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The identification of a selective dopamine D_2 partial agonist, D_3 antagonist displaying high levels of brain exposure

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ABSTRACT

The identification of a highly selective D_2 partial agonist, D_3 antagonist tool molecule which demonstrates high levels of brain exposure and selectivity against an extensive range of dopamine, serotonin, adrenergic, histamine, and muscarinic receptors is described.

A major area of focus in the development of clinically effective antipsychotics has been the study of compounds which display high affinity binding, and low intrinsic activity (IA) partial agonism at the dopamine (DA) D_2 receptor.[1]

Molecules such as aripiprazole **1** (Fig. 1) exhibit partial agonism at the DA D_2 receptor (IA = 0.3[1a]), a profile which is postulated to prevent effective blockade of DA D_2 function from rising above 70% even when receptor occupancies approaching 100% are obtained. This level of blockade falls within the anticipated therapeutic window of greater than 65% required for clinical efficacy, and below 80%, above which adverse events occur. Clinical investigations support this hypothesis with only minimal clinically limiting side effects observed in patients receiving aripiprazole compared with a high adverse event rate in patients receiving a comparably efficacious dose of the potent DA D_2 antagonist haloperidol **2**.[3]

Whilst this interpretation of the data is attractive, in reality aripiprazole displays activities at a number of other receptors including dopamine DA D_3, serotonin 5-HT_{1A}, serotonin 5-HT_{2A}, serotonin 5-HT_{2C}, histamine H_1, and adrenergic α1 subtypes.[2–4] It has been suggested that the partial agonist activity at 5-HT_{1A} and antagonist activity at 5-HT_{2A} and 5-HT_{2C} may contribute towards the improved side effect profile of aripiprazole over high potency DA D_2 antagonists.[5]

Herein, we report the identification of a highly selective DA D_2 partial agonist, DA D_3 antagonist tool molecule **3** (Fig. 2), suitable for use in in vivo studies, to allow delineation of the dopamine aspects of the pharmacology of aripiprazole from activities at other receptors.

Studies towards the identification of a DA D_3 selective PET ligand[6,7] also identified DA D_2 partial agonist, DA D_3 antagonist ligands **4** and **5** (racemic) (Fig. 3, Table 1).

Taking **4** and **5** as starting points we explored the imidazolidinone template structure–activity relationships SAR) via synthesis of structurally related compounds. In the first instance efforts were focused on hexahydro azepine **4** where studies were undertaken to vary the 3-Cl phenyl fragment, the imidazolidinone fragment and the alkyl chain length.

Aryl and heteroaryl imidazolidinone fragments were prepared using the procedure of Vidaluc, Imbert, and co-workers.[8] Alkylation with 2-(hexamethyleneimino) ethyl chloride hydrochloride using sodium hydride in DMF gave compounds **6–18** (Fig. 4).

Initial profiling of compounds **6–18** (Fig. 4, Table 2) revealed that modest changes around the aryl fragment in this series had a significant impact on the mode (agonist vs antagonist) of compound action, affinity and selectivity. In fact, substitution in

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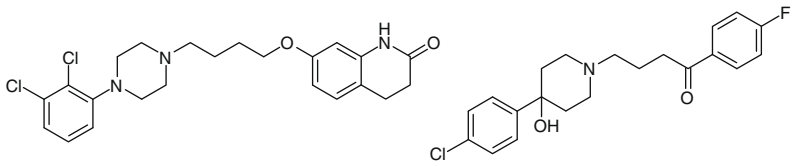
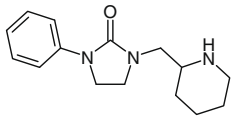


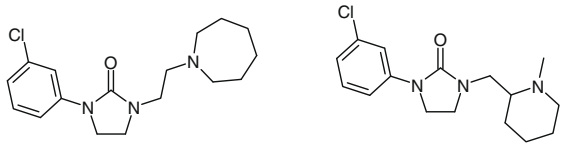
1 2

Figure 1. Aripiprazole **1** and haloperidol **2**.



3

Figure 2. Selective DA D_2 partial agonist, DA D_3 antagonist tool molecule **3**.



4 5

Figure 3. DA D_2 partial agonist starting points.

Table 1
Profiling results for initial hits

Entry	D_2[10] pEC_{50}	D_2[10] (IA)	D_2[10] pK_i	D_3 pEC_{50}	D_3 pK_i
4	9.0	0.75	8.0	<5.5	8.3
5	8.6	0.48	7.8	<5.5	8.4

IA aripiprazole[1b] = 0.60.

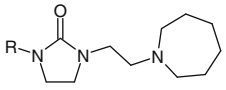


Figure 4. SAR for alternate aryl substitution and replacements.

the 2-position (compound **8**) resulted in a complete loss of dopamine affinity, whereas substitution in the 3-position was generally well tolerated resulting in several compounds showing a high level of affinity for both the DA D_2 and D_3 receptors. It was notable that compound mode of action could be controlled in this series by the choice of 3-substituent with either an antagonist profile at DA D_2 (compounds **11** and **12**) or partial agonist profile with varying degrees of IA (compounds **4**, **9**, **13**, **14** and **17**). Interestingly, 3,5-disubstitution with chlorine, which inferred partial agonism when only 3-substituted (compound **4**), resulted in an antagonist profile (compound **7**). Substitution in the 4-position in this series was less well tolerated and all examples prepared showed a drop in affinity compared with the corresponding 3-substituted derivatives. Addition of a 4-fluorine substituent to the potent 3-chloro partial agonist template also resulted in a drop in affinity as well as a decrease in the IA observed at DA D_2 (compound **16**).

A number of molecules were prepared by introducing a structurally different moiety in place of imidazolidinone, including imidazol-2-one **19** and 1,2,5-thiadiazolidine 1,1-dioxide **20** (Fig. 5, Table 3). In all such derivatives prepared a reduction in affinity for both the DA D_2 and D_3 receptors was observed.

Variation in the length of the alkyl chain in the series was also found to negatively impact affinity for both the DA D_2 and D_3 receptors. The C3 alkyl derivative **21** was prepared via alkylation of 1-(3-chlorophenyl)-2-imidazolidinone[8] with (3-bromopropoxy)-*t*-butyldimethylsilane using sodium hydride in DMF. Subsequent treatment with tetra-*n*-butyl ammonium fluoride gave the corresponding alcohol which was reacted with methanesulfonyl chloride to give the corresponding mesylate. Reaction of the mesylate with hexahydro azepine in the presence of triethylamine and potassium iodide gave **21**.

A substantial reduction in affinity for both the DA D_2 and D_3 receptors was observed for the three carbon linked compound **21** of this series with only weak antagonist activity at both receptors (Fig. 6, Table 4).

Compounds obtained in the exploration around **4** (compounds **6–21**) and which displayed high levels of DA D_2 partial agonism, were assessed to establish their broader selectivity and in vitro pharmacokinetic profile. Unfortunately, significant off target activity at a range of aminergic receptors was identified including serotonin 5-HT_{1A}, serotonin 5-HT_{2A}, and the histamine H_3 receptor. The intrinsic clearance in liver microsomes (Cli) was generally poor across the series. With this data in hand it was decided to refocus attention onto derivatives of **5** as the parent compound had displayed a superior selectivity profile to **4**.

In the first instance des-methyl analogues of **5** were prepared in which the position of the nitrogen within the piperidine ring was varied. These compounds were prepared by reaction of 1-(3-chlorophenyl)-2-imidazolidinone[8] with the corresponding bromomethyl pyridine in the presence of sodium hydride in DMF. Reduction of the pyridine ring using Adams catalyst in ethanol containing acetic acid gave piperidines **22** and **23** (as racemates) and **24** (Fig. 7).

Of the three positional isomers prepared only **22** showed activity at the DA D_2 and D_3 receptors. Pleasingly, the level of intrinsic activity observed for DA D_2 partial agonism for **22** was comparable to that of aripiprazole (Fig. 7, Table 5).

Exploration of the SAR around the aryl fragment was then undertaken for the 2-methylene piperidine system.

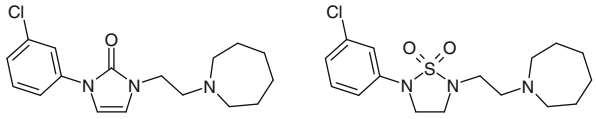
As reported in Table 6, 3-Cl phenyl **22**, phenyl **3** and 3-pyridyl **26** all displayed DA D_2 partial agonism and the 3-CF_3 phenyl **25** showed an antagonist profile. Notably, the 3-pyridyl fragment in this series displayed significantly reduced affinity at both DA D_2 and D_3 compared with the corresponding hexahydro azepine compound **17**; this was rationalized by the highly polar nature of **26** (Fig. 8).

Both enantiomers of **22** displayed DA D_2 partial agonism with a similar IA to aripiprazole, though one enantiomer showed 10-fold greater affinity for the DA D_2 and D_3 receptors. As both enantiomers also displayed similar in vitro metabolic stability it was decided to progress compounds **3** and **22** as racemic mixtures.

Table 2
Profiling results for aryl substitution SAR

Entry	R	D_2^{10} pEC_{50}	D_2^{10} (IA)	D_2^{10} pK_i	D_3 pEC_{50}	D_3 pK_i
4	3-Cl Phenyl	9.0	0.75	8.0	<5.5	8.3
6	4-Cl Phenyl	7.3	0.34	7.0	<5.5	7.1
7	3,5-Di-Cl phenyl	<5.1	—	9.2	<5.5	8.8
8	2-Me Phenyl	<5.1	—	<6.2	<5.5	<6.2
9	3-Me Phenyl	8.0	0.83	7.6	<5.5	7.1
10	4-Me Phenyl	<5.1	—	7.3	<5.5	6.9
11	3-OMe Phenyl	<5.1	—	7.4	<5.5	7.3
12	3-CF_3 Phenyl	<5.1	—	8.8	<5.5	9.0
13	Phenyl	9.2	0.88	8.1	<5.5	7.2
14	3-F Phenyl	9.1	0.83	8.6	<5.5	7.8
15	4-F Phenyl	8.0	0.64	7.2	<5.5	6.6
16	4-F, 3-Cl Phenyl	8.0	0.48	7.8	<5.5	7.6
17	3-Pyridyl	8.1	0.69	7.0	<5.5	7.1
18	4-Pyridyl	<5.5	—	6.2	<5.5	<6.2

IA aripiprazole[1b] = 0.60.



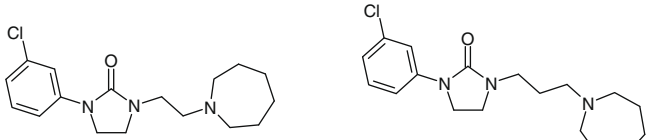
19 **20**

Figure 5. Sample imidazolidinone replacement derivatives.

Table 3
Profiling results for imidazolidinone replacement derivatives

Entry	D_2^{10} pEC_{50}	D_2^{10} (IA)	D_2^{10} pK_i	D_3 pEC_{50}	D_3 pK_i
19	6.9	0.73	6.4	<5.5	<6.2
20	6.9	0.34	6.7	<5.5	6.5

IA aripiprazole[1b] = 0.60.



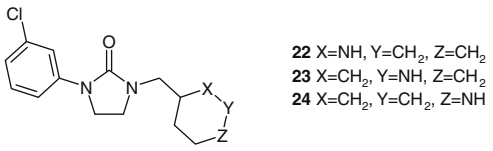
4 **21**

Figure 6. Variation in alkyl chain length.

Table 4
Profiling results for imidazolidinone replacement derivatives

Entry	D_2^{10} pEC_{50}	D_2^{10} (IA)	D_2^{10} pK_i	D_3 pEC_{50}	D_3 pK_i
4	9.0	0.75	8.0	<5.5	8.3
21	<5.1	—	6.2	<5.5	6.9

IA aripiprazole[1b] = 0.60.



22 X=NH, Y=CH_2, Z=CH_2
23 X=CH_2, Y=NH, Z=CH_2
24 X=CH_2, Y=CH_2, Z=NH

Figure 7. Piperidine positional isomers.

Table 5
Profiling results for piperidine nitrogen positional isomers

Entry	D_2^{10} pEC_{50}	D_2^{10} (IA)	D_2^{10} pK_i	D_3 pEC_{50}	D_3 fpK_i
22	9.2	0.60	7.1	<5.5	8.4
23	<5.1	—	<6.2	<5.5	<6.2
24	<5.1	—	<6.2	<5.5	<6.2

IA aripiprazole[1b] = 0.60.

Table 6
Profiling results for aryl substitution SAR

Entry	R	D_2^{10} pEC_{50}	D_2^{10} (IA)	D_2^{10} pK_i	D_3 pEC_{50}	D_3 fpK_i
22	3-Cl Phenyl	9.2	0.60	7.1	<5.5	8.4
25	3-CF_3 Phenyl	<5.1	—	7.7	<5.5	9.6
3	Phenyl	8.3	0.76	7.1	<5.5	7.6
26	3-Pyridyl	6.3	0.60	<6.2	<5.5	<6.2

IA aripiprazole[1b] = 0.60.

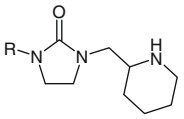
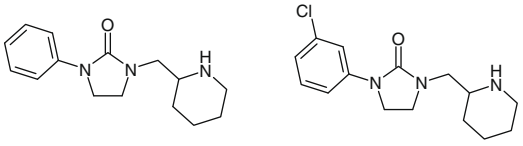


Figure 8. SAR for alternate aryl substitution and replacement.



3 **22**

Figure 9. Compounds selected for physiochemical and in vitro pharmacokinetic profiling.

Compounds **3** and **22** were screened to establish their physiochemical and in vitro pharmacokinetic profile (Fig. 9).

Metabolic stability in vitro for both compounds was comparable (Table 7) However, compound **3** showed a superior P450 inhibition profile to **22**. viz compound **22** CYP2D6 IC_{50} = 1.0 μM as opposed to

Table 7
Physiochemical and in vitro pharmacokinetic data for **3** and **22**

Entry	Solubility μg/mL	log *D* pH 7.4	Cli rat mL/min/g	Cli human mL/min/g	2D6 IC_{50} (μM)
3	106	0.73	22.9	4.5	>10
22	148	1.24	23.7	3.4	1.0

Table 8
Sample selectivity data for **3** and **22**

Entry	5-HT1A pEC_{50}	5-HT1D pEC_{50}	5-HT2A fpK_i	α1B fpK_i	H1 fpK_i	hERG pIC_{50}
3	<5.5	<5.5	<5.6	6.5	<5.7	4.8
22	6.1	6.3	<5.8	<6.6	<5.6	5.1

greater than 10 μM for **3**, both compounds showed inhibition potencies greater than 10 μM at all other isoforms tested.[9]

Initial selectivity profiling against a panel of receptors and channels, chosen to allow a comparison with aripiprazole, revealed **3** displayed a superior profile to **22** (Table 8).

As such, **3** was cross screened against an extensive panel of aminergic receptors (including dopamine, serotonin, adrenergic, histamine, and muscarinic receptors[9]) and liability targets which revealed only modest off-target activity at DA D_1 $fpK_i = 5.3$, α_{1B} adrenergic $fpK_i = 6.5$ and, hERG $pIC_{50} = 4.8$. Notably, **3** was completely selective against the DA D_4 receptor (agonist and antagonist assays). More extensive screening by CEREP against a diverse range of targets revealed no further liabilities.

In light of these results compound **3** was selected for in vivo testing in the rat. As the rat Cli data had suggested, extensive first pass metabolism resulted in low exposure of **3** via the oral route, thus the compound was formulated in labrasol for *subcutaneous* (sc) administration, from which good brain exposure was achieved (Table 9).

The DMPK profile of **3** showed high brain penetration (brain to blood ratio of 5.5) with high concentrations in the brain (667 ng/g) after a single sc administration at 3 mg/kg. The compound exhibited a T_{max} of 0.5 h making it suitable for use in behavioural models, moreover, human serum albumin binding (HAS) was moderate the rat brain tissue binding (BTB) was comparatively low leading to a fraction unbound of 22.3% (Table 10).

In summary, we have prepared a DA D2 partial agonist, DA D3 antagonist tool molecule which shows a high degree of selectivity over an extensive range of dopamine, serotonin, adrenergic, histamine, and muscarinic receptors. Moreover on administration sc **3** achieves high levels of brain exposure with a high free fraction. Details of the in vivo profiling of **3** will be published in due course.

Table 9
In vivo DMPK profile of **3** after sc administration

Dose mg/kg	Br:Bl	C_{max} (Br) ng/g	C_{max} (Bl) ng/mL	T_{max} (h)
3.0	5.5	667	142	0.5

Table 10
Protein and tissue binding profile of **3**

HSA (%)	Plasma rat (%)	BTB rat (%)
41.8	21.5	77.7

Acknowledgments

Abir Khazragi.

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