Bioorganic & Medicinal Chemistry 17 (2009) 5482–5487

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Parallel synthesis of potent dopaminergic *N*-phenyltriazole carboxamides applying a novel click chemistry based phenol linker

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ARTICLE INFO

Article history:
Received 15 April 2009
Revised 15 June 2009
Accepted 18 June 2009
Available online 25 June 2009

Keywords:
Dopamine receptor
D3
Click chemistry
Parallel synthesis
Catch and release
Triazole

ABSTRACT

Taking advantage of our click chemistry based methodology to construct novel SPOS (solid phase organic synthesis) resins, the triazolylmethyl linked catechol **6a** was discovered, which is readily available via copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC) of azidomethyl substituted polystyrene with *O*-propargylcatechol and can be applied for the parallel synthesis of *N*-phenyltriazole carboxamides. As a proof-of-concept, a 'catch-and-release' strategy could be successfully applied for a parallel synthesis of dopaminergic phenyltriazoles of type **2**. A focused model library of 20 test compounds revealing three points of diversity was generated by a three-step SPOS approach. Product purification was performed employing a solid-supported carboxylic acid anhydride as a scavenger. GPCR-ligand binding screening revealed dopamine D3 receptor ligands with K_i values in the single digit nanomolar range.

1. Introduction

The 'click chemistry' concept developed by Sharpless and co-workers focuses on the utilization of highly efficient organic reactions aiming to maximize the synthetic efficiency of selective compound modifications.[1] Within the 'click chemistry' toolbox, the copper catalyzed azide–alkyne [3+2] cycloaddition (CuAAC) proved to be a highly versatile and useful reaction leading to the 1,2,3-triazole heterocycle. Click chemistry has become a powerful method within combinatorial chemistry, and it has found increasing applications in drug discovery and lead optimization programs.[2,3] Click chemistry has been accepted as a highly beneficial tool for parallel synthesis. Because an appropriate linker displaying optimal reactivity and swelling is crucial for the success, we prepared novel, functionalized resins, taking advantage of the concept of click chemistry. Thus, CuAAC of alkynyl-substituted handles with azidomethyl polystyrene led to a new family of highly efficient BAL (backbone amide linker),[4] REM (regenerative Michael acceptor),[5] SPAn (solid/solution-phase annulation)[6] and scavenger resins.[7] 'Click resins' enable solid phase supported reactions to work under nearly perfect conditions fulfilling the requirements of click chemistry.

The dopamine D3 receptor has attracted enormous attention as a potential drug target, since selective D3 ligands are promising agents for the therapy of schizophrenia, Parkinson's disease and drug abuse.[8–10] Extensive structure–activity relationship studies revealed that aryl carboxamides bearing a *N*-alkyl-*N*′-arylpiperazine side chain exhibit preferential D3 recognition.[11–15] Taking advantage of our click chemistry based BAL resins, we reported on a parallel synthesis of biphenyl carboxamides of type **1a**[16] and *N*-benzyl-1,2,3-triazole carboxamides of type **1b**.[17] To further optimize the receptor binding profiles, we intended to approach structural hybrids of type **2** modifying the described *N*-benzyltriazole based ligands towards *N*-phenyl substituted derivatives (Fig. 1).

Unfortunately, the elaborated backbone amide linker strategy including a [3+2]-cycloaddition of a solid-supported propynoic acid amide with benzyl azide could not be applied for the construction of phenyltriazoles since the employed arylazides decomposed at the required temperature of 150 °C. Thus, we planned to compass an alternative strategy for a parallel synthesis of *N*-phenyl-1,2,3-triazole-4-carboxamides of type **2** taking advantage of our click chemistry based methodology to construct novel SPOS (solid phase organic synthesis) resins. Since solid-supported phenol handles proved to be efficient tools for the SPOS of carboxamides via a 'catch-and-release' approach,[18–20] we intended to construct novel triazole linked phenols by applying our highly efficient, reliable and robust click chemistry based concept utilizing the CuAAC.[21,22] The presented methodology should allow an efficient parallel synthesis of *N*-phenyl-1,2,3-triazole carboxamides. This structural feature can be found in a variety of pharmacological relevant compounds like tyrosine phosphatase inhibitors, allosteric mGluR1 antagonists and anti-platelet active agents.[23–25] As a proof-of-concept we intended to construct a focused library of putative dopamine receptor ligands.

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doi:10.1016/j.bmc.2009.06.041

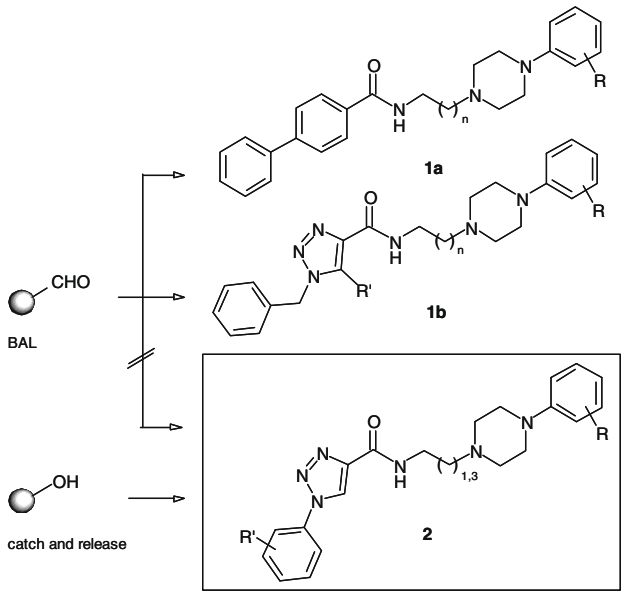
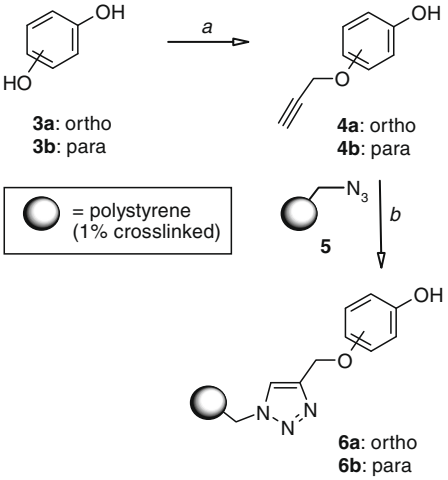


Figure 1. Phenyltriazole carboxamides as a potential novel class of dopamine D3 receptor ligands.

2. Results and discussion

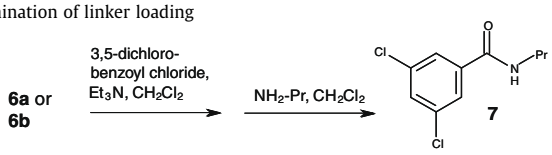
2.1. Chemistry

In contrast to previously used phenylthioethers for the linkage of catch and release functionalities, we tried to take advantage of putatively chemically more stable phenylether based oxa-analogs of type **6** to be functionalized and immobilized via click chemistry. Thus, azidomethyl substituted polystyrene **5**, which is readily available from Merrifield resin, was planned to be used as a starting material. Catechol and hydroquinone (**3a**,**b**) were mono-functionalized by O-propargylation to give the terminal alkynes **4a** and **4b**, respectively. Using CuI as a catalyst, [3+2]-cycloaddition of the immobilized azide **5** and **4a**,**b** resulted in formation of the triazole-resins **6a**,**b** (Scheme 1). To determine the amount of phenol residues available for the aspired 'catch-and-release' strategy, the functional resins **6a**,**b** were acylated with 3,5-dichlorobenzoyl chloride. Subsequent release by aminolysis with *n*-propylamine afforded the carboxamide **7**. After purification by flash chromatography, the yields of **7** indicated a loading of 0.98 and 0.99 mmol/g for catechol and hydroquinone, respectively (Table 1). This data is in high accordance to the loading calculated by elementary analysis of nitrogen. The theoretical loading which was based on the amount of chloromethyl groups in the starting material (1.0–1.2 mmol/g), indicated nearly quantitative conversion.



Scheme 1. Reagents and conditions: (a) propargyl bromide, K_2CO_3, acetone, reflux, 18 h; (b) Cu(I)I, THF/DIPEA, 35 °C, 36 h.

Table 1
Determination of linker loading

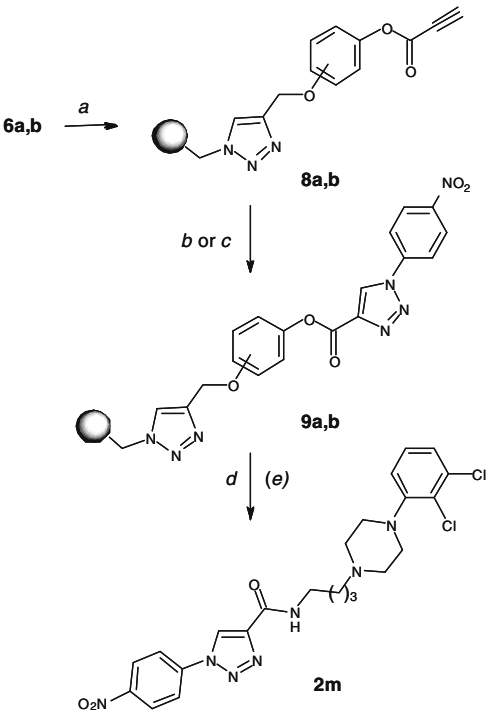


Resin	Yield of **7** (mmol) per g resin	Triazole loading (mmol) per g resin[a]	Theoretical loading[b] (mmol/g)
6a	0.98	0.99	0.85–1.03
6b	0.99	0.96	0.85–1.03

[a] Calculated by elementary analysis of nitrogen.
[b] Calculated on the loading of Merrifield resin (1.0–1.2 mmol/g).

To investigate the reliability and the scope of our solid phase supported method, we elaborated the synthesis of a model compound. An objective of this step was to identify the more efficient one of the two resins **6a**,**b** to optimize suitable reaction conditions for the cycloaddition reaction and to validate the use of a scavenger resin for the removal of the excess of amine reactant after the product cleavage.

In detail, diisopropyl carbodiimide (DIC) promoted acylation of the phenol resins **6a**,**b** with propynoic acid furnished the respective esters **8a**,**b** (Scheme 2). Product formation could be easily monitored by IR spectroscopy indicating the presence of both the ester C=O and C–C triple bond. Cycloaddition with *p*-nitrophenyl azide was performed either in the presence or absence of CuI at



Scheme 2. (a) (1) Propynoic acid, DIC, CH_2Cl_2, 7 h, (2) propynoic acid, DIC, CH_2Cl_2, 14 h; (b) *p*-nitrophenyl azide, CuI, DMF, 120 °C, 24 h; (c) *p*-nitrophenyl azide, DMF, 120 °C, 48 h; (d) *N*-(4-aminobutyl)-*N*′-(dichlorophenyl)piperazine, CH_2Cl_2, 20 °C, 18 h; (e) MP anhydride resin, CH_2Cl_2, 20 h, 24 h.

Table 2
Optimization of SPOS condition

Resin		6a		6b	
Catalyst		CuI[a] (%)	None[b] (%)	CuI[a] (%)	None[b] (%)
Method A[c]	Yield[e]	50	54	43	40
	Purity[f]	>95	>95	>95	>95
Method B[d]	Yield[e]	79	46	63	91
	Purity[f]	98	97	87	86

[a] Reaction time 24 h.
[b] Reaction time 48 h.
[c] Purification by flash chromatography.
[d] Purification by scavenger resin.
[e] Determined by ^{1}H NMR.
[f] Determined by LC–MS (254 nm).

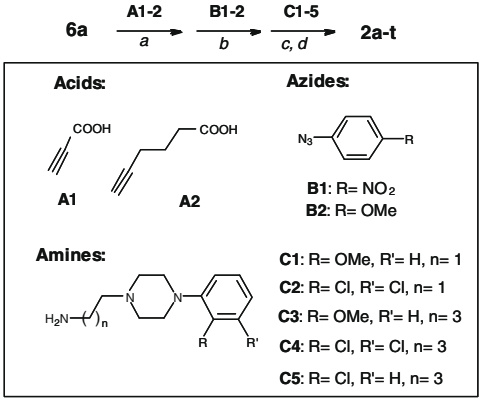
120 °C to give the triazoles **9a**,**b**. Finally, the cleavage with 3 equiv of *N*-(4-aminobutyl)-*N*′-(dichlorophenyl)piperazine afforded the triazole carboxamide **2m**, when product purification was done alternatively by addition of a macroporous anhydride[26] as a scavenger resin or by flash chromatography.

The results of this SPOS validation and optimization are summarized in Table 2. A couple of clear conclusions can be drawn from the obtained results:

(A) Giving comparable yields, the use of CuI as a catalyst allows choosing shorter reaction times. Exclusive formation of the 1,4-disubstituted triazole isomer was observed.
(B) The catechol-based resin **6a** works more efficiently regarding to the obtained product yields and purities.
(C) Application of an amine scavenger resin revealed to be a highly efficient alternative to chromatographic purification.

Using the elaborated solid phase supported methodology described above, five aminoalkylpiperazines, two alkynyl carboxylic acids and two aromatic azides were chosen to construct a three dimensional focused library of putative D3 receptor ligands of type **2**. Following the described procedure including DIC-mediated acylation of the triazolylmethylcatechol (TMC) resin **6a**, CuI-catalyzed cycloaddition and aminolysis followed by removal of the excessive amine, the aryltriazoles **2a**–**t** were obtained. LC–MS derived purities and yields are displayed in Table 3. In general, the obtained

Table 3
Purities, yields and receptor screening results for SPOS products **2a–t**



a: 1) **A1-2**, DIC, CH_2Cl_2, 7h, 2) **A1-2**, DIC, CH_2Cl_2, 14h; b: **B1-2**, CuI, DMF, 120°C, 24h; c: **C1-5**, CH_2Cl_2, 20°C, 18h; d: MP anhydride resin, CH_2Cl_2, 20° C, 24h.

Compound		Purity[a] (%)	Yield (%)	Displacement of radioligand					
				D1	$D2_l$	$D2_s$	D3	D4	α1
2a	A1B1C1	81	88						80
2b	A1B2C1	76	61						56
2c	A2B1C1	79	29						43
2d	A2B2C1	83	46						56
2e	A1B1C2	85	46				52		47
2f	A1B2C2	73	52				40		39
2g	A2B1C2	80	32				39	37	
2h	A2B2C2	82	28				42	41	
2i	A1B1C3	89	82			38	58		73
2j	A1B2C3	95	76				57		64
2k	A2B1C3	87	41		39	52	51		83
2l	A2B2C3	91	64			33	46		72
2m	A1B1C4	81	31		33	53	95		48
2n	A1B2C4	85	88				76		
2o	A2B1C4	75	61			43	76		
2p	A2B2C4	76	66			43	71		
2q	A1B1C5	77	86				61		61
2r	A1B2C5	83	97				58		55
2s	A2B1C5	84	76				51		64
2t	A2B2C5	91	79				38		58
Displacement (%)				<30	30–59			60–79	>80
Color code									

[a] Determined by LC–MS (254 nm).

Table 4
Receptor binding data of selected compounds at human $D2_{long}$, $D2_{short}$, D3 and D4.4 receptors as well as porcine D1 and α1 receptors

Compound	K_i values[a] (nM)					
	pD1	$hD2_{long}$	$hD2_{short}$	hD3	hD4.4	pα1
2a	4100	750	470	180	140	6.5
2m	810	49	53	1.0	280	17
2n	1700	44	68	4.9	320	46
2o	2200	83	47	5.7	240	28
2p	1800	120	42	9.3	200	42

[a] K_i values in nM are based on the means of 2–4 experiments each done in triplicate.

purities of 73–95% allowed biological screening of all 20 library members without further purification steps.

2.2. Biological investigations

A GPCR (G-protein coupled receptor) screening focused on biogenic amine receptors was performed to characterize all library members to determine their ability to bind to the cloned human D2long, D2short, D3 and D4 dopamine receptor subtypes, and the porcine D1 dopamine and α1 adrenergic receptors.[16,27] This was accomplished in a screening system by measuring the displacement of the following radioligands: [^{3}H]spiperone for D2, D3, D4, [^{3}H]SCH23390 for D1 receptors and [^{3}H]prazosin for α1, using 100 nM concentration of the test compounds. The results in Table 3 reflect the percentage of radioligand displacement from the six different GPCRs. None of the compounds was able to bind significantly to the D1 receptor. The affinities to the D2long, D2short and D4 subtypes were weak to moderate. In contrast, the majority of the test compounds showed excellent binding to the D3 receptor. Interestingly, the most potent D3 ligands **2m**,**n**,**o**,**p** feature the piperazinylbutylamine moiety in combination with the dichlorophenyl substituent. For the α1 adreno-receptor, a different structure activity relationship could be observed. Here, the incorporation of a 2-(methoxyphenyl)piperazine moiety leads to highest affinities (compounds **2i**,**k**,**l**).

Equilibrium binding constants (K_i-values) were determined of those compounds showing a percentage displacement greater than 70% at the D3 receptor as well as for **2a**, for which the screening results indicated strong α1 selectivity. The affinity constant (K_i) values are shown in Table 4. The *N*-piperazinylethyl substituted triazole carboxamide **2a** displays low nanomolar affinity (K_i = 6.5 nM) for the α1 receptor in combination with a high selectivity over all tested dopamine receptor subtypes. On the other hand, the *N*-(dichlorophenyl)piperazine with the four-carbon spacer proved to be a scaffold leading to K_i values in the single digit nanomolar range. Within this group, the (*p*-nitrophenyl)triazole carboxamide **2m** (A1B1C4) turned out to be the most potent library member with a K_i(D3) value of 1.0 nM and a 50-fold selectivity over the D2 subtypes. Taking this compound as a starting point for further lead optimization projects, additional structural modifications—preferably at the aryltriazole moiety—should most probably yield subnanomolar D3 ligands.

3. Conclusion

In conclusion, utilizing the triazolylmethylcatechol (TMC) resin **6a**, which is readily available via [3+2]azide–alkyne cycloaddition, a 'catch-and-release' strategy could be successfully applied for a parallel synthesis of dopaminergic phenyltriazoles. A focused library of 20 test compounds revealing three points of diversity was generated by a three-step SPOS approach followed by treatment with macroporous anhydride resin. GPCR-ligand binding assays indicated single digit nanomolar dopamine D3 receptor binding with a superior K_i value of 1.0 nM for the dichlorophenylpiperazine **2m** and α1 receptor preference for the methoxyphenylpiperazine **2a**.

4. Experimental

4.1. General

Polystyrene resins were purchased from NovaBiochem. SPOS were performed manually in a Heidolph Synthesis I equipped with PFA vessels. Absolute solvents were purchased from Acros. Commercially available starting material was used without further purification. IR spectra were registered on JASCO model FTIR 410 instrument via KBr pellet. ^{1}H NMR (360 MHz or 600 MHz) spectra were determined on a Bruker AM 360 or a Bruker AVANCE spectrometer in solution. LC–MS analyses were conducted in an Agilent Binary Gradient System (MeOH/0.1 N aq HCO_2H 10/90–90/10) in combination with ChemStation Software and UV detection at 254 nm using a Zorbax SB-C8 (4.6 mm × 150 mm, 5 μm) with a flow rate of 0.5 mL/min. Mass detection was pointed out with a Bruker Esquire 2000 ion-trap mass spectrometer using an APC ionization source. EI-MS spectra were recorded on FINNIGAN MAT TSQ 70 spectrometer. Flash chromatography was done using silica gel (40–63 μm) as stationary phase. TLC analyses were done on Merck 60 F_{254} glass plates and analyzed by UV light (254 nm) or by iodine vapor.

4.2. Synthesis of linker precursors

Propargyloxyphenols **4a** and **4b** were synthesized starting from catechol (**3a**) and hydroquinone (**3b**), respectively, according to a procedure described in the literature.[28]

4.2.1. 2-Propargyloxyphenol 4a

^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 2.58 (t, J = 2.3 Hz, 1H), 4.79 (d, J = 2.4 Hz, 2H), 6.85–6.91 (m, 1H), 6.95 (dd, J = 7.9 Hz, 1.5 Hz, 1H), 6.97–7.05 (m, 2H). EI-MS (m/z): 148 (M^+).

4.2.2. 4-Propargyloxyphenol 4b

Analytical data are identical to those reported in the literature.[29]

4.3. Synthesis of phenol resins 6a and 6b

Merrifield resin (6.0 g, 1.1 mmol/g) was reacted with NaN_3 (1.3 g, 20 mmol) in DMSO (70 mL) at 70 °C for 48 h. After being cooled to room temperature the resin was sequentially washed with DMSO (3 × 50 mL), H_2O (3 × 50 mL), MeOH (5 × 50 mL), CH_2Cl_2 (3 × 50 mL) and Et_2O (3 × 50 mL), dried under vacuum and analyzed by IR spectroscopy showing a strong absorption at 2090 cm^{-1}. The resulting azidomethyl polystyrene **5** (1.5 g) was shaken together with 2-propargyloxyphenol **4a** or 4-propargyloxyphenol **4b** (0.6 g, 4.1 mmol) and CuI (30 mg, 0.17 mmol) in a mixture of THF/DIPEA 2:1 (20 mL) at 35 °C for 36 h whereas a disappearance of the IR-absorption at 2090 cm^{-1} could be observed. After being cooled to room temperature, the solvents were removed by filtration and a washing procedure including pyridine (3 × 50 mL), THF (3 × 50 mL), MeOH (3 × 50 mL), CH_2Cl_2 (5 × 50 mL) and Et_2O (3 × 50 mL) followed by drying of the resin under vacuum affording the resins **6a** and **6b**.

4.4. Determination of resin capacity

Resins **6a** or **6b** (100 mg) were distributed in teflon vessels and each reacted with 3,5-dichlorobenzoyl chloride (120 mg, 0.55 mmol) in a mixture of Et_3N and CH_2Cl_2 (1/10, 10 mL) at room temperature for 48 h. The resin were thoroughly washed with

DMF (3 × 25 mL), CH_2Cl_2 (3 × 25 mL), Et_2O (2 × 25 mL) and dried by suction. Subsequent addition of 1-propylamine (20 mg, 0.55 mmol) in CH_2Cl_2 (5 mL) was followed by shacking for 18 h at ambient temperature. The cleavage solution was collected by filtration and evaporated. The residue was dried under vacuum and purified by flash chromatography using hexane/ethyl acetate (1/1) to afford 22.5 mg (from **6a**) and 23 mg (from **6b**) of **7**, respectively.

4.5. Synthesis of phenylazide B1

1-Azido-4-methoxybenzene was synthesized from 1-bromo-4-iodobenzene in the presence of CuI and L-proline according to a method described in the literature.[30]

4.6. Synthesis of phenylazide B2

1-Azido-4-nitrobenzene was synthesized from 4-nitroaniline according to a method described in the literature.[31]

4.7. 3D library. Synthesis of *N*-phenyltriazoles 2a–t

TMC resin **6a** (20 × 100 mg, 20 × 0.1 mmol) was distributed into 20 teflon vessels followed by a solution of DIC (each 69 mg, 0.55 mmol) and the corresponding carboxylic acids 10 × **A1** and 10 × **A2** (each 0.5 mmol) in CH_2Cl_2 (5 mL). After agitation for 7 h at room temperature the resins were washed with CH_2Cl_2 and the entire acylation process was repeated with a prolonged reaction time of 14 h. The resins were washed with CH_2Cl_2 (3 × 20 mL) and Et_2O (3 × 20 mL) and subsequently treated with a solution of the corresponding arylazides 10 × **B1** and 10 × **B2** (each 1.0 mmol) and CuI (2 mg, 0.01 mmol) in DMF (1 mL). After agitation at 120 °C for 24 h the resins were washed extensively with hot DMF (10 × 20 mL), CH_2Cl_2 (8 × 25 mL) and Et_2O (2 × 20 mL). A solution of the primary amines **C1**–**C5** (each 0.4 mmol) in CH_2Cl_2 (3 mL) was added and agitation was continued for 18 h at room temperature. Anhydride MP scavenger resin (20 × 130 mg, 20 × 1.0 mmol) was added and the mixtures were shaken for another 24 h. The cleavage solutions were collected by filtration and the resins were washed with CH_2Cl_2 (each 20 mL). After evaporation of the combined solutions, the residues were dried under vacuum over night to afford the crude products **2a**–**t**. The products were analyzed by LC–MS for the determination of the purity. For representative NMR data, see below.

4.8. *N*-{2-[4-(2,3-Dichlorophenyl)piperazin-1-yl]ethyl}-1-(4-nitrophenyl)-1*H*-[1,2,3]triazole-4-carboxamide (2e, A1B1C2)

^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 2.71–2.82 (m, 6H), 3.09–3.18 (m, 4H), 3.65–3.71 (m, 2H), 7.00 (d, *J* = 6.6 Hz, 1H), 7.15–7.21 (m, 2H), 7.74 (br s, 1H), 8.00–8.09 (m, 2H), 8.44–8.53 (m, 2H), 8.67 (s, 1H). APCI-MS (*m*/*z*): 490.1 $(M+1)^+$.

4.9. *N*-{2-[4-(2,3-Dichlorophenyl)piperazin-1-yl]ethyl}-4-[1-(4-nitrophenyl)-1*H*-[1,2,3]triazol-4-yl]butyramide (2g, A2B1C2)

^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 2.10–2.19 (m, 2H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.58–2.66 (m, 2H), 2.67–2.78 (m, 4H), 2.94 (t, 7.2 Hz, 2H), 3.05–3.18 (m, 4H), 3.40–3.50 (m, 2H), 6.98 (ddd, *J* = 7.3 Hz, 2.1 Hz, 1.8 Hz, 1H), 7.14–7.22 (m, 2H), 7.95 (s, 1H), 7.93–8.05 (m, 2H), 8.29–8.40 (m, 2H). APCI-MS (*m*/*z*): 532.8 $(M+1)^+$.

4.10. *N*-{2-[4-(2,3-Dichlorophenyl)piperazin-1-yl]ethyl}-4-[1-(4-methoxyphenyl)-1*H*-[1,2,3]triazol-4-yl]butyramide (2h, A2B2C2)

^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 2.07–2.18 (m, 2H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.63 (t, *J* = 5.7 Hz, 2H), 2.67–2.78 (m, 4H), 2.91 (t, 7.2 Hz, 2H), 3.05–3.18 (m, 4H), 3.41–3.50 (m, 2H), 3.90 (s, 3H), 6.97 (ddd, *J* = 7.3 Hz, 2.1 Hz, 1.8 Hz, 1H), 7.00–7.10 (m, 2H), 7.14–7.22 (m, 2H), 7.59–7.70 (m, 2H), 7.73 (s, 1H). APCI-MS (*m*/*z*): 532.8 $(M+1)^+$.

4.11. *N*-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-4-[1-(4-nitrophenyl)-1*H*-[1,2,3]triazol-4-yl]butyramide (2k, A2B1C3)

^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.60–1.72 (m, 2H), 1.80–2.05 (m, 4H), 2.14 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.2 Hz, 2H), 2.80–3.00 (m, 6H), 3.34–3.50 (m, 6H), 3.88 (s, 3H), 6.85–6.98 (m, 2H), 7.01–7.11 (m, 2H), 8.07–8.18 (m, 2H), 8.33 (s, 1H), 8.33–8.43 (m, 2H). APCI-MS (*m*/*z*): 522.3 $(M+1)^+$.

4.12. *N*-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-4-[1-(4-methoxyphenyl)-1*H*-[1,2,3]triazol-4-yl]butyramide (2l, A2B2C3)

^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.58–1.75 (m, 4H), 2.06–2.17 (m, 2H), 2.33 (dd, *J* = 7.2 Hz, 6.8 Hz, 2H), 2.52–2.60 (m, 2H), 2.72–2.82 (m, 4H), 2.86–2.89 (m, 2H), 3.10–3.22 (m, 4H), 3.27–3.35 (m, 2H), 3.87 (s, 3H), 3.88 (s, 3H), 6.88 (d, *J* = 7.5 Hz, 1H), 6.92–6.97 (m, 3H), 6.96–7.08 (m, 2H), 7.59–7.70 (m, 2H), 8.03 (s, 1H). APCI-MS (*m*/*z*): 507.4 $(M+1)^+$.

4.13. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}-1-(4-nitrophenyl)-1*H*-[1,2,3]triazol-4-carboxamide (2m, A1B1C4)

^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 1.68–1.83 (m, 4H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.65–2.79 (m, 4H), 3.10–3.20 (m, 4H), 3.58 (dd, *J* = 12.4 Hz, 6.3 Hz, 2H), 7.00 (dd, *J* = 6.5 Hz, 2.9 Hz, 1H), 7.13–7.21 (m, 2H), 7.57 (br s, 1H), 8.00–8.10 (m, 2H), 8.43–8.50 (m, 2H), 8.70 (s, 1H). APCI-MS (*m*/*z*): 518.3 $(M+1)^+$.

4.14. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}-1-(4-methoxyphenyl)-1*H*-[1,2,3]triazol-4-carboxamide (2n, A1B2C4)

^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 1.71–1.84 (m, 4H), 2.68–2.75 (m, 2H), 2.81–2.94 (m, 4H), 3.18–3.24 (m, 4H), 3.53 (dd, *J* = 13.2 Hz, 6.6 Hz, 2H), 3.87 (s, 3H), 6.97 (ddd, *J* = 7.5 Hz, 1.8 Hz, 1.2 Hz, 1H), 7.00–7.09 (m, 2H), 7.12–7.21 (m, 2H), 7.59–7.68 (m, 2H), 8.50 (s, 1H). APCI-MS (*m*/*z*): 503.3 $(M+1)^+$.

4.15. Binding studies

Receptor binding studies were carried out as described in literature.[24] In brief, the dopamine D1 receptor assay was done with porcine striatal membranes at a final protein concentration of 60 μg/assay tube and the radioligand [^{3}H]SCH 23390 at 0.3 nM (K_d = 0.95 nM). Competition experiments with the human $D2_{long}$, $D2_{short}$, D3 and D4.4 receptors were run with preparations of membranes from CHO cells expressing the corresponding receptor and [^{3}H]spiperone at a final concentration of 0.5 nM. The assays were carried out with a protein concentration of 3–10 μg/assay tube and K_d values of 0.06–0.07 nM for $D2_{long}$, 0.09–0.15 nM for $D2_{short}$, 0.08–0.23 nM for D3 and 0.22–0.28 nM for D4.4.

The investigation of adrenergic α_1 binding was performed as described in literature.[15] In brief, porcine cortical membranes were subjected to the binding assay at a concentration of 55 μg/assay tube for determination of adrenergic α1 binding utilizing [^{3}H]prazosin at a final concentration of 0.4 with a K_D value of 0.06 nM.

Screening studies were established when using test compounds at a final concentration of 10 μM, 100 nM and 1 nM as triplicates and the assay conditions as described above.

Protein concentration was established by the method of Lowry using bovine serum albumin as standard.[32]

Data analysis of the resulting competition curves was accomplished by non-linear regression analysis using the algorithms in PRISM (GraphPad Software, San Diego, CA). K_i values were derived from the corresponding EC_{50} data utilizing the equation of Cheng and Prusoff.[33]

Acknowledgments

The *Fonds der Chemischen Industrie* is acknowledged for financial support. We thank Dr. J.-C. Schwartz and Dr. P. Sokoloff (INSERM, Paris), Dr. H. H. M. Van Tol (Clarke Institute of Psychiatry, Toronto) and Dr. J. Shine (The Garvan Institute of Medical Research, Sydney) for providing D3, D4.4 and D2 receptor expressing cell lines. This work was supported by the Deutsche Forschungsgemeinschaft (DFG).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.06.041.

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