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Applying a multitarget rational drug design strategy: the first set of modulators with potent and balanced activity toward dopamine D3 receptor and fatty acid amide hydrolase†

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Combining computer-assisted drug design and synthetic efforts, we generated compounds with potent and balanced activities toward both D3 dopamine receptor and fatty acid amide hydrolase (FAAH) enzyme. By concurrently modulating these targets, our compounds hold great potential toward exerting a disease-modifying effect on nicotine addiction and other forms of compulsive behavior.

Tobacco smoking is a chronic syndrome that represents one of the most severe global health threats.[1] While it is the prolonged exposure to harmful substances contained in cigarette smoke that eventually leads to cardiovascular and respiratory conditions, cancer, and other disorders, tobacco addiction is caused by nicotine. Nicotine is a psychoactive alkaloid that elevates the levels of dopamine in areas of the brain connected to reward,[2] thus leading to addiction. Available treatments for nicotine addiction are partially effective in attenuating the symptoms of withdrawal but their success in preventing relapse has been only very limited.[3] Dopamine receptor D3 (DRD3) is a member of the GPCR superfamily that is mainly expressed in the mesolimbocortical system, a neural pathway implicated in reward and motivated behavior.[4] DRD3 has been extensively investigated to develop new medications for nicotine addiction.[5]

In animal models, DRD3 partial agonists decrease the compulsion for nicotine self-administration under reinforcement schedules and prevent the establishment of drug-seeking behavior.[6,7] However, DRD3 modulators do not display any significant effect on the rewarding properties of nicotine, and have only mild effects on withdrawal. It has been suggested, therefore, that an effective medication could be obtained by coupling the modulation of DRD3 with additional effects on other relevant targets.[6] Recent studies have shown that inhibition of the fatty acid amide hydrolase (FAAH) enzyme is effective in counteracting the abuse-related effects of nicotine.[8] In animal models, URB597, a selective FAAH inhibitor,[9] reduces the nicotine-induced elevation of dopamine in the brain, preventing self-administration and preferential behaviours.

Herein, we report on the rational design, synthesis, and biological evaluation of the first set of dual DRD3 partial agonists and FAAH inhibitors.

In Fig. 1, examples of known D3 selective modulators (**1–3**)[5] and FAAH inhibitors (**4–6**)[9] are reported. We realized that it was possible to devise a dual-target pharmacophore model by exploiting the overlap between the pharmacophoric features of DRD3 partial agonists and those of the *O*-aryl carbamate derivatives (Fig. S1 in ESI†).[5,10,11]

Ideally, molecules matching this description should be able to concurrently modulate both targets. Querying 263 annotated structures of *O*-aryl carbamate derivatives and 4298 DRD3

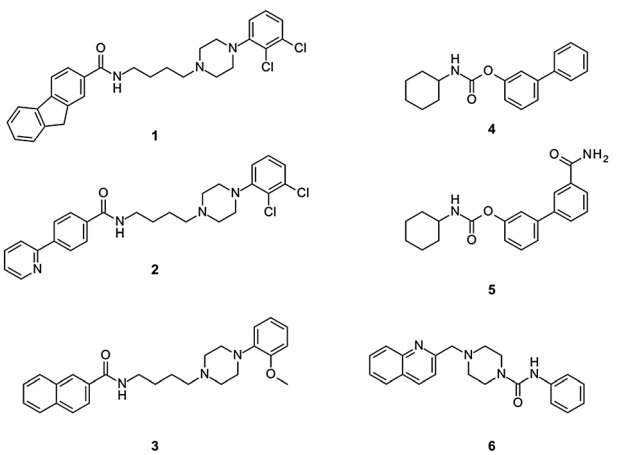

Fig. 1 Known DRD3 antagonists/partial agonists NGB2904 (**1**), CJB090 (**2**) and BP-897 (**3**) and known FAAH inhibitor derivatives URB524 (**4**), URB597 (**5**) and PF-622 (**6**).

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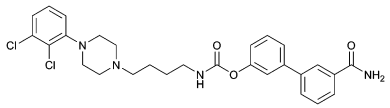
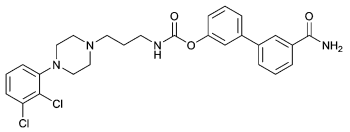
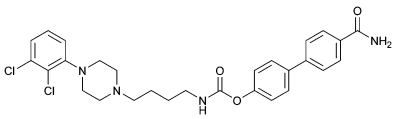
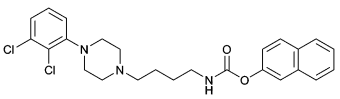
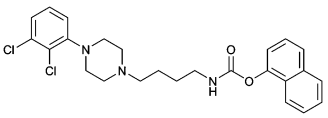
† Electronic supplementary information (ESI) available: Experimental procedures; characterization; spectral data for all compounds. See DOI: 10.1039/c4cc00967c

Table 1 Biological data of activities of known compounds and synthesized molecules

Compound	Structure	rat FAAH IC50 (nM)	Human FAAH IC50 (nM)	DRD3 EC50 (nM)	DRD3% efficacy[a]	DRD2 EC50 (nM)	DRD2% efficacy[b]	Ratio D2/D3	CB1 EC50 (nM)	Ratio CB1/D3
7		0.3	1.6	6.5	51.7	>1000	41.2	>154	0.9	0.1
8		0.1	1.3	3.9	64.8	320.0	61.1	82	0.3	0.1
15		0.7	0.6	1.0	55.6	23.0	25.2	23	14.0	14
16		13.0	2.7	7.7	81.2	240.0	32.3	31	64.0	8
17		22.0	6.1	1.3	50.4	209.0	A	161	420.0	323

[a] *vs.* 300 nM dopamine. [b] *vs.* 3 μM dopamine; N.C., value not calculable, the concentration–response curve shows less than 25% effect at the highest concentration; A, antagonist.

modulators retrieved from ChEMBL,[12] we could not find any match to the combined pharmacophore. Hence, we pursued the generation of novel, purposely conceived compounds. We assembled an *in silico* library of 280 compounds, in which each molecule had a univocal arrangement of chemical features rationally selected to display activity and selectivity on both targets. These compounds were docked into the crystal structures of rat FAAH (r-FAAH)[13] and human DRD3[14] (see ESI† for details).

Two compounds, **7** and **8** (Table 1), displayed good predicted binding affinities at both targets. In r-FAAH, compound **7** adopted the orientation suggested for *O*-aryl carbamates by the crystal structure of URB597-carbamoylated humanized rat FAAH,[15] as well as by quantum mechanical studies carried out on **4**[16] (Fig. 2a). The docked pose of **7** at DRD3 (Fig. 2b) was in good agreement with the binding mode previously proposed for DRD3 selective modulators.[14] The aryl-piperazine is lodged in the same region occupied by eticlopride in the crystal and the *O*-biphenyl moiety projected toward the less conserved region of the pocket. Compound **8** established similar interactions with both targets (see ESI†). Encouraged by these results, we synthesized **7** and **8**.[17,18] The syntheses are reported in the ESI.† The biological activities of the new compounds were evaluated in r-FAAH, human FAAH (h-FAAH) and in a human DRD3 functional assay (see ESI†). Results are reported in Table 1. Known DRD3 modulators **2** and **3** did not show any significant inhibitory activity on r-FAAH and h-FAAH. FAAH inhibitor **5** had no activity on DRD3 (see ESI†). Interestingly, compounds **7** and **8** turned out to be very potent FAAH inhibitors with 0.3 nM and 0.1 nM activities on r-FAAH and 1.6 nM and 1.3 nM activities on h-FAAH, respectively. In agreement with the SAR reported by Mor *et al.*,[19] an elongated substituent on the nitrogen side of the carbamate was beneficial for potency. The presence of a basic nitrogen atom in the lipophilic acyl chain binding pocket was already reported for PF-622 (**6**, Fig. 1) and was not detrimental for activity.[20] At the same time, compounds **7** and **8** showed potent modulatory activity on DRD3, with a partial agonist profile and median effective concentration (EC_{50}) of 6.5 nM and 3.9 nM (see Table 1). The length of the linker did not influence the potency. This first set of data confirmed our initial hypothesis that a seamless combination of the pharmacophoric features of FAAH inhibitors and DRD3 partial agonists in a single molecular entity can lead to dual-target modulators. Next, the compounds were tested against the short isoform of human dopamine D2 receptor (DRD2) to evaluate the DRD2/DRD3 selectivity ratio, as the simultaneous activation of DRD2 and DRD3 could lead to severe side effects.[21] Both derivatives were selective for DRD3, with DRD2/DRD3 affinity ratios greater than 80. The second off-target that we tested was the cannabinoid receptor CB_1. CB_1 receptors are highly expressed in regions of the brain implicated in dopamine-mediated reward,[22,23] and the CB_1 antagonist rimonabant (SR141716A) was evaluated in clinical trials as a potential new medication for nicotine addiction.[24] Despite its effectiveness, rimonabant was not further developed due to neuropsychiatric side effects attributed to the blockage of intrinsic endocannabinoid signalling.[24] CB_1 direct agonism can also be detrimental, because of the pleiotropic functions served by this receptor in the brain and peripheral tissues.[22] Surprisingly, compounds **7** and **8** showed CB_1 activation in the picomolar range (see Table 1). While it is known that **5** has no effect on CB_1,[25] we tested known DRD3 partial

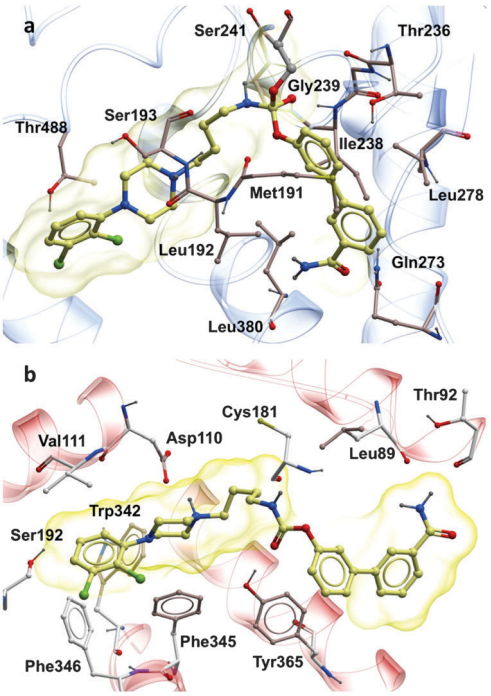


Fig. 2 (a) Docked pose of **7** in the crystal structure of rat FAAH; (b) docked pose of **7** in the crystal structure of human DRD3.

agonists **2** and **3** for CB_1 activity. **3** did not show any activity but **2** turned out to be a rather potent CB_1 agonist with an EC_{50} of 840 nM (Table S1 in the ESI†).

To design-out CB_1 activity, three additional derivatives were prepared by modifying the *O*-aryl group according to Scheme S1 (ESI†).

Since **7** showed a classic partial agonist profile on DRD3 and a greater selectivity toward DRD2 with respect to **8**, the length of the linker was kept at 4 methylene units. To modulate the orientation of the aryl-substituent, we introduced a *p*-biphenyl moiety (**15**). Although the latter moiety was reported to be detrimental for FAAH activity,[9] compound **15** maintained a good potency, showed a partial agonist profile on DRD3, and acquired a small but significant selectivity ratio over CB_1. In this case, the main issue was the selectivity over DRD2, which, dropping from over 150- to 23-fold, was negatively affected by this substitution. Next, considering that: (i) **3** is completely devoid of CB_1 activity and that (ii) the naphthyl group was already reported on both DRD3 modulators[5] and FAAH inhibitors,[26] the two naphthyl-substituted regioisomers **16** and **17** were synthesized. These compounds were endowed with good and balanced activities in the low nanomolar range. However, **16** did not show any improvement in CB_1 selectivity relative to **15**, had only a moderate 31-fold selectivity over DRD2, and the functional assay on DRD3 highlighted an almost full agonist activity profile. Conversely, **17** gave successful results in the CB_1 designing-out effort, showing a good selectivity with a CB_1/DRD3 ratio of over 300-fold and 420 nM EC_{50} on CB_1, over 450-fold lower than the prototype **7**. Together with potent and balanced activities (6.1 nM on h-FAAH and 1.3 nM on DRD3), compound **17** also had 161-fold selectivity over DRD2, a clear partial agonist profile, and interesting physico-chemical calculated features (see Table S1 in the ESI†). The docked poses of compounds **8** and **15–17** at FAAH and DRD3 are reported in the ESI.†

Here, confirming the feasibility of our recently reported strategy,[27] we have combined computational methods and synthetic efforts to successfully discover novel, potent and balanced dual-target molecules. The described compounds are an example of dual modulators rationally designed to display activity toward a GPCR and an enzyme, which are structurally unrelated but involved in a common biological function.[28] While the vast majority of known drugs have been developed as selective modulators of a single target, this approach has shown several limitations in treating complex and multifaceted pathologies.[29] The Multi-Target Directed Ligand (MTDL) strategy is based on the idea that a single molecular entity can be devised to hit multiple targets that cooperate in the framework of the same disease.[30] MTDLs may have a superior therapeutic effect with respect to single target compounds and might prevent unwanted compensations.[31–34] Being able to modulate DRD3 and inhibit FAAH, this class of compounds might hold great potential as disease-modifying agents for the treatment of nicotine addiction.[35,36]

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Notes and references

1 B. A. Syed and K. Chaudhari, *Nat. Rev. Drug Discovery*, 2013, **12**, 97–98.
2 J. J. Escobar-Chávez, C. L. Domínguez-Delgado and I. M. Rodríguez-Cruz, *Drug Des., Dev. Ther.*, 2011, **5**, 211–224.
3 R. Polosa and N. L. Benowitz, *Trends Pharmacol. Sci.*, 2011, **32**, 281–289.
4 B. J. Everitt and T. W. Robbins, *Nat. Neurosci.*, 2005, **8**, 1481–1489.
5 F. Micheli, *ChemMedChem*, 2011, **6**, 1152–1162.
6 M. Mugnaini, L. Iavarone, P. Cavallini, C. Griffante, B. Oliosi, C. Savoia, J. Beaver, E. A. Rabiner, F. Micheli, C. Heidbreder, A. Andorn, E. Merlo Pich and M. Bani, *Neuropsychopharmacology*, 2013, **38**, 302–312.
7 A. Hackling, R. Ghosh, S. Perachon, A. Mann, H. D. Holtje, C. G. Wermuth, J. C. Schwartz, W. Sippl, P. Sokoloff and H. Stark, *J. Med. Chem.*, 2003, **46**, 3883–3899.
8 M. Scherma, L. V. Panlilio, P. Fadda, L. Fattore, I. Gamaleddin, B. Le Foll, Z. Justinova, E. Mikics, J. Haller, J. Medalie, J. Stroik, C. Barnes, S. Yasar, G. Tanda, D. Piomelli, W. Fratta and S. R. Goldberg, *J. Pharmacol. Exp. Ther.*, 2008, **327**, 482–490.
9 M. Mor, S. Rivara, A. Lodola, P. V. Plazzi, G. Tarzia, A. Duranti, A. Tontini, G. Piersanti, S. Kathuria and D. Piomelli, *J. Med. Chem.*, 2004, **47**, 4998–5008.
10 H. Deng, *Expert Opin. Drug Discovery*, 2010, **5**, 961–993.
11 A. H. Newman, T. Beuming, A. K. Banala, P. Donthamsetti, K. Pongetti, A. LaBounty, B. Levy, J. Cao, M. Michino, R. R. Luedtke, J. A. Javitch and L. Shi, *J. Med. Chem.*, 2012, **55**, 6689–6699.
12 A. Gaulton, L. J. Bellis, A. P. Bento, J. Chambers, M. Davies, A. Hersey, Y. Light, S. McGlinchey, D. Michalovich, B. Al-Lazikani and J. P. Overington, *Nucleic Acids Res.*, 2012, **40**, D1100–D1107.
13 X. Min, S. T. Thibault, A. C. Porter, D. J. Gustin, T. J. Carlson, H. Xu, M. Lindstrom, G. Xu, C. Uyeda, Z. Ma, Y. Li, F. Kayser, N. P. Walker and Z. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 7379–7384.
14 E. Y. Chien, W. Liu, Q. Zhao, V. Katritch, G. W. Han, M. A. Hanson, L. Shi, A. H. Newman, J. A. Javitch, V. Cherezov and R. C. Stevens, *Science*, 2010, **330**, 1091–1095.

15 M. Mileni, S. Kamtekar, D. C. Wood, T. E. Benson, B. F. Cravatt and R. C. Stevens, *J. Mol. Biol.*, 2010, **400**, 743–754.
16 A. Lodola, M. Mor, S. Rivara, C. Christov, G. Tarzia, D. Piomelli and A. J. Mulholland, *Chem. Commun.*, 2008, 214–216.
17 A. Del Zotto, F. Amoroso, W. Baratta and P. Rigo, *Eur. J. Org. Chem.*, 2008, 110–116.
18 H. J. Knolker and T. Braxmeier, *Tetrahedron Lett.*, 1996, **4039**, 5861–5864.
19 M. Mor, A. Lodola, S. Rivara, F. Vacondio, A. Duranti, A. Tontini, S. Sanchini, G. Piersanti, J. R. Clapper, A. R. King, G. Tarzia and D. Piomelli, *J. Med. Chem.*, 2008, **51**, 3487–3498.
20 K. Ahn, D. S. Johnson, L. R. Fitzgerald, M. Liimatta, A. Arendse, T. Stevenson, E. T. Lund, R. a. Nugent, T. K. Nomanbhoy, J. P. Alexander and B. F. Cravatt, *Biochemistry*, 2007, **46**, 13019–13030.
21 J. N. Joyce and M. J. Millan, *Drug Discovery Today*, 2005, **10**, 917–925.
22 V. Di Marzo, *Nat. Rev. Drug Discovery*, 2008, **7**, 438–455.
23 R. Maldonado and F. Berrendero, *Curr. Drug Targets*, 2010, **11**, 440–449.
24 F. A. Moreira and J. A. Crippa, *Rev. Bras. Psiquiatr.*, 2009, **31**, 145–153.
25 D. Piomelli, G. Tarzia, A. Duranti, A. Tontini, M. Mor, T. R. Compton, O. Dasse, E. P. Monaghan, J. A. Parrott and D. Putman, *CNS Drug Rev.*, 2006, **12**, 21–38.
26 G. Tarzia, A. Duranti, A. Tontini, G. Piersanti, M. Mor, S. Rivara, P. V. Plazzi, C. Park, S. Kathuria and D. Piomelli, *J. Med. Chem.*, 2003, **46**, 2352–2360.
27 G. Bottegoni, A. D. Favia, M. Recanatini and A. Cavalli, *Drug Discovery Today*, 2012, **17**, 23–34.
28 J. U. Peters, *J. Med. Chem.*, 2013, **56**, 8955–8971, DOI: 10.1021/jm400856t.
29 P. Csermely, V. Agoston and S. Pongor, *Trends Pharmacol. Sci.*, 2005, **26**, 178–182.
30 A. Cavalli, M. L. Bolognesi, A. Minarini, M. Rosini, V. Tumiatti, M. Recanatini and C. Melchiorre, *J. Med. Chem.*, 2008, **51**, 347–372.
31 K. Mohr, J. Schmitz, R. Schrage, C. Trankle and U. Holzgrabe, *Angew. Chem., Int. Ed.*, 2013, **52**, 508–516.
32 S. Butini, S. Gemma, G. Campiani, S. Franceschini, F. Trotta, M. Borriello, N. Ceres, S. Ros, S. S. Coccone, M. Bernetti, M. De Angelis, M. Brindisi, V. Nacci, I. Fiorini, E. Novellino, A. Cagnotto, T. Mennini, K. Sandager-Nielsen, J. T. Andreasen, J. Scheel-Kruger, J. D. Mikkelsen and C. Fattorusso, *J. Med. Chem.*, 2009, **52**, 151–169.
33 R. Morphy, C. Kay and Z. Rankovic, *Drug Discovery Today*, 2004, **9**, 641–651.
34 A. L. Hopkins, *Nat. Chem. Biol.*, 2008, **4**, 682–690.
35 A. H. Newman, J. Cao, C. J. Bennett, M. J. Robarge, R. A. Freeman and R. R. Luedtke, *Bioorg. Med. Chem. Lett.*, 2003, **13**, 2179–2183.
36 F. J. Garcia-Ladona and B. F. Cox, *CNS Drug Rev.*, 2003, **9**, 141–158.