Discovery and Lead Optimization of Atropisomer D1 Agonists with Reduced Desensitization

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Supporting Information

Contents

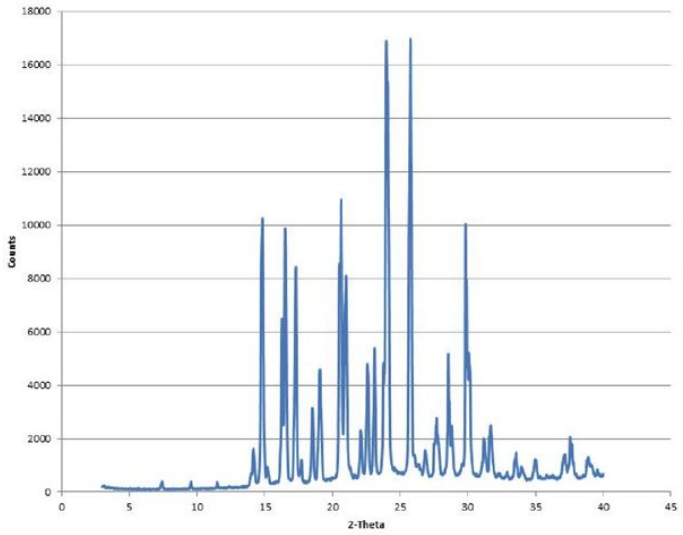
Animal Welfare Statement

All procedures performed on animals in these experiments were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institute of Health (NIH: 8th edition) and approved by an Institutional Animal Care and Use Committee (IACUC).

Abbreviations List

Dopamine (DA), central nervous system (CNS), Parkinson’s Disease (PD), intravenously (IV), positive allosteric modulators (PAMs), high throughput screen (HTS), homogenous time-resolved fluorescence (HTRF), Human embryonic kidney (HEK), human D1 (hD1), human D1 receptor (hD1r), structure activity relationship (SAR), matched molecular pair (MMP), multi-parameter optimization (MPO), multi-drug resistance protein (MDR), breast cancer resistance protein (BCRP), polar surface area (PSA), hydrogen bond donor (HBD), area under the curve (AUC), human liver microsome (HLM), human hepatocyte (hHep), lipophilic efficiency (LipE), pharmacokinetics (PK), locomotor activity (LMA), brain/plasma (B/P), fraction unbound in brain (Fb,u), fraction unbound in plasma (Fp,u), concentration unbound in brain (Cb,u), and concentration unbound in plasma (Cp,u).

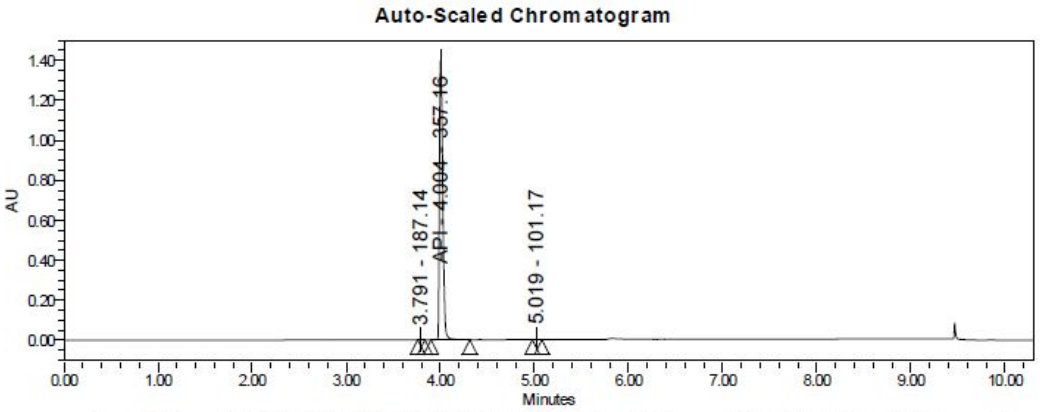
PXRD of Compound 31

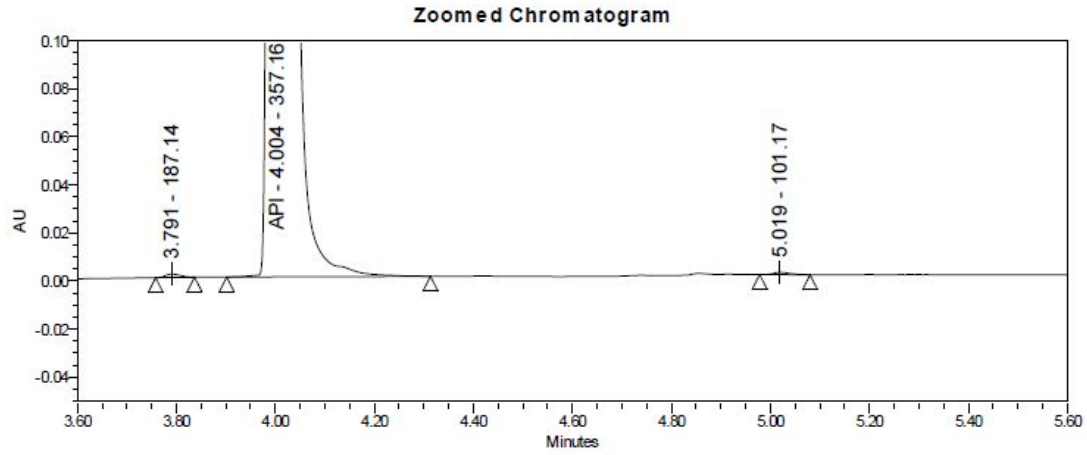


Purity Report on Compounds 29 (racemic) and 31 (enantiomer)

Compound 29

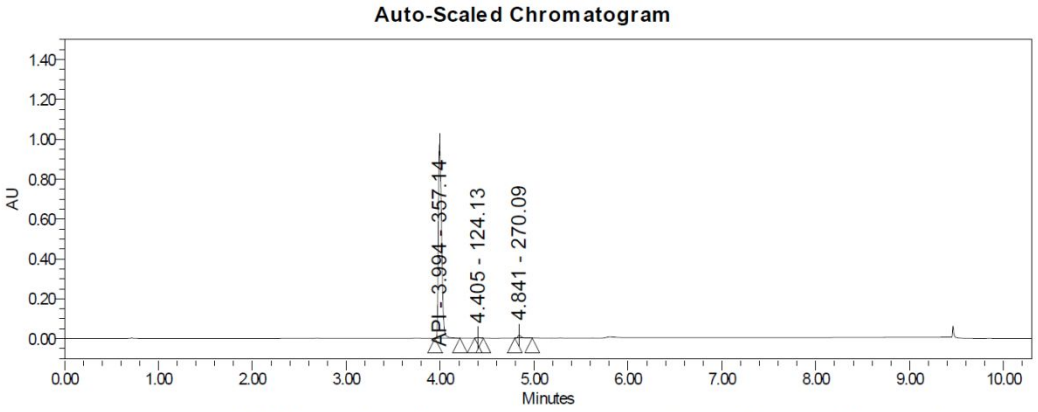
Final Purity by HPLC: 99.50%



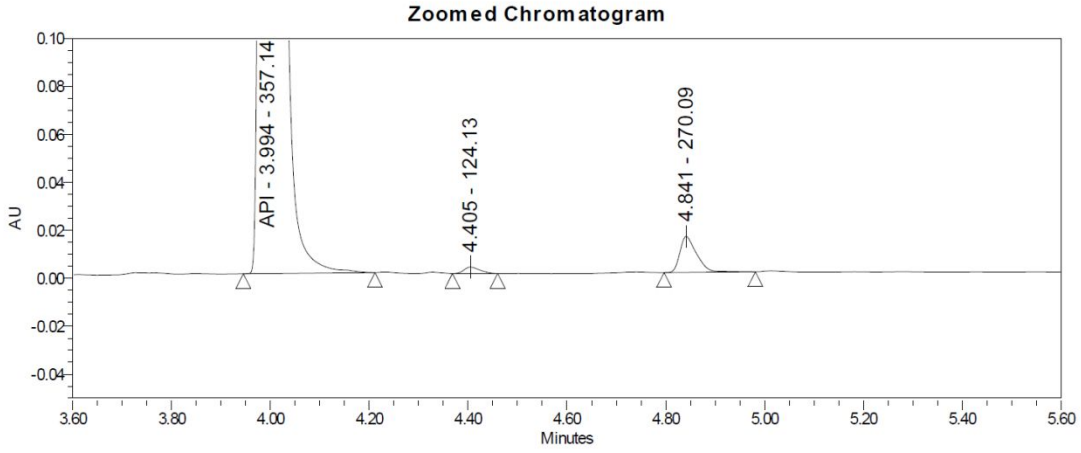


Compound 31

Final Purity: 98.24%.



Sample Name PF-06256142-00-0003; Vial 1:A,3; Injection 1; Channel PDA Ch1 210nm@4.8nm; Date Acquired 08-Oct-2018 16:24:01 EDT



HPLC Method

Method A:

System Used: Waters Acquity with PDA detector and solvent switcher

Time	%Buffer	%Acetonitrile
0.00	95	5
8.2	0	100
8.7	0	100
8.80	95	5
10.30	95	5

Flow Rate: 0.5 ml/min

Column Temp: 45° C

Inj Vol: Varies from 1-2 µl

Detection: UV-210 nm (200-400 nm collected)

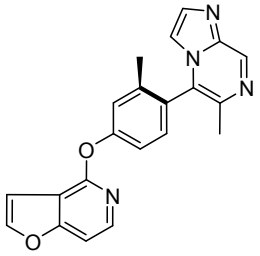
(10pts/sec with 4.8 nm bandwidth)

Method	Buffer	Column
4	0.1% Methanesulfonic Acid (pH~2)	Waters Cortecs 2.1 x 50 mm 1.6 um

VCD of Compound 30

VCD Experimental

Compound **30** and the racemate **29** were dissolved in $CDCl_3$ (3.3mg/0.1mL and 4.7mg/0.14mL respectively) and placed in a 100 µm pathlength cell with BaF_2 windows. IR and VCD spectra were recorded on a Chiral*IR*™ VCD spectrometer (BioTools, Inc.) equipped with Dual*PEM* accessory, with 4 cm^{-1} resolution, 10-h collection for both samples, and instrument optimized at 1400 cm^{-1}. The IR of the solvent was collected for 46 scans. The solvent-subtracted IR and racemate subtracted VCD spectra are shown.



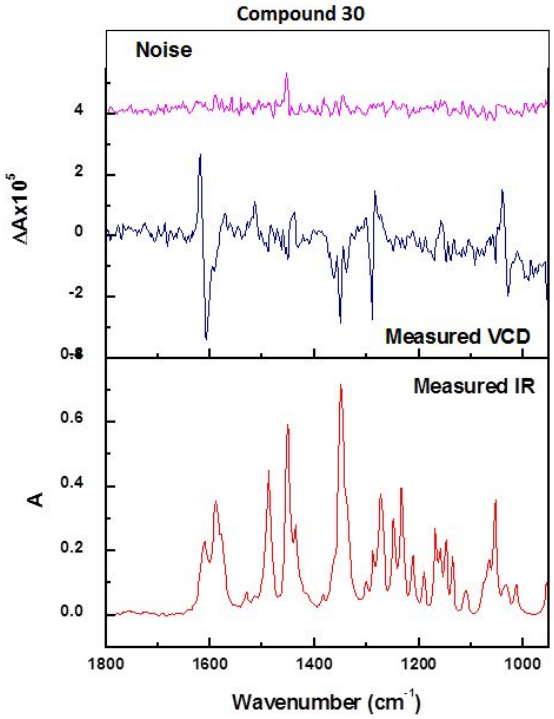
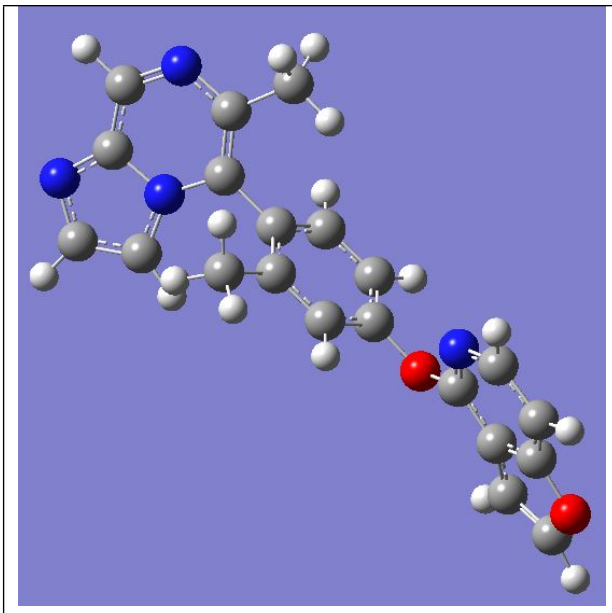


Figure 1. IR (lower frame) and VCD (upper frame) spectra of compound **30** in $CDCl_3$ (3.3mg/0.1mL); 100-μm path-length cell with BaF_2 windows; 10 h collection for sample and racemate; instrument optimized at 1400 cm^{-1}. Solvent-subtracted IR and racemate subtracted VCD spectra are shown. Uppermost trace is the VCD noise.

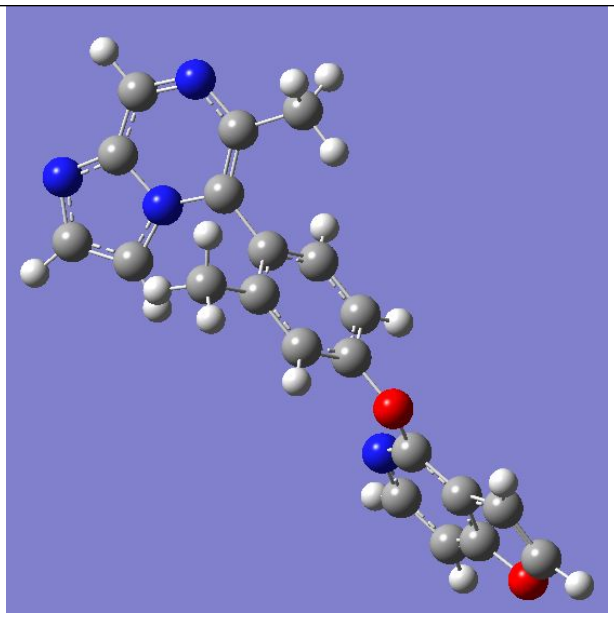
VCD Theoretical Calculations:

The (*R*)-configuration shown above was built with Hyperchem (Hypercube, Inc., Gainesville, FL). A conformational search was carried out with Hyperchem for the entire structure at the molecular mechanics level. Geometry optimization, frequency, IR and VCD intensity calculations were carried out at the DFT level (B3LYP functional/ 6-31G(d) basis set) with Gaussian 09 (Gaussian Inc., Wallingford, CT). The calculated frequencies were scaled by 0.97 and the IR and VCD intensities were converted to Lorentzian bands with 6-cm^{-1} half-width for comparison to experiment.

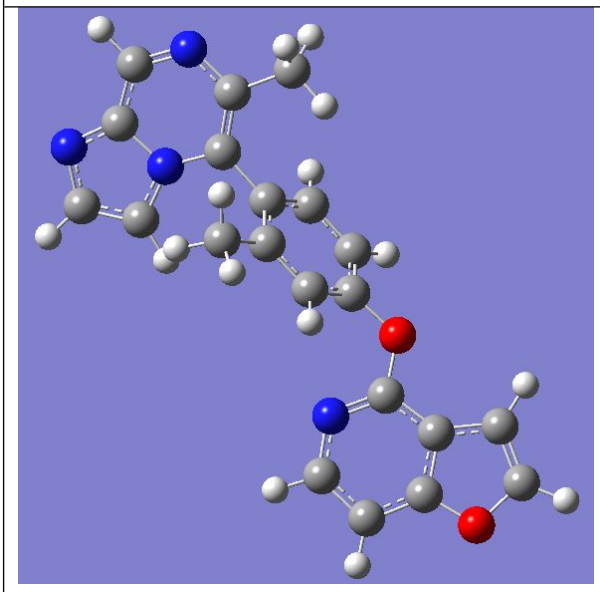
Gaussian calculations resulted in four conformers that have energies within 1 kcal/mol from the lowest-energy conformer. The other conformers have energies more than 3 kcal/mol higher than the lowest-energy conformer. The optimized geometries of the four low-energy conformers of the (*R*) - configuration are shown in Figure 2, and the comparison of the observed VCD and IR spectra with those of the four low-energy conformers are shown in Figure 3. Based on the overall agreement in VCD pattern for the observed and the Boltzmann sum of the calculated spectra of the four lowest-energy conformers (Figure 4), ***the absolute configuration of compound 30 is assigned as (R)- (M enantiomer) .*** The assignment was evaluated by Compare VOA program, and the confidence level of the assignment is 100% based on current database that includes 89 previous correct assignments for different chiral structures.



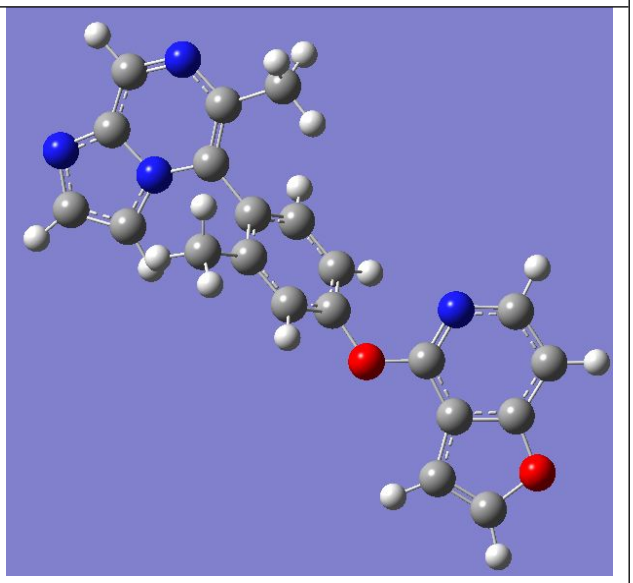
conf1, RE = 0.11 kcal/mol (23%)



conf2, RE = 0 kcal/mol (27%)



conf7, RE = 0.024 kcal/mol (26%)



conf8, RE = 0.058 kcal/mol (24%)

Figure 2. Optimized geometries of the four calculated lowest-energy conformers of the (*R*) -(=M) configuration of Compound **30**

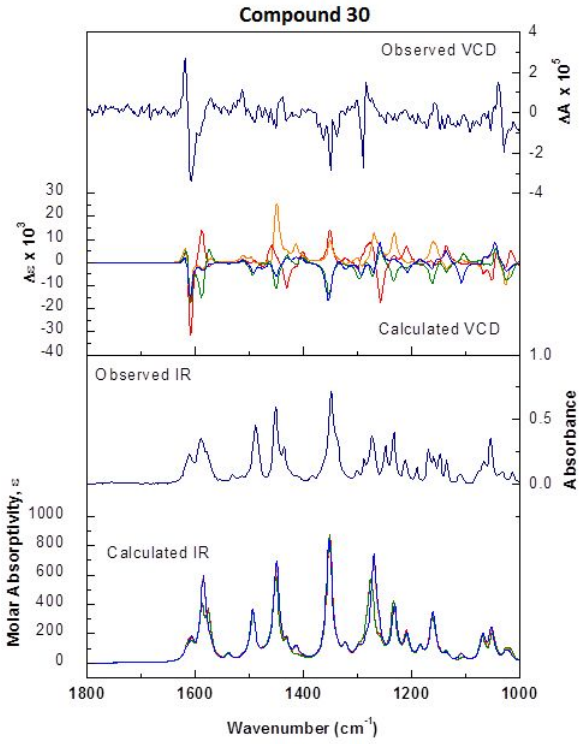


***Figure 3*.** IR (lower frame) and VCD (upper frame) spectra observed for compound **30** (right axes) compared with calculated spectra for the four calculated lowest-energy conformations for the (*R*) - (=M) configuration (left axes).

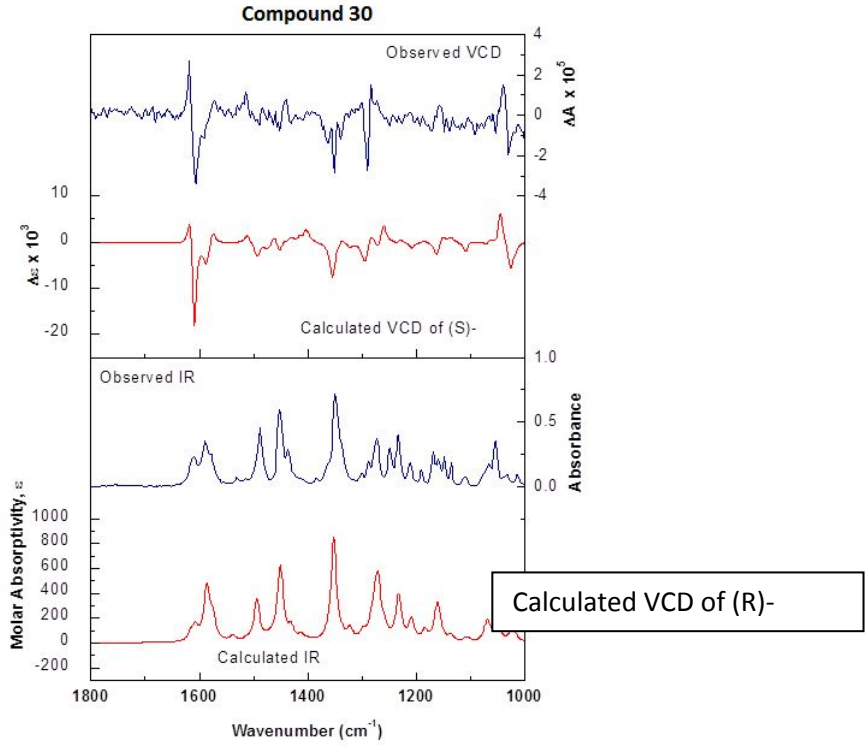


Figure 4. IR (lower frame) and VCD (upper frame) spectra observed for compound 30 (right axes) compared with the Boltzmann-population-weighted calculated spectra for the four lowest-energy conformations of the (*R*) - (=M) configuration (left axes).

Compound 31 Light Atom X-Ray Chrystalography

SUMMARY:

- The structure was solved in the P2(1)2(1)2(1) space group
- The asymmetric unit is comprised of one molecule of compound 31
- R value 3.3%
- Absolute configuration (see report text)

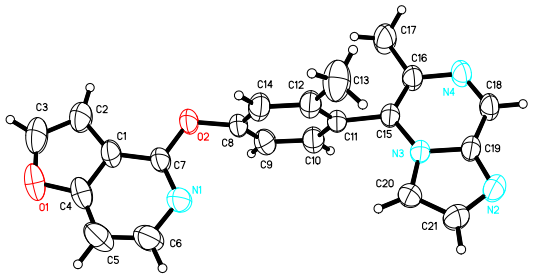


Figure 1. ORTEP with ellipsoids drawn at 50% confidence level.

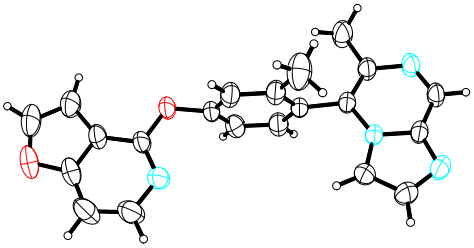


Figure 2. ORTEP with ellipsoids drawn at 50% confidence level.

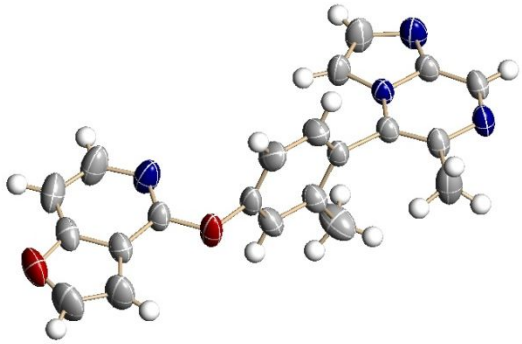


Figure 3

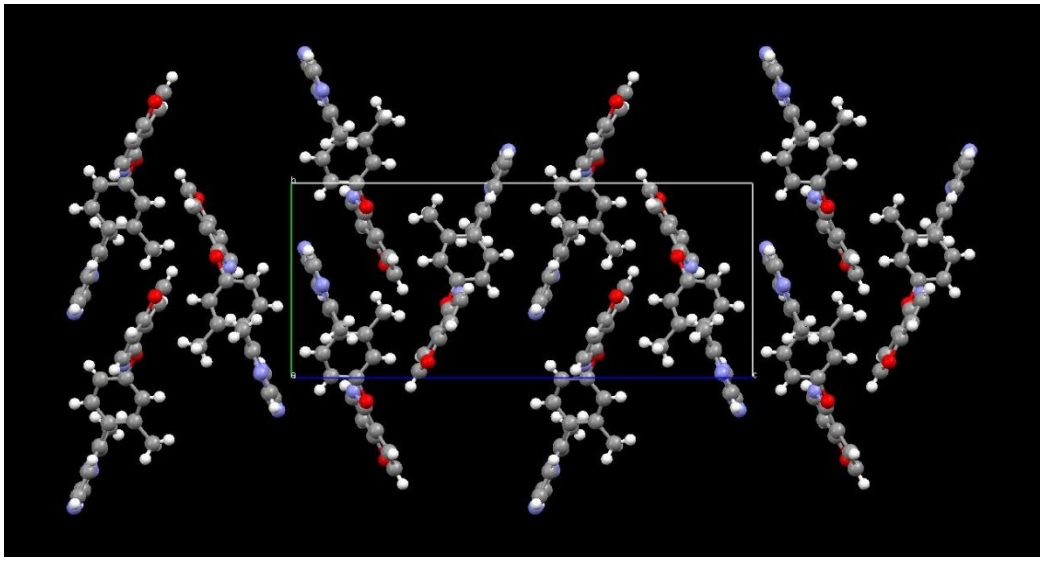


Figure 4. Packing diagram

EXPERIMENTAL:

Data collection was performed on a Bruker APEX diffractometer at room temperature. Data collection consisted of 3 omega scans and low angle and three at high angle; each with 0.5 step. In addition, 2 phi scans were collected to improve the quality of the absorption correction. The structure was solved by direct methods using SHELX software suite in the space group P2(1)2(1)2(1) . The structure was subsequently refined by the full-matrix least squares method. All non-hydrogen atoms were found and refined using anisotropic displacement parameters. Hydrogen atoms were placed in calculated positions and were allowed to ride on their carrier atoms. The final refinement included isotropic displacement parameters for all hydrogen atoms. The compound is assumed enantiopure, having been separated through chiral HPLC. Analysis of the absolute structure using likelihood methods (Hooft 2008) was performed using PLATON (Spek 2010). The results indicate that the absolute structure has been correctly assigned. The method calculates that the probability that the structure is assigned correctly is 100.0% . The Hooft parameter is reported as -0.03 with an esd of 0.10.

The final R-index was 3.8%. A final difference Fourier revealed no missing or misplaced electron density. Structure assigned as the aS atropisomer.

Pertinent crystal, data collection and refinement are summarized in table 1. Atomic coordinates, bond lengths, bond angles, Torsion angles and displacement parameters are listed in tables 2 –6.

X-Ray Software and References

SHELXTL, Version 5.1, Bruker AXS, 1997
PLATON, A.L. Spek, J. Appl. Cryst. 2003, 36, 7-13.
MERCURY, C.F. Macrae, P.R. Edington, P. McCabe, E. Pidcock, G.P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Cryst.* **39**, 453-457, 2006.
R.W.W. Hooft et al. *J. Appl. Cryst.* (2008). 41. 96-103.
H.D. Flack, *Acta Cryst.* 1983, **A**39, 867-881.

Table 1. Crystal data and structure refinement for compound **31**.

Identification code	z070	
Crystallization	EtOAc	
Empirical formula	C21 H16 N4 O2	
Formula weight	356.38	
Temperature	273(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	P2(1)2(1)2(1)	
Unit cell dimensions	a = 7.4652(2) Å	α= 90°.
	b = 10.0278(3) Å	β= 90°.
	c = 23.8026(9) Å	γ = 90°.
Volume	1781.85(10) $Å^3$	
Z	4	
Density (calculated)	1.328 Mg/m^3	
Absorption coefficient	0.718 mm^{-1}	
F(000)	744	
Crystal size	0.35 x 0.20 x 0.10 mm^3	
Theta range for data collection	3.71 to 67.23°.	
Index ranges	-8<=h<=8, -11<=k<=8, -28<=l<=27	
Reflections collected	10748	
Independent reflections	3051 [R(int) = 0.0329]	
Completeness to theta = 67.23°	98.1 %	
Absorption correction	Empirical	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	3051 / 0 / 247	
Goodness-of-fit on F^2	1.020	
Final R indices [I>2sigma(I)]	R1 = 0.0335, wR2 = 0.0899	
R indices (all data)	R1 = 0.0371, wR2 = 0.0932	
Absolute structure parameter	0.0(3)	
Extinction coefficient	0.0046(4)	
Largest diff. peak and hole	0.108 and -0.129 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for compound 31. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	4046(2)	-2710(2)	1831(1)	50(1)
C(2)	3247(3)	-3807(2)	2115(1)	74(1)
C(3)	4586(4)	-4647(2)	2230(1)	92(1)
C(4)	5861(3)	-2981(2)	1800(1)	57(1)
C(5)	7067(2)	-2137(2)	1555(1)	73(1)
C(6)	6344(3)	-1001(2)	1334(1)	80(1)
C(7)	3493(2)	-1508(2)	1588(1)	48(1)
C(8)	1118(2)	8(2)	1410(1)	51(1)
C(9)	581(3)	86(2)	860(1)	60(1)
C(10)	-216(3)	1258(2)	676(1)	55(1)
C(11)	-482(2)	2321(2)	1038(1)	42(1)
C(12)	105(2)	2237(2)	1596(1)	49(1)
C(13)	-173(4)	3374(2)	1997(1)	81(1)
C(14)	906(2)	1060(2)	1773(1)	53(1)
C(15)	-1484(2)	3513(2)	841(1)	43(1)
C(16)	-3301(2)	3640(2)	847(1)	50(1)
C(17)	-4492(3)	2543(2)	1060(1)	83(1)
C(18)	-3235(2)	5770(2)	485(1)	47(1)
C(19)	-1356(2)	5744(2)	459(1)	46(1)
C(20)	1287(2)	4815(2)	592(1)	58(1)
C(21)	1440(3)	6076(2)	379(1)	71(1)
N(1)	4582(2)	-682(2)	1347(1)	65(1)
N(2)	-173(2)	6661(2)	293(1)	63(1)
N(3)	-514(2)	4597(1)	644(1)	43(1)
N(4)	-4180(2)	4768(2)	669(1)	52(1)
O(1)	6213(2)	-4181(2)	2045(1)	80(1)
O(2)	1714(2)	-1219(1)	1626(1)	65(1)

Table 3. Bond lengths [Å] and angles [°] for compound 31.

C(1)-C(4)	1.383(2)	C(18)-C(19)	1.404(2)
C(1)-C(7)	1.400(2)	C(19)-N(2)	1.334(2)
C(1)-C(2)	1.422(3)	C(19)-N(3)	1.383(2)
C(2)-C(3)	1.336(3)	C(20)-C(21)	1.367(3)
C(3)-O(1)	1.374(3)	C(20)-N(3)	1.368(2)
C(4)-O(1)	1.363(2)	C(21)-N(2)	1.355(2)
C(4)-C(5)	1.366(3)		
C(5)-C(6)	1.365(3)	C(4)-C(1)-C(7)	115.83(17)
C(6)-N(1)	1.354(3)	C(4)-C(1)-C(2)	106.54(17)
C(7)-N(1)	1.294(2)	C(7)-C(1)-C(2)	137.62(17)
C(7)-O(2)	1.3622(19)	C(3)-C(2)-C(1)	105.7(2)
C(8)-C(9)	1.372(3)	C(2)-C(3)-O(1)	112.4(2)
C(8)-C(14)	1.372(3)	O(1)-C(4)-C(5)	127.06(17)
C(8)-O(2)	1.4054(19)	O(1)-C(4)-C(1)	109.80(19)
C(9)-C(10)	1.389(2)	C(5)-C(4)-C(1)	123.14(17)
C(10)-C(11)	1.386(2)	C(6)-C(5)-C(4)	114.86(17)
C(11)-C(12)	1.400(2)	N(1)-C(6)-C(5)	124.9(2)
C(11)-C(15)	1.486(2)	N(1)-C(7)-O(2)	120.38(15)
C(12)-C(14)	1.389(2)	N(1)-C(7)-C(1)	123.28(15)
C(12)-C(13)	1.501(3)	O(2)-C(7)-C(1)	116.32(15)
C(15)-C(16)	1.362(2)	C(9)-C(8)-C(14)	121.59(16)
C(15)-N(3)	1.388(2)	C(9)-C(8)-O(2)	119.39(18)
C(16)-N(4)	1.375(2)	C(14)-C(8)-O(2)	118.64(16)
C(16)-C(17)	1.503(2)	C(8)-C(9)-C(10)	118.29(18)
C(18)-N(4)	1.303(2)	C(11)-C(10)-C(9)	121.09(16)

C(10)-C(11)-C(12)	119.95(15)	C(7)-O(2)-C(8)	118.07(13)
C(10)-C(11)-C(15)	119.64(15)		
C(12)-C(11)-C(15)	120.32(15)		
C(14)-C(12)-C(11)	118.23(16)		
C(14)-C(12)-C(13)	120.82(16)		
C(11)-C(12)-C(13)	120.92(15)		
C(8)-C(14)-C(12)	120.82(16)		
C(16)-C(15)-N(3)	116.70(14)		
C(16)-C(15)-C(11)	124.97(15)		
N(3)-C(15)-C(11)	118.32(13)		
C(15)-C(16)-N(4)	123.30(15)		
C(15)-C(16)-C(17)	121.58(15)		
N(4)-C(16)-C(17)	115.11(13)		
N(4)-C(18)-C(19)	122.77(15)		
N(2)-C(19)-N(3)	111.49(14)		
N(2)-C(19)-C(18)	131.40(15)		
N(3)-C(19)-C(18)	117.10(14)		
C(21)-C(20)-N(3)	105.24(16)		
N(2)-C(21)-C(20)	112.49(16)		
C(7)-N(1)-C(6)	117.96(17)		
C(19)-N(2)-C(21)	104.19(14)		
C(20)-N(3)-C(19)	106.59(14)		
C(20)-N(3)-C(15)	131.94(14)		
C(19)-N(3)-C(15)	121.46(12)		
C(18)-N(4)-C(16)	118.66(13)		
C(4)-O(1)-C(3)	105.52(16)		

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for compound **31**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	50(1)	37(1)	63(1)	4(1)	-6(1)	10(1)
C(2)	72(1)	48(1)	101(2)	26(1)	4(1)	9(1)
C(3)	103(2)	57(1)	115(2)	33(1)	-6(2)	24(1)
C(4)	59(1)	45(1)	66(1)	-7(1)	-13(1)	19(1)
C(5)	44(1)	66(1)	108(2)	-10(1)	2(1)	12(1)
C(6)	49(1)	58(1)	133(2)	11(1)	22(1)	3(1)
C(7)	42(1)	34(1)	68(1)	7(1)	3(1)	8(1)
C(8)	40(1)	39(1)	74(1)	20(1)	8(1)	10(1)
C(9)	70(1)	44(1)	68(1)	4(1)	14(1)	18(1)
C(10)	63(1)	49(1)	54(1)	7(1)	3(1)	13(1)
C(11)	36(1)	36(1)	55(1)	11(1)	2(1)	4(1)
C(12)	49(1)	39(1)	59(1)	8(1)	-7(1)	2(1)
C(13)	116(2)	53(1)	73(1)	-6(1)	-23(1)	11(1)
C(14)	49(1)	47(1)	63(1)	15(1)	-9(1)	2(1)
C(15)	41(1)	34(1)	52(1)	9(1)	0(1)	4(1)
C(16)	38(1)	40(1)	73(1)	13(1)	-4(1)	1(1)
C(17)	47(1)	56(1)	146(2)	39(1)	3(1)	-1(1)
C(18)	43(1)	38(1)	62(1)	9(1)	-9(1)	7(1)
C(19)	46(1)	34(1)	57(1)	12(1)	-1(1)	4(1)
C(20)	37(1)	54(1)	84(1)	21(1)	6(1)	4(1)
C(21)	45(1)	59(1)	108(2)	29(1)	9(1)	-4(1)
N(1)	52(1)	45(1)	99(1)	16(1)	19(1)	7(1)
N(2)	53(1)	45(1)	89(1)	25(1)	4(1)	-1(1)
N(3)	37(1)	37(1)	55(1)	12(1)	1(1)	4(1)
N(4)	39(1)	43(1)	75(1)	12(1)	-7(1)	5(1)
O(1)	80(1)	62(1)	98(1)	12(1)	-20(1)	32(1)
O(2)	44(1)	46(1)	106(1)	35(1)	12(1)	12(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3) for compound 31.

	x	y	z	U(eq)
H(2)	2041	-3917	2202	88
H(3)	4434	-5456	2415	110
H(5)	8287	-2322	1540	87
H(6)	7120	-402	1162	96
H(9)	748	-629	616	72
H(10)	-578	1331	303	66
H(13A)	-1432	3492	2063	121
H(13B)	316	4175	1838	121
H(13C)	418	3181	2346	121
H(14)	1303	983	2142	64
H(17A)	-4900	2012	750	124
H(17B)	-5505	2924	1250	124
H(17C)	-3833	1991	1317	124
H(18)	-3830	6534	365	57
H(20)	2210	4230	683	70
H(21)	2529	6486	302	85

Measurment of compound 31 Racemization Kinetics

Racemization data at 195 °C

Data For Flow Run at 195 °C				
Sample #	Recorded Temperature (°C)	Recorded Time (min)	Measured %ee	ln %ee
0	control	0	99.0	4.60
1	195 C	3.06	86.0	4.45
2	195 C	3.08	85.2	4.45
3	195 C	4.04	77.4	4.35
4	195 C	4.07	77.8	4.35
5	195 C	5.12	73.8	4.30
6	195 C	5.12	74.0	4.30
7	195 C	6.13	69.8	4.25
8	195 C	6.11	70.6	4.26
9	195 C	8.18	63.2	4.15
10	195 C	8.16	62.8	4.14
11	195 C	10.24	56.2	4.03
12	195 C	10.19	56.6	4.04
13	195 C	12.24	50.2	3.92
14	195 C	12.28	49.4	3.90
15	195 C	15.47	43.4	3.77
16	195 C	15.51	42.4	3.75
17	195 C	20.66	32.0	3.47
18	195 C	20.62	33.4	3.51
19	195 C	30.58	21.8	3.08
20	195 C	30.50	22.0	3.09
21	195 C	32.50	17.0	2.83
22	195 C	32.46	17.2	2.84

Racemization data at 200 °C

Data For Flow Run at 200 °C				
Sample #	Recorded Temperature (°C)	Recorded Time (min)	Measured %ee	ln %ee
0	control	0	99.0	4.60
1	200 C	3.01	74.8	4.31
2	200 C	3.00	74.0	4.30
3	200 C	3.98	70.2	4.25
4	200 C	3.97	71.0	4.26
5	200 C	5.03	65.0	4.17
6	200 C	5.00	65.0	4.17
7	200 C	6.01	60.6	4.10
8	200 C	6.03	60.0	4.09
9	200 C	8.02	50.8	3.93
10	200 C	8.02	51.0	3.93
11	200 C	10.05	45.2	3.81
12	200 C	10.04	45.0	3.81
13	200 C	12.03	38.0	3.64
14	200 C	12.02	40.0	3.69
15	200 C	15.18	31.6	3.45
16	200 C	15.20	32.8	3.49
17	200 C	20.33	21.6	3.07
18	200 C	20.23	20.2	3.01
19	200 C	30.15	12.4	2.52
20	200 C	30.08	12.0	2.48
21	200 C	31.88	9.4	2.24
22	200 C	31.98	9.6	2.26

Racemization Data at 205 °C

Data For Flow Run at 205 °C				
Sample #	Recorded Temperature (°C)	Recorded Time (min)	Measured %ee	ln %ee
0	control	0	99	4.60
1	205 C	2.55	75.6	4.33
2	205 C	2.52	75.0	4.32
3	205 C	3.00	71.2	4.27
4	205 C	3.02	71.0	4.26
5	205 C	4.00	64.2	4.16
6	205 C	3.97	63.0	4.14
7	205 C	5.00	56.2	4.03
8	205 C	5.01	57.0	4.04
9	205 C	6.03	49.8	3.91
10	205 C	5.99	50.6	3.92
11	205 C	8.01	41.6	3.73
12	205 C	7.98	42.0	3.74
13	205 C	10.00	33.2	3.50
14	205 C	10.00	32.2	3.47
15	205 C	11.95	27.4	3.31
16	205 C	11.97	27.6	3.32
17	205 C	15.07	20.0	3.00
18	205 C	15.10	19.8	2.99
19	205 C	20.15	12.4	2.52
20	205 C	20.14	12.8	2.55
21	205 C	29.76	5.6	1.72
22	205 C	29.76	5.2	1.65

Racemization data at 210 °C

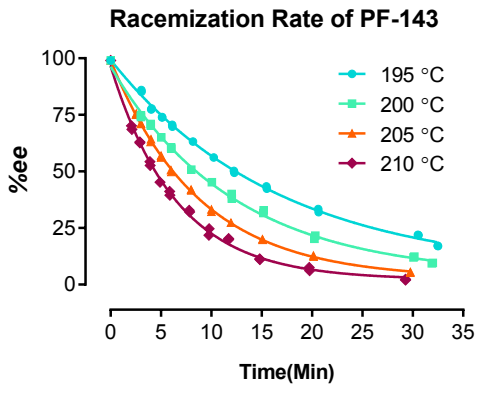
Data For Flow Run at 210 °C				
Sample #	Recorded Temperature (°C)	Recorded Time (min)	Measured %ee	ln %ee
0	control	0	100	4.61
1	210 C	2.08	70.1	4.25
2	210 C	2.11	68.6	4.23
3	210 C	2.90	63.0	4.14
4	210 C	2.90	62.6	4.14
5	210 C	3.90	54.3	3.99
6	210 C	3.92	52.6	3.96
7	210 C	4.90	45.2	3.81
8	210 C	4.91	45.2	3.81
9	210 C	5.86	41.2	3.72
10	210 C	5.88	39.5	3.68
11	210 C	7.84	32.1	3.47
12	210 C	7.80	32.8	3.49
13	210 C	9.79	24.7	3.21
14	210 C	9.77	21.8	3.08
15	210 C	11.71	19.9	2.99
16	210 C	11.72	20.3	3.01
17	210 C	14.81	11.3	2.42
18	210 C	14.83	11.1	2.41
19	210 C	19.72	7.5	2.01
20	210 C	19.75	6.2	1.83
21	210 C	29.28	2.4	0.89
22	210 C	29.29	2.0	0.69

Atropisomer Racemization Kinetics Discussion

Understanding that the energetics of atropisomer interconversion would be a relevant for both synthetic chemistry and any therapeutic advancement for compounds in this hindered biaryl bond series, we used a streamlined segmented flow method[36] to experimentally determine accurate $\Delta G^{\ddagger}$ and $T_{1/2}$ for racemization in solvents of interest (see SI for data). The kinetics governing the racemization of the less potent enantiomer **30** were measured using a segmented flow reactor in a solution of 3- methyl-butane-1-ol spiked with 3% toluene to assist UV detection of the segments.[36] After an initial temperature scouting run to determine optimal temperatures, compound **30** (>99% starting *ee*) was heated in duplicate to 195, 200, 205 and 210°C for 11 predefined time intervals between 2.5 and 30 min. Samples were analyzed by chiral chromatography to determine %*ee* (Figure 6A). The first order rate constants, for the rate of racemization,[37] were obtained by plotting ln(%ee) vs. time at each temperature (Figure 6B) where the slope = $-k_{rac}$ (Figure 6C). The $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ of racemization was solved by applying Eyring Equation (Figure 6D and 8E). From the slope of the line in the Eyring plot we extrapolated what compound **30**'s $\Delta G^{\ddagger}$ and $T_{1/2}$ at would be in solution at temperatures ranging from 0-250 °C (Figure 6F). For example,

using this approach, we determined these parameters for **31** and chiral intermediates leading to **31.** Knowing that these compounds can be resolved early in the synthesis and then handled in the laboratory for short periods of time at temperatures up to 100 °C in solvents of interest (extrapolated $T_{1/2}$ = 18 days in) without risk without significant erosion of enantiopurity, was enabling to route development efforts.

A



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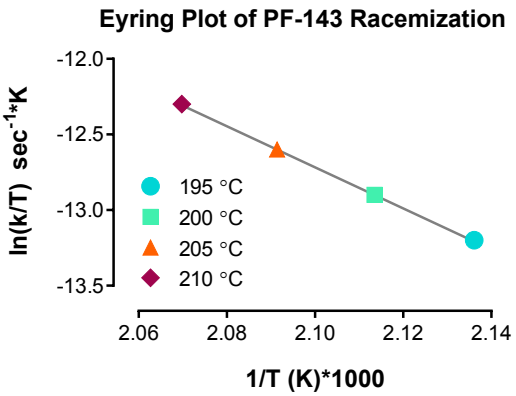

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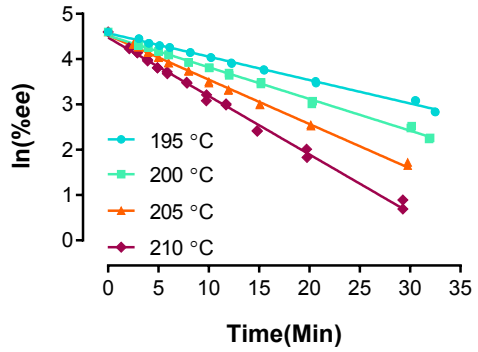

E

Measured Activation Parameters

ΔH‡	ΔS‡	units
26.8	-0.01621	kcal/mol & kcal/mol•K

C

Entry	T (°C)	K_{rac} (min^{-1})	R^2	$t_{1/2}$ (min)
1	210	0.052	0.995	13.4
2	205	0.070	0.996	9.9
3	200	0.098	0.997	7.1
4	195	0.129	0.994	5.4

Extrapolations from Y = -13489X + 15.604

F

T (°C)	ΔG kcal/mol	$T_{1/2}$	units
0	31.2	37620	years
25	31.6	548	years
37	31.8	91.6	years
60	32.2	4.2	years
100	32.9	18.0	days
250	35.4	35	seconds

Figure 6: (A and B) Plots depicting the erosion of %*ee* versus time in a 3- methyl-butane-1-ol/toluene solution (97:3) at 4 different temperatures at 12 different time points generated in duplicate; (C) Table summarizing the measured first-order rate constants for the racemization of compound **30** in solution; (D) Eyring plot for the atropo isomerization of compound **30** in solution; (E) extrapolated activation parameters for compound **30** in solution; (F) Calculated $\Delta G^{\ddagger}$ and $T_{1/2}$ at temperatures ranging from 0-250 °C.

Method: Mouse Locomotor Activity (LMA)

All procedures performed on these animals were in accordance with regulations and established guidelines and were reviewed and approved by Pfizer Institutional Animal Care and Use Committee. Naïve male CD-1 mice, 14-16 grams at delivery from Charles River Laboratories, were given one week to acclimate to the facilities prior to study initiation. They were socially housed four per cage in vent-rack cages and allowed free access to food and water. Mice were maintained on a 12 hour light/dark (0600 h/1800 h) cycle under controlled temperature and humidity conditions. Average weight upon testing was 20-25 grams.

Compound 29 (free base; in house) was dissolved in 5% dimethyl sulfoxide + 5% Cremophor EL + 90% sterile water or sterile saline 1 molar equivalent of hydrochloric acid. R (+)-SCH-23390 (hydrochloride, Sigma-Aldrich, MO, USA) was corrected for the weight of the salt and dissolved in sterile water. Mice were dosed subcutaneously in 10 mL/kg volumes.

On testing day, mice were brought to the testing room one hour prior to study initiation. Mice were habituated to the chamber (20 cm X 20 cm X 30 cm acrylic chamber in a sound-attenuating testing box) for 90 minutes prior to compound administration. For the compound 6 alone study, mice were given a dose of Compound 29, or vehicle, then returned to the chamber and data (beam breaks) were collected (Versamax software; AccuScan Instruments; Columbus, OH) over two hours. For the combination study, SCH-23390 and compound 29, the same procedures were followed above, however, prior to treatment with compound 29 or vehicle mice were dosed with either a dose of SCH-23390 or vehicle and returned to the chamber for 30 minutes prior to compound 29 or vehicle dosing. Data (cumulative beam breaks over 2 hours, n=8/group) were compared using a one-way ANOVA followed by a Dunnett's post-test (GraphPad Prism software (ver 7); San Diego, CA).

Method: D1R Desensitization Studies in Rat Primary Neurons

Primary rat striatal neurons were isolated from embryonic day 18 (E18) mice by standard neuronal isolation procedures. Isolated neurons are plated at a density of 30,000 cells/well in poly-ornithine/laminin coated 96 well plates (BD Falcon), and cultured in Neurobasal media supplemented with B27, 1x Glutamax, and penicillin/streptomycin (100 U/mL) (all from Invitrogen) and incubated at 37°C in 5% CO2 for 14-16 d prior to assay. To assess D1 agonists-induced receptor desensitization, neurons were pretreated with 10 ?M of agonists or 0.1% DMSO (Vehicle) in serum free Neurobasal media for 90 min. After the pretreatment, cells were washed twice at 5 min intervals with 250 ?l/well fresh Neurobasal media. The residual D1Rs cAMP signaling ability was then measured by 30 min stimulation with 1 ?M SKF-81297, a D1 full agonist (in the presence of 250 ?M IBMX). The concentration of cAMP accumulation was determined using the Cisbio cAMP dynamic2 kit (Cisbio) according to the manufacturer protocol. The concentration of cAMP (nM) from treated wells was interpolated from a cAMP standard curve run in the same assay. The percent desensitization was calculated as the percent decrease in cAMP relative to vehicle control.

D1 Radioligand Binding Assay

Binding assays were performed using a stably expressed human D1R LTK cell line. Basic assay parameters were determined from saturation binding studies where the Kd using [3H]-SCH23390 (Perkin Elmer NET930001MC) was found to be 1.3 nM. Tissue concentration curve studies determined the optimal amount of tissue to be 2.4 mg/ml. These ligand and tissue concentrations were used in time course studies where it was determined to be linear with equilibrium being reached at 30 minutes at 37

°C. Ki values were determined by homogenizing 2.4mg/ml of hD1R tissue in 50mM Tris (pH 7.4 at 4 °C) containing 2.0mM MgCl2, and spun in a centrifuge at 40,000 × g for 10 min. The pellet was then resuspended in assay buffer (50mM Tris (pH 7.4 at RT) containing 4mM MgSO4 and 0.5mM EDTA). Incubations were initiated by the addition of 200 µl of tissue to a 96-well plate containing test drugs (2.5 µl) and 0.5 nM [3H]-SCH23390 (50ul) for a final assay volume of 250 µl. Non-specific binding was determined by radioligand binding in the presence of a saturating concentration of (+)-butaclamol (10 µM). After the 30 minute incubation period at 37 °C, assay samples were rapidly filtered through Unifilter-96 GF/B PEI-coated filter plates and rinsed with ice-cold 50mM Tris buffer (pH 7.4 at 4 °C). Membrane radioligand levels were determined by liquid scintillation counting of the filter plates in 50 µl Ecolume. The IC50 value (concentration at which 50% inhibition of specific binding occurs) was calculated by linear regression of the concentration-response data (10 concentrations at half log increments) in Activity Base (IDBS). Ki values were calculated according to the Cheng-Prusoff equation. Reported values are the mean of results obtained across at least three independent experiments (n ≥ 3) each assayed in triplicate unless otherwise noted.

D1 Binding Ki 95% confidence intervals

Cmpd #	D1 hK_i[b] (nM) (95% CI)
1	243 (122-363)
6	2,242 (1702-2782)
17	254 (125-385)
18	1,680[e] (n=1)
19	563[f] (303-823)
20	121[f] (59.2-183.3)
21	376 (196-556)
22	244 (356-844)
23	56.3 (8.7-121)
24	98.9 (57.9-139.9)
25	38.1 (15.1-61.1)
26	8.5 (6.5-23.5)
27	6.8 (5.9-7.9)
28	2,346 (2036-2656)
29	8.9 (4.8-13.2)
30	21.6 (13.7-29.5)

31	11.9 (2.2-21.8)

[e]N = 1; [f]N = 2.

Functional cAMP Assays

For the cAMP assay, the expression of the clone was confirmed by sequencing. Stable HEK293T cells expressing hD1R (wide type or mutant) were grown in high glucose DMEM (Invitrogen 11995-065), 10% fetal bovine serum dialyzed (Invitrogen 26400–044), 1 × MEM NEAA (Invitrogen 1140), 25 mM HEPES (Invitrogen 15630), 1 × penicillin/streptomycin (Invitrogen 15070-063) and 500 µg/mL genenticin (Invitrogen 10131-035) at 37 °C and 5% CO_2. At 72–96 h post seeding, cells were rinsed with phosphate buffered saline and 0.25% trypsin-EDTA was added to dislodge the cells. Media was then added and cells were centrifuged and media removed. The cell pellets were resuspended in Cell Culture Freezing Medium (Invitrogen 12648-056) at a density of 40 million cells/ml. One ml aliquots of the cells were made in Cryo-vials and frozen at −80 °C for use in the hD1R HTRF cAMP assay. Frozen cells were quickly thawed, re-suspended in warm media and allowed to sit for 5 min prior to centrifugation (1000 rpm) at room temperature. Media was removed and the cell pellet was re-suspended in PBS containing 500 µM isobutylmethylxanthine (IBMX) to inhibit PDE activity. Using a Multidrop Combi (Thermo Scientific), 5 µL cells/well at a cell density of approximately 1000 cells/well were added to the assay plate (Greiner 784085) which contained 5 µl of test compound. To control for subtle cell density plating differences, each plate also contained positive controls of 5 µM dopamine (final concentration) and negative controls of 0.5% DMSO (final concentration). Cells and compounds were incubated at room temperature for 30 min. Working solutions of cAMP-D2 and anti-cAMP cryptate were prepared according to Cisbio instructions. Using the Multidrop Combi, 5 µL cAMP-D2 working solution was added to the assay plate containing the test compound and cells. Using the Multidrop Combi, 5 µL anti-cAMP-cryptate working solutions was added to assay plates containing test compound, cells, and cAMP-D2. Assay plates were incubated for 1 h at room temperature, then read using an Envision plate reader (Perkin Elmer) using Cisbio recommended settings. A time resolved and ratiometric emissions measurement (665 nm/620 nm) was obtained, which was then converted to cAMP concentrations using a standard curve. A cAMP standard curve was generated using cAMP stock solution provided in the Cisbio kit, which was then used to convert the raw ratio data to cAMP concentrations. EC_{50} values were determined using a logistic 4 parameter fit model to an 11 point concentration response curve with half-log increments. The percent efficacy for each curve was determined by the maximum asymptote of that fitted curve, and expressed as a percent of the maximum response produced by the positive controls (5 µM dopamine) on each plate. Reported values are the mean of results obtained across at least three independent experiments ($n \geq 3$) each assayed in triplicate. The large file HTS screen protocol varied slightly from the subsequent SAR protocol in following areas: the HTS screen was run in 384 well plates containing 14 compounds/well and in the presence of an EC_{10} of dopamine present in all assays wells. Additionally, the ratiometric emissions measurement (665 nm/620 nm) was used to calculate % effect directly without the conversion to cAMP from the standard curve. Potential hits were de-convoluted and re-tested in the assay for confirmation at 20 µM compound concentration in a single compound/well format and without EC_{10} of Dopamine.

Summary of functional data in both “cAMP” and “ratio” modes including the standard deviation plus 95% confidence interval

cpd #	D1 functional cAMP mode EC50 (uM)				D1 functional ratio mode ASMAX (%)			
	GeoMean (EC50)	Count	STDev	95% CI	Average (%)	Count	STDev	95%CI
1	0.2324	341	0.2039	0.2324 ± 0.022	100.245	341	9.743	100.245 ± 1
6	2.2929	40	4.4503	2.2929 ± 1.4	36.57	39	8.044	36.57 ± 2.5
21	1.1408	57	0.8699	1.1408 ± 0.23	73.25	57	11.368	73.25 ± 3
18	2.4111	1			11.28	1		
23	1.3332	3	1.0707	1.3332 ± 1.2	64.294	3	9.729	64.294 ± 11
24	0.8211	8	0.6457	0.8211 ± 0.45	61.183	8	8.544	61.183 ± 5.9
17	1.2324	1			33.898	1		
22	0.4159	35	1.4106	0.4159 ± 0.47	81.959	35	13.927	81.959 ± 4.6
25	0.2623	2	0.1023		53.969	2	1.828	
26	0.1226	7	0.0875	0.1226 ± 0.065	62.293	7	12.59	62.293 ± 9.3
27	0.0636	3	0.1842	0.0636 ± 0.21	64.420	3	9.97	64.42 ± 11
19	1.1080	1			7.339	1		
20	0.8955	1			17.626	1		
29	0.1064	25	0.0743	0.1064 ± 0.029	86.353	25	8.10	86.353 ± 3.2
28	5.4846	6	3.4939	5.4846 ± 2.8	26.327	6	9.51	26.327 ± 7.6
31	0.0328	30	0.0261	0.0328 ± 0.0093	91.465	30	11.90	91.465 ± 4.3
30	0.1407	6	0.0278	0.1407 ± 0.022	88.783	6	7.39	88.783 ± 5.9

Molecular Smiles Strings For All Final Compounds

Compound #	Smiles
6	n1(nccc1-c2ccc(cc2)Oc3nccc4occc43)C
17	n1(nccc1-c2c(cc(cc2)Oc3nccc4occc43)C)C
18	n1(nccc1-c2ccc(cc2)Oc3nccc4occc43)C(C)(C)C
19	n1(nccc1-c2c(cc(cc2)Oc3nccc4occc43)C)C(C)(C)C
20	n1(ncc(c1-c2c(cc(cc2)Oc3nccc4occc43)C)C)C(C)(C)C
21	n1(c(nc2c1ccnc2)C)-c3ccc(cc3)Oc4nccc5occc54
22	n1(c(nc2c1ccnc2)C)-c3c(cc(cc3)Oc4nccc5occc54)C
23	n2c(c1c(occ1)cc2)Oc3ccc(cc3)-c5c4c(cncc4)ccc5OC
24	n21c(ncc1)cccc2-c3ccc(cc3)Oc4nccc5occc54
25	n21c(ncc1)cccc2-c3c(cc(cc3)Oc4nccc5occc54)C
26	n21c(ncc1)ccc(c2-c3ccc(cc3)Oc4nccc5occc54)C
27	n21c(ncc1)ccc(c2-c3c(cc(cc3)Oc4nccc5occc54)C)C
28	n21ncnc1cncc2-c3c(cc(cc3)Oc4nccc5occc54)C
29	n21c(ncc1)cnc(c2-c3c(cc(cc3)Oc4nccc5occc54)C)C

30	n21c(ncc1)cnc(c2-c3c(cc(cc3)Oc4nccc5occc54)C)C
31	n21c(ncc1)cnc(c2-c3c(cc(cc3)Oc4nccc5occc54)C)C