylbutyl	311.6	2.8	111.3	99	75.9	**3g**
4h	3,4-Dimethoxybenzyl	>250	360		91	82.1	**3h**
4i	2,6-Dichlorobenzyl	6	13	0.5	87	78.1	**3i**
4j	2-Methylphenyl	>250	25		83	83.6	**3j**
4k	4-Ethylphenyl	>250	5		93	92.6	**3k**
4l	4-(Acetylamino)phenyl	>250	3		87	92.6	**3l**
4m	3-Fluorophenyl	>250	2		94	88.6	**3m**
4n	3,4-Difluorophenyl	165	6	28	92	83.9	**3n**
4o	3,5-Difluorophenyl	>250	27		93	81.7	**3o**
4p	3-(Trifluoromethyl)phenyl	>250	33		98	91.1	**3p**
4q	3,5-Dichlorophenyl	178	7	25	95	91.8	**3q**
4r	4-Chloro-2-nitrophenyl	>250	50		82	71.4	**3r**
4s	2-Methyl-5-nitrophenyl	146	5	29	92	70.8	**3s**
4t	3-(Trifluoromethoxy)phenyl	340	6	57	94	82.8	**3t**
4u	2-Benzoylphenyl	>250	268		75	87.4	**3u**
4v	4-Phenoxyphenyl	219	1.6	136	98	94.6	**3v**
4w	4-Ethoxyphenyl	62	1.7	37	96	94.9	**3w**
4x	3,4-Dimethoxyphenyl	125	3	42	94	92.0	**3x**
4y[c]	4-Benzoylphenyl	488[c]	0.4[c]	1220[c]	96	89.1	**3y**
4z[c]	4-Chlorophenyl	284[c]	2[c]	142[c]	91	92.0	**3z**

[a] Ratio calculated were applicable.
[b] Relative purity as determined by HPLC analysis using the 100% method with UV-detection.
[c] Structures/affinities described previously.[1,5]

analysis of all library members assured a good to excellent quality throughout the set of synthesized compounds.

3. Discussion

The above demonstrated synthesis using polymer-bound carboxylic acid equivalents permit the fast and reliable preparation of milligram quantities of test samples for biological evaluation. On the one hand, the number of compounds achievable by this procedure is considerably lower than in combinatorial solid-phase synthesis approaches. But on the other hand, the quality of the compounds in terms of reliable data sets obtained at least equals the typical purity of compounds from classical organic synthesis projects. Like in solid-phase synthesis, the PASP synthesis performed enables parallel product isolation because the excess of reagent that is applied to drive reactions to completion can be removed by filtration. In contrary to solid-phase synthesis, the reagents are polymer-bound and not the product, saving a final cleavage reaction that might lead to product contamination, typically by TFA salt formation. Therefore, this intermediate technique allows the fast and convenient access of compounds intended for the determination of quantitative structure–activity relationships in drug research in high yield and purity.

Making use of an already refined pharmacophore model for the differentiation of D_2 and D_3 receptor affinities, the compounds obtained in this study display the desired properties in varying degrees. Whereas the affinity for the D_3 receptor subtype is generally good, compounds **4a**,**b**,**h**, and **u** display moderate binding only having K_i values in the concentration range of 123 nM–0.9 μM. The majority of the novel compounds turned out to possess K_i values in the single digit nanomolar concentration range and can therefore be considered as high affinity ligands. The type of compounds sought after should at the same time be able to discriminate between the closely related D_2 and D_3 receptor subtypes. As it has already been predicted, compounds with a stretched overall topology should favor D_3 receptor binding over D_2 interaction.[5] In the series described here, compounds **4d**,**g**,**t**,**v**,**x**, and **y** show the desirable profile and enable the calculation of a selectivity ratio. Generally speaking, smaller aromatic rings have proven to be a good choice for the exchange of the naphthyl moiety of BP 897 (**1**). Various benzoic acid derivatives could be good D_3 receptor ligands, but the tendency of the system to favor an extended aromatic region can directly be judged from the comparison of the highly analog compounds **4v** and **w** or **4u** and **y**. Replacement of an aromatic phenoxy substituent in **4v** by an ethoxy group in **4w** leaves the affinity toward D_3 receptors unaltered (K_i 1.6 nM and 1.7 nM, respectively). At the same time the

affinity for the D_2 receptor drops by the factor 3.5. Since both compounds were obtained in the same high purity of 95%, this result can be taken to draw the conclusion that the additional or extended aromatic system can be accommodated by the topology of the binding site of the D_3 receptor subtype whereas it reduces affinity for D_2 receptor binding significantly.

Entry **4d** serves as an example for the hypothesis, that a lipophilic, but not necessarily aromatic residue adjacent to the favorable amide linkage is required. The distance and flexibility of the aliphatic lipophilic substituents relative to the hydrogen bond acceptor carbonyl group is important to render the useful D_3 receptor ligand. Elongation of the aliphatic chain diminishes affinity, as can be concluded from the weaker binding of analogs **4e**–**g**.

Test compounds **4g** and **l** exhibit affinity toward the D_3 receptor subtype in the single digit nanomolar concentration range without displaying considerable affinity for D_2 receptors. This is surprising in the case of compound **4m**. The comparably small fluoro substituent is not likely to alter the size of the aromatic substituent significantly. The fact that a second fluoro substituent in **4n** leads to detectable D_2 receptor binding renders this data not informative to explain the desired lack of D_2 receptor subtype binding. Lipophilicity and solubility of **4m** and **n** are likely to be very similar and differences in binding affinities are not understood. Because compound **4m** is predicted to have unwanted effects concerning reproduction in the web-based toxicity risk assessment using the OSIRIS property explorer (available at http://www.actelion.com/; ©2001 Thomas Sander), this compound will not be followed up. In contrary, the nonhalogenated amide derivative **4l** with similar affinity profile is not under suspicion of possessing perilous chemical functionalities. At the same time **4l** is predicted to have a favorable cLog *P* of 2.9 and a fair overall drug-likeness score of 0.74, using the same convenient tool for biomedical property estimation. Nevertheless, one has to mention that the biological data are determined in a rough high throughput screening which may lead to a different interpretation with a more careful inspection of pharmacological profiles for different aminergic receptor modulations.

4. Conclusion

The identification of an easily accessible diversity site on a lead structure with already high affinity for dopamine receptors effortlessly led to the synthesis of novel dopamine D_3 receptor ligands. Parallel derivatization of a suitable amino scaffold by use of polymer bound activated carboxylic acid equivalents yielded adequately pure carboxamides without the need of further purification steps. A naked-eye control of the 2,3,5,6-tetrafluoro-4-hydroxy-benzoic acid linker fixation was implemented and a high degree of automation in this synthetic approach (e.g., washing steps of the resins, HPLC analysis) facilitates the preparation of desired test compounds. The results of this screen corresponds to the anticipated gain in knowledge and will be subjected to virtual database screening contributing to our ongoing investigation of BP 897 analogs. While the pharmacophore model used proved to be useful to identify novel compounds with desirable properties, not all structure–activity relationships observed are understood on this basis. An iterative refinement of the model used will have to be undertaken and the results of this study will contribute to a deeper understanding of SAR in this field. The PASP synthesis protocol suggested for rapid lead analog generation could be a reliable and attractive tool for this purpose in medicinal chemistry projects.

5. Experimental

5.1. General experimental

The presented set of structures was part of a 70 member library, prepared in parallel. All compounds were subjected to HPLC analysis. According to the commonly accepted '80/80-Standard' criteria (http://paragon.acs.org/paragon/ShowDocServlet?contentId=paragon/menu_content/authorchecklist/cc_authguide.pdf) ten sample structures out of this set were analyzed in more detail. ^{1}H NMR spectra were recorded on a JEOL ECLIPSE + 500 spectrometer, using tetramethylsilane as an internal standard. The purity of the latter compounds was deduced from ^{1}H NMR data as well as evaluated by HPLC, using a Dionex Summit HPLC-system with a UV detector set at 254 nm and CC 125/4 Nucleodur™ 100-5 C18 ec columns, supplied by Macherey-Nagel. For analytical evaluation the following eluent systems were used: A (H_2O/TFA, 100:0.05) and B (methanol/TFA, 100:0.05). Compounds were dissolved in methanol/water and injected at a flow rate of 1 mL/min. The applied gradient system started with a ratio A/B of 9:1 and was run to 1:9 1 min after injection over the following 30 min. High resolution MS data were obtained on a Micromass Autospec (ESI, methanol (1/1, v/v) infusion at 10 μL/min with polyethylene glycol as reference) instrument. TLC reaction control was performed on Macherey-Nagel Polygram™ Sil G/UV254 precoated microplates, spots were visualized under UV-illumination at 254 nm. IR spectra were recorded on a Nicolet 510P FT-IR spectrometer.

5.2. General procedure for the attachment of the linker onto aminomethylated polystyrene

Aminomethylated polystyrene (6.8 g) (Novabiochem batch A25711, loading level = 1.40 mmol/g) were suspended in 78 mL DMF and 10 mg bromophenol blue were added. The suspension turned purple due to the basic aminofunctions. In a second flask 3.7 g 2,3,5,6-tetrafluoro-4-hydroxy-benzoic acid hydrate (Sigma Aldrich 36,385-5; 16.2 mmol, 1.7 equiv) were dissolved in 14 mL DMF. 2.25 mL DIC (14.4 mmol, 1.5 equiv) were then added and the mixture was shaken for 10 min. After that, a solution of 1.94 g 1-hydroxybenzotriazole (14.4 mmol, 1.5 equiv) dissolved in 3.6 mL DMF was added and shaken for 3 h. The resulting solution was than poured into the suspension of the resin and shaken for 16 h. The color of the suspended beads

turned slowly to yellow, which indicated the absence of amino functionalities. The resin was washed three times with 100 mL DMF. IR control experiments (resin sample washed with DMF, THF and dichloromethane) showed the expected amido band at 1652 cm^{-1}, but also a signal at 1763 cm^{-1} due to unwanted, but expected formation of the ester. Therefore the resin was placed into 78 mL DMF and 1.04 mL piperidine were added slowly. The mixture was shaken for 90 min and the resin was washed three times with 100 mL DMF. To remove previously formed piperidine salts, the resin was swollen in 78 mL DMF and 9 mL 2 M HCl were slowly added. After shaking the suspension for another 90 min, the solvent was removed via filtration and the remaining resin was washed intensively (3×80 mL DMF, 3×80 mL THF, and 3×80 mL dichloromethane) and dried under vacuo. IR control showed the complete disappearance of the unwanted ester band at 1763 cm^{-1}. The obtained resin displayed a loading level of 1.11 mmol/g (yield 102.5%).

5.3. General procedure for the preparation of the polymer-bound carboxylic acids residues 3a–z

Five hundred milligrams of the previously described resin were swollen in 10 mL DMF and 2 equiv (1.12 mmol) of the particular carboxylic acid were added to the suspension, followed by 13.5 mg DMAP (0.11 mmol, 2 equiv). The mixture was shaken for 5 min and 171 μL DIC (1.1 mmol, 2 equiv) were added. The reaction vial was than sealed and shaken for 16 h. After extensive washing (3×30 mL DMF, 3×30 mL THF, and 3×30 mL dichloromethane), the resin was dried in vacuo. IR control showed a characteristic band in the range of 1750–1790 cm^{-1}. The obtained yields were in between 83% and 98% (loading level 0.73–0.98 mmol/g).

5.4. General procedure for the transfer of the polymer-bound carboxylic acid residues 3a–z onto 2

Two hundred milligrams of the appropriate resin **3a**–**z** (approx. 0.16 mmol) were split and put into the reaction vessels of the ares-block of the ACT Vanguard synthesizer and pre-swollen in 1.5 mL per vessel of freshly distilled THF. After the preparation of a stock solution of **2** (concentration of 6 mg of **2** in 1 mL THF), 0.84 mL (5 mg of **2**, 0.017 mmol, approx. 0.2 equiv) of this stock solution were added to the suspension of the resin in each vessel. The block was than flushed with argon and sealed. After 16 h of shaking, TLC indicated complete conversion of the amine **2** and the resin was filtered off and washed ten times with 1.5 mL of THF. All fractions were collected in appropriate glass tubes put into the cleavage block. The organic fractions were combined, evaporated, and dried in vacuo. All yields were in between 70% and 96%.

5.5. Compound 4k (4-ethyl-*N*-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.32 (t, 1H, J = 5.4 Hz), 7.76 (d, 2H, J = 8.3 Hz), 7.27 (d, 2H, J = 8.1 Hz), 6.91 (m, 2H), 6.86 (m, 2H), 3.76 (s, 3H), 3.28 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.64 (q, 2H, J = 7.6 Hz), 2.34 (m, 2H), 1.50 (m, 4H), 1.19 (t, 3H, J = 7.6 Hz). HRESI-MS found 396.2651; requires 396.2648. HPLC = 92.6%.

5.6. Compound 4l (4-acetylamino-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 10.08 (s, 1H), 8.27 (t, 1H, J = 5.6 Hz), 7.78 (d, 2H, J = 8.9 Hz), 7.62 (d, 2H, J = 8.7 Hz), 6.91 (m, 2H), 6.86 (m, 2H), 3.76 (s, 3H), 3.28 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.34 (m, 2H), 2.06 (s, 3H), 1.53 (m, 4H). HRESI-MS found 425.2553; requires 425.2539. HPLC = 92.6%.

5.7. Compound 4p (*N*-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]butyl}-3-(trifluoromethyl)benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.69 (t, 1H, J = 5.6 Hz), 8.18 (m, 1H), 8.15 (d, 1H, J = 7.8 Hz), 7.89 (d, 1H, J = 8.02 Hz), 7.71 (t, 1H, J = 7.8 Hz), 6.92 (m, 2H), 6.86 (m, 2H), 3.76 (s, 3H), 3.28 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.36 (t, 2H, J = 7.0 Hz), 1.55 (m, 4H). HRESI-MS found 436.2212; requires 436.2214. HPLC = 91.1%.

5.8. Compound 4q (3,5-dichloro-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.64 (t, 1H, J = 5.5 Hz), 7.86 (d, 2H, J = 2.1 Hz), 7.78 (t, 1H, J = 2.0 Hz), 6.92 (m, 2H), 6.86 (m, 2H), 3.76 (s, 3H), 3.28 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.34 (t, 2H, J = 7.0 Hz), 1.56 (m, 2H), 1.50 (m, 2H). HRESI-MS found 436.1559; requires 436.1584. HPLC = 91.8%.

5.9. Compound 4u (2-benzoyl-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 7.70 (d, 1H, J = 7.3 Hz), 7.51 (m, 2H), 7.32 (m, 4H), 7.23 (d, 2H, J = 6.9 Hz), 7.06 (s, 1H), 6.91 (m, 2H), 6.85 (m, 2H), 3.76 (s, 3H), 3.27 (m, 6H overlapping H_2O), 2.90 (m, 4H), 2.42 (m, 2H), 1.44 (m, 2H), 1.37 (m, 2H). HRESI-MS found 472.2600; requires 472.2566. HPLC = 87.4%.

5.10. Compound 4v (*N*-{4-[4-(2-methoxyphenyl)-piperazin-1-yl]butyl}-4-phenoxybenzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.36 (t, 1H, J = 5.6 Hz), 7.86 (d, 2H, J = 8.7 Hz), 7.43 (t, 2H, J = 8.0 Hz), 7.20 (t, 1H, J = 7.8 Hz), 7.07 (d, 2H, J = 8.5 Hz), 7.02 (d, 2H, J = 8.9 Hz), 6.92 (m, 2H), 6.86 (m, 2H), 3.76 (s, 3H), 3.28 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.34 (t, 2H, J = 6.9 Hz), 1.53 (m, 4H). HRESI-MS found 460.2600; requires 460.2588. HPLC = 94.6%.

5.11. Compound 4w (4-ethoxy-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.24 (t, 1H, J = 5.8 Hz), 7.80 (d, 2H, J = 8.9 Hz), 6.95 (d, 2H,

J = 8.9 Hz), 6.92 (m, 2H), 6.86 (m, 2H), 4.08 (q, 2H, J = 7.0 Hz), 3.76 (s, 3H), 3.26 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.34 (m, 2H), 1.52 (m, 4H), 1.33 (t, 3H, J = 7.0 Hz). HRESI-MS found 412.2600; requires 813.3718. HPLC = 94.9%.

5.12. Compound 4x (3,4-dimethoxy-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.27 (t, 1H, J = 5.4 Hz), 7.46 (dd, 1H, J = 2.1/8.3 Hz), 7.43 (d, 1H, J = 2.1 Hz), 7.00 (d, 1H, J = 8.5 Hz), 6.92 (m, 2H), 6.86 (m, 2H), 3.80 (s, 6H), 3.77 (s, 3H), 3.28 (m overlapping H_2O, 6H), 2.95 (m, 4H), 2.34 (t, 2H, J = 7.1 Hz), 1.52 (m, 4H). HRESI-MS found 428.2549; requires 428.2519. HPLC = 92.0%.

5.13. Compound 4y (4-benzoyl-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.63 (t, 1H, J = 5.5 Hz), 7.99 (d, 2H, J = 8.5 Hz), 7.79 (d, 2H, J = 8.5 Hz), 7.74 (d, 2H, J = 8.3 Hz), 7.70 (t, 1H, J = 7.5 Hz), 7.58 (t, 2H, J = 7.6 Hz), 6.92 (m, 2H), 6.86 (m, 2H), 3.76 (s, 3H), 3.31 (m overlapping H_2O, 6H), 2.94 (m, 4H), 2.36 (t, 2H, J = 7.0 Hz), 1.75 (m, 2H), 1.55 (m, 2H) (identical with reported data[5] with minor deviations). HRESI-MS found 472.2600; requires 472.2612. HPLC = 89.1%.

5.14. Compound 4z (4-chloro-*N*-{4-[4-(2-methoxyphenyl)piperazin-1-yl]butyl}benzamide)

^{1}H NMR δ_H (ppm) (500 MHz, DMSO-d_6, Me_4Si): 8.50 (t, 1H, J = 5.3 Hz), 7.86 (d, 2H, J = 8.7 Hz), 7.53 (d, 2H, J = 8.5 Hz), 6.92 (m, 2H), 6.87 (m, 2H), 3.76 (s, 3H), 3.27 (m overlapping H_2O, 6H), 2.96 (m, 4H), 2.42 (m, 2H), 1.54 (m, 4H) (identical with reported data[5] with minor deviations). HRESI-MS found 402.1948; requires 402.1973. HPLC = 92.0%.

5.15. Determination of dopamine receptor subtype binding

Pharmacological testing was performed as described previously with slight modifications.[5] In brief, for the D_3 receptor, binding was performed with ^{3}H spiperone using 5 μg of membrane suspension (prepared from stable human D_3 transfected CHO cell line). For the D_2 receptor, binding was performed with ^{3}H spiperone using 10 μg of membrane suspension (prepared from stable human D_{2S} transfected HEK293 cell line). All tests were performed at least in duplicate, mean values are given.

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