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Click chemistry based solid phase supported synthesis of dopaminergic phenylacetylenes

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Abstract—'Click resins' enable solid phase supported reactions to work under nearly perfect conditions fulfilling the requirements of click chemistry. Utilizing the formylpyrrolylmethyltriazole (FPMT) linker **6**, which is readily available via copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC), a BAL strategy could be successfully applied for a parallel synthesis of dopaminergic phenylacetylens. A focused library of 20 test compounds revealing three points of diversity was generated by a four-step SPOS approach including microwave assisted Sonogashira coupling. GPCR-ligand binding assays indicated excellent dopamine D3 and D4 receptor binding affinities which were identified to cause a partial agonist activity for the most potent test compounds **2c**,**e**,**i**,**k**.

1. Introduction

The concept of click chemistry, which was established by K. Barry Sharpless a few years ago, has been exploited as a highly beneficial tool for the process of lead development in drug discovery.[1,2] Click reactions must be modular, wide in scope, provide high yields, work in both small and large scale applications and lack significant by-products (or result in inoffensive or easily removable by-products).[3] The required process characteristics include simple reaction conditions, readily available starting materials and reagents, and should require no or easily removable solvents. Any purification should be non-chromatographic, and the products generated must be stable under physiological conditions. Interestingly, the same requirements hold true for a parallel approach in drug discovery, since combinatorial chemistry is highly sensible on the reliability of the reactions used to construct the new chemical bonds.[4] In conjunction with a programme to design super-potent dopamine D3 receptor ligands employing click chemistry,[5,6] we herein describe that 'click resins' enable solid phase supported reactions[7] to work under nearly perfect conditions fulfilling the requirements of click chemistry very impressively. Intending to investigate the introduction of an alkynyl subunit as a pharmacophoric element, we planned to take advantage of a concept of privileged structures[8] when a careful bioisosteric replacement should be capable of providing ligands for more than one receptor.[9–12] For family A GPCRs, we identified carboxamides of type **1** as such a privileged structure serving also as potential PET imaging agents.[13–16] On the basis of lead compound **1**, we herein present a solid phase supported construction of a focused library of type **2**. Furthermore, data on their GPCR binding and functional properties are provided (Chart 1).

2. Results and discussion

2.1. Chemistry

The backbone amide linker (BAL) strategy for the preparation of C-terminally modified and cyclic peptides, as well as non-peptidic compounds, has been established by Barany and co-workers.[17,18] During the last years, the versatility of BAL linkage has been largely proved, especially in solid phase peptide synthesis. On the basis of this concept, we established a first series of click based backbone amide linkers, when the electron-donating properties of the enamine functionality of indole-3-carbaldehyde facilitated an extraordinary smooth and high yielding liberation of the final products.[19] A formal

Keywords: Click resins; Click chemistry; Copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC); BAL strategy; Microwave assisted Sonogashira coupling; GPCR; Dopamine D3 receptor binding; Dopamine D4 receptor binding; Partial agonist activity.
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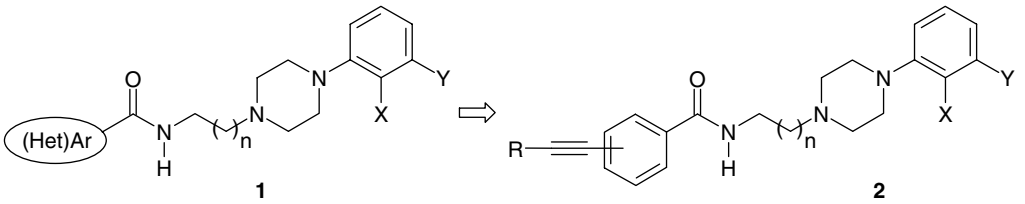
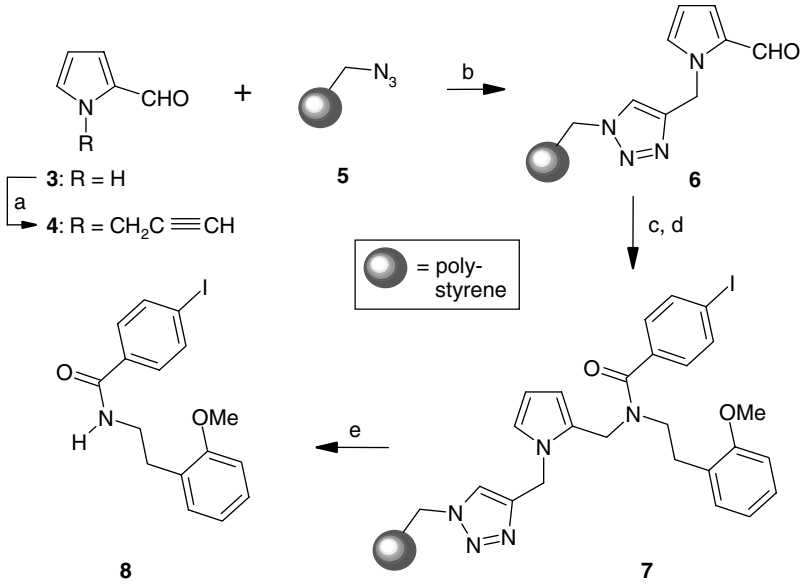


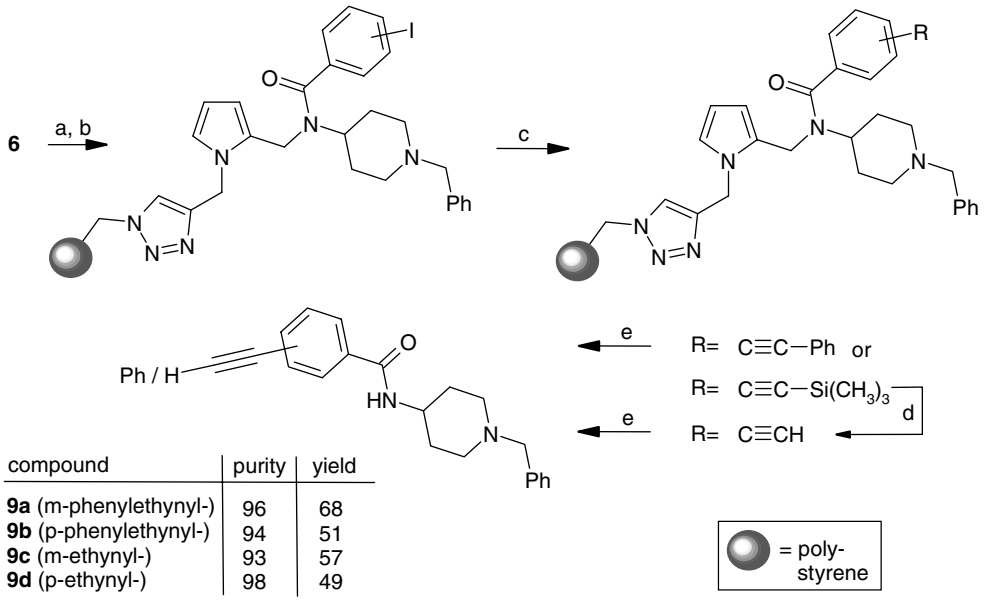
Chart 1.

extension of the more aromatic indole moiety (I_A = 146) by the less aromatic pyrrole (I_A = 85) should lead to an even greater electron-donating effect of the heterocyclic nitrogen.[20] To facilitate a wide application of click based resins in drug discovery, there is a need for robust and orthogonally stable linkers that are not only cleaved in high yields under mild conditions but are also easily accessible. Thus, our plan of synthesis started from pyrrole-2-carbaldehyde as a commercially available building block that could be readily functionalized by N-alkylation.[21] Thus, treatment of the heterocyclic building block **3** with propargyl bromide afforded the alkyne **4** which was reacted with azidomethyl polystyrene resin (**5**) in presence of copper iodide in THF/DIPEA (Scheme 1).[22–24] After a preliminary determination of the loading by elemental analysis (nitrogen content) indicated a linker density of 1.0 mmol/g and, thus, complete [3 + 2] azide-alkyne cycloaddition, we tried to investigate the loading and the reactivity of the new formylpyrrolylmethyltriazole (FPMT) resin **6** by initiating a reaction sequence of reductive amination, acylation and acidic cleavage. As a representative primary amine, we chose 2-methoxyphenylethylamine which was added to the functionalized resin **6** in dichoromethane. After treatment with $NaBH(OAc)_3$ at room temperature and washing, the amine functionalized resin was acylated with 4-iodobenzoyl chloride to give the immobilized target compound **7**. Finally, TFA induced hydrolysis (2%) resulted in formation of the arylcarboxamide **8**. Careful analysis by LC/MS indicated high yield (88%) and, even more important, exceptionally high purity (>99%).

To investigate the reliability and the scope of our solid phase supported method, we elaborated the synthesis of a model library. A further objective of this step was to identify and optimize suitable reaction conditions for an attachment of alkyne derived subunits which should play a key role in our structure–activity relationship studies. As a primary amine, we chose *N*-benzyl-4-aminopiperidine which could be reductively immobilized onto the click resin **6** in presence of $NaBH(OAc)_3$ (Scheme 2). The reaction was considered to be complete when an absorption band at 1650 cm^{-1} had disappeared. Amide bond formation with 4-iodobenzoic acid and its 3-substituted regioisomer was promoted by using HOAt/DIC when formation of resin bound carboxamide could be qualitatively ascertained by FTIR displaying the appearance of the diagnostic absorption band at 1620 cm^{-1}. For the introduction of an alkyne unit, palladium catalyzed cross-coupling reaction under Sonogashira conditions had to be elaborated. Employing phenylacetylene or trimethylsilylacetylene as an acetylene precursor, cross-coupling reaction could be observed by FTIR when the catalyst system $Pd(PPh_3)_2Cl_2$/Cu(I)I was used.[25] To accelerate the reac-



Scheme 1. Reagents and conditions: (a) propargyl bromide, K_2CO_3, DMF, rt, 16 h; (b) Cu(I)I, THF/DIPEA, 35 °C, 36 h; (c) (1) 2-(2-methoxyphenyl)ethylamine, dichloromethane, rt, 30 min; (2) $Na(OAc)_3BH$, rt, 24 h; (d) 4-iodobenzoyl chloride, Et_3N, dichloromethane, rt, 2 h; (d) TFA (2% in dichloromethane), rt, 2 h.

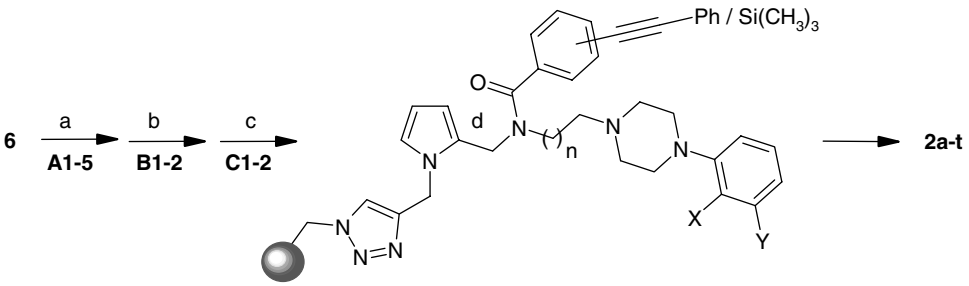


compound	purity	yield
9a (m-phenylethynyl-)	96	68
9b (p-phenylethynyl-)	94	51
9c (m-ethynyl-)	93	57
9d (p-ethynyl-)	98	49

Scheme 2. Reagents and conditions: (a) (1) 1-Benzyl-4-aminopiperidine, dichloromethane, rt, 30 min; (2) $Na(OAc)_3BH$, rt, 24 h; (b) 3-/4-iodobenzoic acid, HOAt, DIC, DMF, dichloromethane, rt, 28 h; (c) **C1**–**2**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, microwave, 120 °C, 15 min; (d) TBAF, THF, rt, 7 h; (e) TFA (2% in dichloromethane), rt, 2 h.

tion, microwave assistance turned out to be highly beneficial. Upon exposition to microwave irradiation at 120 °C, substantial conversion could be observed after 15 min.[26] In order to obtain the free acetylene functionality, desilylation was promoted by tetrabutylammonium fluoride in THF when a significant absorption at 3200 cm^{-1} in the IR spectra indicated a complete reaction. Finally, all the products were cleaved by using 2% TFA in dichloromethane. Purities of the crude products were assessed by LC/MS. Using the above-mentioned synthetic route, all the crude products were obtained with a high degree of purity (93–98%) as assessed by LC/MS. Any trace impurities present could be attributed to residual coupling agents. Thus, the strategy elaborated promised to be a robust methodology for the further development of a larger three-dimensional library based on the FPMT resin system.

As an extension of our recent investigations revealing that conjugated alkynes can substantially improve molecular recognition between GPCRs and receptor ligands,[27–29] a focused library of compounds described by the general formula **2** should be constructed. Using the solid phase supported methodology described above, five amines, two benzoic acids and two alkyne derivatives were chosen as attractive building blocks. Because phenylpiperazine moieties are known to effectively bind to biogenic amine receptors, especially the dopamine D3 subtype, the amines **A1**–**5** were selected for the reductive amination step. Both *para*- and *meta*-iodobenzoic acids (**B1**–**2**) were chosen for the second diversity point, in order to investigate the influence of the orientation of the acetylene substituent with respect to the amide. For the Sonogashira coupling, phenylacetylene and trimethylsilylacetylene were selected as commercially available building blocks when removal of the trimethylsilyl-protecting group was envisaged after the palladium promoted coupling step. The synthesis was initiated by reductive amination of the click resin **6** followed by acylation of the resin bound secondary amines when using HOAt/DIC as activating agents (Scheme 3). Sonogashira coupling was performed with the corresponding acetylenes **C1**–**2** in presence of $Pd(PPh_3)_2Cl_2$, Cu(I)I and irradiated via microwave for 15 min at 120 °C. The trimethylsilyl group was removed by using a 1 M solution of tetrabutylammonium fluoride in THF. Finally all the



Scheme 3. Reagents and conditions: (a) (1) **A1**–**5**, dichloromethane, rt, 30 min; (2) $Na(OAc)_3BH$, rt, 24 h; (b) **B1**–**2**, HOAt, DIC, DMF, dichloromethane, rt, 28 h; (c) **C1**–**2**, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, microwave, 120 °C, 15 min; (d) for **C1**: (1) TBAF, THF, rt, 7 h; (2) TFA (2% in dichloromethane), rt, 2 h; for **C2**: TFA (2% in dichloromethane), rt, 2 h.

products were released from the solid support by 2% TFA in dichloromethane. All reaction steps were randomly monitored by IR spectroscopy (see Chart 2).

LC/MS analyses of the crude products are displayed in Table 1. With exception of the derivative **2c** that displays a purity of 83%, purities >90% were obtained for

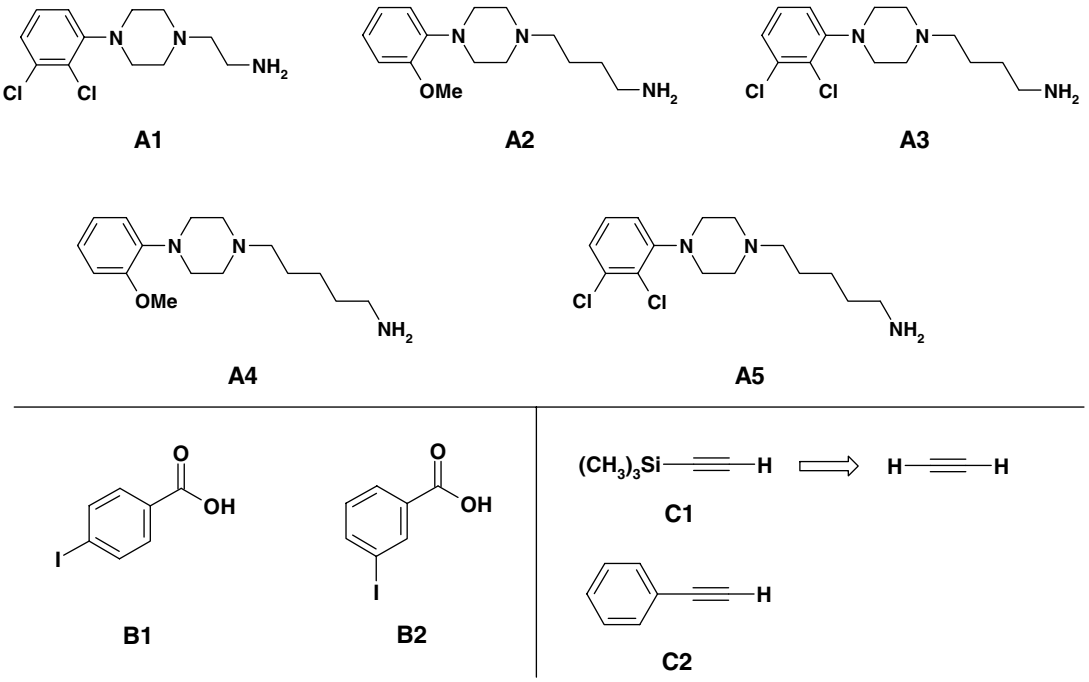
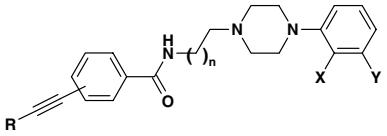


Chart 2. Building blocks.

Table 1. Building blocks and analytical data for the compound library **2a–t**



Compound	Code	Subst. pattern	R =	*n* =	X =	Y =	Purity[a] (%)	Yield[b] (%)	Rt[c] (min)
2a	A1B1C1	*para*	H	1	Cl	Cl	93	39	17.5
2b	A1B1C2	*para*	Ph	1	Cl	Cl	95	42	19.8
2c	A1B2C1	*meta*	H	1	Cl	Cl	83	32	18.9
2d	A1B2C2	*meta*	Ph	1	Cl	Cl	97	44	19.6
2e	A2B1C1	*para*	H	3	MeO	H	100	50	15.5
2f	A2B1C2	*para*	Ph	3	MeO	H	98	54	18.6
2g	A2B2C1	*meta*	H	3	MeO	H	95	63	15.6
2h	A2B2C2	*meta*	Ph	3	MeO	H	98	61	18.7
2i	A3B1C1	*para*	H	3	Cl	Cl	95	66	17.2
2j	A3B1C2	*para*	Ph	3	Cl	Cl	97	42	19.6
2k	A3B2C1	*meta*	H	3	Cl	Cl	93	63	17.4
2l	A3B2C2	*meta*	Ph	3	Cl	Cl	92	47	19.7
2m	A4B1C1	*para*	H	4	MeO	H	96	48	15.8
2n	A4B1C2	*para*	Ph	4	MeO	H	92	57	18.8
2o	A4B2C1	*meta*	H	4	MeO	H	92	58	15.8
2p	A4B2C2	*meta*	Ph	4	MeO	H	93	55	18.8
2q	A5B1C1	*para*	H	4	Cl	Cl	91	32	17.8
2r	A5B1C2	*para*	Ph	4	Cl	Cl	91	41	19.9
2s	A5B2C1	*meta*	H	4	Cl	Cl	92	32	17.9
2t	A5B2C2	*meta*	Ph	4	Cl	Cl	92	32	19.9

[a] Purities were determined by LC/MS analysis employing UV detection at 254 nm.
[b] Given values refer to crude yields.
[c] Exact HPLC conditions are given in Section 4.

all final products. The yields varied between 32% and 66%. The results demonstrate that the pyrrole resin provides an ideal support for preparing libraries of carboxamides. The novel BAL type linker **6** proved to be compatible with the reaction conditions that had to be applied facilitating a smooth and high yielding cleavage, as well. Taking also into account the convenient preparation, of **6**, the requirements of click chemistry have been clearly fulfilled.

2.2. Biological investigations

A biogenic amine receptor screening of the phenylacetylenes **2a**–**t** was performed without further purifications when their ability to bind to the cloned human $D2_{long}$, $D2_{short}$, D3 and D4 dopamine receptors, the porcine D1 dopamine and α1 adrenergic receptors, and the serotonin receptor subtypes 5-$HT1_A$, 5-HT2 was evaluated in vitro.[14,30] This was accomplished in a screening system by measuring the displacement of the following radioligands: [^{3}H]spiperone for D2, D3, D4, [^{3}H]SCH 23390 for D1 receptors, [^{3}H]prazosin for α1, [^{3}H]8-hydroxy-DPAT and [^{3}H]ketanserin for 5-$HT1_A$ and 5-HT2, respectively, and using 10 μM, 100 nM and 1 nM concentrations of the test compounds. The results in Table 2 reflect the displacement data at a final concentration of 100 nM of the test compound.

Starting from the privileged structure **1**, the extension of the benzamide π-system by introduction of an alkyne subunit led to substantial and specific GPCR recognition. The results of the screening indicate that the subfamily of dichlorophenylpiperazines derived from the building block **A3** exerted the highest relative affinities for the dopamine D3 receptor. High relative displacements at the D3 receptor were also observed for some

Table 2. Screening of receptor binding: relative displacement of the radioligand by the test compounds **2a**–**t** utilizing human $D2_{long}$, $D2_{short}$, D3 and D4.4 receptors as well as porcine D1, 5-$HT1_A$, 5-HT2 and α1 receptors[a]

		displacement of radioligand [%]							
Cmpd.	Code	D1	$D2_{long}$	$D2_{short}$	D3	D4	α1	5-$HT1_A$	5-HT2
2a	A1B1C1	4	21	31	64	81	64	14	0
2b	A1B1C2	4	1	7	61	9	30	15	0
2c	A1B2C1	10	21	41	63	94	57	76	13
2d	A1B2C2	3	5	7	28	8	9	32	0
2e	A2B1C1	6	3	7	90	35	63	52	8
2f	A2B1C2	1	4	14	66	15	14	3	9
2g	A2B2C1	6	12	15	78	69	66	86	1
2h	A2B2C2	2	6	31	83	46	55	29	0
2i	A3B1C1	11	39	51	97	34	43	33	33
2j	A3B1C2	16	19	27	100	29	5	30	0
2k	A3B2C1	9	40	53	97	56	45	49	15
2l	A3B2C2	0	8	6	91	18	9	41	5
2m	A4B1C1	10	27	43	70	53	71	40	14
2n	A4B1C2	7	3	10	45	35	36	0	14
2o	A4B2C1	10	18	23	35	35	69	17	19
2p	A4B2C2	7	29	34	46	45	88	1	4
2q	A5B1C1	3	35	57	80	30	40	12	32
2r	A5B1C2	0	2	11	47	11	8	6	12
2s	A5B2C1	4	12	31	80	26	33	2	19
2t	A5B2C2	0	2	0	22	5	7	28	8

displacement %	<30%	31-70%	71-79%	80-90%	91-100%
color code					

[a] Displacement of the radioligand at a final concentration of the test compound of 100 nM determined as triplicate in (%).

members of the methoxyphenylpiperazine **A2** (**A2B1C1**, **A2B2C2**) and the five-carbon spacer containing amine **A5** (**A5B1C1**, **A5B2C1**) derived subfamilies. In addition, high levels of displacements for other library members were observed for other biogenic amine receptor subtypes. For example, the test compounds **2a** (**A1B1C1**) and **2c** (**A1B2C1**) with a two-carbon spacer showed strong binding at the D4 receptor, while the meta-substituted alkynylcarboxamides **2p** (**A4B2C2**) and **2g** (**A2B2C1**) showed significant affinities for α1 and 5-HT1$_A$ receptors, respectively.

The equilibrium binding constants (K_i) were then determined for members for the subfamilies **A1**, **A2**, **A3**, and for those compounds showing a percentage displacement greater than 80%.[31] The affinity constant (K_i) values are shown in Table 3. Test compounds **2e** (**A2B1C1**), **2i** (**A3B1C1**), **2j** (**A3B1C2**), **2k** (**A3B2C1**) and **2q** (**A5B1C1**) display high affinity for the dopamine D3 receptor, especially the 4-ethynylcarboxamide **2i** displaying sub-nanomolar binding affinity (K_i = 0.31 nM). Thus, as has been noted in previous studies,[11] a 2,3-dichlorophenylpiperazine moiety with a four-carbon spacer proved to be the scaffold leading to the highest affinity for the D3 receptor. It is important to note that D3 affinity is superior for the terminal acetylenes when compared to the phenylacetylene derivatives. Furthermore, it was interesting to see that *para*-substituted phenylacetylene derivatives display higher binding affinities than the respective *meta*-regioisomers. Two obvious explanations can apply for this fact; the electronic density distribution due to the resonance effect between the carbonyl function and the triple bond in *para* position is different to the *meta*-isomers. Additionally, the geometry of the two π-systems is different, where probably the *meta*-derivatives could induce repulsive steric interactions with the GPCR binding sites. On the other hand in *para* position, even a sterically demanding phenylacetylene unit is tolerated well by the D3 receptor. Thus, the test compound **2j** (**A3B1C2**) displayed a K_i of 3.2 nM for D3 and exceptional subtype selectivity (>200). The piperazinylethyl substituted carboxamide **2c** (**A1B2C1**) turned out to exhibit high D4 selectivity (50-fold over D3, and ~100-fold over D2). To exclude that the superior D4 binding of **2c** was due to a chemical impurity, determination of the binding affinity was repeated after chromatographic purification revealing a similar K_i value of 0.45 nM.

As a measure of functional activity, ligand efficacy of the test compounds **2c, 2e, 2i** and **2k** displaying exceptional high binding (K_i values ⩽1 nM) was determined by a mitogenesis assay measuring the rate of [^{3}H]thymidine incorporation into growing CHO dhfr$^-$ cells and a CHO10001 cell line stably expressing the human D3 and D4.2 receptor, respectively.[15] The data listed in Table 4 clearly show substantial D3 ligand efficacy of the test compounds **2e**, **2i** and **2k** with EC$_{50}$ values in

Table 4. Intrinsic activities of selected test compounds and the reference quinpirole derived from the stimulating effect on mitogenesis of D3 and D4.2 receptor expressing cells[a]

Compound	Code	Receptor subtype	EC$_{50}$ value[b] (nM)	Intrinsic act.[c] (%)
2c	A1B2C1	D4	0.73	49
2e	A2B1C1	D3	2.1	57
2i	A3B1C1	D3	2.0	48
2k	A3B2C1	D3	3.5	57
Quinpirole		D3	5.2	100
		D4	10	100

[a] Determined with CHO dhfr$^-$ mutant and CHO10001 cells stably expressing the human D3 and D4.2 receptor, respectively.
[b] EC$_{50}$ values derived from a mean curve of four independent experiments.
[c] Rate of incorporation of [^{3}H]thymidine as evidence for mitogenesis activity relative to the maximal effect of the full agonist quinpirole (=100%) used as a reference.

Table 3. Receptor binding data of selected compounds at human D2$_{long}$, D2$_{short}$, D3 and D4.4 receptors as well as porcine D1, 5-HT1$_A$ and α1 receptors

Compound	Code	K_i values in (nM)[a]						
		[^{3}H]SCH 23390	[^{3}H]spiperone				[^{3}H]prazo-sin	[^{3}H]WAY 100635
		pD1	hD2$_{long}$	hD2$_{short}$	hD3	hD4	pα1	p5-HT1A
2a	A1B1C1	3500	69	48	31	8.0	8.4	n.d.
2b	A1B1C2	5500	4700	9900	29	22	48	n.d.
2c[b]	A1B2C1	1500	120	83	50	0.97 (0.45)	12	11
2d	A1B2C2	3100	1200	1400	68	12	74	n.d.
2e	A2B1C1	3000	250	160	1.0	110	6.5	17
2f	A2B1C2	16,000	1400	190	10	220	98	n.d.
2g	A2B2C1	1500	140	78	6.2	16	4.9	5.4
2h	A2B2C2	5900	1200	280	22	170	35	n.d.
2i	A3B1C1	910	15	27	0.31	120	16	99
2j	A3B1C2	4700	780	840	3.2	330	200	n.d.
2k	A3B2C1	730	15	24	0.83	40	15	35
2l	A3B2C2	1800	210	240	7.5	190	88	n.d.
2p	A4B2C2	590	31	27	37	35	1.5	n.d.
2q	A5B1C1	770	23	11	1.5	69	14	n.d.
2s	A5B2C1	860	51	23	5.6	100	30	n.d.

[a] K_i values in nM are based on the means of 2–4 experiments each done in triplicate. n.d. not determined.
[b] Binding affinity of compound **2c** was also determined after flash-chromatographic purification. Resulting K_i values are given in parentheses.

the low nanomolar range (48–57%, $EC_{50} = 2.0 - 3.5$ nM). The alkynylbenzamide **2c** proved to behave as a D4 partial agonist (49%, $EC_{50} = 0.73$ nM). Due to recent observations indicating that D3 and D4 partial agonists are of potential therapeutical interest for the treatment of cocain addiction and erectile dysfunction, respectively, our findings could be a starting point for further biomedical investigations.

3. Conclusion

In conclusion, utilizing the FPTM resin **6**, which is readily available via [3 + 2]azide-alkyne cycloaddition, a BAL strategy could be successfully applied for a parallel synthesis of dopaminergic phenylacetylens. A focused library of 20 test compounds revealing three points of diversity was generated by a four-step SPOS approach including microwave assisted Sonogashira coupling. GPCR-ligand binding assays indicated excellent dopamine D3 and D4 receptor binding affinities which were identified to cause a partial agonist activity for the most potent test compounds **2c**,**e**,**i**,**k**.

4. Experimental

4.1. General

Polystyrene resins were purchased from NovaBiochem. SPOS were performed manually in a Heidolph Synthesis I equipped with PFA vessels. Microwave assisted syntheses were conducted with a CEM Discover focussed microwave oven. 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) spectra were determined on a BRUCKER AM 360 or a BRUCKER AVANCE spectrometer in solution. LC/MS analyses were conducted in an Agilent Binary Gradient System (MeOH/0.1 N aq HCOOH, 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 Brucker 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 vapour.

4.2. 1-Propargylpyrrole-2-carbaldehyde (4)

Pyrrole-2-carbaldehyde (8.0 g, 84 mmol), propargyl bromide (12.0 g, 0.10 mol) and K_2CO_3 (5.0 g, 36 mmol) in DMF (50 mL) were stirred at room temperature for 16 h. The mixture was treated with a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The organic layer was washed with brine, dried with Na_2SO_4 and concentrated under vacuum to give **4** (6.0 g, 71%) as a yellow solid. Analytical data are identical to those reported in Ref. 21.

4.3. FPMT resin (6)

Merrifield resin (3.0 g, 1.1 mmol/g) was reacted with NaN_3 (0.60 g, 9.2 mmol), in DMSO (35 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** was shaken together with 1-propargylpyrrole-2-carbaldehyde **4** (1.0 g, 7.5 mmol) and CuI (20 mg, 0.11 mmol) in a mixture of THF/DIPEA 2:1 (35 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 drying of the resin under vacuum afforded the resin **6** (IR: 1650 cm^{-1} for the C=O group).

4.4. Determination of resin capacity

Resin **6** (100 mg) was distributed in a Teflon vessel and reacted with a solution of 2-methoxyphenylethylamine (83 mg, 0.55 mmol) in CH_2Cl_2 (10 mL) for 30 min. After addition of $NaBH(OAc)_3$ (117 mg, 0.55 mmol) the resin was agitated at room temperature for 24 h. The resin was thoroughly washed with MeOH (3× 25 mL), CH_2Cl_2 (3× 25 mL), Et_2O (2× 25 mL) and dried by suction. IR analysis revealed the disappearance of the band at 1650 cm^{-1} indicating a complete reaction. Acylation was accomplished by the addition of 4-iodobenzoyl chloride (147 mg, 0.55 mmol) and Et_3N (57 mg, 0.55 mmol) in CH_2Cl_2 (5 mL) and agitation for 18 h at room temperature. After a sequentially filtering and washing procedure with MeOH (3× 25 mL), CH_2Cl_2 (3× 25 mL) and Et_2O (3× 25 mL) the IR spectra showed an absorption band at 1617 cm^{-1} for the C=O of the carboxamide. Subsequently, the resin was treated with 2% TFA in CH_2Cl_2 (10 mL) for 2 h. After collection of the cleavage solution the resin was rinsed with CH_2Cl_2 and the combined filtrates were washed with an aq solution of $NaHCO_3$, dried with Na_2SO_4 and evaporated under reduced pressure. The residue was dried under high vacuum overnight to afford 33.5 mg (88%) of crude product **8** which was analyzed by LC/MS system with UV detection (254 nm) indicating a purity of >99%.

4.5. Model library

4.5.1. Reductive amination. Resin **6** (4× 100 mg) was distributed into six vessels and a solution of 1-benzyl-4-aminopiperidine (4× 105 mg, 4× 0.55 mmol) in CH_2Cl_2 (6 mL) was added. Agitation for 30 min at room temperature was followed by addition of $NaBH(OAc)_3$ (4× 233 mg, 4× 1.1 mmol). The mixture was agitated at room temperature for 24 h. After filtration of the

solvent, the resins were thoroughly washed with MeOH, CH_2Cl_2 and Et_2O and dried by suction.

4.5.2. Acylation. The resulting six batches of immobilized amines were acylated using 3-iodobenzoic acid (2× 135 mg, 2× 0.55 mmol) or 4-iodobenzoic acid (2× 135 mg, 2× 0.55 mmol), HOAt (4× 75 mg, 4× 0.55 mmol), DIC (4× 69 mg, 4× 0.55 mmol) dissolved in a mixture of DMF/CH_2Cl_2 (2:3, 5 mL). Reaction mixtures were agitated for 28 h at room temperature, washed with DMF, MeOH, CH_2Cl_2, Et_2O and dried by suction. FTIR spectra showed signal at 1650 cm^{-1} for the C═O of the carboxamide group.

4.5.3. Pd-coupling. The resins were transferred to four microwave tubes and $Pd(PPh_3)_2Cl_2$ (4× 3.9 mg, 4× 5.5 μmol), CuI (4× 2.1 mg, 4× 11 μmol), Et_3N (4× 2 mL), DMF (4× 0.5 mL) and the corresponding alkyne **C1**–**C2** (4× 0.22 mmol) were added. The tubes were capped, heated in the microwave cavity for 15 min at 120 °C, transferred to six vessels and sequentially filtered and washed with DMF, THF, CH_2Cl_2 and Et_2O.

4.5.4. Deprotection. The three **C1**-derived silylalkynes were deprotected by addition of tetrabutylammonium fluoride (1.0 M in THF, 2× 2.2 mL) followed by agitation at room temperature for 7 h. The resins were washed with THF, MeOH, CH_2Cl_2 and Et_2O and dried under reduced pressure.

4.5.5. Cleavage. Products were cleaved from the resins by agitation with 2% TFA in CH_2Cl_2 (10 mL) at room temperature for 2 h. Each resin was filtered and rinsed with CH_2Cl_2, the combined filtrates were collected and washed with a saturated solution of $NaHCO_3$. The organic layer was separated, dried with Na_2SO_4 and the solvent was evaporated under reduced pressure. The resulting residues were dried under high vacuum overnight to afford the crude products which were analyzed by LC/MS for the determination of the purity. For representative analytical data, see below.

4.5.6. *N*-(1-Benzylpiperidin-4-yl)-4-phenylethynylbenzamide 9b. ^1H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.81–1.96 (m, 2H), 2.07–2.18 (m, 2H), 2.43–2.56 (m, 2H), 3.15–3.27 (m, 2H), 3.85 (s, 2H), 4.07–4.20 (m, 1H), 6.34 (d, J = 7.9 Hz, 1H), 7.35–7.45 (m, 8H), 7.55–7.63 (m, 4H), 7.77 (d, J = 8.2 Hz, 2H). APCI-MS (*m*/*z*): 395.2 $(M+1)^+$.

4.5.7. *N*-(1-Benzylpiperidin-4-yl)-3-ethynylbenzamide 9c. ^1H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 1.86–1.98 (m, 2H), 2.05–2.17 (m, 2H), 2.48–2.60 (m, 2H), 3.14 (s, 1H), 3.20–3.32 (m, 2H), 3.90 (s, 2H), 4.08–4.19 (m, 1H), 6.55 (br s, 1H), 7.30–7.46 (m, 6H), 7.61 (d, J = 7.2 Hz, 1H), 7.74–7.81 (d, J = 7.2 Hz, 1H), 7.89 (s, 1H). APCI-MS (*m*/*z*): 319.2 $(M+1)^+$.

4.6. 3D library: synthesis of ethynylphenyl carboxamides 2a–t

Resin **6** (20× 100 mg, 20× 0.1 mmol) was distributed into 20 Teflon vessels followed by a solution of the corresponding primary amines 4 × **A1**, 4 × **A2**, 4 × **A3**, 4 × **A4**, 4 × **A5** (each 0.5 mmol) in CH_2Cl_2 (5 mL). After agitation for 30 min at room temperature $NaBH(OAc)_3$ (0.233 g, 1.0 mmol) was added and shaking was continued for 24 h. The resins were extensively washed with MeOH, CH_2Cl_2, Et_2O and the corresponding acids 10 × **B1** and 10 × **B2** (each 0.5 mmol), HOAt (20× 74 mg, 20× 0.5 mmol) and DIC (20× 63 mg, 0.5 mmol) in a mixture of DMF/CH_2Cl_2 (2:3, 5 mL) were added. The resins were agitated at room temperature for 28 h, washed with DMF, MeOH, CH_2Cl_2 and Et_2O. The resins were transferred into 10 mL microwave glass tubes (pyrex) and the corresponding acetylenes 10 × **C1** or 10 × **C2** (each 0.2 mol) were added together with $Pd(PPh_3)_2Cl_2$ (20× 3.5 mg, 20× 0.005 mmol), CuI (20× 1.9 mg, 20× 0.011 mmol), DMF (0.5 mL) and Et_3N (2 mL). The tubes were sealed and heated under microwave irradiation at 120 °C for 15 min. After being cooled to ambient temperature, the reaction mixtures were filtered and washed with DMF, THF, MeOH, CH_2Cl_2 and Et_2O. In case of the 10 (trimethylsilyl)acetylenes, deprotection was accomplished by addition of tetrabutylammonium fluoride (1.0 M in THF, 10× 0.2 mmol) in THF (2 mL) and agitation at room temperature for 7 h. The resins were washed with THF, MeOH, CH_2Cl_2 and Et_2O. Finally, all products were cleaved from the solid support by reaction with 2% TFA in CH_2Cl_2 (10 mL) at room temperature for 2 h. Each resin was filtered and rinsed with CH_2Cl_2, the combined filtrates were collected and washed with $NaHCO_3$ (saturated aqueous solution), the organic phase was separated, dried with Na_2SO_4 and the solvent evaporated under vacuum. The residues were dried under vacuum overnight 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.

4.6.1. *N*-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-4-ethynylbenzamide (2e, A2B1C1). ^1H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.72–1.83 (m, 4H), 2.63–2.73 (m, 2H), 2.81–2.93 (m, 4H), 3.10–3.19 (m, 4H), 3.21 (s, 1H), 3.48–3.57 (m, 2H), 3.88 (s, 3H), 6.88–6.99 (m, 3H), 7.02–7.08 (m, 1H), 7.57 (d, J = 8.4 Hz, 2H), 7.79 (d, J = 8.4 Hz, 2H). APCI-MS (*m*/*z*): 392.3 $(M+1)^+$.

4.6.2. *N*-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-4-phenylethynylbenzamide (2f, A2B1C2). ^1H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.73–1.85 (m, 4H), 2.65–2.73 (m, 2H), 2.82–2.95 (m, 4H), 3.14–3.25 (m, 4H), 3.50–3.59 (m, 2H), 3.89 (s, 3H), 6.89 (d, J = 8.1 Hz, 1H), 6.93–6.97 (m, 2H), 6.98–7.08 (m, 3H), 7.37–7.42 (m, 3H), 7.55–7.59 (m, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.83 (d, J = 8.2 Hz, 2H). APCI-MS (*m*/*z*): 468.3 $(M+1)^+$.

4.6.3. *N*-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-3-ethynylbenzamide (2g, A2B2C1). ^1H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.70–1.80 (m, 4H), 2.61–2.69 (m, 2H), 2.78–2.89 (m, 4H), 3.11 (s, 1H), 3.13–3.21 (m, 4H), 3.49–3.56 (m, 2H), 3.85 (s, 3H), 6.89 (d,

J = 7.2Hz, 1H), 6.93–6.97 (m, 2H), 7.0–7.07 (m, 1H), 7.41 (dd, J = 7.9, 7.5 Hz, 1H), 7.60–7.64 (m, 1H), 7.80–7.84 (m, 1H), 7.91 (t, J = 1.4 Hz, 1H). APCI-MS (m/z): 392.3 $(M+1)^+$.

4.6.4. *N*-{5-[4-(2-Methoxyphenyl)piperazin-1-yl]pentyl}-3-phenylethynylbenzamide (2h, A2B2C2). ^{1}H NMR: ($CDCl_3$, 360 MHz) δ (ppm) = 1.43–1.53 (m, 2H), 1.59–1.76 (m, 4H), 2.48 (t, J = 7.5 Hz, 2H), 2.65–2.77 (m, 4H), 3.08–3.22 (m, 4H), 3.51 (dd, J = 12.9, 6.8 Hz, 2H), 3.89 (s, 3H), 6.17 (br s, 1H), 6.88 (d, J = 8.6 Hz, 1H), 6.92–7.05 (m, 3H), 7.36–7.42 (m, 3H), 7.45 (dd, J = 7.9, 7.5 Hz, 1H), 7.54–7.59 (m, 2H), 7.65–7.69 (m, 1H), 7.74–7.81 (m, 1H), 7.93 (t, J = 1.4 Hz, 1H). APCI-MS (m/z): 482.3 $(M+1)^+$.

4.6.5. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}-4-ethynylbenzamide (2i, A3B1C1). ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) 0 1.70–1.84 (m, 4H), 2.69–2.78 (m, 2H), 2.81–2.99 (m, 4H), 3.10–3.19 (m, 4H), 3.21 (s, 1H), 3.47–3.56 (m, 2H), 6.92–7.0 (m, 1H), 7.15–7.24 (m, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.78 (d, J = 8.4 Hz, 2H). APCI-MS (m/z): 430.5 $(M+1)^+$.

4.6.6. *N*-{4-[4-(2,3-Dichlorophenyl)piperazin-1-yl]butyl}-3-ethynylbenzamide (2k, A3B2C1). ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 1.67–1.78 (m, 4H), 2.53 (dd, J = 7.2, 6 Hz, 2H), 2.63–2.78 (m, 4H), 2.99–3.09 (m, 4H), 3.14 (s, 1H), 3.47–3.55 (m, 2H), 6.91 (d, J = 6.6 Hz, 1H), 7.13–7.21 (m, 2H), 7.41 (dd, J = 7.8, 7.2 Hz, 1H), 7.61 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.87 (s, 1H). APCI-MS (m/z): 430.4 $(M+1)^+$.

4.6.7. *N*-{5-[4-(2,3-Dichlorophenyl)piperazin-1-yl]pentyl}-4-ethynylbenzamide (2q, A5B1C1). ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 1.44–1.51 (m, 2H), 1.63–1.73 (m, 4H), 2.50–2.61 (m, 2H), 2.67–2.92 (m, 4H), 3.09–3.18 (m, 4H), 3.21 (s, 1H), 3.5 (dd, J = 13.2, 6.6 Hz, 2H), 6.97 (dd, J = 7.8, 1.8 Hz, 1H), 7.15–7.21 (m, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H). APCI-MS (m/z): 444.5 $(M+1)^+$.

4.6.8. *N*-{5-[4-(2,3-Dichlorophenyl)piperazin-1-yl]pentyl}-3-ethynylbenzamide (2s, A5B2C1). ^{1}H NMR: ($CDCl_3$, 600 MHz) δ (ppm) = 1.44–1.52 (m, 2H), 1.62–1.73 (m, 4H), 2.49–2.62 (m, 2H), 2.65–2.90 (m, 4H), 3.08–3.20 (m, 5H), 3.50 (dd, J = 13.2, 6.6 Hz, 2H), 6.23 (br s, 1H), 6.98 (dd, J = 7.2, 1.8 Hz, 1H), 7.15–7.21 (m, 2H), 7.43 (t, J = 7.8 Hz, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.8 (d, J = 7.8 Hz, 1 H), 7.88–7.90 (s, 1H). APCI-MS (m/z): 444.5 $(M+1)^+$.

4.7. Binding studies

Receptor binding studies were carried out as described in the literature.[29] 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 0.1 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 $D2_{short}$, 0.08–0.25 nM for D3 and 0.22–0.28 nM for D4.4.

The investigation of serotonin 5-$HT1_A$ and 5-HT2 as well as adrenergic α_1 binding was performed as described in the literature.[15] In brief, porcine cortical membranes were subjected to the binding assay at a concentration of 80 μg/assay tube for determination of 5-$HT1_A$ and 5-HT2 binding utilizing [^{3}H]WAY100635 and [^{3}H]ketanserin each at a final concentration of 0.1 and 0.5 nM with K_D values of 0.03–0.04 nM (for 5-$HT1_A$) and 1.5–2.1 nM (for 5-HT2). Cortical membranes at 55 μg/assay tube and the radioligand [^{3}H]prazosin at a final concentration of 0.1 nM were applied to determine adrenergic α1 binding with K_D values 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 (5-$HT1_A$ screening was done with the radioligand [^{3}H]8-OH-DPAT at a concentration of 0.5 nM).

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.[31]

4.8. Functional experiments

Determination of the stimulating effect of the test compounds on mitogenesis as a functional assay was done with a CHO dhfr$^-$ cell line stably transfected with the human dopamine D3 receptor and with a CHO 10001 cell line stably expressing the human dopamine D4.2 receptor according to literature.[15] The concentration of the radiotracer was 0.02 μCi/well of [^{3}H]thymidine (specific activity 25 Ci/mmol, Amersham Biosciences) and the incubation was 4 or 2 h for D3 and D4 expressing cells, respectively.

Experimental data resulting from the mitogenesis assay were each normalized and then combined to get a mean curve. Nonlinear regression analysis of this curve provided the EC_{50} value as a measure of potency. The top value of the curve represented the maximal intrinsic activity which was correlated to the effect of the full agonist quinpirole (100%).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.038.

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