Published in final edited form as:

Bioorg Med Chem Lett. 2015 February 1; 25(3): 519–523. doi:10.1016/j.bmcl.2014.12.023.

Synthesis, Pharmacological Evaluation and Molecular Modeling Studies of Triazole Containing Dopamine D₃ Receptor Ligands

Xin Peng[†], Qi Wang[†], Yogesh Mishra^{*}, Jinbin Xu[†], David E. Reichert[†], Maninder Malik^{*}, Michelle Taylor^{*}, Robert R. Luedtke^{*}, and Robert H. Mach^{*,†,‡,§}

[†] Department of Radiology, Washington University School of Medicine, St. Louis, MO 63110

[‡] Department of Cell Biology & Physiology, Washington University School of Medicine, St. Louis, MO 63110

[§] Department of Biochemistry & Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110

^{*} Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107, USA.

Abstract

A series of 2-methoxyphenyl piperazine analogues containing a triazole ring were synthesized and their *in vitro* binding affinities at human dopamine D₂ and D₃ receptors were evaluated.

Compounds **5b**, **5c**, **5d**, and **4g**, demonstrate high affinity for dopamine D₃ receptors and moderate selectivity for the dopamine D₃ versus D₂ receptor subtypes. To further examine their potential as therapeutic agents, their intrinsic efficacy at both D₂ and D₃ receptors was determined using a forskolin-dependent adenylyl cyclase inhibition assay. Affinity at dopamine D₄ and serotonin 5-HT_{1A} receptors was also determined. In addition, information from previous molecular modeling studies of the binding of a panel of 163 structurally-related benzamide analogues at dopamine D₂ and D₃ receptors was applied to this series of compounds. The results of the modeling studies were consistent with our previous experimental data. More importantly, the modeling study results explained why the replacement of the amide linkage with the hetero-aromatic ring leads to a reduction in the affinity of these compounds at D₃ receptors.

Keywords

dopamine receptors; dopamine D₃ receptor selective ligands; triazole; molecular modeling

© 2014 Elsevier Ltd. All rights reserved.

^{*}To whom correspondence should be addressed. Robert H. Mach, Ph.D. University of Pennsylvania Chemistry Building, Room 283 231 S. 34th St Philadelphia, PA 19104 rmach@mail.med.upenn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary material

Supplementary data (experimental procedures, characterization of all compounds and biological assays protocols) associated with this article can be found, in the online version, at

Dopamine is a neurotransmitter that plays a number of important roles in the central nervous system (CNS) by activating both presynaptic and postsynaptic dopamine receptors. Dopamine receptors belong to the family of seven transmembrane domain G-protein coupled receptors (GPCRs) and are classified into two types: D₁-like and D₂-like receptors. D₁-like receptors consist of D₁ and D₅ receptors, and D₂-like receptors consist of D₂, D₃ and D₄ receptors. Agonist activation of D₁-like receptors leads to an increase of adenylyl cyclase activity, whereas activation of D₂-like receptors leads to adenylyl cyclase inhibition.¹

Experimental evidence has suggested that changes in the expression of D₃ receptors may be associated with several CNS diseases and behavioral disorders, including psychostimulant abuse. For instance, increased CNS D₃ receptor expression was found in human cocaine overdose victims when compared with age-matched controls.² In addition, several reports suggest that D₃ receptor antagonists and partial agonists attenuate cocaine's rewarding effects.³⁻⁵ An up-regulation of D₃ receptors has been found in animal models of dyskinesia⁶⁻⁸ and D₃ receptor selective compounds were found to attenuate L-DOPA-induced dyskinetic-like involuntary movements in hemi-parkinsonian rats, suggesting that D₃ receptors could be therapeutic targets for L-DOPA-induced dyskinesia.⁹ Therefore, highly selective D₃ receptor ligands represent potential pharmacotherapeutic agents for the treatment of D₃-related CNS disorders.

There is substantial amino acid sequence homology between the transmembrane helices of the D₂ and D₃ receptors (78% homology)¹⁰, which makes it difficult to develop highly selective D₃ receptor ligands. Our group has developed several benzamide analogues as D₃-selective ligands as candidates including (**Figure 1**): **WC 10** and **WC 44**.¹¹ Tritium-labeled **WC 10** has been used for quantitative autoradiography studies.¹²

In this work, we explored new synthons as replacements for the amide linkage in benzamide based D₃ receptor ligands. 5-Membered heterocycle ring, such as triazole, has been reported to represent an isosteric substitution of an amide linkage.^{13, 14} In addition, the hetero atoms in the triazole ring may participate in hydrogen bonding and dipole-dipole interactions with receptors,^{14, 15} which could result in a more favorable receptor binding affinity. Gmeiner et al first synthesized a series of analogues with 1,2,3-triazole linkers as ligands for GPCR receptors.¹⁶ Micheli et al reported 1,2,4-Triazol-3-yl-thiopropyl-tetrahydrobenzazepines as D₃ antagonists.¹⁷ Carro et al has explored 1,4-disubstituted triazole analogues as D₃ ligands.¹⁸ We have synthesized a series of 1,2,3-triazole based D₃ receptor ligands with reversed substitution pattern from Carro et al's report. The aryl substitutions are on N1 position of the triazole in our series of ligands. Moreover, we explored varied carbon linkages in conjunction with triazole moiety. This letter reports the synthesis, evaluation, and SAR studies of 1,2,3-triazole based D₃ receptor ligands and molecular modeling studies with these ligands.

These analogues were evaluated using *in vitro* receptor binding assays to determine the binding affinity and selectivity at D₂ and D₃ receptors. Additional assays, such as dopamine D₄, 5-HT_{1A} and the intrinsic efficacy of selected ligands at D₂ and D₃ receptors were also conducted at D₃-selective compounds.

Our group had previously generated comprehensive three dimensional quantitative structure–activity relationship (3D-QSAR) models for a set of 163 D₂/D₃ benzamide compounds based on homology models of the D₂ and D₃ receptors.¹⁹ The homology model of the D₃ receptor is consistent with the D₃ X-ray crystal structure²⁰ (PDB code: 3PBL) (RMSD of the model to the X-ray structure is 2.88 Å, which is lower than the resolution of the X-ray structure). The 3DQSAR models for the compound library also showed excellent correlation and predictive power. Thus, we used the new series of triazole phenylpiperazine analogues as another external test set to evaluate the accuracy of our QSAR models. Moreover, QSAR has provided insights on the different binding mode of this series of ligands versus our previous benzamide ligands.

The target compounds were synthesized using click chemistry as depicted in **Schemes 1**. Anilines were first converted to the corresponding diazoniums using 1:1 HCl/H₂O as the solvent, in the presence of sodium nitrite. The transformation of diazoniums to azides was then achieved by treating with sodium azide, using sodium acetate to neutralize the excess HCl.

The substituted 4-phenylpiperazinyl aryl-1-yne with different carbon spacers were synthesized by *N*-alkylation of 1-(2-methoxyphenyl)-piperazine and corresponding tosylates, using acetonitrile as the solvent and sodium carbonate as the base. Tosylates were converted from corresponding substituted alcohols by stirring with *p*-TsCl and triethylamine in dichloromethane.

The target triazole analogues were synthesized by click reactions using classic Cu(I)-catalyzed conditions reported by Fokin et al.²¹

The triazole analogues were evaluated for affinity at human D₂ and D₃ dopamine receptors expressed in stably transfected HEK cells (**Table 1**) using a competitive inhibition binding assay, which was previously reported.²² The ligand binding selectivity is calculated as K_i (D₂)/ K_i (D₃). Analogues that exhibited >10-fold binding selectivity for dopamine D₃ receptors compared to the D₂ receptor subtype were further evaluated for a) affinity at D₄ dopamine receptors and 5-HT_{1A} serotonin receptors; b) intrinsic efficacy at D₂ and D₃ receptors using a forskolin-dependent adenylyl cyclase inhibition assay.

The radioligand binding analysis identified several triazole analogues with 10-fold selectivity for D₃ versus D₂ receptors including compounds **4b**, **5b**, **3c**, **4c**, **5c**, **4f**, **4g**, and **5g**. Among these compounds, **4b**, **5b**, **4g**, and **5g** have the highest affinity, with K_i values for the D₃ receptor 4 nM.

Compound **4a**, which is the reversed substituted analogue of compound **15** in Carro et al's paper,¹⁸ shows a much higher affinity at D₃ receptor and higher D₂/D₃ selectivity compared to **15** (**Figure 2**).

Another observation from the experimental data is that varying the length of the carbon spacer group while maintaining the same aromatic substituents changes both D₂ and D₃ receptor affinity. It was found that triazole analogues with a three-carbon spacer generally exhibits lower D₂ and D₃ affinity than their four or five-carbon-spacer analogues. When the

carbon spacer group increases from four to five, D₂ receptor affinities generally increase to greater extent than D₃ receptor affinities. It leads to the fact that although most of the five-carbon spacer compounds have the highest D₃ receptor affinity, they exhibit lower D₂ versus D₃ selectivity.

The structure-activity relationship was further explored by changing the substituents on the aromatic ring. It was found that compounds with highest affinity and/or selectivity are *para*-substituted. For example: **3c**, **4c**, **5c**, **5d**, **5f**, **4g**, and **5g**.

Unfortunately, when amide bonds were replaced with triazoles, both D₃ receptor affinity and selectivity decreased. For example, **WC 10** (K_i D₃ = 0.8 nM, D₂/D₃ = 43) versus the triazole analogue **4f** (K_i D₃ = 13.9 nM, D₂/D₃ = 12).

Compounds **4b**, **5b**, **3c**, **4c**, **5c**, **4f**, **4g**, and **5g** were selected for further binding assays because their selectivity for the D₃ versus the D₂ receptor • 10-fold. The affinity of these compounds was found to be low at the D₄ dopamine receptor subtype. Additionally, as we previously reported, D₃-selective benzamide analogues can exhibit high binding affinity at 5-HT_{1A} receptors.¹¹ Unfortunately, the triazole analogues did not improve this property. All of the eight compounds we tested except **3c** bind to 5-HT_{1A} receptors with high affinity. It is necessary to point out that the replacement of amide with triazoles increases the ligands' logD value.

An adenylyl cyclase inhibition assay was used to determine the intrinsic activity of compounds **4b**, **5b**, **3c**, **4c**, **5c**, **4f**, **4g**, and **5g**. All test compounds were used at a concentration equal to 10 × the calculated K_i value; therefore 90% of the D₂ or D₃ binding sites were occupied (**Table 2**). For each compound, the inhibition was compared to the intrinsic activity of quinpirole (full agonist) and haloperidol (antagonist). All of the compounds were found to be partial agonists at D₂ dopamine receptors, with the intrinsic activity ranging from approximately 31-75% of the full agonist. However, for D₃ receptor a broader range of intrinsic activity was observed (from 13% efficacy to a 107% full agonist). Interestingly, compound **4f** is a full agonist at D₃ receptors, while its benzamide analogue, **WC 10** is a D₃ receptor antagonist/weak partial agonist (18.7%).¹¹

Computational molecular modeling studies were initiated to begin to define at a molecular level how triazole phenylpiperazines bind to the D₂ or D₃ receptors. The Comparative Molecular Similarity Index Analysis (CoMSIA1) for both D₂ and D₃ provided results in good agreement with the experimental values for ligand-receptor affinity (**Supplementary Table 2**). Because their values only span one to two orders of magnitude, no statistical analysis was performed. However, a) the residuals are all reasonably small and b) overall trends for the compounds' predicted affinities agree with the experimental binding data on both D₂ and D₃. These modeling studies indicate that the compounds have similar binding modes at the D₂ receptor site as their benzamide counterparts (**WC** series), while losing about 10-fold affinity at D₃ site.

In the molecular modeling study, both CoMSIA1 models utilized a combination of steric, electrostatic, hydrophobic and hydrogen bond donor and acceptor fields. An analysis of the

contribution of each of these components suggests that hydrophobic interactions and hydrogen binding are the major determinant factors for the compounds' binding affinities.

The replacement of the amide bond in **WC** series with triazole was found to reduce both the affinity and selectivity to D₃ receptors compared to the **WC** series (**WC 10** vs. **4f**). A closer examination of our D₃ receptor-ligand binding model reveals that, besides the salt bridge between the highly conserved residue Asp3.32 in the transmembrane (TM) 3 helix of the receptor and the protonated nitrogen on the piperidine scaffold, the benzamide group on **WC 10** potentially forms a critical hydrogen bond with the D₃ receptor (**Figure 3**). This hydrogen bond is between the amine on the benzamide group and the carbonyl of the backbone from C181 on extracellular loop (EL) 2 of D₃. C181 is a highly conserved cysteine residue, which is involved in the formation of a disulfide bond between EL2 and the third transmembrane (TM 3) spanning helix. This disulfide bond plays an important role in the stabilization of the GPCR structure. Consequently, the ligand is securely anchored at the binding site, with its benzamide group extending toward the extracellular opening of the binding pocket. The extension of this amine-containing scaffold into a second binding pocket is crucial for high D₃ receptor binding affinity.²⁰

This second binding pocket is delineated by the residues at the junction of EL1, EL2 and the interfaces of TM helices 1, 2 and 7 and opens to the extracellular regions. This region was described in the dopamine D₃ crystal structure paper as a secondary binding pocket,²⁰ and recently, as a allosteric site for dopamine D₃ receptors.²³ The four-carbon linker on the ligand scaffold is conformationally flexible. The benzamide hydrogen bond anchors the ligand and helps to orient it toward the secondary binding pocket. Therefore, interactions between the substituents on the benzene ring with amino acid residues in the secondary binding site become possible.

The loss of this hydrogen bonding in the triazole analogues eliminates an important interaction that is essential for guiding the aryl moiety (of the arylamide) towards a second binding pocket (**Figure 4**). The importance of the carboxamide linker for achieving D₃ vs. D₂ receptor binding affinity and selectivity has been previously reported.²⁴ The current work further defines the role that this pivotal pharmacophore plays in the development of dopamine D₃ receptor selective ligands.

Alignments for the analogues **WC 10**, **3f**, **4f** and **5f** on the molecular field maps of CoMSIA1 derived for D₃ shows that longer carbon spacer compounds (**4f** and **5f**) have the aromatic ring sticking into the hydrophobic favorable regions within the second binding pocket (**Figure 5B**). Since hydrophobic interactions are a major contributor to the ligand affinity, we would expect the compounds with the longer spacer chain to bind with higher affinity. This also partially explains why a substituent in the *para* position of the aromatic ring enhances activity. However, in our D₂ receptor model, this class of compounds binds to the D₂ receptor with an L-shaped carbon spacer chain. As a result, the alignment of the triazole analogues overlaps quite well with **WC 10** (**Figure 5A**). Therefore, these analogues have comparable binding affinities with the **WC** series at the D₂ receptor. These modeling studies suggest that the origins of D₂ vs. D₃ receptor subtype selectivity for the ligands arise primarily from contour differences of the two binding sites. Therefore ligands must exploit

subtle topographical differences within the two ligand binding sites to achieve high selectivity between the D₂ and D₃ dopamine receptor subtypes.

In summary, we have synthesized and evaluated a series of triazole analogues which showed moderate to high affinity for D₃ receptors and variable selectivity for D₃ versus D₂ receptors. For this series of compounds we identified several compounds that exhibited high D₃ binding affinity (<4.0 nM) with moderate D₃ versus D₂ receptor binding selectivity. The SAR could be partially explained by our previously generated 3D-QSAR models for selective dopamine receptor ligands. This study expands the SAR of the D₃-selective ligands and our knowledge on the binding modes of dopamine receptors. The development of new class of compounds for dopamine D₃ receptors is ongoing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grants DA29840 (RHM), DA23957 and DA13584 (RRL) and Integrated Research Training Program of Excellence in Radiochemistry (60096D).

Abbreviations

3D-QSAR models	three dimensional quantitative structure–activity relationship models
CoMSIA	Comparative Molecular Similarity Index Analysis
EL1	Extracellular Loop 1
EL2	Extracellular Loop 2
GPCR	G-protein coupled receptor
RMSD	Root Mean-Square Deviation
SAR	Structure–Activity Relationship
TM	Transmembrane

REFERENCES

1. Joyce JN, Millan MJ. Dopamine D₃ receptor antagonists as therapeutic agents. *Drug Discov Today*. 2005; 10:917–25. [PubMed: 15993811]
2. Staley JK, Mash DC. Adaptive increase in D₃ dopamine receptors in the brain reward circuits of human cocaine fatalities. *J Neurosci*. 1996; 16:6100–6. [PubMed: 8815892]
3. Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW. Nucleus accumbens D_{2/3} receptors predict trait impulsivity and cocaine reinforcement. *Science*. 2007; 315:1267–70. [PubMed: 17332411]
4. Di Ciano P. Drug seeking under a second-order schedule of reinforcement depends on dopamine D₃ receptors in the basolateral amygdala. *Behav Neurosci*. 2008; 122:129–39. [PubMed: 18298256]
5. Chen L, Xu M. Dopamine D₁ and D₃ receptors are differentially involved in cue-elicited cocaine seeking. *J Neurochem*. 2010; 114:530–41. [PubMed: 20456009]

6. Bordet R, Ridray S, Carboni S, Diaz J, Sokoloff P, Schwartz JC. Induction of dopamine D3 receptor expression as a mechanism of behavioral sensitization to levodopa. *Proc Natl Acad Sci U S A*. 1997; 94:3363–7. [PubMed: 9096399]
7. Bordet R, Ridray S, Schwartz JC, Sokoloff P. Involvement of the direct striatonigral pathway in levodopa-induced sensitization in 6-hydroxydopamine-lesioned rats. *Eur J Neurosci*. 2000; 12:2117–23. [PubMed: 10886351]
8. Bordet R. [Central dopamine receptors: general considerations (Part 1)]. *Rev Neurol (Paris)*. 2004; 160:862–70. [PubMed: 15454880]
9. Kumar R, Riddle LR, Griffin SA, Chu W, Vangveravong S, Neisewander J, Mach RH, Luedtke RR. Evaluation of D2 and D3 dopamine receptor selective compounds on L-dopa-dependent abnormal involuntary movements in rats. *Neuropharmacology*. 2009; 56:956–69. [PubMed: 19371586]
10. Shi L, Javitch JA. The binding site of aminergic G protein-coupled receptors: the transmembrane segments and second extracellular loop. *Annu Rev Pharmacol Toxicol*. 2002; 42:437–67. [PubMed: 11807179]
11. Chu W, Tu Z, McElveen E, Xu J, Taylor M, Luedtke RR, Mach RH. Synthesis and in vitro binding of N-phenyl piperazine analogs as potential dopamine D3 receptor ligands. *Bioorg Med Chem*. 2005; 13:77–87. [PubMed: 15582454]
12. Xu J, Chu W, Tu Z, Jones LA, Luedtke RR, Perlmutter JS, Mintun MA, Mach RH. [(3)H]4-(Dimethylamino)-N-[4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl]benzamide, a selective radioligand for dopamine D(3) receptors. I. In vitro characterization. *Synapse*. 2009; 63:717–28. [PubMed: 19425052]
13. Tron GC, Pirali T, Billington RA, Canonico PL, Sorba G, Genazzani AA. Click chemistry reactions in medicinal chemistry: applications of the 1,3-dipolar cycloaddition between azides and alkynes. *Med Res Rev*. 2008; 28:278–308. [PubMed: 17763363]
14. Bock VD, Hiemstra H, van Maarseveen JH. Cu-I-catalyzed alkyne-azide “click” cycloadditions from a mechanistic and synthetic perspective. *European Journal of Organic Chemistry*. 2006:51–68.
15. Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. *Drug Discov Today*. 2003; 8:1128–37. [PubMed: 14678739]
16. Loaiza PR, Lober S, Hubner H, Gmeiner P. Click chemistry on solid phase: parallel synthesis of N-benzyltriazole carboxamides as super-potent G-protein coupled receptor ligands. *J Comb Chem*. 2006; 8:252–61. [PubMed: 16529521]
17. Micheli F, Bonanomi G, Blaney FE, Braggio S, Capelli AM, Checchia A, Curcuruto O, Damiani F, Fabio RD, Donati D, Gentile G, Gribble A, Hamprecht D, Tedesco G, Terreni S, Tarsi L, Lightfoot A, Stemp G, Macdonald G, Smith 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 CR Jr, Hagan JJ, Heidbreder C. 1,2,4-triazol-3-yl-thiopropyl-tetrahydrobenzazepines: a series of potent and selective dopamine D(3) receptor antagonists. *J Med Chem*. 2007; 50:5076–89. [PubMed: 17867665]
18. Insua I, Alvarado M, Masaguer CF, Iglesias A, Brea J, Loza MI, Carro L. Synthesis and binding affinity of new 1,4-disubstituted triazoles as potential dopamine D(3) receptor ligands. *Bioorg Med Chem Lett*. 2013; 23:5586–91. [PubMed: 24012118]
19. Wang Q, Mach RH, Luedtke RR, Reichert DE. Subtype selectivity of dopamine receptor ligands: insights from structure and ligand-based methods. *J Chem Inf Model*. 2010; 50:1970–85. [PubMed: 20936866]
20. Chien EY, Liu W, Zhao Q, Katritch V, Han GW, Hanson MA, Shi L, Newman AH, Javitch JA, Cherezov V, Stevens RC. Structure of the human dopamine D3 receptor in complex with a D2/D3 selective antagonist. *Science*. 2010; 330:1091–5. [PubMed: 21097933]
21. Himo F, Lovell T, Hilgraf R, Rostovtsev VV, Noodleman L, Sharpless KB, Fokin VV. Copper(I)-catalyzed synthesis of azoles. DFT study predicts unprecedented reactivity and intermediates. *J Am Chem Soc*. 2005; 127:210–6. [PubMed: 15631470]
22. Luedtke RR, Freeman RA, Boundy VA, Martin MW, Huang Y, Mach RH. Characterization of (125)I-IABN, a novel azabicyclononane benzamide selective for D2-like dopamine receptors. *Synapse*. 2000; 38:438–49. [PubMed: 11044891]

23. Lane JR, Chubukov P, Liu W, Canals M, Cherezov V, Abagyan R, Stevens RC, Katritch V. Structure-based ligand discovery targeting orthosteric and allosteric pockets of dopamine receptors. *Mol Pharmacol*. 2013; 84:794–807. [PubMed: 24021214]
24. Banala AK, Levy BA, Khatri SS, Furman CA, Roof RA, Mishra Y, Griffin SA, Sibley DR, Luedtke RR, Newman AH. N-(3-Fluoro-4-(4-(2-methoxy or 2,3-dichlorophenyl)piperazine-1-yl)butyl)arylcarboxamides as Selective Dopamine D3 Receptor Ligands: Critical Role of the Carboxamide Linker for D3 Receptor Selectivity. *J Med Chem*. 2011

WC 10

$D_3 = 0.8 \pm 0.1$ nM
 $D_2 = 34.4 \pm 3.3$ nM
 $D_2/D_3 = 43$

WC 44

$D_3 = 2.4 \pm 0.4$ nM
 $D_2 = 54.5 \pm 4.4$ nM
 $D_2/D_3 = 23$

Figure 1.
Structure and D_2/D_3 Receptor Binding Data for Compounds WC 10 and WC 44.

Figure 2.
Comparison between Compound 4a and 15

Figure 3. Binding of WC 10 to the Human D₃ Dopamine Receptor

The binding of **WC 10** and the human D₃ receptor model is shown. A stick model is shown for the backbone atoms of the D₃ receptor. Oxygen is depicted in red and the nitrogen atoms are depicted in blue. The hydrogen bond between the C181 carbonyl and the amide nitrogen of **WC 10** is shown as a dashed green line. This hydrogen bond appears to be a pivotal interaction for the binding of arylamide phenylpiperazines. The triazole and isoxazole analogues are unable to make this pivotal hydrogen bond. Therefore an interaction that is essential for guiding the aryl moiety of the benzamide towards a second binding pocket has been eliminated.

Figure 4. Binding Modes for Triazole Analogues of WC 10 with Varying Carbon Spacer Lengths in the Human Dopamine D₃ Receptor Binding Site

A comparison of the alignments of the triazole analogues (**3f**, **4f**, **5f**), with **WC 10** in the D₃ dopamine receptor binding site is shown. Oxygen is depicted in red and the nitrogen atoms are depicted in blue. The hydrogen bond between the carbonyl group of C181 and the amide nitrogen of **WC 10** is shown as a dashed green line. A composite comparison of the binding **3f**, **4f** and **5f** in the D₃ receptor binding site is shown.

A. D₂ Receptor

B. D₃ Receptor

Figure 5. Structural Alignment of WC and the Triazole Analogues as They Occupy the D₂ and D₃ Dopamine Receptor Binding Sites

A structural alignment of **WC 10** with triazole compounds **3f**, **4f** and **5f** is shown as they occupy the D₂ and D₃ dopamine receptor binding sites. Oxygen is depicted in red and nitrogen atoms are depicted in blue. Carbon atoms are colored in white for **WC 10**, in cyan for **3f**, in yellow for **4f**, and in green for **5f**. Hydrogen atoms are undisplayed for clarity. The contours are displayed at the 80% favored and 20% disfavored level. **A.** The triazole analogues **3f**, **4f**, and **5f** aligned together with **WC 10** at the D₂ receptor binding pocket. Each of the ligands assumes a bent configuration. **B.** The triazole analogues (**3f**, **4f**, and **5f**) together with **WC 10** are illustrated by a stick model in the hydrophobic molecular field map

based on our previously generated CoMSIA1 model for D₃ receptor. When bound to the D₃ receptor, each of the ligands assume a more linear configuration than that observed for D₂ receptor binding. Increased binding affinity is correlated with more hydrophobic near orange, less hydrophobic near violet.

Reagents and Conditions:

- (a) 1:1 HCl, NaNO₂; CH₃COONa, NaN₃
- (b) *p*-TsCl, Et₃N, CH₂Cl₂, 0°C
- (c) 1-(2-methoxyphenyl)piperazine, NaCO₃, CH₃CN, reflux
- (d) Azides (**1a - 1h**), 5mol% CuSO₄, 10mol% Sodium Ascorbate, 1:1 *tert*-Butanol: H₂O

^aSynthesis of 2d is different from that of 2a and 2b. The procedure is described in supplementary information

Scheme 1.
Synthesis of Triazole Analogues

Table 1

D₂, D₃, D₃, 5-HT_{1A} Affinities and Log D Values of Triazole Analogues

Ar	Carbon Spacer	Compound number	D ₂ (K _i nM)	D ₃ (K _i nM)	D ₂ /D ₃ Ratio	D ₄ (K _i nM)	D ₄ /D ₃ Ratio	5-HT _{1A} (K _i nM)	Log D ^b
3	3a	64.0 ± 11.4	22.2 ± 3.8	2.9				3.8
	4	4a	30.9 ± 3.9	4.7 ± 0.4	6.6				3.9
	5	5a	19.4 ± 2.5	3.1 ± 0.2	6.3				4.7
3	3b	79.3 ± 5.3	36.3 ± 1.3	2.2				3.5
	4	4b	42.3 ± 5.2	3.7 ± 0.5	11.5	875 ± 117	237	6.0 ± 1.3	3.7
	5	5b	17.7 ± 0.7	1.8 ± 0.3	9.8	186 ± 23	103	5.8 ± 1.5	4.4
3	3c	158 ± 15	8.9 ± 2.1	17.7	90 ± 19	10	7.4 ± 0.5	5.2
	4	4c	94.3 ± 12.7	8.2 ± 1.8	11.6	395 ± 13	48	1.3 ± 0.2	5.4
	5	5c	109 ± 19	6.3 ± 1.2	17.3	163 ± 10	26	153 ± 12	6.1
3	3d	73.5 ± 9	26.5 ± 1.1	2.8				3.8
	4	4d	46.7 ± 1.2	6.5 ± 0.7	7.2				4.0
	5	5d	23.7 ± 4.2	3.2 ± 0.5	7.4				4.8
3	3e	272 ± 42	93.1 ± 8.8	2.9				3.7
	4	4e	139 ± 3	25.4 ± 2.3	5.5				3.8
	5	5e	62.7 ± 7.6	21.1 ± 1.9	3.0				4.6

Ar	Carbon Spacer	Compound number	D ₂ (K _i nM)	D ₃ (K _i nM)	D ₂ /D ₃ Ratio	D ₁ (K _i nM)	D ₄ /D ₃ Ratio	5-HT _{1A} (K _i nM)	Log D ^b
3	3f	162 ± 13	29.6 ± 5.2	5.5				3.9
	4	4f	171 ± 13	13.9 ± 1.9	12.4	758 ± 23	55	9.4 ± 1.1	4.0
5	5f	62.6 ± 6.1	6.7 ± 0.6	9.3				4.8
	3	3g	44.1 ± 2.0	9.6 ± 2.3	4.6				4.2
4	4g	37.7 ± 4.2	2.1 ± 0.3	18.1	200 ± 26	95	5.3 ± 0.6	4.4
	5	5g	26.6 ± 3.6	2.3 ± 0.3	11.6	227 ± 14	99	3.9 ± 0.9	5.2
3	3h	12.8 ± 1.7	6.8 ± 0.84	1.9				3.0
	4	4h	9.4 ± 1.1	2.9 ± 0.6	3.2				3.1

^b Calculated using ACD log D software, Advanced Chemistry Development, Toronto, Canada. All K_i values for the affinity at D₂ and D₃ receptors are reported as nanomolar values and are the mean value ± S.E.M. for n > 3.

Table 2Intrinsic Activity of Selected Analogues at Dopamine D₂ and D₃ Receptors^c

Compound	hD ₂ HEK	hD ₃ HEK
Haloperidol	-0.6 ± 1.6	4.0 ± 5.5
WC 10	33.5 ± 3.1	18.7 ± 2.2
WC 44	35.3 ± 1.0	96.2 ± 4.2
3c	53.8 ± 6.1	50.5 ± 9.9
4b	49.0 ± 3.2	48.2 ± 5.4
4c	67.8 ± 5.3	72.4 ± 4.4
4f	38.8 ± 7.6	107.0 ± 19.3
4g	41.1 ± 8.8	39.4 ± 5.0
5b	43.1 ± 5.4	25.9 ± 6.3
5c	31.4 ± 5.0	67.8 ± 15.6
5g	75.4 ± 5.5	82.2 ± 10.3
Quinpirole	100	100

^cThe intrinsic activity of the test compounds was evaluated by determining the percent inhibition of a forskolin-dependent whole cell adenylyl cyclase assay. The results were normalized to the percent inhibition obtained using the full agonist quinpirole at human D₂ (1 μM) and D₃ (100 nM) receptors expressed in stably transfected HEK 293 cells. For D₂ receptors the maximum inhibition was ±90% and for D₃ receptors the maximum inhibition ranged from 38 to 53%. The test drug was used at a concentration equal to approximately 10× the K_i value that was determined from the radioligand binding analysis. The mean ± the S.E.M. values are reported for n = 3.