ACS Medicinal Chemistry Letters

Letter

Structure-Functional-Selectivity Relationship Studies of Novel Apomorphine Analogs to Develop D1R/D2R Biased Ligands

Hyejin Park, Aarti N. Urs, Joseph Zimmerman, Chuan Liu, Qiu Wang, and Nikhil M Urs

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.9b00575 • Publication Date (Web): 06 Jan 2020

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Structure-Functional-Selectivity Relationship Studies of Novel Apomorphine Analogs to Develop D1R/D2R Biased Ligands

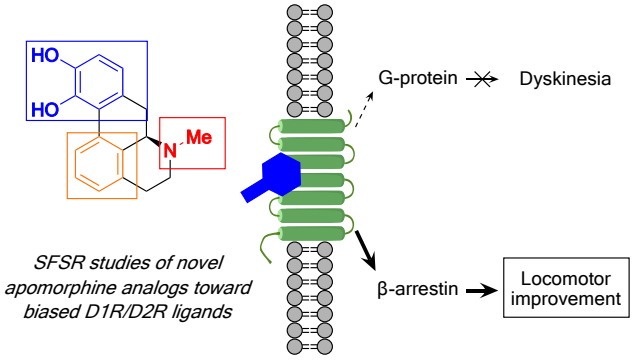
Hyejin Park,[+§] Aarti N. Urs,[‡§] Joseph Zimmerman,[‡] Chuan Liu,[+] Qiu Wang[+]* and Nikhil M. Urs[‡]*

[+]Department of Chemistry, Duke University, Durham, NC 27708

[‡]Department of Pharmacology and Therapeutics, University of Florida, Gainesville FL 32610

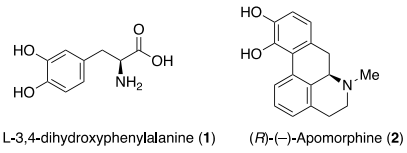
KEYWORDS: apomorphine analog, D1R/D2R, biased ligands, structure-functional-selectivity relationships (SFSR)

ABSTRACT: Loss of dopamine neurons is central to the manifestation of Parkinson's disease motor symptoms. The dopamine precursor L-DOPA, the most commonly used therapeutic agent for Parkinson's disease, can restore normal movement yet cause side-effects such as dyskinesias upon prolonged administration. Dopamine D1 and D2 receptors activate G-protein- and arrestin-dependent signaling pathways that regulate various dopamine-dependent functions including locomotion. Studies have shown that shifting the balance of dopamine receptor signaling toward the arrestin pathway can be beneficial for inducing normal movement, while reducing dyskinesias. However, simultaneous activation of both D1 and D2Rs is required for robust locomotor activity. Thus, it is desirable to develop ligands targeting both D1 and D2Rs and their functional selectivity. Here, we report structure-functional-selectivity relationships (SFSR) studies of novel apomorphine analogs to identify structural motifs responsible for biased activity at both D1 and D2Rs.



Parkinson's disease (PD) is a neurodegenerative disorder caused by degeneration of dopamine neurons and loss of dopamine signaling in the striatum.[1] The loss of dopaminergic neurons in PD causes motor dysregulation, resulting in tremor, rigidity and slowed movements.[2] Dopamine signaling is mediated by two main G-protein-coupled receptors (GPCRs), D1-like (D1R and D5R) and D2-like (D2R, D3R, D4R) receptors, both of which have been therapeutic targets for developing antiparkinsonian and antipsychotic drugs.[3] Although D1 and D2 receptors are involved in similar biological activities, they couple and activate different G-protein complexes, either increasing (D1-like) or decreasing (D2-like) the production of 3`, 5`- cyclic Adenosine Mono Phosphate (cAMP). These receptors are found in similar or unique anatomical regions in the central nervous system, and respond to dopamine with different sensitivity. Thus, targeting different dopamine receptors have distinct therapeutic outcome and clinical efficacy.[3-5]

PD is commonly treated with the dopamine precursor L-3,4-dihydroxyphenylalanine (L-DOPA) or dopamine receptor agonists such as apomorphine (**Figure 1**). However, chronic treatment with L-DOPA, (*R*)-apomorphine or other dopamine receptor agonists often leads to abnormal involuntary movements called dyskinesias (L-DOPA induced dyskinesia, LIDs).[6] In addition to the canonical G-protein coupled pathway, dopamine also activates a distinct β-arrestin mediated pathway.[7, 8] Previous studies have shown that overactivation of the G-protein mediated pathway is associated with the manifestation of dyskinesias.[9] On the other hand, β-arrestin 2 signaling may act as a major contributor in alleviating locomotor abnormalities,[10, 11] as the recruitment of β-arrestin 2 not only desensitizes G-protein signaling, but also activates a G-protein-independent signaling pathway that can induce beneficial locomotor effects.[3, 12, 13] These studies suggest that the side effects of dopamine receptor agonist drugs can be repressed by reducing activation of the G-protein pathway.



L-3,4-dihydroxyphenylalanine (**1**) (*R*)-(–)-Apomorphine (**2**)

Figure 1. Structures of L-DOPA and (*R*)-(–)-Apomorphine

Selective activation of a signaling pathway in GPCRs can be achieved using functionally selective, or so called biased ligands.[14] These biased ligands can selectively activate a single or subset of signaling cascades responsible for therapeutic effects, in contrast to traditional agonists that stimulate all possible responses upon binding to a receptor.[15-17] Since L-DOPA, apomorphine or other dopamine receptor agonists induce dyskinesias and activate both signaling pathways, development of β-arrestin biased agonists provides a strategic therapeutic approach to treat motor symptoms of PD without inducing dyskinesias.[18] There are a number of reported D1R G-protein pathway selective ligands,[19-22] which show therapeutic efficacy but their dyskinesia activity has not been reported. However, there are no known β-arrestin biased D1R agonists to date. Conversely, β-arrestin biased D2R agonists have been reported.[23] Herein, our approach is to target both D1 and D2Rs, since the activation of both D1 and D2Rs is required for a robust locomotor response. We therefore focused our efforts on parent drug scaffolds that are dual D1/D2R agonists.

Our search for novel dual D1/D2R β-arrestin biased agonists started from (*R*)-(–)-apomorphine as a core scaffold (**Figure 2**). Apomorphine, a non-selective unbiased D1R and D2R agonist, has been clinically used to treat PD motor symptoms.[24] We decided to start from (*R*)-(–)-apomorphine in search for novel dual D1/D2R β-arrestin biased agonists for two main reasons. First, with apomorphine as a known PD drug, its structurally close analogs are expected to retain dopamine receptor affinity and therapeutic properties. Second, there have been some structure-activity relationship (SAR) studies in the literature for apomorphine analogs,[25, 26] although there are no structure-functional-selectivity relationship (SFSR) studies.

Herein, we report the synthesis of novel apomorphine analogs and the SFSR examination on how various structural modifications affect biased signaling at D1 and D2Rs. To determine whether modifying various structural elements of apomorphine could result in biased signaling, we divided its scaffold into three primary regions (**Figure 2**). These studies will provide insights toward discovery of β-arrestin biased analogs of apomorphine that can potentially activate locomotion without inducing dyskinesias.

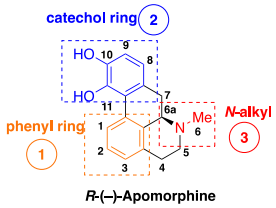
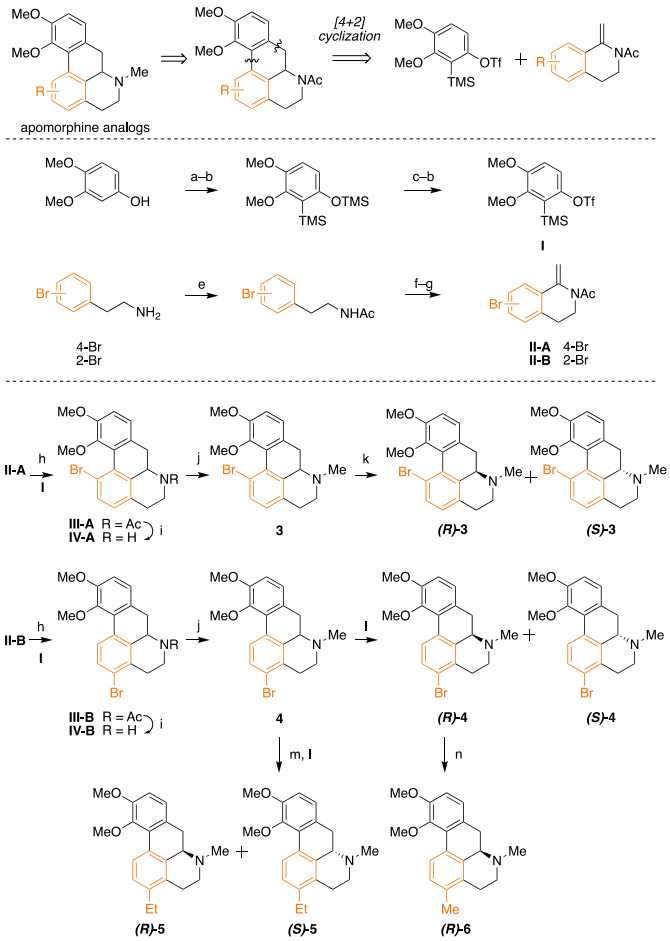


Figure 2. Three regions of apomorphine (**2**) for SFSR studies.

Our studies began with the phenyl ring, which was the least explored structural moiety in previous studies. We chose to create an array of analogs with substituents at either the C-1 or C-3 position, including bromine and alkyl groups. The synthesis of these compounds was achieved by a regioselective [4+2] cyclization of aryne precursor and isoquinoline derivative as a key step to afford the core aporphine ring as described in **Scheme 1**. The aryne precursor **I** and the isoquinoline derivatives **II-A** and **II-B** were synthesized following a previously reported procedure.[27] The CsF-promoted [4+2]-cyclization of isoquinoline **II-A** and **II-B** with 2-trimethylsilylaryl triflate **I** delivered the core intermediates **III-A** and **III-B**, which were hydrolyzed to free amines **IV-A** and **IV-B** under microwave heating. Finally, reductive methylation yielded analogs **3** and **4** which bear a bromine at C-1 and at C-3, respectively. To examine the effects of C-substituents on the phenyl ring, we next performed a cross-coupling reaction of racemic **4** for the synthesis of ethyl-substituted apomorphine derivative **5**, as well as a coupling reaction of ***(R)*-4** for the synthesis of ***(R)*-6**. This synthetic route gave a racemic mixture of apomorphine analogs. Yet the racemic mixture has shown to be less than half as potent as the (*R*)-(–) isomer,[28] since the (*S*)-(+) enantiomer is known to be a DA receptor antagonist that blocks the (*R*)-(–) isomer actions.[29-31] Thus, we separated two enantiomers via either chiral resolution with (+)-DBTA[32] or chiral HPLC to generate enantiopure novel apomorphine analogs ***(R)*-3** and ***(S)*-3, *(R)*-4** and ***(S)*-4,** as well as ***(R)*-5** and ***(S)*-5,** for comparison in the SFSR studies.

All compounds were evaluated for their activities for stimulating G-protein signaling using a cAMP level-dependent chemiluminescent sensor (GloSensor assay, Promega) and β-arrestin 2 recruitment (Bioluminiscence Resonance Energy Transfer or BRET assay) at D1 and D2 receptors using standard *in vitro* dose-response assays (see SI). The results in the tables show the potency (EC_{50}, pEC_{50}) and efficacy (E_{max}) values calculated using the sigmoidal dose-response function in GraphPad Prism 7.0. The percent response values were normalized to dopamine. Transduction coefficients i.e. log (τ/KA) were calculated for each compound at both pathways (G-protein and β-arrestin 2) based on the Black and Leff operational model, where KA is the equilibrium dissociation constant and τ is the agonist efficacy. Bias factors were calculated based on the method of Kenakin et al,[33] where Δlog (τ/KA) for each compound was calculated in relation to the reference agonist dopamine, and the ΔΔlog (τ/KA) i.e bias factor was calculated by subtracting the relative transduction coefficients Δlog (τ/KA) of the two pathways for each compound.

Scheme 1. Synthesis of novel apomorphine analogs 3–6.

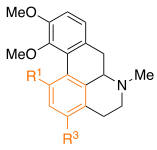


(a) HMDS, 80 °C, 2 h (quant.); (b) LDA, TMSCl, THF, 20 h (96%); (c) TBAF, THF, 0 °C, 15 min (70%); (d) Tf_2O, pyridine, DCM, 0 °C, 18 h (46%); (e) Ac_2O, pyridine, 70 °C, 2 h (97–99%); (f) $(ClOC)_2$, $FeCl_3$, H_2SO_4, DCM, MeOH; (g) Ac_2O, pyridine, 60 °C, 3 h (11–33%); (h) CsF, MeCN, 80 °C, 24 h (26–51%); (i) LiOH, EtOH/H_2O, MW, 180 °C, 40 min (59–83%); (j) CH_2O, $NaBH_4$, MeOH, 1.5 h (43–55%); (k) (+)-DBTA, EtOAc/*i*PrOH; (l) Chiral HPLC (Lux® 5μm Celluclose-1, 250 × 4.6 mm); (m) $Pd(dppf)Cl_2$, $Zn(Et)_2$, 1,4-dioxane, 70 ºC, 23 h (39%, one step); (n) $Pd(dppf)Cl_2$, $Zn(Me)_2$, 1,4-dioxane, 70 ºC, 17 h (29%).

As shown in **Table 1**, both enantiomers of bromine and alkyl substituted apomorphine analogs showed a general reduction in activity in all assays for both receptors. However, compounds **(*R*)-3** and **(*R*)-4** showed weak bias toward G-protein signaling at D1Rs while having no recruitment of β-arrestin 2. Thus, we decided to test antagonist activities of both enantiomers of compounds **3–6** at both D1R and D2Rs (**Fig 3, Table 2**). Interestingly, compounds **(*S*)-3** and **(*S*)-4** showed biased antagonism (comparing IC_{50}'s only) for β-arrestin 2 at both D1 and D2 receptors. The (*S*)-enantiomer of apomorphine is known to be an antagonist at dopamine receptors,[29-31] but this is the first study that shows *biased* antagonism of the (*S*)-enantiomer.

It is known that apomorphine's activity as a dopamine receptor agonist originates from the catechol ring and phenethylamine moiety.[34] Thus, substitutions on the catechol ring may influence the binding of apomorphine at D1/D2R

Table 1. Agonist activities of apomorphine analogs with substituents on C-1 and C-3 of phenyl ring on D1R and D2R.



Cmpd	(S)/(R)	R^1	R^3	β-arrestin			cAMP			
				EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	Bias G
				D1R						
Apo	(R)	H	H	520.8	6.3 ± 0.87	13.02 ± 4.9	3.77	8.4 ± 0.15	94.92 ± 3.17	41.50
(*R*)-3	(R)	Br	H	>10,000	<5.00	N.D.	239.2	6.62 ± 0.45	26.61 ± 3.67	N.C.
(*S*)-3	(S)	Br	H	>10,000	<5.00	N.D.	>10,000	<5.00	N.D.	N.C.
(*R*)-4	(R)	H	Br	>10,000	<5.00	N.D.	150.2	6.82 ± 0.89	19.98 ± 4.96	N.C.
(*S*)-4	(S)	H	Br	>10,000	<5.00	N.D.	385.9	6.41 ± 1.56	0.6325 ± 1.48	N.C.
(*R*)-5	(R)	H	Et	36.86	7.43 ± 2.1	13.67 ± 1.88	>10,000	<5.00	N.D.	N.C
(*S*)-5	(S)	H	Et	246.8	6.60 ± 0.79	15.04 ± 1.65	505.1	6.30 ± 0.65	9.204 ± 2.02	0
(*R*)-6	(R)	H	Me	0.8	9.10 ± 0.45	14.17 ± 0.9	87.44	7.06 ± 0.84	5.099 ± 1.03	N.C
				D2R						
Apo	(R)	H	H	10.1	8.0 ± 0.08	75.0 ± 2.43	1.61	8.8 ± 0.56	60 ± 7.7	2.16
(*R*)-3	(R)	Br	H	55.37	7.26 ± 3.11	7.28 ± 2.25	>10,000	<5.00	N.D.	N.C
(*S*)-3	(S)	Br	H	>10,000	<5.00	N.D.	>10,000	<5.00	N.D.	N.C
(*R*)-4	(R)	H	Br	>10,000	<5.00	N.D.	>10,000	<5.00	N.D.	N.C
(*S*)-4	(S)	H	Br	>10,000	<5.00	N.D.	>10,000	<5.00	N.D.	N.C
(*R*)-5	(R)	H	Et	>10,000	<5.00	N.D.	>10,000	<5.00	N.D.	N.C
(*S*)-5	(S)	H	Et	613.5	6.21 ± 2.86	7.33 ± 2.86	>10,000	<5.00	N.D.	N.C
(*R*)-6	(R)	H	Me	2561	5.59 ± 2.48	9.51 ± 3.6	>10,000	<5.00	N.D.	N.C

EC_{50} and E_{max} values are from three independent experiments performed in duplicate or triplicate. E_{max} values are calculated as % response normalized to dopamine. N.C. not calculable, N.D. not determined.

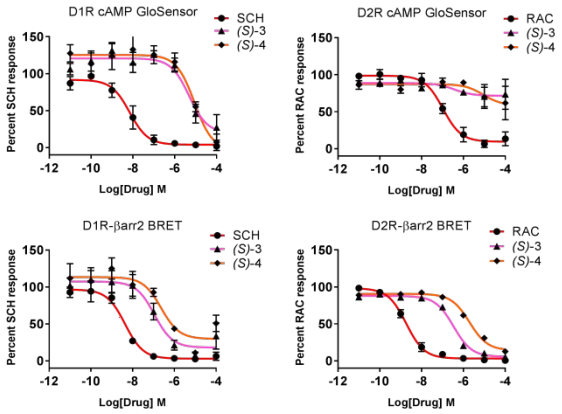


Figure 3. Dose response curves of antagonist activities of bromine substituted apomorphine analogs. *In vitro* GloSensor and BRET antagonist assays at D1Rs and D2Rs for bromine substituted Apo analogs ***(S)*-3** and ***(S)*-4** in HEK-293 cells. D1R antagonist SCH23390 (SCH) and D1R antagonist raclopride (RAC) were used as reference antagonists. Data are presented as percent of the total SCH or RAC response (mean ± SEM).

toward biased signaling. To probe this hypothesis, a series of C-9 substituted apomorphine analogs were prepared by direct functionalization of apomorphine (**Scheme 2**).

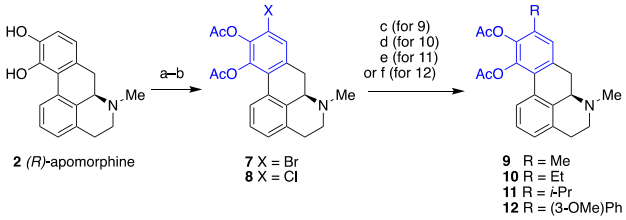
First, the treatment of (*R*)-(–)-apomorphine (**2**) with *N*-bromosuccinimide (NBS) and *N*-chlorosuccinimide (NCS) followed by *O*-acetyl protection readily gave the brominated and chlorinated apomorphine analogs **7** and **8**, respectively (**Scheme 2**). Subsequent coupling reactions of bromoarene **7** afforded apomorphine derivatives **9–12** bearing an alkyl or aryl substituent at C-9 position on the catechol ring (See SI).

Table 2. Antagonist activities of apomorphine analogs with substituents on C-1 and C-3 of phenyl ring on D1R and D2R.

Cmpd	(S)/(R)	R_1	R_2	β-arrestin			cAMP		
				IC_{50} (nM)	pIC_{50} ± SEM	E_{max} (%) ± SEM	IC_{50} (nM)	pIC_{50} ± SEM	E_{max} (%) ± SEM
				D1R					
SCH				4.34	8.36 ± 0.14	93.7 ± 5.23	6.96	8.16 ± 0.16	87.8 ± 6.1
(*R*)-3	(R)	Br	H	4080	5.39 ± 0.44	105.46 ± 9.87	>10,000	4.83 ± 0.24	113.67 ± 5.95
(*S*)-3	(S)	Br	H	110.7	7.0 ± 0.26	90.0 ± 10	3887	5.41 ± 0.25	101.94 ± 6.04
(*R*)-4	(R)	H	Br	815.7	5.83 ± 0.51	69.97 ± 11.1	5115	5.29 ± 0.34	94.27 ± 7.44
(*S*)-4	(S)	H	Br	232.4	6.6 ± 0.32	83.73 ± 11.1	7461	5.13 ± 0.24	112.5 ± 6.9
(*R*)-5	(R)	H	Et	1603	5.8 ± 0.58	71.73 ± 12.5	8590	5.07 ± 0.3	106.47 ± 8.17
(*S*)-5	(S)	H	Et	1522	5.82 ± 0.62	80.48 ± 11.8	>10,000	<5.00	N.D
(*R*)-6	(R)	H	Me	1427	5.85 ± 0.4	78.36 ± 9.78	>10,000	<5.00	N.D
				D2R					
RAC				1.83	8.74 ± 0.07	95 ± 2.76	104.2	7 ± 0.14	89.28 ± 4.9
(*R*)-3	(R)	Br	H	30.35	7.52 ± 0.83	67.4 ± 12.3	0.124	9.91 ± 3.5	13.83 ± 7.7
(*S*)-3	(S)	Br	H	337.5	6.5 ± 0.32	82.33± 9	365.2	6.44±0.94	17.26±6.9
(*R*)-4	(R)	H	Br	45.07	7.35 ± 0.76	71.5 ± 11.1	3222	5.49 ± 0.65	53.88 ± 5.55
(*S*)-4	(S)	H	Br	1957	5.71 ± 0.3	76.53 ± 9.9	8900	5.05±0.74	28.6 ± 12.92
(*R*)-5	(R)	H	Et	28.2	7.55 ± 0.98	60.82 ± 13.4	N.C.	N.C.	N.C.
(*S*)-5	(S)	H	Et	40.77	7.39 ± 0.56	83.47 ± 10.2	>10,000	<5.00	N.D
(*R*)-6	(R)	H	Me	3629	5.44 ± 0.51	96.73 ± 7.75	N.C.	N.C.	N.C.

IC_{50} and E_{max} values are from three independent experiments performed in duplicate or triplicate. E_{max} values are normalized to SCH23390 (SCH) as an antagonist reference for D1 receptor or raclopride (RAC) as an antagonist reference for D2 receptor. N.C. not calculatable. N.D. Not determined.

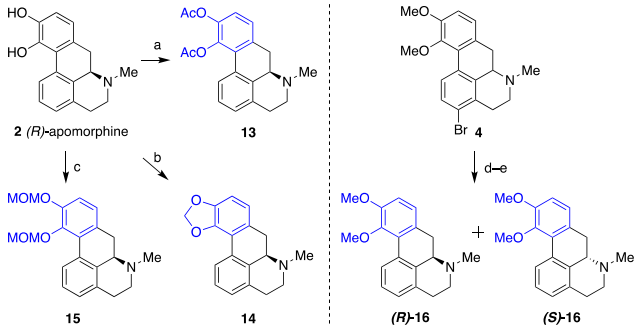
Scheme 2. C-9 Functionalization of apomorphine.



(a) NBS, TFA, 0 ºC, then Ac_2O, pyridine (51%); (b) NCS, TFA, 0 ºC, then Ac_2O, pyridine (49%); (c) **7**, $Pd(dppf)Cl_2$, $Zn(Me)_2$, 1,4-dioxane, 70 ºC, 24 h (57%); (d) **7**, $Pd(dppf)Cl_2$, $Zn(Et)_2$, 1,4-dioxane, 70 ºC, 24 h (35%); (e) **7**, $Pd(dppf)Cl_2$, $Zn(iPr)_2$, 1,4-dioxane, 70 ºC, 20 h (19%); (f) **7**, 3-methoxyphenylboronic acid, $Pd(OAc)_2$, RuPhos, K_3PO_4, THF, H_2O, rt, 17 h (17%).

Meanwhile, we tested the effects of various catechol protecting groups, as several protecting groups have been used in our design and synthesis of novel apomorphine analogs. It is also known that apomorphine, containing the 10, 11-dihydroxy group, is prone to auto-oxidation and has limited bioavailability,[24, 35] both of which may be improved upon the presence of a protecting group. As outlined in **Scheme 3**, we prepared apomorphine derivatives **13–16** which bear acetyl, methylene, methoxy methyl (MOM), or methyl protecting groups, starting from either commercially available (*R*)-(–)-apomorphine (**2**) or apomorphine analog **4**.

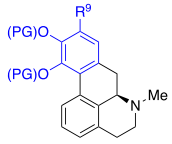
Scheme 3. Catechol protection of apomorphine.



(a) Ac_2O, pyridine, rt, 5 h (quant); (b) KOH, CH_2Br_2, DMSO, 80 ºC, 4.5 h (61%); (c) NaOH, MOMCl, CH_2Cl_2, 0 °C, 16 h (5%); (d) $RuCl_2(p\text{-cymene})_2$, K_2CO_3, *i*-PrOH, 100 ºC, 24 h (92%); (e) Chiral HPLC (Lux® 5µm Celluclose-1, 250 × 4.6 mm).

Overall, increasing steric bulkiness of the substituents on C-9 led to pronounced loss of activity of β-arrestin 2 recruitment at both D1R (no activity) and D2R (E_{max}< 36%) (**Table 3**). Some analogs such as **9** exhibited low bias for G-protein activity (7.2 for D1R and 1.78 for D2R), which is the opposite selectivity we desire. Taken together, these results indicate that halogen and alkyl substituents on the catechol ring largely do not contribute to functional selectivity, but rather reduce agonism for both G-protein and β-arrestin 2 at dopamine receptors.

Table 3. Agonist activities of apomorphine analogs with C-9 modifications on the catechol ring on D1R and D2R.



Cmpd	PG	R^9	β-arrestin			cAMP			
			EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	Bias G
			D1R						
Apo	H	H	520.8	6.3 ± 0.87	13.02 ± 4.9	3.77	8.4 ± 0.15	94.92 ± 3.17	41.50
7	Ac	Br	>10000	<5	N.D	333.9	6.45 ± 0.54	37.94 ± 8.29	N.C
8	Ac	Cl	>10000	<5	N.D	194.3	6.71 ± 0.48	43.25 ± 8.5	N.C
9	Ac	Me	14.45	7.84 ± 1.32	3.69 ± 0.33	1555	5.81 ± 0.1	67.04 ± 3.19	7.2
10	Ac	Et	0.38	9.42 ± 2.1	3.77 ± 0.37	1548	5.81 ± 0.57	17.49 ± 4.71	0
11	Ac	*i*-Pr	>10000	<5	N.D	951.4	6.02 ± 0.99	17.09 ± 7.84	N.C
12	Ac	3-OMePh	41.36	7.38 ± 2.64	3.5 ± 2.6	669.1	6.18 ± 0.36	24.9 ± 4	N.C
13	Ac	H	>10000	<5	N.D	20.4	7.69 ± 0.23	84.07 ± 8.04	N.C
			D2R						
Apo	H		10.1	8.0 ± 0.08	75.0 ±2 .43	1.61	8.8 ± 0.56	60 ± 7.7	2.16
7	Ac	Br	1412	5.9 ± 0.43	6.47 ± 1.32	>10000	<5	N.D	N.C
8	Ac	Cl	604	6.22 ± 0.68	6.7 ± 2.0	>10000	<5	N.D	N.C
9	Ac	Me	1812	5.74 ± 0.1	36.32 ± 1.32	579.7	6.24 ± 0.16	94.84 ± 6.46	1.78
10	Ac	Et	1636	5.79 ± 0.24	16.04 ± 1.77	3586	5.45 ± 0.25	94.75 ± 11.44	0.66
11	Ac	*i*-Pr	>10000	<5	N.D	>10000	<5	N.D	N.C
12	Ac	3-OMePh	N.A	N.A	N.A	>10000	<5	N.D	N.C
13	Ac	H	111.2	7.0 ± 0.18	66.9 ± 5.5	12.86	7.9 ± 0.27	71.2 ± 8.1	1.2

EC_{50} and E_{max} values are from three independent experiments performed in duplicate or triplicate. E_{max} values are calculated as % response normalized to dopamine. N.A. no activity at maximum concentration tested (10^{-4} M), N.C. not calculable, N.D. not determined.

Table 4. Agonist activities of apomorphine analogs with modifications on the catechol ring on D1R and D2R.

Cmpd	PG	β-arrestin			cAMP			
		EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	Bias G
		D1R						
14	$-CH_2-$	>10000	<5	N.D	112.2	7.0 ± 1.8	8.3 ± 6.2	N.C
15	MOM	8.45	8.1 ± 1.26	5.74 ± 3.1	>10000	<5	N.D	N.C
(*R*)-16	Me	N.C	N.C	N.C	N.C	N.C	N.C	N.C
(*S*)-16	Me	N.C	N.C	N.C	N.C	N.C	N.C	N.C
		D2R						
14	$-CH_2-$	102	7.0 ± 1.84	3.6 ± 2.8	>10000	<5	N.D	N.C
15	MOM	0.3	9.5 ± 2.0	2.0 ± 1.9	8236	5.08 ± 0.19	140	35.81
(*R*)-16	Me	N.C	N.C	N.C	1913	5.7 ± 0.6	75.1 ± 19.4	N.C
(*S*)-16	Me	N.C	N.C	N.C	1496	5.8 ± 0.2	107.6 ± 10.0	N.C

EC_{50} and E_{max} values are from three independent experiments performed in duplicate or triplicate. E_{max} values are calculated as % response normalized to dopamine. N.C. not calculable, N.D. not determined.

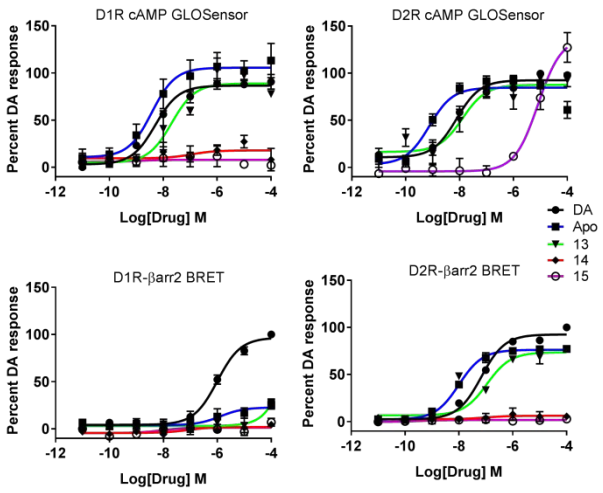
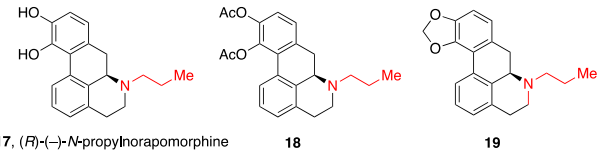


Figure 4. Dose response curves comparing apomorphine (Apo) and catechol protected analogs. *In vitro* GloSensor and BRET agonist assays at D1Rs and D2Rs for catechol protected Apo analogs (**13–15**) in HEK-293 cells. Data are presented as percent of the total DA response (mean ± SEM).

Among the three protecting groups, the *O*-acetyl-protected **13** (**Table 4**) but not the methylene protected (**14**), methoxy methyl protected (**15**) or methyl protected (**16**) apomorphine, had the least change in activities in all assays (**Figure 4**, green line and **Table 4**) compared to apomorphine (**Figure 4**, blue line and **Table 4**). Thus, **13** could be a desirable derivative for further modifications, as it preserves activity relatively well compared to other protected analogs, and the diester derivatives have shown to prevent oxidation and prolong the half-life of apomorphine.[36, 37]

Lastly, we decided to study the *N*-alkyl substitution effects on functional selectivity. Previous studies on *N*-alkyl substitutions of apomorphine showed that (*R*)-*N*-propylnorapomorphine (NPA, **17**) has a higher affinity at D2 receptor, with selectivity over D1 receptor.[35, 38, 39] However, there were no functional selectivity profiles available. Here we chose to evaluate a set of derivatives of NPA **17–19** for their functional selectivity on D1 and D2 receptors (**Scheme 4** and **Table 5**).

Scheme 4. *N*-propylnorapomorphine (NPA) and analogs.



17, (*R*)-(–)-*N*-propylnorapomorphine **18** **19**

In our study, *N*-propyl substitution (NPA) showed almost 100-fold higher potency at D2R (pEC_{50}: 10.4 ± 0.09) over D1R (pEC_{50}: 8.96 ± 0.15) at the G-protein pathway, which is consistent with previous findings.[38] Interestingly, NPA (**17**) also showed increased efficacy and potency for β-arrestin 2 recruitment at D2R (pEC_{50}: 8.93 ± 0.04 E_{max}: 93.83 ± 1.74) over D1R (pEC_{50}: 5.73 ± 0.11, E_{max}: 72.23 ± 4.91) (**Table 5** and **Figure 5**). However, NPA (**17**) showed increased efficacy for β-arrestin 2 recruitment at D1R when compared to apomorphine (**Figure 5** blue line and **Table 5**, D1R E_{max}: 13.02 ± 4.9). Compared to the acetyl protected *N*-methyl analog **13,** the acetyl protected *N*-propyl analog **18** exhibited greater efficacy and potency at both D1 and D2Rs for β-arrestin 2 recruitment and G-protein signaling (**Table 5**). Methylene-dioxy protection of the *N*-propyl substituted apomorphine (**19**, MDO-NPA) led to reduced activity at all pathways but to a lesser degree (**Table 5, Figure 5**) than apomorphine analogs **14**–**16**.

In this study, we employed (*R*)-apomorphine, a known agonist of dopamine receptors, as our parent scaffold, synthesized a series of novel analogs, and evaluated their SFSR toward identifying β-arrestin biased analogs for D1R/D2R. We systematically examined substitutions on different regions of the apomorphine scaffold and performed cell-based screening assays for G-protein versus β-arrestin recruitment activity at D1 and D2Rs.

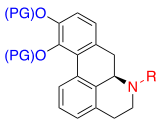
The SFSR studies of the substitution on the catechol group indicate that among various protecting groups (**13–16**), *O*-acetylation resulted in the least change in activities of apomorphine, compared to methylene, methoxy methyl, or methyl groups. Modifications at C-9 position of the catechol ring (**7–12**) largely inhibited arrestin activity although compounds such as **9** were slightly biased for the Gαs-cAMP pathway.

The SFSR studies of the lower phenyl ring revealed that substitutions at either the C-1 or C-3 position generally abolished activity at both pathways for both receptors. Yet these findings are confounded by the fact that these analogs bear methyl protected catechol ring, while the methyl protection group itself reduces activity at all pathways except for G-protein at D2R. Interestingly, **(*S*)-3** and **(*S*)-4** showed β-arrestin biased antagonism (IC_{50} only) at both D1 and D2 receptors. Although these results do not align with our main interest of discovery of β-arrestin biased agonists, such compounds might be useful for conditions of hyperdopaminergia that are associated with psychiatric disorders such as schizophrenia or ADHD, which will be investigated in our future work.

The SFSR studies of the *N*-alkyl chain were performed on the *N*-propyl analogs of apomorphine, based on previous findings that show NPA is a selective D2 agonist.[38] We show that NPA has picomolar potency for the D2R G-protein pathway but nanomolar potency for D1R G-protein and D2R β-arrestin 2 pathways. At low doses NPA produces catalepsy and inhibition of locomotor activity, whereas higher doses it increases locomotor activity. This phenomenon can be explained by the higher potency observed at the D2R G-protein and arrestin pathways compared to D1R, especially at lower picomolar doses. Such low doses might preferentially activate presynaptic D2 autoreceptors causing catalepsy. Although NPA showed potent activation of the D2R β-arrestin 2 pathway, it did show higher efficacy than apomorphine at the D1R β-arrestin 2 pathway, suggesting that the *N*-alkyl chain may play an important role in functional selectivity for the arrestin pathway, which will be examined in the future studies.

In summary, these novel apomorphine-based analogs provide valuable chemical tools and SFSR information in deciphering receptor pharmacology implicated in Parkinson's disease and other dopamine-related disorders.

Table 5. Agonist activities of *N*-propylnorapomorphine analogs on D1R and D2R



Cmpd	R	PG	β-arrestin			cAMP			
			EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	EC_{50} (nM)	pEC_{50} ± SEM	E_{max} (%) ± SEM	Bias G
			D1R						
Apo	Methyl	H	520.8	6.3 ± 0.87	13.02 ± 4.9	3.77	8.4 ± 0.15	94.92 ± 3.17	41.50
13	Methyl	Ac	>10000	<5	N.D	20.4	7.69 ± 0.23	84.07 ± 8.04	N.C
17	Propyl	H	1884	5.73 ± 0.11	72.23 ± 4.91	1.1	8.96 ± 0.15	84.87 ± 5.7	19.32
18	Propyl	Ac	5496	5.3 ± 0.3	52.5 ± 13.74	31.7	7.5 ± 0.12	88.8 ± 4	0.79
19	Propyl	$-CH_2-$	1949	5.71 ± 0.91	7.44 ± 3.1	717.5	6.14±0.16	57.8 ± 4.1	0
			D2R						
Apo	Methyl	H	10.1	8.0 ± 0.08	75.0 ± 2.43	1.61	8.8 ± 0.56	60 ± 7.7	2.16
13	Methyl	Ac	111.2	7.0 ± 0.18	66.9 ± 5.5	12.86	7.9 ± 0.27	71.2 ± 8.1	1.2
17	Propyl	H	1.18	8.93 ± 0.04	93.83 ± 1.74	0.04	10.4 ± 0.09	94.38 ± 3.42	0.85
18	Propyl	Ac	6.34	8.2 ± 0.04	92.12 ± 1.6	0.373	9.43 ± 0.13	86.52 ± 4.3	0.6
19	Propyl	$-CH_2-$	520	6.3 ± 0.05	79.41	7.7	8.11 ± 0.13	85.43 ± 5.17	2.7

EC_{50} and E_{max} values were calculated with % response normalized to dopamine. N.C. not calculable. N.D. not determined.

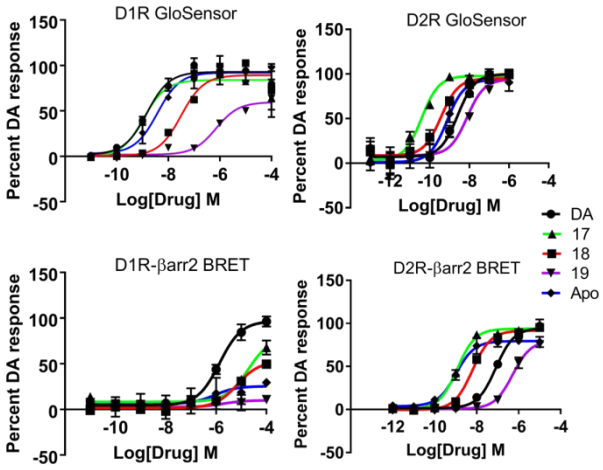


Figure 5. Dose response curves for *N*-propyl substituted apomorphine analogs. *In vitro* GloSensor and BRET agonist assays at D1Rs and D2Rs for *N*-propyl Apo analogs **17** (NPA), **18** and **19** in HEK-293 cells. Data are presented as percent of the total DA response (mean ± SEM).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Experimental and characterization data for all compounds and biological experiment procedures

AUTHOR INFORMATION

Corresponding Author

Nikhil M. Urs: nikhilurs@ufl.edu
Qiu Wang: qiu.wang@duke.edu

§H. Park and A. N. Urs contributed equally to this work.

ACKNOWLEDGMENT

We thank the financial support to this project from the Michael J. Fox Foundation (MJFF #11764 to N.U.) and the Alfred P. Sloan Foundation (to Q.W.).

ABBREVIATIONS

PD, Parkinson's disease; GPCR, G protein-coupled receptor; SFSR, structure-functional-selectivity relationship; D1R, dopamine D1 receptor; D2R, dopamine D2 receptor; BRET, β-arrestin bioluminiscence resonance energy transfer; L-DOPA, L-3,4-dihydroxyphenylalanine; DCM, dichloromethane; LDA, lithium diisopropylamide; DBTA, dibenzoyl tartaric acid; APO, apomorphine; SCH, SCH23390; RAC, raclopride; NPA, *N*-propylnorapomorphine.

REFERENCES

(1) Ehringer, H., and Hornykiewicz, O. (1998) Distribution of noradrenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system, *Parkinsonism Relat. Disord. 4*, 53–57.

(2) Cavalli, A., Bolognesi, M. L., Minarini, A., Rosini, M., Tumiatti, V., Recanatini, M., and Melchiorre, C. (2008) Multi-target-directed ligands to combat neurodegenerative diseases, *J. Med. Chem. 51*, 347–372.

(3) Zhang, A., Neumeyer, J. L., and Baldessarini, R. J. (2007) Recent progress in development of dopamine receptor subtype-selective agents- potential therapeutics for neurological and psychiatric disorders, *Chem. Rev. 107*, 274–302.

(4) Kebabian, J. W., and Calne, D. B. (1979) Multiple receptors for dopamine, *Nature 277*, 93–96.

(5) Seeman, P., and Van Tol, H. H. (1994) Dopamine receptor pharmacology, *Trends Pharmacol. Sci. 15*, 264–270.

(6) Delfino, M. A., Stefano, A. V., Ferrario, J. E., Taravini, I. R., Murer, M. G., and Gershanik, O. S. (2004) Behavioral sensitization to different dopamine agonists in a parkinsonian rodent model of drug-induced dyskinesias, *Behav. Brain. Res. 152*, 297–306.

(7) Shen, W. W. (1999) A history of antipsychotic drug development, *Compr. Psychiat 40*, 407–414.

(8) Beaulieu, J. M., and Gainetdinov, R. R. (2011) The physiology, signaling, and pharmacology of dopamine receptors, *Pharmacol. Rev. 63*, 182–217.

(9) Huot, P., Johnston, T. H., Koprich, J. B., Fox, S. H., and Brotchie, J. M. (2013) The pharmacology of L-DOPA-induced dyskinesia in Parkinson's disease, *Pharmacol. Rev. 65*, 171–222.

(10) Urs, N. M., Bido, S., Peterson, S. M., Daigle, T. L., Bass, C. E., Gainetdinov, R. R., Bezard, E., and Caron, M. G. (2015) Targeting β-arrestin2 in the treatment of L-DOPA-induced dyskinesia in Parkinson's disease, *Proc. Natl. Acad. Sci. U.S.A. 112*, E2517–2526.

(11) Santini, E., Valjent, E., Usiello, A., Carta, M., Borgkvist, A., Girault, J. A., Herve, D., Greengard, P., and Fisone, G. (2007) Critical involvement of cAMP/DARPP-32 and extracellular signal-regulated protein kinase signaling in L-DOPA-induced dyskinesia, *J. Neurosci. 27*, 6995–7005.

(12) McCorvy, J. D., Butler, K. V., Kelly, B., Rechsteiner, K., Karpiak, J., Betz, R. M., Kormos, B. L., Shoichet, B. K., Dror, R. O., Jin, J., and Roth, B. L. (2018) Structure-inspired design of β-arrestin-biased ligands for aminergic GPCRs, *Nat. Chem. Biol. 14*, 126-134.

(13) Urs, N. M., Daigle, T. L., and Caron, M. G. (2011) A dopamine D1 receptor-dependent β-arrestin signaling complex potentially regulates morphine-induced psychomotor activation but not reward in mice, *Neuropsychopharmacology 36*, 551-558.

(14) Urban, J. D., Clarke, W. P., von Zastrow, M., Nichols, D. E., Kobilka, B., Weinstein, H., Javitch, J. A., Roth, B. L., Christopoulos, A., Sexton, P. M., Miller, K. J., Spedding, M., and Mailman, R. B. (2007) Functional selectivity and classical concepts of quantitative pharmacology, *J. Pharmacol. Exp. Ther. 320*, 1-13.

(15) Mailman, R. B. (2007) GPCR functional selectivity has therapeutic impact, *Trends Pharmacol. Sci. 28*, 390–396.

(16) Hodavance, S. Y., Gareri, C., Torok, R. D., and Rockman, H. A. (2016) G Protein-coupled receptor biased agonism, *J. Cardiovasc. Pharmacol. 67*, 193–202.

(17) Kenakin, T., and Christopoulos, A. (2012) Signalling bias in new drug discovery: detection, quantification and therapeutic impact, *Nat. Rev. Drug Discov. 12*, 205.

(18) Lefkowitz, R. J., and Shenoy, S. K. (2005) Transduction of receptor signals by β-arrestins, *Science 308*, 512–517.

(19) Conroy, J. L., Free, R. B., and Sibley, D. R. (2015) Identification of G protein-biased agonists that fail to recruit β-arrestin or promote internalization of the D1 dopamine receptor, *ACS Chem. Neurosci. 6*, 681–692.

(20) Gray, D. L., Allen, J. A., Mente, S., O'Connor, R. E., DeMarco, G. J., Efremov, I., Tierney, P., Volfson, D., Davoren, J., Guilmette, E., Salafia, M., Kozak, R., and Ehlers, M. D. (2018) Impaired β-arrestin recruitment and reduced desensitization by non-catechol agonists of the D1 dopamine receptor, *Nat. Commun. 9*, 674.

(21) Martini, M. L., Liu, J., Ray, C., Yu, X., Huang, X. P., Urs, A., Urs, N., McCorvy, J. D., Caron, M. G., Roth, B. L., and Jin, J. (2019) Defining Structure-Functional Selectivity Relationships (SFSR) for a Class of Non-Catechol Dopamine D1 Receptor Agonists, *J. Med. Chem. 62*, 3753–3772.

(22) Martini, M. L., Ray, C., Yu, X., Liu, J., Pogorelov, V. M., Wetsel, W. C., Huang, X. P., McCorvy, J. D., Caron, M. G., and Jin, J. (2019) Designing Functionally Selective Noncatechol Dopamine D1 Receptor Agonists with Potent In Vivo Antiparkinsonian Activity, *ACS Chem. Neurosci. 10*, 4160–4182.

(23) Allen, J. A., Yost, J. M., Setola, V., Chen, X., Sassano, M. F., Chen, M., Peterson, S., Yadav, P. N., Huang, X.-p., Feng, B., Jensen, N. H., Che, X., Bai, X., Frye, S. V., Wetsel, W. C., Caron, M. G., Javitch, J. A., Roth, B. L., and Jin, J. (2011) Discovery of β-arrestin–biased dopamine D2 ligands for probing signal transduction pathways essential for antipsychotic efficacy, *Proc. Natl. Acad. Sci. U.S.A. 108*, 18488–18493.

(24) Muguet, D., Broussolle, E., and Chazot, G. (1995) Apomorphine in patients with Parkinson's disease, *Biomed. Pharmacother. 49*, 197–209.

(25) Subramony, J. A. (2006) Apomorphine in dopaminergic therapy, *Mol. Pharm. 3*, 380–385.

(26) Zhang, A., Zhang, Y., Branfman, A. R., Baldessarini, R. J., and Neumeyer, J. L. (2007) Advances in development of dopaminergic aporphinoids, *J. Med. Chem. 50*, 171–181.

(27) Raminelli, C., Muraca, A., Perecim, G., and Rodrigues, A. (2017) Convergent Total Synthesis of (±)-Apomorphine via Benzyne Chemistry: Insights into the Mechanisms Involved in the Key Step, *Synthesis 49*, 3546–3557.

(28) Schoenfeld, R. I., Neumeyer, J. L., Dafeldecker, W., and Roffler-Tarlov, S. (1975) Comparison of structural and stereoisomers of apomorphine on stereotyped sniffing behavior of the rat, *Eur. J. Pharmacol. 30*, 63–68.

(29) Seeman, P., Watanabe, M., Grigoriadis, D., Tedesco, J. L., George, S. R., Svensson, U., Nilsson, J. L., and Neumeyer, J. L. (1985) Dopamine D2 receptor binding sites for agonists. A tetrahedral model, *Mol. Pharmacol. 28*, 391.

(30) Neumeyer, J. L., Reischig, D., Arana, G. W., Campbell, A., Baldessarini, R. J., Kula, N. S., and Watling, K. J. (1983) Aporphines. 48. Enantioselectivity of (*R*)-(-)- and (*S*)-(+)-*N*-*n*-propylnorapomorphine on dopamine receptors, *J. Med. Chem. 26*, 516–521.

(31) Goldman, M. E., and Kebabian, J. W. (1984) Aporphine enantiomers - interactions with D-1 and D-2 dopamine receptors, *Mol. Pharmacol. 25*, 18–23.

(32) Shi, X.-X., Ni, F., Shang, H.-X., Yan, M.-L., and Su, J.-Q. (2006) Racemization of (*S*)-(+)-10,11-dimethoxyaporphine and (*S*)-(+)-aporphine: efficient preparations of (*R*)-(−)-apomorphine and (R)-(−)-aporphine via a recycle process of resolution, *Tetrahedron: Asym. 17*, 2210–2215.

(33) Kenakin, T., Watson, C., Muniz-Medina, V., Christopoulos, A., and Novick, S. (2012) A Simple Method for Quantifying Functional Selectivity and Agonist Bias, *ACS Chem. Neurosci. 3*, 193–203.

(34) Ernst, A. M. (1965) Relation between the action of dopamine and apomorphine and their *O*-methylated derivatives upon the CNS, *Psychopharmacologia 7*, 391–399.

(35) Campbell, A., Baldessarini, R. J., Ram, V. J., and Neumeyer, J. L. (1982) Behavioral effects of (-)10,11-methylenedioxy-*N*-n-propylnoraporphine, an orally effective long-acting agent active at central dopamine receptors, and analogous aporphines, *Neuropharmacology 21*, 953-961.

(36) Borgman, R. J., Baldessarini, R. J., and Walton, K. G. (1976) Diester derivatives as apomorphine prodrugs *J. Med. Chem. 19*, 717–719.

(37) Borkar, N., Li, B., Holm, R., Hakansson, A. E., Mullertz, A., Yang, M., and Mu, H. (2015) Lipophilic prodrugs of apomorphine I: Preparation, characterisation, and in vitro enzymatic hydrolysis in biorelevant media, *Eur. J. Pharm. Biopharm. 89*, 216–223.

(38) Gao, Y. G., Ram, V. J., Campbell, A., Kula, N. S., Baldessarini, R. J., and Neumeyer, J. L. (1990) Synthesis and structural requirements of N-substituted norapomorphines for affinity

and activity at dopamine D-1, D-2, and agonist receptor sites in rat-brain, *J. Med. Chem. 33*, 39–44.

(39) Neumeyer, J. L., Gao, Y. G., Kula, N. S., and Baldessarini, R. J. (1990) Synthesis and dopamine receptor affinity of (*R*)-(-)-2-fluoro-*N*-normal-propylnorapomorphine - a highly potent and selective dopamine-D2 agonist, *J. Med. Chem. 33*, 3122–3124.