Bioorganic & Medicinal Chemistry Letters 19 (2009) 4011–4013

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Dopamine D_3 receptor antagonists: The quest for a potentially selective PET ligand. Part two: Lead optimization

Fabrizio Micheli [a,*], Ian Holmes [b,*], Luca Arista [c], Giorgio Bonanomi [a], Simone Braggio [a], Francesca Cardullo [a], Romano Di Fabio [a], Daniele Donati [d], Gabriella Gentile [a], Dieter Hamprecht [e], Silvia Terreni [a], Christian Heidbreder [f], Chiara Savoia [a], Cristiana Griffante [a], Angela Worby [g]

[a] GlaxoSmithKline, Neurosciences Centre of Excellence for Drug Discovery, Medicine Research Centre, Verona, Italy
[b] Cancer Therapeutics, Melbourne, Australia
[c] Novartis Institute Research, Basel, CH, Switzerland
[d] Nerviano Medical Science, Nerviano, MI, Italy
[e] Boehringer Ingelheim, MI, Italy
[f] Altria Client Services, Richmond, VA, USA
[g] GlaxoSmithKline, Molecular Discovery Research, New Frontiers Science Park, Harlow, UK

ARTICLE INFO

Article history:
Received 19 May 2009
Revised 5 June 2009
Accepted 8 June 2009
Available online 13 June 2009

Keywords:
Dopamine D_3
Antagonist
PET
Ligand

ABSTRACT

The lead optimization process to identify new selective dopamine D_3 receptor antagonists is reported. DMPK parameters and binding data suggest that selective D_3 receptor antagonists as potential PET ligands might have been identified.

Dopamine receptors are key elements in neuronal functions. They are subdivided into two classes, the first one containing D_1 and D_5 receptors, and the second one consisting of the D_2, D_3 and D_4 receptors.[1]

Following the isolation and characterization of the cDNA for the dopamine DA D_3 receptor,[2] a number of DA D_3 receptor antagonists both selective and non selective have been recently reported.[3] Growing evidence suggests that selective antagonists at the DA D_3 receptor can reduce the reinforcing efficacy of drugs of abuse, significantly improve drug-induced learning deficits without altering the normal learning process in non-impaired rats, and show efficacy in animal models of schizophrenia.[4]

Following the identification of a class of low molecular weight selective dopamine DA D_3 receptor antagonists with potential as PET ligands,[5] a lead optimization program was initiated to identify the ideal PET candidate molecule.

The screening cascade of the lead identification phase was revised accordingly. Each new chemical entity (NCE) was assessed for its agonistic versus antagonistic properties using a functional GTPγS assay expressing the human DA D_3 receptor:[6] all the compounds reported in this manuscript proved to be antagonists at the D_3 receptor.

As for some previously reported lead optimization programmes for DA D_3 selective antagonists,[6–8] it was considered important to obtain 100-fold selectivity versus DA D_2 (functional assays) and a 100-fold selectivity versus the hERG ion channel (Dofetilide binding assay).[6]

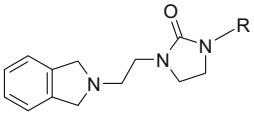
In addition, brain penetration, free fraction in tissue, and plasma protein binding were measured for selected derivatives.

Starting from the previously identified iso-indoline (**1**, Table 1),[5] which had a 40-fold selectivity over the DA D_2 receptor, it was decided to keep the iso-indoline fixed and to explore the SAR of the aromatic ring. The replacement of the Chlorine with a trifluoro methyl group (**2**) led to similar potency at the D_3 receptor, but to a slight decrease in selectivity. The introduction of a bulky iso-propyl substituent (**3**) produced a detrimental effect on both potency and selectivity. The bis-dichloro derivative (**4**), increased potency, but had almost no selectivity, whereas the introduction of a different 3,4 pattern of substitution (**5**) led to a decrease in desired activity, but produced a 50-fold increase in selectivity versus the DA D_2 receptor. The completion of the exploration (**6–13**) of simple substituents on the phenyl ring showed that, in this series, only the 3-CN group (**10**) provided results comparable to the original starting

* Corresponding authors. Tel.: +39 045 8218515; fax: +39 045 8218196 (F.M.).
E-mail address: fm20244@gsk.com (F. Micheli).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.06.028

Table 1
hERG and affinity results



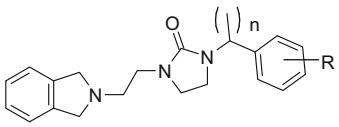
Entry	R	D_3 fpKi	D_2 fpKi	hERG pIC_{50}
1	3-Cl phenyl	8.6	7.0	5.5
2	3-CF3 phenyl	8.6	7.3	6.1
3	3-*i*-Pr phenyl	8.2	7.1	5.3
4	3,5-diCl phenyl	8.9	8.2	5.8
5	3-CN, 4-F phenyl	7.8	6.1	5.0
6	3,4-diCl phenyl	7.9	6.6	6.0
7	3-OCF3 phenyl	8.8	7.4	6.2
8	3-Cl, 4-F phenyl	7.7	6.8	5.3
9	3-O-*i*-Pr phenyl	7.4	6.5	5.4
10	3-CN phenyl	8.6	7.0	5.5
11	3-Cl, 5-F phenyl	8.8	7.5	5.5
12	3-Cl, 6-F phenyl	8.2	6.8	4.7
13	4-*t*-Bu phenyl	7.0	6.2	6.0
14	1-Benzothien-5-yl	7.2	6.6	5.7
15	2-Methyl-1,3-benzothiazol-5-yl	6.6	<5.0	5.4
16	5-Methyl-2-thienyl	6.7	6.3	4.5
17	1-Benzothien-3-yl	6.9	5.9	5.1
18	1-Benzofuran-5-yl	6.9	6.0	5.1
19	1-Acetyl-1H-indol-5-yl	6.3	<5.0	5.0
20	6-Methyl-3-pyridinyl	7.2	<5.0	4.5
21	4-(2-Pyridinyl)-3-thienyl	6.7	<5.0	nt

SEM for D_3 GTPγS, and HERG data sets is ± 0.1. SEM for the D_2 GTPγS data is ± 0.2. nt = Not tested.

point (**1**), with no improvement in affinity at the DA D_3 receptor. Derivative **10** showed a slightly reduced Cli both in human and rat (1.6 and 23.5 ml/min/g, respectively) compared to derivative **1** (8.9 and 36.9 ml/min/g, respectively) and also had a P450 profile with IC_{50} potencies greater than 5 μM on all tested isoforms. Accordingly, it could have offered a suitable pharmacokinetic (PK) profile for further testing. However, it failed to satisfy all the requirements of the initial screening cascade and was therefore not progressed further. A strategy to replace the substituted phenyl ring with different heterocycles was, therefore, implemented (**14–21**, Table 1). No major improvement of selectivity was observed for this sub-series, apart from derivative **20** which proved to be active at the D_3 receptor only. However, its low affinity at the D_3 receptor did not permit further progression.

A second strategy, still keeping the unsubstituted iso-indoline fixed, moved the aromatic ring one atom away from the imidazolinone ring (**22–29**, Table 2).

Table 2
hERG and affinity results



Entry	R	*n*	D_3 fpKi	D_2 fpKi	hERG pIC_{50}
22	H	0	6.2	<5.0	4.6
23	3-Cl	0	6.9	6.3	5.6
24	3-CF_3	0	7.9	6.6	5.2
25	3,4-diCl	0	7.1	<5.0	6.1
26	2,5-diCl	0	6.8	6.1	6.4
27	4-Cl	0	6.8	<5.0	5.7
28	2-Cl	0	6.3	6.4	5.7
29	3-CF_3	1	6.3	<5.0	5.9

SEM for D_3 GTPγS, and HERG data sets is ± 0.1. SEM for the D_2 GTPγS data is ± 0.2.

Unfortunately, none of the prepared derivatives fulfilled the criteria of the screening cascade and the series was abandoned.

At this point of the exploration, the decision to abandon the iso-indoline scaffold was taken and two different templates were chosen as potential alternatives: substituted piperidine (Table 3) and substituted piperazines (Table 4).

Interestingly, while alkyl substituted piperidines (**30–32**) had no major impact on selectivity, the 4-trifluoromethyl derivative **33** showed promising properties in this series (e.g., 30-fold selectivity over the DA D_2 receptor).

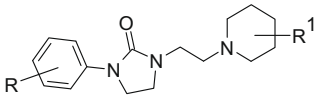
The corresponding 3-Cl derivative (**35**) was then prepared. This compound showed a 60-fold selectivity over the DA D_2 receptor. This derivative also showed very high Cli both on human and rat liver microsomes (17.6 and 25.8 ml/min/g, respectively) and also had a P450 profile with IC_{50} potencies greater than 2 μM on all tested isoforms.

Accordingly, the 3-CN derivative (**36**) was prepared, showing for the first time a 100-fold selectivity over the DA D_2 receptor and a more than 1000-fold selectivity over the hERG channel.

The more hydrophilic cyano derivative had a better Cli profile (4.9 and 11.5 ml/min/g with human and rat microsomes, respectively) and a comparable P450 profile. Further exploration within this class (**37–40**) also led to the identification of a compound (**38**) endowed with a better selectivity (126-fold), similar DMPK properties (Cli = 9.1 and 10.4 ml/min/g on h- and r-microsomes, respectively), but with a lower affinity at the DA D_3 receptor.

Promising results were also obtained with the piperazine scaffold, where both the 3-CN (**41**, Table 4) and 3-trifluoro methoxy

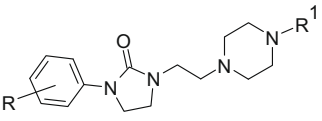
Table 3
hERG and affinity results



Entry	R	R^1	D_3 fpKi	D_2 fpKi	hERG pIC_{50}
30	3-Cl	3-Me	8.2	7.7	5.5
31	3-Cl	2-Me	8.1	7.6	5.7
32	3-Cl	4-Me	8.6	7.8	5.8
33	3-OH	4-CF_3	8.9	7.4	4.8
34	3-I	4-CF_3	9.0	7.9	6.7
35	3-Cl	4-CF_3	8.8	7.0	5.9
36	3-CN	4-CF_3	9.0	7.0	5.1
37	3-OCF_3	4-CF_3	8.3	6.8	5.4
38	3-CN, 4-F	4-CF_3	8.1	6.0	5.2
39	3-OMe	4-CF_3	8.0	6.8	4.6
40	3-Cl, 2-F	4-CF_3	7.4	<5.0	4.9

SEM for D_3 GTPγS, and HERG data sets is ± 0.1. SEM for the D_2 GTPγS data is ± 0.2.

Table 4
hERG and affinity results



Entry	R	R^1	D_3 fpKi	D_2 fpKi	hERG pIC_{50}
41	3-CN	*i*-Pr	8.4	6.5	4.9
42	3-OCF_3	*i*-Pr	8.5	6.6	5.8
43	3-CN, 4-F	*i*-Pr	6.9	<5.0	4.8
44	3-Cl, 2-F	*i*-Pr	6.8	<5.0	4.7
45	3-OMe	*i*-Pr	6.8	5.6	4.2
46	3-Cl	*i*-Pr	8.1	6.7	5.2
47	3-Cl	Me	6.8	6.3	5.4

SEM for D_3 GTPγS, and HERG data sets is ± 0.1. SEM for the D_2 GTPγS data is ± 0.2.

(**42**) derivatives showed good affinity at the DA D_3 receptor and good (80-fold) selectivity over the DA D_2 receptor. These piperazine derivatives also showed very promising DMPK profiles as reported below.

Derivative **41** showed the lowest Cli values ever observed in the imidazolinone scaffold (<0.5 and 0.9 ml/min/kg on h-and r-microsomes, respectively) and similar results were achieved by derivative **42** (<0.5 and 3.7 ml/min/kg on h-and r-microsomes, respectively). Both derivatives showed P450 values with IC_{50}s greater than 7 μM on all tested isoforms.

In the light of these results, the piperazine class was selected for further optimization as a potential PET ligand. Derivative **36** was selected for in vivo testing[9] in the rat. Its DMPK profile was in agreement with the desired intravenous (i.v.) profile, showing a high brain penetration (brain to blood ratio = 6.4) with high levels in the brain (258 ng/g) after a single administration at 1 mg/kg, despite a very high blood clearance (78 mL/min/kg equivalent to 92% of the liver blood flow). The distribution volume was moderate (Vd = 5.4 l/kg) with an acceptable half-life ($T_{1/2}$ = 1.7 h).

Moreover, the brain tissue binding of **36** was rather low, leading to a brain fraction unbound = 15.1%. The levels of protein binding were low in rat and pig plasma too (72% and 61%, respectively).

To fully complete the characterization of this derivative, filtration binding affinities at the human DA D_3 and D_2 receptors were performed. Filtration binding assay were also used to determine the affinity of the compound in rat and pig native tissues; the results are reported in Table 5.

These data further highlighted the potentiality of derivative **36** in terms of affinity as a potential PET ligand both in human and in preclinical species.

Data in Table 6 further strengthen its value.

Accordingly, this compound was further characterized as a potential PET ligand and this will be reported in future communications.

Table 5
Affinity results and SEM for D_3 and D_2 filtration binding

	h DA D_3	h DA D_2	Rat D_3 native tissue	Pig D_3 native tissue
pK_i	9.1 ± 0.2	6.8 ± 0.2	9.1 ± 0.1	9.1 ± 0.01

Table 6
Summary of DMPKdata for compound **36**

Entry	Brain/blood ratio	Vd (l/kg)	Clb (ml/min/kg)	$T_{1/2}$ (h)
36	6.4	5.4	78	1.7

Acknowledgments

We would like to thank Adolfo Prandi for the preparation of some products and intermediates. We would like to acknowledge Dr. Giovanna Tedesco and Dr. Anna Maria Capelli (GSK MDR, Verona) for their computational support. We would also like to thank Dr. Carla Marchioro's group for the structural and analytical support received.

References and notes

1. Schultz, W. *Ann. Rev. Neurosci.* **2007**, *30*, 259.
2. Sokoloff, P.; Giros, B.; Martres, M. P.; Bouthenet, M. L.; Schwartz, J. C. *Nature* **1990**, *347*, 146.
3. (a) Micheli, F.; Heidbreder, C. A. *Exp. Opin. Ther. Patent* **2008**, *18*, 821; (b) Micheli, F.; Heidbreder, C. *Recent Patents CNS Drug Disc.* **2006**, *1*, 271; (c) Zhang, A.; Kan, Y.; Li, F. *Exp. Opin. Ther. Patent* **2006**, *16*, 587.
4. Heidbreder, C. A.; Gardner, E. L.; Xi, Z.-X.; Thanos, P. K.; Mugnaini, M.; Hagan, J. J., ; Ashby, C. R., Jr. *Brain Res. Rev.* **2005**, *49*, 77.
5. Holmes, I.P.; Micheli, F.; Gaines, S.; Lorthioir, O.; Watson, S. P.; Di Fabio, R.; Gentile, G.; Heidbreder, C.; Savoia, C.; Worby, A. *Bioorg. Med. Chem. Lett.* **2009**, doi:org/10.1016/j.bmcl.2009.06.043.
6. Micheli, F.; Bonanomi, G.; Blaney, F. E.; Braggio, S.; Capelli, A. M.; Checchia, A.; Curcuruto, O.; Damiani, F.; Di-Fabio, R.; Donati, D.; Gentile, G.; Gribble, A.; Hamprecht, D.; Tedesco, G.; Terreni, S.; Tarsi, L.; Lightfoot, A.; Pecoraro, M.; Petrone, M.; Perini, O.; Piner, J.; Rossi, T.; Worby, A.; Pilla, M.; Valerio, E.; Griffante, C.; Mugnaini, M.; Wood, M.; Scott, C.; Andreoli, M.; Lacroix, L.; Schwarz, A.; Gozzi, A.; Bifone, A.; Ashby, C. R., Jr.; Hagan, J. J.; Heidbreder, C. *J. Med. Chem.* **2007**, *50*, 5076.
7. Micheli, F.; Bonanomi, G.; Braggio, S.; Capelli, A. M.; Celestini, P.; Damiani, F.; Di Fabio, R.; Donati, D.; Gagliardi, S.; Gentile, G.; Hamprecht, D.; Petrone, M.; Radaelli, S.; Tedesco, G.; Terreni, S.; Worby, A.; Heidbreder, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 901.
8. Micheli, F.; Bonanomi, G.; Braggio, S.; Capelli, A. M.; Damiani, F.; Di Fabio, R.; Donati, D.; Gentile, G.; Hamprecht, D.; Perini, O.; Petrone, M.; Tedesco, G.; Terreni, S.; Worby, A.; Heidbreder, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 907.
9. The research complied with national legislation and with company policy on the Care and Use of Animals and with related codes of practice.