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Design, synthesis, and evaluation of N-(4-(4-phenyl piperazin-1-yl)butyl)-4-(thiophen-3-yl)benzamides as selective dopamine D_3 receptor ligands

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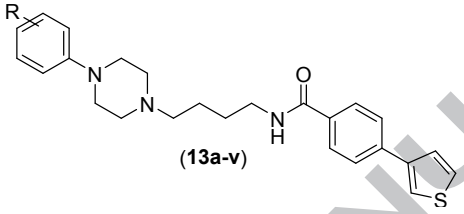

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ABSTRACT

As part of our on-going effort to explore the role of dopamine receptors in drug addiction and identify potential novel therapies for this condition, we have a identified a series of N-(4-(4-phenyl piperazin-1-yl)butyl)-4-(thiophen-3-yl)benzamide D_3 ligands. Members of this class are highly selective for D_3 versus D_2, and we have identified two compounds (**13g** and **13r**) whose rat in vivo IV pharmacokinetic properties that indicate that they are suitable for assessment in in vivo efficacy models of substance use disorders.

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Drug addiction is a major societal and medical issue. The scope of this issue in the U.S. was quantified in the National Survey on Drug Use and Health which reported that in 2014 over 27 million people over the age of 12 had consumed an illicit drug in the past 30 days. The same survey indicated that there were >7.1 million people in the U.S. suffering from addiction to illicit drugs (e.g. cocaine, heroin, oxycodone, etc.) and that the annual societal cost of dealing with this issue exceeds $600 billion.[1] The impact on individual health and wellness is also significant, as illicit drug use is linked to increased rates of numerous diseases and conditions including cardiovascular disease, stroke, cancer, lung disease, as well as HIV and hepatitis infection.[2] The impact of drug addiction extends beyond the patient, as family members, friends, and co-workers often deal with the impact of the patient's drug use. Unfortunately, there are few treatment options available to address drug addiction, and the substantial risk of relapse further complicates patient care.

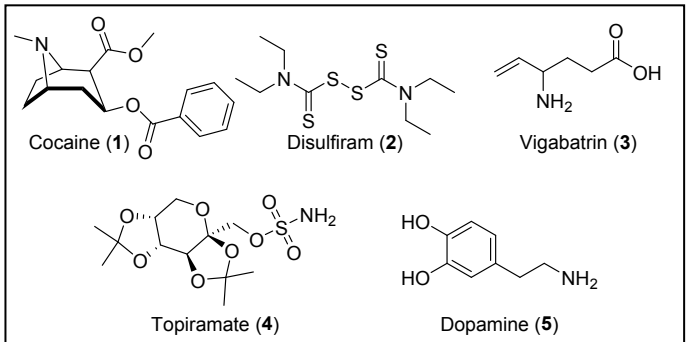


Figure 1:Structures of Cocaine (**1**), Disulfiram (**2**), Vigabatrin (**3**), Topiramate (**4**), and Dopamine (**5**).

Cocaine (**1**) is one of the most commonly abused illicit drugs. This addictive psychostimulant was isolated from the coca plant (Erythroxylon coca) over 100 years ago,[3] and while there are some validated medical uses (e.g. local anesthetic for surgeries of the eyes, ears, nose, and throat), its primary use is as a recreational, illicit drug. As of 2014, over 1.5 million people in the U.S. reported being current user of cocaine, which can be either injected or snorted as the HCl salt or smoked as the free base (crack cocaine). Irrespective of the delivery mechanism, once cocaine enters the brain, it interferes with the mesocorticolimbic dopamine (MCL-DA) system. Under normal conditions, dopamine is released by pre-synaptic neurons and interacts with dopamine receptors on post-synaptic neurons. The dopamine transporters (DAT) clear dopamine from the synapse.[4] Cocaine interferes with this process by preventing dopamine uptake by DAT, and the resulting increase in synaptic dopamine amplifies dopamine signaling and creates the euphoric feeling associated with cocaine exposure. Chronic cocaine exposure leads to changes in expression of DAT and dopamine receptors. These changes in dopamine receptor expression may contribute to the increasing level of cocaine required to deliver the same euphoric effects as chronic use continues. In addition to euphoria, cocaine also has negative impact on the decision making process, which often leads to feeling of anxiety, panic, paranoia, and violent behavior. It also increases heart rate, blood pressure, and body temperature.[5] As the cycle of cocaine exposure continues, the dose required by addicts rises, and the risk of overdose increases. The impact of cocaine overdoses is evident in the 2011 Drug Abuse Warning Network (DAWN) report which revealed that over 500,000 patients required hospital emergency department services as a result of cocaine use.[6]

While the magnitude of this problem is clear, therapeutic options are limited. Behavioral interventions, such as contingency management programs provide rewards for abstinence have produced positive benefits in some patients. Similarly, cognitive-behavioral therapies that teach recovering addicts to recognize and avoid situations that will trigger the desire to use cocaine can be beneficial. These methods, however, are limited by high costs and the lack of sufficient resources to address the full scope of patients requiring treatment.[7] Ideally, pharmacological treatments could be paired with behavioral intervention programs, but there are no FDA approved medicines for the treatment of cocaine addiction. Several research teams have focused on developing novel therapies or reapplying existing therapies to the problem of substance abuse. Disulfiram (**2**), a medication approved for the treatment of alcoholism, has shown promise in a limited patient population, but the mechanism of action of this medication remains a mystery.[8] The antiepileptic irreversible inhibitor of GABA transaminase Vigabatrin (**3**),[9] and the AMPA/Kainate receptor antagonist Topiramate (**4**)[10] have also been examined in clinical trials, but neither have been approved for use as treatments for cocaine addiction. There have also been reports of positive results in animal studies with compounds that selectively target the neurokinin-1 receptor,[11] the cysteine glutamate antiporter,[12] and the 5-HT_{2C} serotonin receptor.[13]

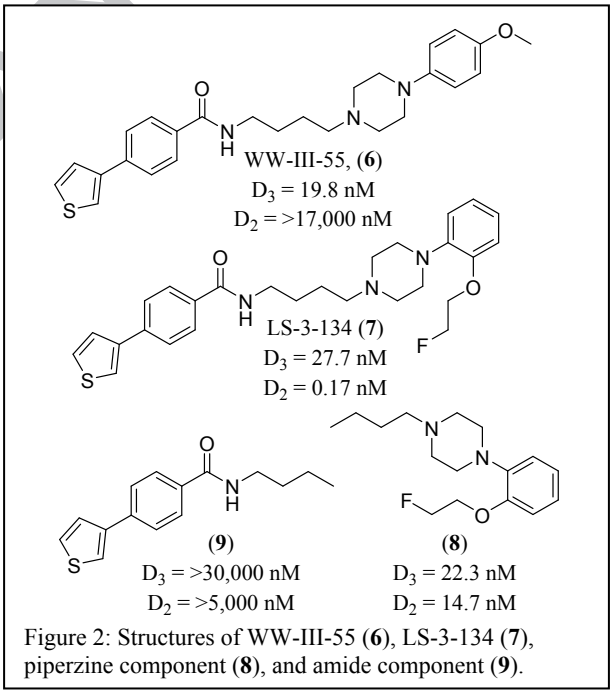
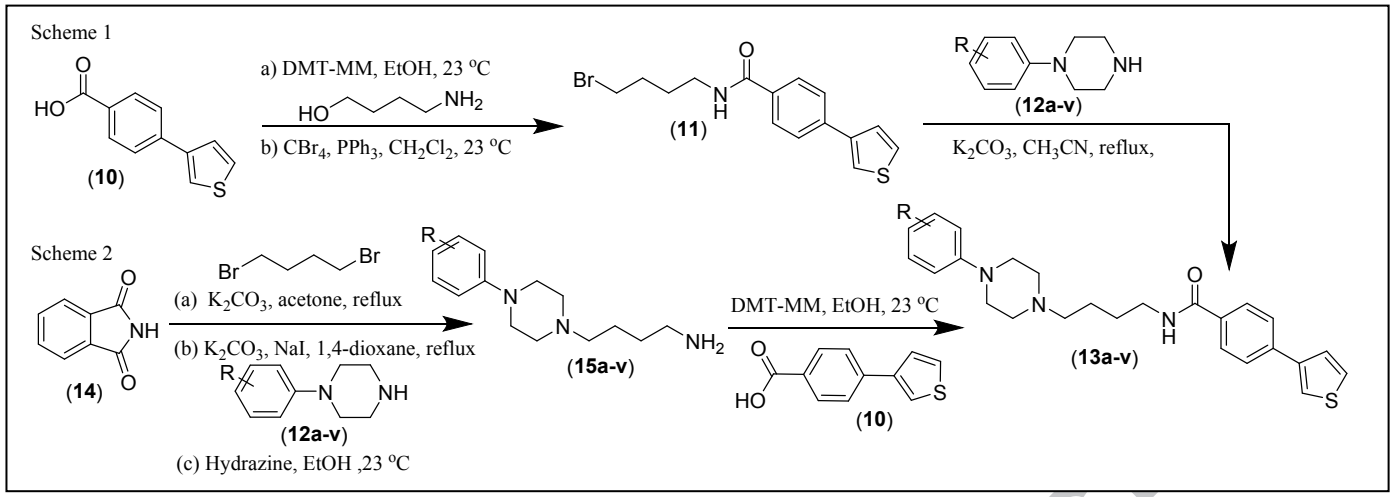


Figure 2: Structures of WW-III-55 (**6**), LS-3-134 (**7**), piperzine component (**8**), and amide component (**9**).

As part of our on-going efforts to identify potential novel therapies for the treatment of cocaine addiction, we have been exploring the impact of modulating dopamine signaling using dopamine D_3 receptor ligands. Dopamine (**5**), a neurotransmitter that is synthesized in the brain and the periphery, has been linked to a wide range of physiology. In the periphery, for example, this compound can act as a vasodilator, modulate renal sodium excretion, and impact urine output. In the central nervous system, dopamine (**5**) is known to have a significant impact on learning, movement, and behavioral motivations.[14] At the cellular level, dopamine initiates signaling via the action of dopamine receptors.



The dopamine family of G-protein coupled receptors (GPCRs) has 5 members designated D_1, D_2, D_3, D_4, and D_5 that can be further sub-divided into two classes, D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) receptors. These classifications are based on genetic organization, amino acid homology and pharmacological properties.[15] The D_3 receptor has been the subject of intense interest as a potential therapeutic target for the treatment of cocaine addiction based on multiple lines of evidence. It is known, for example, that both chronic and acute exposure to cocaine leads to increased D_3 expression in the nucleus accumbens. This region is responsible for cognitive processing including behaviors associated with cocaine addiction.[16] In addition, D_3 is highly expressed in key regions of the brain linked to cocaine addiction. Multiple reviews describing the role of D_3 in cocaine addiction are available.[17] The D_2 receptor has also been implicated in cocaine addiction. This target has been explored as a potential therapeutic target,[18] but modulation of D_2 signaling is also associated with serious side effects (e.g. extrapyamidal symptoms, catalepsy).[19] These risks lead us to focus on the development of compounds with a high degree of selectivity for D_3 over the D_2 dopamine receptor.

We have previously identified WW-III-55 (**6**) as a potent D_3 ligand that is highly selective for D_3 over D_2 (D_3 K_i = 19.8 nM, D_2 K_i >17,000 nM, D_2/D_3 > 858)[20] and a fluorinated analog (**7**) that when radiolabeled with an ^{18}F can be used in PET tracer studies.[21] As part of these studies, we hypothesized that the phenylpiperazine region is critical to binding, while the high degree of selectivity observed in these compounds was driven by the incorporation of the phenyl thiophene region. We based these hypotheses on the observations that the n-butylpiperazine component (**8**) binds to both D_2 (K_i = 14.7 nM) and D_3 (K_i = 22.3 nM) with similar affinity, while the amide component (**9**) binds with low affinity at these dopamine receptors. In addition, our previously reported molecular dynamics and docking studies of the (**7**) and (**8**) show that the piperazine nitrogens of these compounds undergo a key electrostatic interaction with $Asp^{3.32}$ that is not available to the amide component (**9**).[22]

We recently turned our attention to developing a better understanding of the impact of the piperazine region of WW-III-55 (**6**) with the aim of identifying compounds with *in vivo* pharmacokinetic properties supportive of oral dosing in rat models of cocaine addiction. A series of analogs was prepared beginning with either 4-(thiophen-3-yl)benzoic acid (**10**, scheme 1) or phthalimide (**14**, scheme 2). Specifically, 4-(thiophen-3-yl)benzoic acid (**10**) is converted to an amide by reaction with 4-aminobutan-1-ol using the coupling agent 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride (DMT-MM), and the resulting compound is converted to a primary bromide (**11**) using carbon tetrabromide and triphenylphosphine. Nucleophilic displacement of the bromide with piperazines (**12a-v**) under basic conditions (K_2CO_3, CH_3CN) provides the desired products (**13a-v**). Alternatively, alkylation of phthalimide (**14**) with 1,4-dibromobutane under basic conditions (K_2CO_3, acetone) produces the corresponding primary alkyl bromide. This is followed by nucleophilic displacement of the remaining bromide with piperazines (**12a-v**) under basic conditions (K_2CO_3, 1,4-dioxane) in the presence of sodium iodide. Removal of the phthalimide with hydrazine provide the primary amine intermediate (**15a-v**), which can be coupled with 4-(thiophen-3-yl)benzoic acid (**10**) using DMT-MM to provide the desired final compounds (**13a-v**).

Table 1: 1st tier *In vitro* pharmacology of (**13a-v**)

#	R	D_3	D_2	D_2/D_3	RLM $T_{1/2}$
		K_i (nM)			(min.)
13a	H	8	8434	1054	24.6
13b	2-Cl	0.55	169	309	3.5
13c	3-Cl	8.3	12046	1451	28.1
13d	4-Cl	32.7	27359	836	50.2
13e	2,3-Cl	0.89	63.1	70.8	14.4
13f	2,4-Cl	3.8	757	198	18
13g	3,5-Cl	0.44	2049	4615	>60
13h*	2-OCH_3	0.20	38.6	193	2.4
13i	3-OCH_3	18.0	1832	102	9.3
13j*	4-OCH_3	19.8	17098	862	>60
13k*	2-OH	16.7	3449	206	9.4
13l	3-OH	0.47	140	301	5.1
13m	4-OH	13.6	4753	349	11.4
13n	2-CH_3	0.68	190	281	3.7
13o	3-CH_3	21.9	16318	744	22.3
13p	4 CH_3	36.1	24994	692	7.5
13q	2-CN	0.25	92.9	369	2
13r	3-CN	0.50	743	1486	>60
13s	4-CN	2,378	>20000	8.4	13.2
13t	2-CF_3	1.5	261	172	4
13u	3-CF_3	0.41	99.7	243	16.9
13v	4-CF_3	78.3	>30000	384	>60

*13h, 13j and 13k were previously by Mach et. al. [20, 23]

In vitro competitive D_3 and D_2 radioligand binding assays and rat liver microsome (RLM) stability studies were used to identify potential lead compounds for further examination. As indicated in

Table 1, electron donating and electron withdrawing substituents on the phenyl ring of the aryl piperazine were well tolerated by the D_3 dopamine receptor as indicated by the potent binding observed (K_i < 100 nM). There are, however, notable differences in D_3 binding affinity. In the 2-position, for example, analogs with electron donating and withdrawing substituents (**13b**, **13h**, **13n**, **13q**, **13t**) demonstrated D_3 receptor K_i values between 0.2 nM and 2.0 nM with the notable exception of the 2-OH analog (**13k**, D_3 K_i = 16.7 nM). Sub-nanomolar potency was observed in several analogs with substituents in the 3-postion of the phenyl ring of the aryl piperazine (**13l**, 3-OH, D_3 K_i = 0.47 nM, **13r,** 3-CN, D_3 K_i = 0.50 nM, **13u**, 3-CF_3 D_3 K_i = 0.41 nM), but other analogs bound with substantially lower affinity (**13c**, 3-Cl, D_3 K_i = 8.3 nM, **13i**, 3-OCH_3 D_3 K_i = 18.0 nM, **13o**, 3-CH_3, D_3 K_i = 21.9 nM). Analogs with substituents in the 4-position displayed the lowest level of D_3 binding affinity, but the majority of analogs examined had D_3 receptor K_i values <100 nM. Electron withdrawing (**13d**, 4-Cl, D_3 K_i = 32.7 nM, **13v**, 4-CF_3, D_3 K_i = 78.3 nM) and electron donating substituents (**13j**, 4-OCH_3, D_3 K_i = 19.8 nM, **13m**, 4-OH, D_3 K_i = 13.6 nM, **13p**, 4-CH_3, D_3 K_i = 36.1 nM) were well tolerated, but the 4-CN analog (13s) bound with substantially lower affinity (D_3 K_i = 2378). This loss of binding affinity may be the result of unfavorable steric interactions or possibly the result of negative electrostatic interaction between the highly polarized cyano group and amino acid residues in the D_3 receptor binding pocket.

The impact of the same structural changes on D_2 binding demonstrated some key differences in the structure activity relationship (SAR) of this receptor in comparison to D_3. Substitution in the 4-position of the phenyl ring of the aryl piperazine lead to substantial losses in D_2 receptor binding irrespective of the electron character of the substituent. Electron withdrawing substituents in the 2-position produced (**13b**, **13q** and **13t**) moderate affinity (K_i = 169 nM, 92.9 nM, and 261 nM respectively), while electron donating groups produced mixed results. The non-polar 2-methyl (**13n**) and 2-methoxy (**13h**) substituents demonstrated moderate D_2 receptor binding (K_i = 190 nM and 38.6 nM), but the more polar 2-hydroxy substituent (**13k**) has substantially lower affinity (K_i = 3449 nM). Finally, substitution in the 3-position only produced moderate affinity when an OH (**13l**, K_i = 140 nM) or CF_3 (**13u**, K_i = 99.7 nM) moiety was installed. Other substituents, both electron withdrawing and electron donating lead to decreased in D_2 binding affinity (K_i = 743 to 16318 nM). One possible explanation for these observations may be that the 3-OH and the CF_3 group are both acting as hydrogen bond acceptors in the D_2 receptor binding site, thereby stabilizing the interaction.

The impact of the differences in the SAR of D_3 and D_2 binding sites are evident when the binding affinities of individual compounds at both receptors are considered. While we have previously demonstrated that the 4-(thiophen-3-yl)benzene moiety is a significant driver of D_2/D_3 receptor selectivity, it is clear that the phenyl piperazine region of the molecule can also have a significant impact on selectivity. Altering the type and position of functional groups in this region of the molecule produces compounds with significantly different D_2/D_3 binding selectivity profiles. The 4-methoxy analog (**13j**), for example, is 862 fold more potent at D_3 vs D_2, but the 2-methoxy (**13h**, previously reported as OS-3-106[20]) and 3-methoxy (**13i**) analogs are significantly less selective (193 fold and 102 fold D_3 vs. D_2 selectivity respectively). In addition, varying the selection of

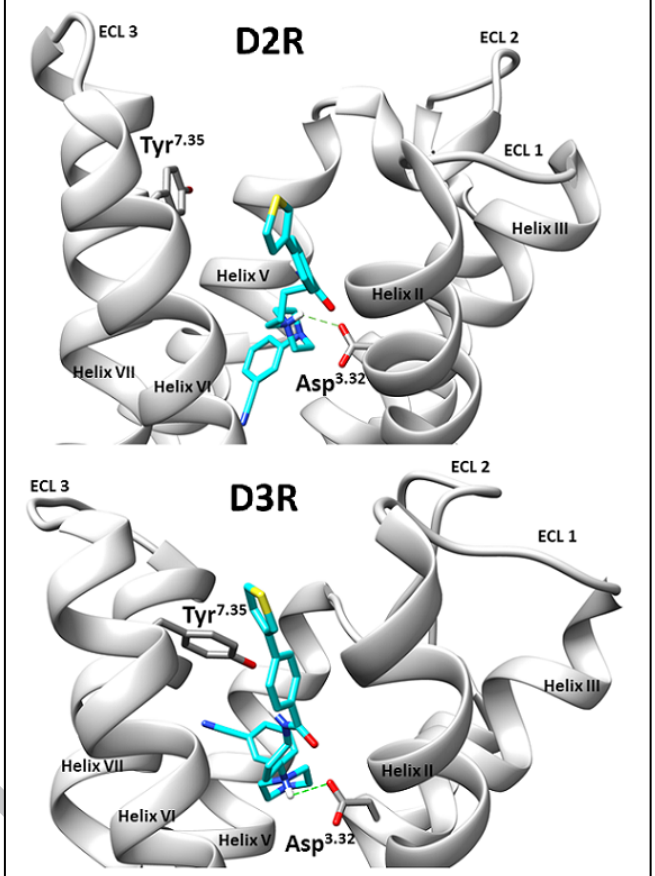


Figure 3: Docking pose of (**13r**) in D_2 (top) and D_3 (bottom). Salt bridges are shown as green dashed lines. Only key hydrogen atoms are shown for clarity.

substituents in the 2-position produces compounds with D_2/D_3 selectivity profiles ranging from 172 to 369-fold, while some 3-substituted analogs are >1400-fold selective for D_3 over D_2 receptor binding (3-CN: **13r** and 3-Cl: **13c**). The highest degree of D_3/D_2 selectivity was observed when two chlorine atoms were appended to the phenyl ring of the phenyl piperazine, but positioning of these substituents was critical to selectivity. Specifically, the 2,3-dichloro analog (**13e**) is 70-fold selective for D_3 vs. D_2, the 2,4-dichloro analog (**13f**) is 2.8 times more selective (198 fold), and the 3,5-dichloro (**13g**) analog is over 65 times more selective (4615 fold) than the 2,3-dichloro analog (**13e**). The differences in D_3 and D_2 SAR allowed us to identify multiple compounds with D_3 binding potency below 10 nM and >1000 fold selectivity vs. D_2 (**13a**, **13c**, **13g**, and **13r**).

To understand how the pockets of D_2 and D_3 provide selective binding of these ligands, we used Autodock vina to dock (**13r**) on the crystal structures of D_2 (PDB code 6C38[24]) and D_3 (PDB code 3PBL[25]). The highest energy poses with an interaction between the protonated nitrogen and Asp 3.32 and orthosteric position of phenylpiperazine pharmacophore are shown in Figure 3. A source of better affinity for the ligand for D_3 may be the potential interaction with Tyr 7.35. In D_3, the amide oxygen of (**13r**) could form a hydrogen bond with Tyr 7.35 in some configurations of the linker rotatable bonds. In three copies of 300 ns molecular dynamics simulation of this molecule in complex with D_3

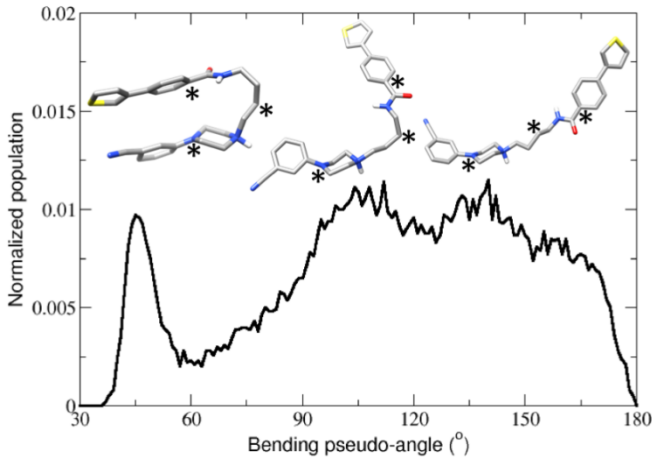


Figure 4: Histogram of the bending pseudo-angle of the ligand (**13r**) in the trajectory of 1 microsecond simulation. Representative molecular conformations corresponding to each region of the histogram are shown with asterisks indicating the atoms that form the pseudo-angle.

(unpublished results), we observed the formation of this new interaction. We have also previously reported that the overall ligand conformation might be important to D_3 binding affinity.[22] In this instance, we studied the conformational ensemble of (**13r**) in a 1 microsecond molecular dynamics (MD) simulation. A bending pseudo-angle between three atoms in the benzamide region and the phenyl piperazine region (denoted by asterisk in Figure 4) was measured as representative of the overall structure. The histogram of this pseudo-angle shows small population of the folded conformation in the MD ensemble in comparison to the large population of the extended and bent conformations (Figure 4), similar to the conformation population distribution of other high affinity ligands in our previous work,[22] suggesting that extended conformation of (**13r**) could explain its high affinity for D_3.

Table 2: 2nd tier *In vitro* pharmacology of (**13g**) and (**13r**)

#	HLM $T_{1/2}$	D_1	D_4	D_5	CYP 2C9	CYP 2D6	CYP 3A4
	(min)	Ki (nM)			IC_{50} (nM)		
13g	60	>10000	>10,000	>10000		>10000	>10000
13r	60	>10000	806	>10000	>10000	>10000	>10000

We further characterized all of the analogs by determining their stability ($T_{1/2}$) in rat liver microsomes (RLMs). These *in vitro* experiments can be used as predictive tools to identify compounds that may have an *in vivo* pharmacokinetic profile capable of supporting *in vivo* efficacy studies.[26] RLM stability ($T_{1/2}$) of this series ranged from as low as 2.4 minutes to as long as 60 minutes. Only 2 compounds, (**13g**) and (**13r**), had RLM $T_{1/2}$ of 60 minutes and D_3 selectivity over D_2 >1000-fold. These compounds were selected for further evaluation. As seen in Table 2, *in vitro* human liver microsome (HLM) studies indicated that both of these compounds are highly stable ($T_{1/2}$ = 60 min.) and had minimal interactions the major metabolic Cyp450 enzymes (Cyp 3A4, 2D6, and 2C9).

We also examined the activity of (**13g**) and (**13r**) in D_3 functional assays using a forskolin-dependent adenylyl cyclase inhibition assay and a β-arrestin binding assay with quinpirole and haloperidol as a prototypic full agonist and antagonist, respectively. Compounds were tested for efficacy in these assay at a dose equal to 10x their D_3 Ki values in order to ensure >90% receptor occupancy and the results were compared with the impact of the full agonist. While neither compound demonstrated activity in the β-arrestin binding assay, both compounds demonstrated partial agonism in the forskolin-dependent adenylyl cyclase inhibition assay. (**13g**) and (**13r**) produced 36.5% (mean ±15.5 S.E.M., n =3) and 19.4% (mean ±5.4 S.E.M., n=3) of the activity of the maximum response observed with quinpirole.

In addition, *in vitro* screening for D_1, D_4, and D_5 binding indicated that both compounds have a high degree of selectivity for D_3 over these three dopamine receptors (**13r** has the lowest selectivity versus D_4 but it is >1600-fold selective for D_3 over D_4). Further evaluation via the Psychoactive Drug Screening Program (PDSP) provided *in vitro* selectivity data for a wide range of targets (Receptors: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7, α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}, benzodiazepine, β_1, β_2, β_3, GABA-A, H_1, H_2, H_3, H_4, M_1, M_2, M_3, M_4, M_5, δ-Opioid, κ-Opioid, μ-Opioid, Sigma-1, Sigma-2. Transporters: dopamine, norepinephrine, serotonin.). Both compounds were highly selective for D_3 over the majority of targets (K_i's > 10,000 nM), but (**13g**) displayed moderate affinity at 5-HT_{1a} (245 nM, 556 fold vs D_3) and (**13r**) had affinity for 5-HT_{2C} (20 nM, 40 fold vs D_3), 5-HT_7 (187 nM, 374 fold vs D_3), H_1, (3112 nM, 6224 fold vs D_3), and SERT (118 nM, 236-fold vs D_3).

Table 3: Rat pharmacokinetic properties of (**13g**) and (**13r**)

#	IV Dose	AUC	$T_{1/2\beta}$*	CL	Vss
	(mg/kg)	(ug/ml*h)	(h)	(mL/min/kg)	(L/kg)
13g	1	0.21	15.3	66.11	55.67
13r	1	0.22	10.9	74.44	28.57

*Terminal $T_{1/2}$

In vivo pharmacokinetic (PK) assessment of both (**13g**) and (**13r**) was conducted in Sprague Dawley rats. As noted in table 3, long terminal $T_{1/2}$'s were observed with both compounds (15.3 and 10.9 hours respectively) when an IV dose of 1 mg/kg was administered. The volume of distribution of (**13g**) was nearly double that of (**13r**), while clearance and AUC were similar. Finally, protein binding studies indicated that both compounds are highly protein bound (>99%).

In summary, we have prepared a series of N-(4-(4-phenyl piperazin-1-yl)butyl)-4-(thiophen-3-yl)benzamide and evaluated their properties in a range of *in vitro* assays. Our studies demonstrate that the aryl piperazine region of the molecule can have a substantial impact on D_3 vs D_2 selectivity, as various substituent patterns produced selectivity ranging from 70.8- to 4615-fold. In addition, we identified two compounds (**13g** and **13r**) that are highly selective for D_3 across a range of pharmacological targets. In addition, rat *in vivo* IV PK studies that indicate that these compounds are suitable for further study to determine brain exposure in advanced PK studies followed by assessment in *in vivo efficacy* models of substance use disorders.

Graphical Abstract

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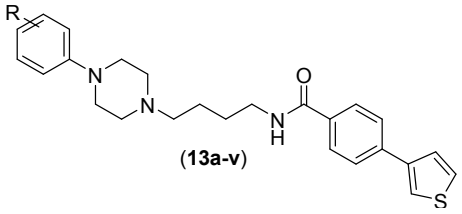

(**13a-v**)

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