Supporting Information

ADME-Guided Design and Synthesis of Aryloxanyl Pyrazolone Derivatives to Block Mutant Superoxide Dismutase 1 (SOD1) Cytotoxicity and Protein Aggregation: Potential Application for the Treatment of Amyotrophic Lateral Sclerosis

Tian Chen[1], Radhia Benmohamed[2], Jinho Kim[3], Karen Smith[3], Daniel Amante[3], Richard I. Morimoto[5], Donald R. Kirsch[2], Robert J. Ferrante[4], Richard B. Silverman[1,6]*

[1]Department of Chemistry Northwestern University, Evanston, Illinois 60208-3113 USA, [2]Cambria Pharmaceuticals, Cambridge, Massachusetts, 02142 USA, [3] Neurological Surgery, Neurology, and Neurobiology Departments, University of Pittsburgh, Pittsburgh, PA 15213, USA and the Geriatric Research Educational and Clinical Center (00-GR-H), V.A. Pittsburgh Healthcare System, 7180 Highland Drive, Pittsburgh, PA 15206, USA, [5]Department of Molecular Biosciences, Rice Institute for Biomedical Research, Northwestern University, Evanston, Illinois 60208-3500 USA, and [6]Department of Molecular Biosciences, Chemistry of Life Processes Institute, Center for Molecular Innovation and Drug Discovery, Northwestern University, Evanston, Illinois 60208-3113 USA.

Table of Contents

Experimental details and data for 35, **36**, **39**, **41**, and **42-47.**

2-Bromo-*N*-methoxy-*N*-methylacetamide (36). [1] *N*,*O*-Dimethylhydroxylamine hydrochloride (16.0 g, 164 mmol) and K_2CO_3 (50.0 g, 362 mmol) were dissolved and stirred in Et_2O (200 mL) and H_2O (200 mL) at 0 ^oC. Bromoacetyl bromide (15.68 mL, 181 mmol) was added dropwise to the reaction at 0 ^oC, which was stirred for another 30 min after the ice bath was removed. The layers were separated, and the aqueous layer was extracted with Et_2O (2 × 200 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated in vacuo to afford **36** (22.0 g, 74%) as a yellow oil. Compound **36** was directly used in the next step without further purification. ^1H NMR (500 MHz, $CDCl_3$, δ): 4.02 (s, 2H), 3.80 (s, 3H), 3.23 (s, 3H).

Biphenyl-3-ol (39). 3-Bromophenol (1.05 g, 6.07 mmol), phenylboronic acid (1.48 g, 12.1 mmol), K_2CO_3 (2.00 g, 14.5 mmol), and $PdCl_2(PPh_3)$ (1/200 eq.) were added to a solution of dioxane/H_2O (20 mL/5 mL). The resulting solution was refluxed for 16 h. The reaction mixture was then partitioned between Et_2O and water, and the aqueous phase was extracted with Et_2O. The combined organic layer was evaporated to dryness

and purified by flash column chromatography (ethyl acetate/hexanes = 1/9) to give **39** (1.02 g, 98%) as a transparent oil. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.57 (d, *J* = 7.2 Hz, 2H), 7.44 (t, *J* = 7.6 Hz, 2H), 7.37-7.30 (m, 2H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.07-7.06 (m, 1H), 6.83-6.81 (m, 1H), 4.94 (br, 1H).

5-Phenylbiphenyl-3-ol (41). 3,5-Dibromophenol (1.0 g, 3.97 mmol), phenylboronic acid (2 g, 16.4 mmol), K_2CO_3 (2.7 g, 19.5 mmol), and $PdCl_2(PPh_3)$ (1/200 eq.) were added to a solution of dioxane/H_2O (20 mL/ 5 mL). The resulting solution was allowed to reflux for 16 h. The reaction mixture was then partitioned between Et_2O and water, and the aqueous phase was extracted with Et_2O. The combined organic layer was evaporated to dryness and purified by flash column chromatography (ethyl acetate/hexanes = 1/9) to give **41** (0.83 g, 83%) as a transparent oil. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.61 (d, *J* = 7.2 Hz, 4H), 7.43 (t, *J* = 7.6 Hz, 4H), 7.37-7.33 (m, 3H), 7.07 (s, 2H), 6.10 (br s, 1H).

Weinreb amide method B1.

2-(3,5-Dichlorophenoxy)acetic acid (35). To a solution of 3,5-dichlorophenol (60.0 g, 366 mmol) in EtOH (25 mL) was added NaOEt (21 wt% in EtOH, 137 mL, 362 mmol) at room temperature. The reaction was left stirring for another 10 min. To the reaction mixture, ethyl bromoacetate (20.5 mL, 370 mmol) was added. The reaction was gently brought to 70 ^{o}C overnight. NaOH (1 N in H_2O, 420 mL, 420 mmol), and 100 mL H_2O was added. The reaction mixture was then brought to 90 ^{o}C for 4 h. After ethyl 2-(3,5-dichlorophenoxy)acetate had disappeared by TLC analysis (ethyl acetate/hexanes = 1/9), the solution was cooled. Then it was washed with chloroform (3 × 500 mL), adjusted to pH 3 with HCl (1 N), extracted with chloroform (5 × 500mL), and concentrated to dryness to give the 3,5-dichlorophenoxyacetic acid **35** (70.0 g, 86%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 10.78 (br s, 1H), 7.03 (s, 1H), 6.83 (s, 2H), 4.68 (s, 2H).

2-(3,5-Dichlorophenoxy)-*N*-methoxy-*N*-methylacetamide (42). To a solution of *N*,*O*-dimethylhydroxylamine HCl salt (5.30 g, 54.33 mmol) in DCM (250 mL) was added DIEA (24 mL, 137.8 mmol) at room temperature. The reaction mixture was stirred for 5 min. Acetyl chloride (13.0 g, 54.28 mmol) was then added in DCM drop by drop at 0 ^{o}C. The reaction mixture was stirred at room temperature for another 30 min. The resulting reaction solution was washed with HCl (1 N) and concentrated under vacuum. The crude solid product was washed with Et_2O to give **42** (12 g, 84%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.99 (s, 1H), 6.84 (s, 2H), 4.80 (s, 2H), 3.77 (s, 3H), 3.25 (s, 3H).

Weinreb amide method B2.

2-(3,5-Dichlorophenoxy)-*N*-methoxy-*N*-methylacetamide (42). To a solution of phenol (5.27 g, 32.33 mmol) in EtOH (10 mL) was added NaOEt (21 wt% in EtOH, 12.1 mL, 32.41 mmol) at room temperature. The reaction mixture was stirred for 10 min. Compound **39** (5.87 g, 32.25 mmol) was then gently added at room temperature. After the resulting solution was stirred at 70 ^{o}C overnight, the reaction mixture was cooled, poured into HCl (0.25 M), and the aqueous layer was extracted with EtOAc. The

combined organic layer was concentrated in vacuo and reconstituted in $CHCl_3$. The precipitate was filtered and washed with $CHCl_3$. Wienreb amide **42** (4.53 g, 53%) was obtained as a white solid.

2-(3,5-Difluorophenoxy)-*N*-methoxy-*N*-methylacetamide (43). Analogous to **42**, compound **43** was prepared via method B2. 3,5-Difluorophenol (1.00 g, 7.69 mmol) was treated with NaOEt (21 wt% in EtOH, 2.90 mL, 7.69 mmol) and **36** (1.40 g, 7.69 mmol). Further purification by flash chromatography (ethyl acetate/hexanes = 1/2) was applied to give **42** (1.24 g, 70%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 6.49-6.42 (m, 3H), 4.81 (s, 2H), 3.77 (s, 3H), 3.24 (s, 3H).

2-(3,5-Dibromophenoxy)-*N*-methoxy-*N*-methylacetamide (44). Analogous to **42**, **44** was prepared via method B2. 3,5-Dibromophenol (1.00 g, 3.97 mmol) was treated with NaOEt (21 wt% in EtOH, 1.50 mL, 4.01 mmol) and **36** (0.72 g, 3.96 mmol). Further purification by flash chromatography (ethyl acetate/hexanes = 1/2) was applied to give **44** (1.07 g, 76%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.28 (s, 1H), 7.04 (s, 2H), 4.80 (s, 2H), 3.77 (s, 3H), 3.25 (s, 3H).

2-(3-Bromophenoxy)-*N*-methoxy-*N*-methylacetamide (45). Analogous to **42**, compound **45** was prepared via method B2. 3-Bromophenol (2.50 g, 14.5 mmol) was treated with NaOEt (21 wt% in EtOH, 5.40 mL, 14.5 mmol) and **36** (2.66 g, 14.6 mmol). Further purification by flash chromatography (ethyl acetate/hexanes = 1/2) was applied to give **45** (1.07 g, 61%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.16-7.09 (m, 3H), 6.90-6.88 (m, 1H), 4.80 (s, 2H), 3.76 (s, 3H), 3.24 (s, 3H).

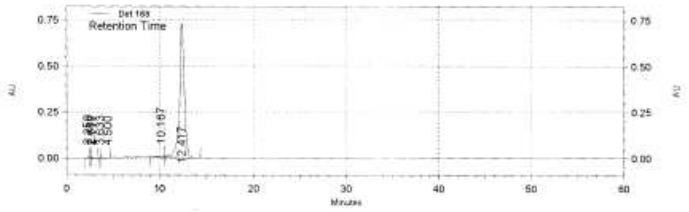
2-(Biphenyl-3-yloxy)-*N*-methoxy-*N*-methylacetamide (46). Analogous to **42**, compound **46** was prepared via method B2. Biphenyl-3-ol (1.02 g, 5.96 mmol) was treated with NaOEt (21 wt% in EtOH, 2.22 mL, 5.95 mmol) and **36** (1.08 g, 5.93 mmol). Further purification by flash chromatography (ethyl acetate/hexanes = 1/2) was applied to give **46** (0.95 g, 59%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.57 (d, J = 7.2 Hz, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.60-7.32 (m, 2H), 7.20 (s, 1H), 7.15-7.09 (m, 1H), 6.93-6.91(m, 1H), 4.88 (s, 2H), 3.77 (s, 3H), 3.24 (s, 3H).

2-(5-Phenylbiphenyl-3-yloxy)-*N*-methoxy-*N*-methylacetamide (47). Analogous to **42**, **47** was prepared via method B2. 5-Phenylbiphenyl-3-ol (0.81 g, 3.29 mmol) was treated with NaOEt (21 wt% in EtOH, 1.25 mL, 3.35 mmol) and **36** (0.60 g, 3.30 mmol). Further purification by flash chromatography (ethyl acetate/hexanes = 1/2) was applied to give **47** (0.71 g, 62%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.63 (d, J = 7.2 Hz, 4H), 7.46-7.43 (m, 5H), 7.38-7.35 (m, 2H), 7.17 (s, 2H), 4.92 (s, 2H), 3.77 (s, 3H), 3.25 (s, 3H).

HPLC data and spectra for 1-19.

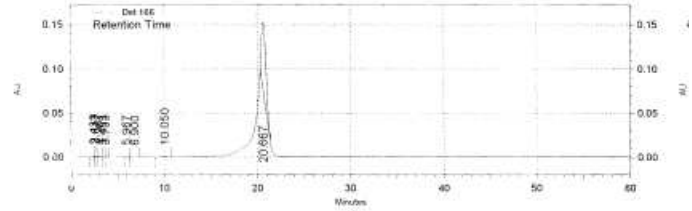
5-((4-Chlorophenylthio)methyl)-1*H*-pyrazol-3(2*H*)-one (1)

HPLC method A (isocratic; MeCN:H_2O 30:70, 60 min; 0.1% TFA): r.t. = 12.42 min, purity = 97.1%.



Time	Area	Area %	Height	Height %
2.250	120287	0.45	5378	0.71
2.600	33115	0.12	4474	0.59
2.717	95979	0.36	5109	0.67
3.633	35978	0.14	4960	0.65
4.500	47747	0.18	835	0.11
10.167	443085	1.66	6932	0.91
12.417	25873335	97.09	730016	96.35
Totals	26649526	100.00	757704	100.00

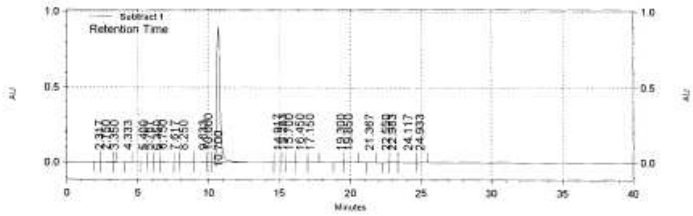
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 20.67 min, purity = 97.0%.



Time	Area	Area %	Height	Height %
2.433	14744	0.56	1050	1.25
2.617	16792	0.64	1507	1.80
2.967	22526	0.86	1760	2.10
3.483	3106	0.12	159	0.19
3.733	2134	0.08	135	0.16
5.967	2611	0.10	166	0.20
6.900	5365	0.20	125	0.15
10.050	10936	0.42	306	0.37
20.667	2552632	97.03	78525	93.78
Totals	2630846	100.00	83733	100.00

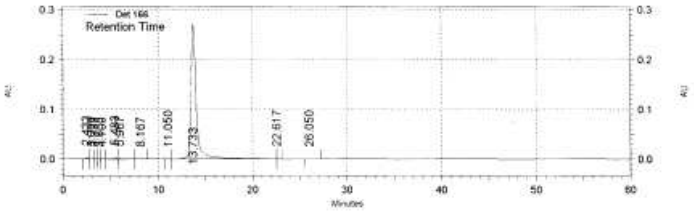
5-((3,5-Dichlorophenylthio)methyl)-1*H*-pyrazol-3(2*H*)-one (2)

HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 10.70 min, purity = 98.4%.



Time	Area	Area %	Height	Height %
2.317	18196	0.12	1240	0.14
2.750	58929	0.38	1436	0.16
3.350	10407	0.07	1154	0.13
4.333	1392	0.01	91	0.01
5.400	1223	0.01	89	0.01
5.767	1211	0.01	73	0.01
6.350	1913	0.01	134	0.01
6.750	58347	0.38	3603	0.39
7.617	1837	0.01	114	0.01
8.250	5551	0.04	212	0.02
9.633	28622	0.19	1900	0.21
10.000	5746	0.04	339	0.04
10.700	15206319	98.36	901686	98.59
14.917	10841	0.07	442	0.05
15.283	5354	0.03	349	0.04
15.700	10280	0.07	511	0.06
16.450	6099	0.04	177	0.02
17.150	2585	0.02	83	0.01
19.300	4726	0.03	215	0.02
19.850	7492	0.05	265	0.03
21.367	1359	0.01	72	0.01
22.550	1496	0.01	79	0.01
22.983	2768	0.02	118	0.01
24.117	5017	0.03	107	0.01
24.933	2035	0.01	85	0.01
Totals	15459745	100.00	914574	100.00

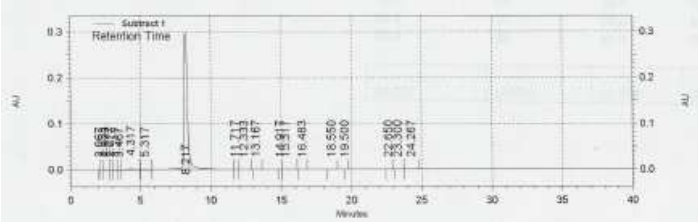
HPLC method B (isocratic; MeOH:H_2O 60:40, 60 min; 0.1% TFA): r.t. = 13.73 min, purity = 97.9%.



Time	Area	Area %	Height	Height %
2.433	36987	0.34	2616	0.93
3.000	13127	0.12	504	0.18
3.333	7043	0.06	419	0.15
3.833	5493	0.05	298	0.11
4.100	8853	0.08	315	0.11
5.483	85567	0.79	3619	1.29
5.967	50606	0.46	1396	0.50
8.167	12567	0.12	285	0.10
11.050	2456	0.02	87	0.03
13.733	10660193	97.87	271788	96.53
22.617	1124	0.01	68	0.02
26.050	8674	0.08	176	0.06
Totals	10892590	100.00	281571	100.00

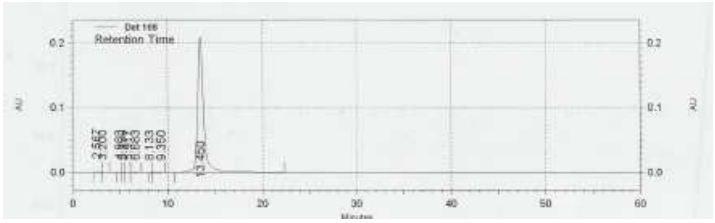
5-(4-Chlorophenylthio)-1*H*-pyrazol-3(2*H*)-one-$^{15}N_2$ (3)

HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 8.22 min, purity = 97.4%.



Time	Area	Area %	Height	Height %
2.067	331	0.01	113	0.04
2.333	3341	0.06	483	0.16
2.533	8362	0.16	866	0.28
2.817	238	0.00	33	0.01
3.217	3866	0.07	345	0.11
3.467	5788	0.11	660	0.22
4.317	75599	1.43	3776	1.24
5.317	20574	0.39	806	0.26
8.217	5139711	97.35	296840	97.31
11.717	1824	0.03	97	0.03
12.333	4295	0.08	164	0.05
13.167	4751	0.09	343	0.11
14.917	274	0.01	32	0.01
15.317	3236	0.06	132	0.04
16.483	531	0.01	32	0.01
18.550	2716	0.05	143	0.05
19.500	340	0.01	24	0.01
22.650	430	0.01	33	0.01
23.300	1231	0.02	71	0.02
24.267	2322	0.04	68	0.02
Totals	5279760	100.00	305061	100.00

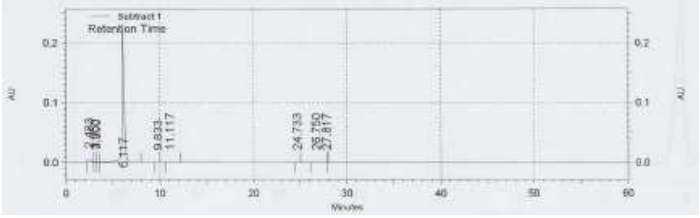
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 13.45 min, purity = 99.1%.



Time	Area	Area %	Height	Height %
2.567	25518	0.28	1952	0.92
3.200	4279	0.05	165	0.08
4.983	1512	0.02	96	0.05
5.350	1567	0.02	96	0.05
5.917	2814	0.03	120	0.06
6.683	14890	0.16	585	0.27
8.133	4145	0.05	275	0.13
9.350	27452	0.30	294	0.14
13.450	9121841	99.11	209685	98.32
Totals	9204018	100.00	213268	100.00

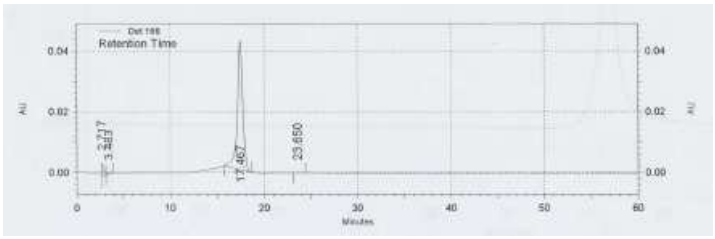
5-(4-Chlorophenylsulfinyl)-1*H*-pyrazol-3(2*H*)-one (4)

HPLC method A (isocratic; MeCN:H_2O 25:75, 60 min; 0.1% TFA): r.t. = 6.12 min, purity = 95.3%.



Time	Area	Area %	Height	Height %
2.483	117168	3.14	3759	1.59
3.050	23295	0.62	1835	0.78
3.300	15131	0.40	1072	0.45
6.117	3562696	95.34	228415	96.91
9.833	605	0.02	31	0.01
11.117	10044	0.27	379	0.16
24.733	975	0.03	48	0.02
26.750	6985	0.19	142	0.06
27.817	126	0.00	25	0.01
Totals	3737025	100.00	235706	100.00

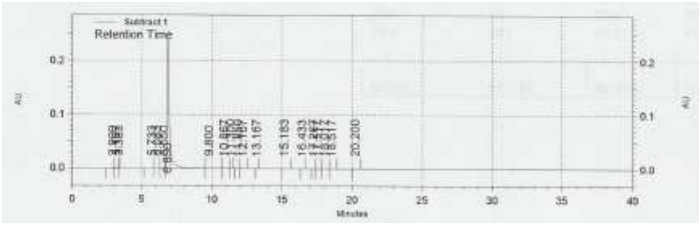
HPLC method B (isocratic; MeOH:H_2O 30:70, 60 min; 0.1% TFA): r.t. = 17.47 min, purity = 95.6%.



Time	Area	Area %	Height	Height %
2.717	58740	3.37	4743	9.93
3.483	13569	0.78	529	1.11
17.467	1665946	95.63	42376	88.76
23.650	3736	0.21	93	0.19
Totals	1741991	100.00	47741	100.00

5-(4-Chloro-2,5-dimethylphenylsulfinyl)-1*H*-pyrazol-3(2*H*)-one (5)

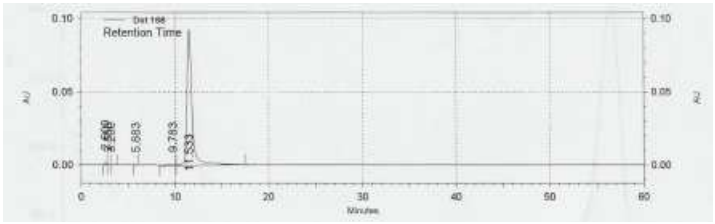
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 6.85 min, purity = 97.7%.



Time	Area	Area %	Height	Height %
2.900	10943	0.39	452	0.18
3.167	8252	0.30	819	0.32
3.383	1044	0.04	372	0.14
5.733	4220	0.15	163	0.06
6.083	4246	0.15	174	0.07
6.550	7445	0.27	665	0.26
6.850	2712696	97.70	253091	98.36
9.800	11808	0.43	286	0.11
10.867	2238	0.08	139	0.05
11.300	192	0.01	29	0.01
11.850	602	0.02	66	0.03

12.167	698	0.03	57	0.02
13.167	190	0.01	24	0.01
15.183	1816	0.07	139	0.05
16.433	301	0.01	32	0.01
17.267	1902	0.07	201	0.08
17.517	2305	0.08	162	0.06
18.017	2332	0.08	170	0.07
18.517	872	0.03	69	0.03
20.200	2393	0.09	189	0.07
Totals	2776495	100.00	257299	100.00

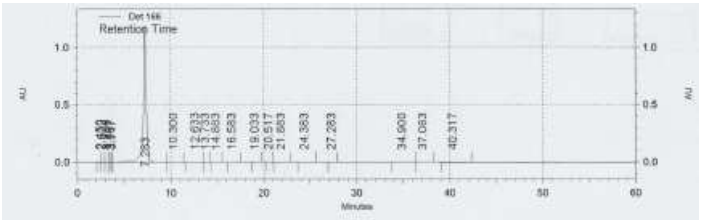
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 11.53 min, purity = 97.4%.



Time	Area	Area %	Height	Height %
2.600	20962	0.59	1827	1.91
2.883	2901	0.08	191	0.20
3.200	3707	0.10	129	0.13
5.883	1163	0.03	65	0.07
9.783	64945	1.82	786	0.82
11.533	3473602	97.37	92802	96.87
Totals	3567280	100.00	95800	100.00

5-(4-Chlorophenylsulfonyl)-1*H*-pyrazol-3(2*H*)-one (6)

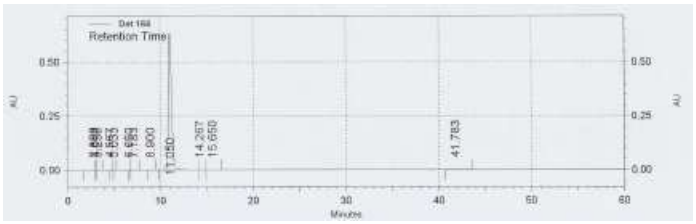
HPLC method A (isocratic; MeCN:H_2O 30:70, 60 min; 0.1% TFA): r.t. = 7.28 min, purity = 98.1%.



Time	Area	Area %	Height	Height %
2.450	95548	0.37	4963	0.41
2.633	53797	0.21	4336	0.36
3.200	59593	0.23	3611	0.30
3.567	28819	0.11	2205	0.18
3.717	26582	0.10	4113	0.34
7.283	25659359	98.07	1185929	97.97
10.300	29154	0.11	693	0.06
12.633	64997	0.25	2135	0.18
13.733	1992	0.01	84	0.01
14.883	2707	0.01	67	0.01
16.583	6590	0.03	204	0.02
19.033	2003	0.01	59	0.00
20.517	1344	0.01	46	0.00

21.883	8116	0.03	207	0.02
24.383	17931	0.07	374	0.03
27.283	1327	0.01	46	0.00
34.900	36932	0.14	557	0.05
37.083	9632	0.04	176	0.01
40.317	58926	0.23	738	0.06
Totals	26165349	100.00	1210543	100.00

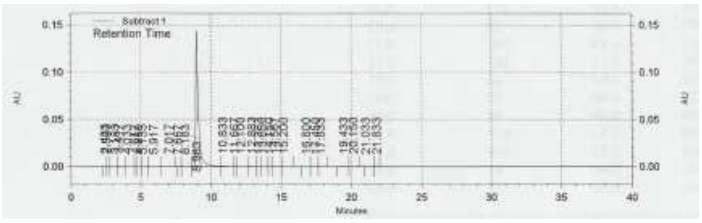
HPLC method B (isocratic; MeOH:H_2O 40:60, 60 min; 0.1% TFA): r.t. = 11.05 min, purity = 99.3%.



Time	Area	Area %	Height	Height %
2.800	35743	0.24	1195	0.19
3.017	4437	0.03	685	0.11
3.250	9198	0.06	561	0.09
4.567	615	0.00	23	0.00
5.033	277	0.00	30	0.00
6.650	152	0.00	20	0.00
7.183	1770	0.01	65	0.01
8.900	2664	0.02	147	0.02
11.050	14900471	99.26	633179	99.42
14.267	4921	0.03	183	0.03
15.650	12581	0.08	276	0.04
41.783	39245	0.26	524	0.08
Totals	15012074	100.00	636888	100.00

5-(3,5-Dichlorophenylsulfonyl)-1*H*-pyrazol-3(2*H*)-one (7)

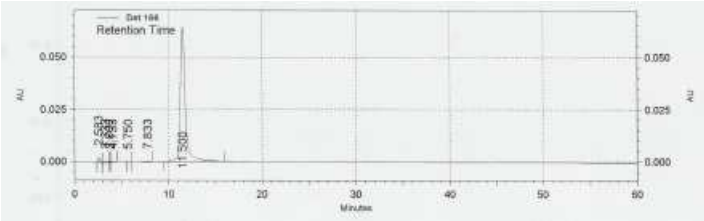
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 8.98 min, purity = 95.3%.



Time	Area	Area %	Height	Height %
2.433	2569	0.13	359	0.24
2.583	1556	0.08	315	0.21
3.183	4923	0.25	274	0.18
3.417	8642	0.43	749	0.50
4.033	4968	0.25	191	0.13
4.617	850	0.04	91	0.06
4.850	1124	0.06	79	0.05
5.133	1086	0.05	53	0.04
5.917	1958	0.10	60	0.04
7.017	2851	0.14	244	0.16
7.667	425	0.02	33	0.02

8.183	1126	0.06	55	0.04
8.983	1897634	95.30	143856	96.11
10.833	18112	0.91	829	0.55
11.667	1466	0.07	136	0.09
12.100	5673	0.28	163	0.11
12.883	8430	0.42	523	0.35
13.383	2101	0.11	143	0.10
13.650	2935	0.15	157	0.10
14.150	1057	0.05	72	0.05
14.567	3841	0.19	160	0.11
15.200	2596	0.13	165	0.11
16.800	725	0.04	44	0.03
17.450	1529	0.08	77	0.05
17.833	1789	0.09	84	0.06
19.433	6553	0.33	497	0.33
20.150	3268	0.16	194	0.13
21.033	1112	0.06	41	0.03
21.833	360	0.02	27	0.02
Totals	1991259	100.00	149671	100.00

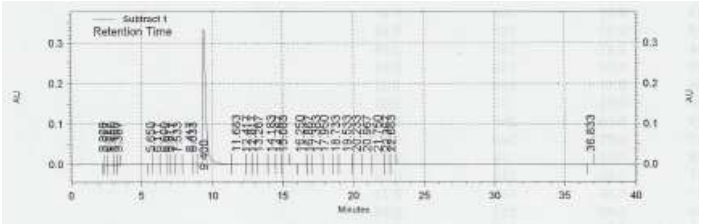
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 11.50 min, purity = 95.8%.



Time	Area	Area %	Height	Height %
2.583	35291	1.48	2135	3.13
3.333	27398	1.15	845	1.24
3.800	6302	0.26	455	0.67
4.133	7034	0.29	301	0.44
5.750	1205	0.05	56	0.08
7.833	24004	1.01	406	0.60
11.500	2285475	95.76	63956	93.84
Totals	2386709	100.00	68154	100.00

5-(4-Chloro-2,5-dimethylphenylsulfonyl)-1*H*-pyrazol-3(2*H*)-one (8)

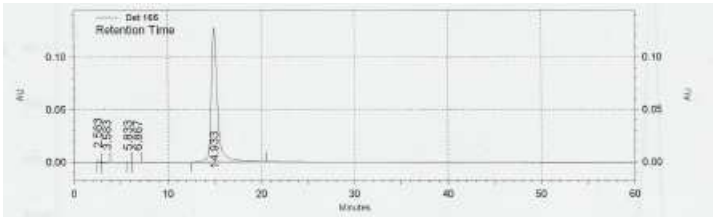
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 9.40 min, purity = 95.4%.



Time	Area	Area %	Height	Height %
2.300	1797	0.04	499	0.14
2.517	8762	0.17	1121	0.32
2.850	52708	1.05	2337	0.67
3.150	34233	0.68	2446	0.70
3.367	18692	0.37	2122	0.61
5.650	678	0.01	62	0.02
6.117	1645	0.03	89	0.03
6.600	3300	0.07	166	0.05
6.917	1150	0.02	94	0.03
7.217	1477	0.03	94	0.03

7.533	2106	0.04	114	0.03
8.417	4949	0.10	241	0.07
8.633	2572	0.05	163	0.05
9.400	4778629	95.44	335838	96.02
11.683	35937	0.72	1149	0.33
12.417	5092	0.10	231	0.07
12.817	3611	0.07	181	0.05
13.267	4383	0.09	166	0.05
14.183	2525	0.05	128	0.04
14.767	3484	0.07	266	0.08
15.083	1882	0.04	138	0.04
16.250	6586	0.13	434	0.12
16.867	600	0.01	60	0.02
17.383	4265	0.09	264	0.08
17.950	3267	0.07	242	0.07
18.733	419	0.01	34	0.01
19.533	3227	0.06	126	0.04
20.233	8021	0.16	471	0.13
20.967	4348	0.09	153	0.04
21.750	3916	0.08	159	0.05
22.367	1185	0.02	62	0.02
22.683	743	0.01	47	0.01
36.833	674	0.01	44	0.01
Totals	5006863	100.00	349741	100.00

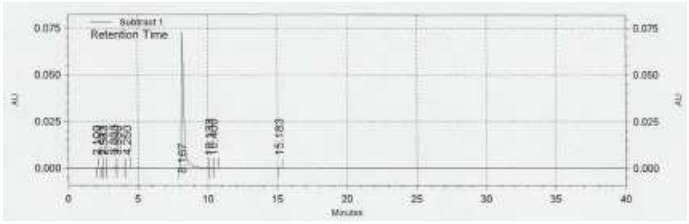
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 14.93 min, purity = 98.9%.



Time	Area	Area %	Height	Height %
2.583	37769	0.62	2220	1.69
3.583	23905	0.39	415	0.32
5.833	977	0.02	53	0.04
6.867	1960	0.03	62	0.05
14.933	6017625	98.94	128374	97.90
Totals	6082236	100.00	131124	100.00

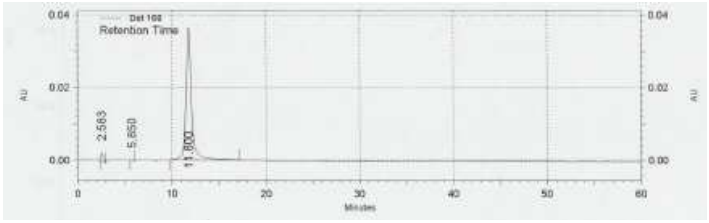
5-((4-Chlorophenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (9)

HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 8.17 min, purity = 98.2%.



Time	Area	Area %	Height	Height %
2.100	1207	0.13	178	0.24
2.417	2468	0.27	341	0.46
2.583	1061	0.11	210	0.28
3.333	7733	0.84	612	0.82
3.550	2200	0.24	109	0.15
4.250	479	0.05	40	0.05
8.167	906120	98.19	72854	97.80
10.133	835	0.09	62	0.08
10.400	327	0.04	39	0.05
15.183	387	0.04	46	0.06
Totals	922817	100.00	74491	100.00

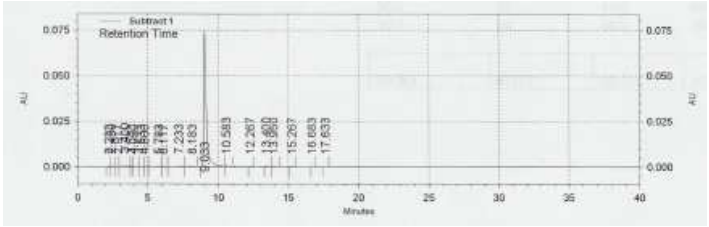
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 11.80 min, purity = 97.4%.



Time	Area	Area %	Height	Height %
2.583	39066	2.51	2264	5.82
5.850	1385	0.09	82	0.21
11.800	1515620	97.40	36531	93.97
Totals	1556071	100.00	38877	100.00

5-((4-Ethylphenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (10)

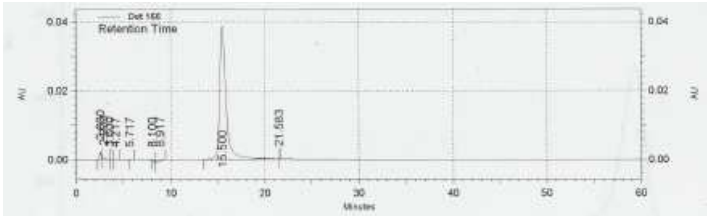
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 9.03 min, purity = 95.3%.



Time	Area	Area %	Height	Height %
2.233	2163	0.22	204	0.26
2.450	3452	0.35	365	0.47
2.917	682	0.07	67	0.09
3.400	11650	1.19	686	0.89
3.833	1448	0.15	131	0.17
4.050	2287	0.23	124	0.16
4.533	1416	0.14	80	0.10
4.800	695	0.07	52	0.07
5.783	3216	0.33	133	0.17
6.117	1886	0.19	110	0.14
7.233	4989	0.51	244	0.32

8.183	2681	0.27	93	0.12
9.033	931801	95.33	74386	96.13
10.583	3339	0.34	245	0.32
12.267	889	0.09	81	0.10
13.400	1714	0.18	160	0.21
13.950	816	0.08	47	0.06
15.267	1282	0.13	95	0.12
16.683	399	0.04	29	0.04
17.633	606	0.06	46	0.06
Totals	977411	100.00	77378	100.00

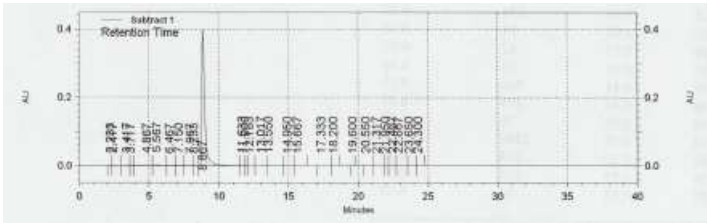
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 15.50 min, purity = 96.5%.



Time	Area	Area %	Height	Height %
2.600	25072	1.31	2115	4.98
2.933	12469	0.65	586	1.38
3.600	1535	0.08	92	0.22
4.217	1131	0.06	52	0.12
5.717	1596	0.08	59	0.14
8.100	3968	0.21	290	0.68
8.917	20965	1.09	510	1.20
15.500	1854074	96.52	38704	91.22
21.583	132	0.01	23	0.05
Totals	1920942	100.00	42431	100.00

5-((3-Ethylphenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (11)

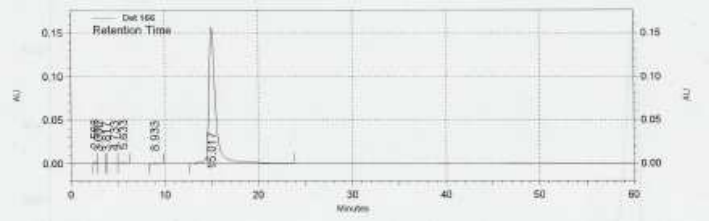
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 8.87 min, purity = 95.0%.



Time	Area	Area %	Height	Height %
2.233	9615	0.16	1021	0.25
2.417	9086	0.15	797	0.19
3.417	14417	0.25	1104	0.27
3.717	3772	0.06	239	0.06
4.867	22268	0.38	686	0.16
5.567	47078	0.80	3088	0.74
6.467	7156	0.12	250	0.06
7.150	13959	0.24	878	0.21
7.967	15885	0.27	433	0.10
8.233	5872	0.10	414	0.10

8.867	5570714	94.96	398367	95.78
11.633	12897	0.22	836	0.20
11.900	4392	0.07	426	0.10
12.183	11473	0.20	513	0.12
13.017	20812	0.35	1147	0.28
13.550	28846	0.49	1708	0.41
14.950	8217	0.14	456	0.11
15.667	5082	0.09	271	0.07
17.333	2663	0.05	94	0.02
18.200	1589	0.03	87	0.02
19.600	493	0.01	49	0.01
20.550	8199	0.14	560	0.13
21.317	8456	0.14	479	0.12
21.950	1190	0.02	70	0.02
22.467	16574	0.28	1140	0.27
22.867	11821	0.20	614	0.15
23.650	2676	0.05	134	0.03
24.300	1407	0.02	74	0.02
Totals	5866609	100.00	415935	100.00

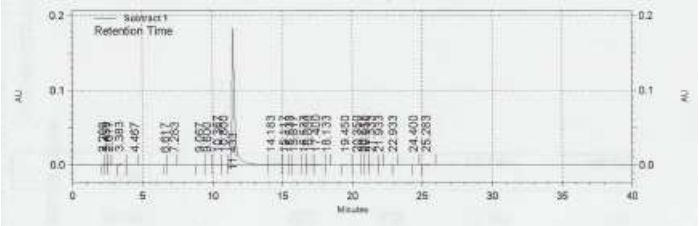
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 15.02 min, purity = 98.3%.



Time	Area	Area %	Height	Height %
2.583	32599	0.40	2571	1.58
3.000	5762	0.07	155	0.10
3.817	1241	0.02	106	0.07
4.733	17352	0.21	628	0.39
5.633	52002	0.64	2181	1.34
8.933	31930	0.39	738	0.45
15.017	7946935	98.26	156334	96.08
Totals	8087821	100.00	162713	100.00

5-((3-*tert*-Butylphenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (12)

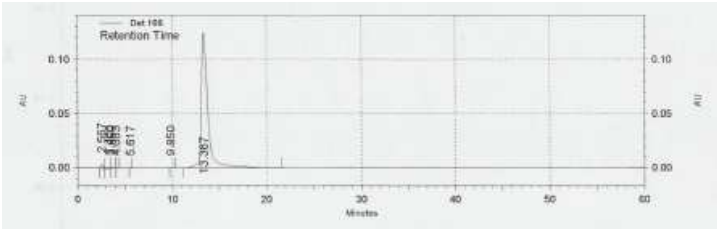
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 11.43 min, purity = 95.7%.



Time	Area	Area %	Height	Height %
2.200	2273	0.09	375	0.20
2.450	8775	0.34	959	0.50
2.617	10812	0.42	1563	0.82
3.383	23732	0.91	1485	0.78
4.467	7763	0.30	187	0.10
6.617	169	0.01	23	0.01
7.283	512	0.02	23	0.01
9.067	929	0.04	47	0.02
9.600	812	0.03	54	0.03
10.367	1964	0.08	117	0.06
10.800	1055	0.04	64	0.03

	11.433	2492549	95.73	183270	95.88
	14.183	11322	0.43	389	0.20
	15.117	4440	0.17	219	0.11
	15.533	1543	0.06	115	0.06
	15.817	3276	0.13	123	0.06
	16.533	1303	0.05	90	0.05
	16.850	4563	0.18	315	0.16
	17.400	8664	0.33	631	0.33
	18.133	365	0.01	33	0.02
	19.450	2410	0.09	186	0.10
	20.350	2217	0.09	158	0.08
	20.717	695	0.03	57	0.03
	20.950	936	0.04	63	0.03
	21.333	1641	0.06	92	0.05
	21.933	346	0.01	34	0.02
	22.933	847	0.03	61	0.03
	24.400	495	0.02	36	0.02
	25.283	7222	0.28	377	0.20
JTJ ENTER LANGUAGE=PCL	Totals	2603630	100.00	191146	100.00

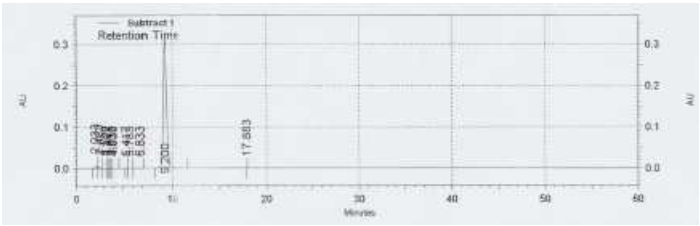
HPLC method B (isocratic; MeOH:H_2O 60:40, 60 min; 0.1% TFA): r.t. = 13.37 min, purity = 98.3%.



Time	Area	Area %	Height	Height %
2.567	54473	0.92	3050	2.36
3.400	25697	0.43	713	0.55
3.650	13800	0.23	523	0.40
4.083	2562	0.04	231	0.18
5.617	1304	0.02	137	0.11
9.850	964	0.02	50	0.04
13.367	5823516	98.33	124592	96.36
Totals	5922316	100.00	129296	100.00

5-((3,5-Dichlorophenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (13)

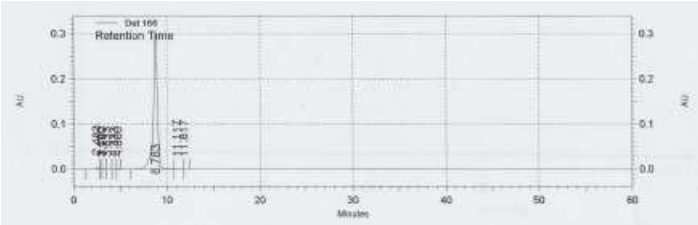
HPLC method A (isocratic; MeCN:H_2O 40:60, 60 min; 0.1% TFA): r.t. = 9.20 min, purity = 97.7%.



Time	Area	Area %	Height	Height %
2.033	87837	1.19	5721	1.68
2.483	54482	0.74	3655	1.07
3.100	6197	0.08	332	0.10
3.367	5209	0.07	332	0.10
3.617	1841	0.02	289	0.08
3.850	4402	0.06	506	0.15
4.033	3430	0.05	161	0.05
5.417	676	0.01	69	0.02
5.783	1838	0.02	88	0.03
6.833	3723	0.05	71	0.02
9.200	7198243	97.70	329030	96.69
17.883	105	0.00	26	0.01

Totals	7367983	100.00	340280	100.00

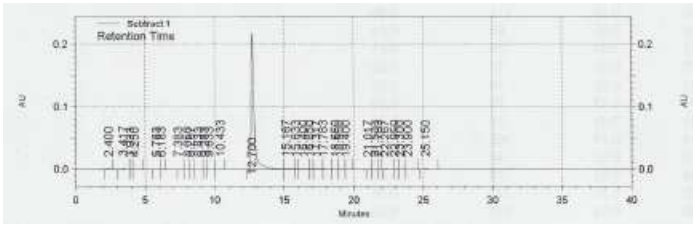
HPLC method B (isocratic; MeOH:H_2O 65:35, 60 min; 0.1% TFA): r.t. = 8.78 min, purity = 98.2%.



Time	Area	Area %	Height	Height %
2.483	70871	0.78	3574	1.16
3.000	7351	0.08	771	0.25
3.150	15928	0.17	758	0.25
3.633	22167	0.24	590	0.19
4.333	20057	0.22	687	0.22
4.800	15561	0.17	751	0.24
8.783	8958239	98.17	301622	97.55
11.117	13868	0.15	389	0.13
11.817	1306	0.01	67	0.02
Totals	9125348	100.00	309209	100.00

5-((3,5-Bis(trifluoromethyl)phenoxy)methyl-1*H*-pyrazol-3(2*H*)-one (14)

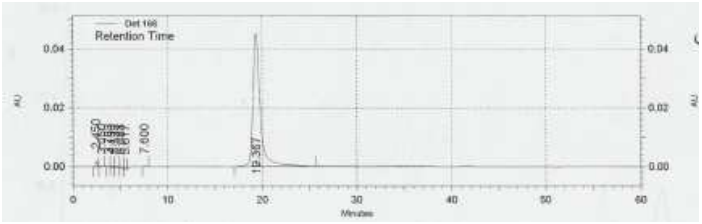
HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 12.70 min, purity = 96.0%.



Time	Area	Area %	Height	Height %
2.400	12985	0.39	665	0.30
3.417	25081	0.76	1707	0.76
3.933	3000	0.09	250	0.11
4.250	4973	0.15	169	0.08
5.783	1578	0.05	70	0.03
6.183	707	0.02	66	0.03
7.383	726	0.02	43	0.02
8.050	915	0.03	71	0.03
8.267	486	0.01	46	0.02
8.833	2283	0.07	159	0.07
9.283	457	0.01	49	0.02
9.633	731	0.02	44	0.02

10.433	1181	0.04	59	0.03
12.700	3158490	96.00	217534	96.83
15.167	22073	0.67	1088	0.48
15.833	2858	0.09	220	0.10
16.400	9646	0.29	240	0.11
16.850	2592	0.08	178	0.08
17.317	8153	0.25	350	0.16
17.783	3308	0.10	133	0.06
18.650	1856	0.06	95	0.04
18.950	1831	0.06	75	0.03
19.400	917	0.03	53	0.02
21.017	845	0.03	78	0.03
21.533	872	0.03	57	0.03
21.783	251	0.01	29	0.01
22.267	5474	0.17	368	0.16
22.950	673	0.02	46	0.02
23.300	584	0.02	39	0.02
23.900	3323	0.10	137	0.06
25.150	11226	0.34	537	0.24
Totals	3290075	100.00	224655	100.00

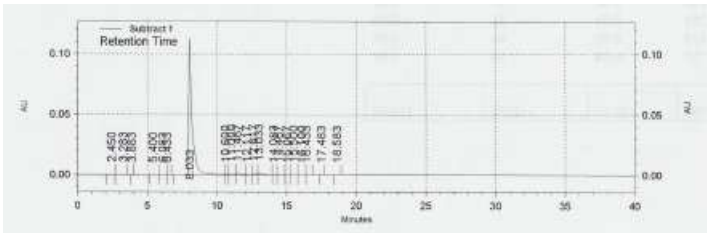
HPLC method B (isocratic; MeOH:H_2O 60:40, 60 min; 0.1% TFA): r.t. = 19.37 min, purity = 96.6%.



Time	Area	Area %	Height	Height %
2.450	23581	0.93	1991	4.00
3.050	5797	0.23	233	0.47
3.783	3488	0.14	172	0.35
4.133	7626	0.30	303	0.61
4.533	15520	0.61	414	0.83
5.083	15867	0.63	602	1.21
5.617	11722	0.46	700	1.41
7.600	2182	0.09	116	0.23
19.367	2449268	96.62	45286	90.90
Totals	2535051	100.00	49817	100.00

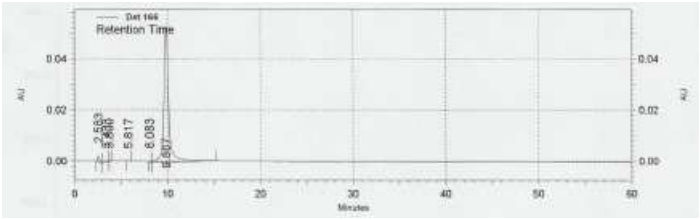
5-((3,5-Difluorophenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (15)

HPLC method A (gradient; MeCN:H_2O 30:70 to 80:20, 0 to 20 min, MeCN:H_2O 80:20 to 30:70, 20 to 40 min; 0.1% TFA): r.t. = 8.03 min, purity = 95.1%.



Time	Area	Area %	Height	Height %
2.450	2881	0.15	213	0.18
3.283	5973	0.31	293	0.25
3.883	98	0.01	23	0.02
5.400	1091	0.06	52	0.04
6.083	1519	0.08	84	0.07
6.433	339	0.02	46	0.04
8.033	1859218	95.12	112677	95.22
10.600	2714	0.14	228	0.19
11.000	9142	0.47	388	0.33
11.467	17702	0.91	970	0.82
12.117	3555	0.18	212	0.18
12.617	13814	0.71	1029	0.87
13.033	26683	1.37	1512	1.28
14.083	1673	0.09	105	0.09
14.467	3035	0.16	166	0.14
15.067	1093	0.06	60	0.05
15.500	1816	0.09	114	0.10
16.100	914	0.05	57	0.05
16.433	284	0.01	21	0.02
17.483	435	0.02	44	0.04
18.583	725	0.04	42	0.04
Totals	1954704	100.00	118336	100.00

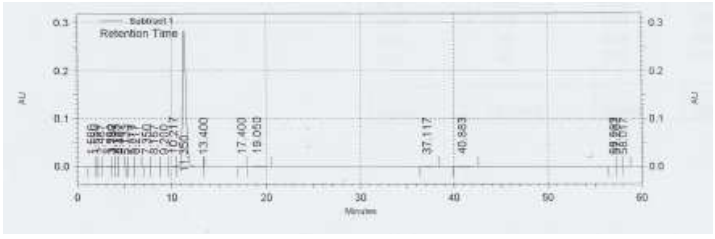
HPLC method B (isocratic; MeOH:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 9.87 min, purity = 96.8%.



Time	Area	Area %	Height	Height %
2.583	36859	1.86	2487	4.41
3.433	20549	1.03	491	0.87
3.800	1511	0.08	184	0.33
5.817	1531	0.08	78	0.14
8.083	2744	0.14	214	0.38
9.867	1922320	96.82	52905	93.87
Totals	1985514	100.00	56359	100.00

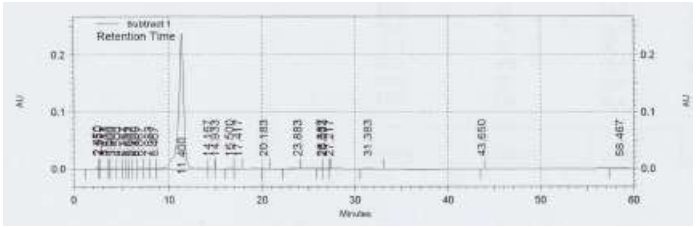
5-((3,5-Dibromophenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (16)

HPLC method A (isocratic; MeCN:H_2O 40:60, 60 min; 0.1% TFA): r.t. = 11.35 min, purity = 96.2%.



Time	Area	Area %	Height	Height %
1.500	11717	0.16	327	0.11
1.850	7874	0.11	810	0.28
2.467	13036	0.18	888	0.30
3.550	43079	0.61	832	0.28
3.767	12180	0.17	1037	0.35
4.133	5155	0.07	301	0.10
4.467	4987	0.07	244	0.08
5.133	335	0.00	31	0.01
5.817	2915	0.04	160	0.05
6.217	3285	0.05	192	0.07
7.350	11418	0.16	691	0.23
8.167	9807	0.14	491	0.17
9.200	1666	0.02	77	0.03
10.217	41180	0.58	1896	0.64
11.350	6840735	96.24	284415	96.62
13.400	114	0.00	30	0.01
17.400	5416	0.08	176	0.06
19.050	49742	0.70	1020	0.35
37.117	12082	0.17	206	0.07
40.883	23358	0.33	292	0.10
56.983	3566	0.05	102	0.03
57.267	3208	0.05	96	0.03
58.017	1226	0.02	56	0.02
Totals	7108081	100.00	294370	100.00

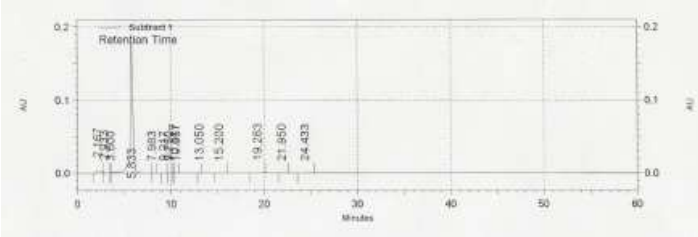
HPLC method B (isocratic; MeOH:H_2O 65:35, 60 min; 0.1% TFA): r.t. = 11.40 min, purity = 97.9%.



Time	Area	Area %	Height	Height %
2.450	16814	0.18	1808	0.74
2.550	10996	0.11	1519	0.62
3.117	25679	0.27	946	0.39
3.500	5995	0.06	377	0.15
3.900	11170	0.12	354	0.14
4.500	9967	0.10	324	0.13
5.167	6706	0.07	303	0.12
5.633	3550	0.04	245	0.10
5.867	4875	0.05	239	0.10
6.400	5476	0.06	206	0.08
6.867	8095	0.08	228	0.09
7.883	8861	0.09	244	0.10
8.467	11862	0.12	392	0.16
11.400	9374683	97.86	237012	96.55
14.167	3150	0.03	135	0.05
14.933	81	0.00	25	0.01
16.500	4249	0.04	125	0.05
17.417	2615	0.03	82	0.03
20.183	1568	0.02	56	0.02
23.883	4551	0.05	63	0.03
26.367	1595	0.02	73	0.03
26.533	1547	0.02	67	0.03
27.217	91	0.00	22	0.01
31.383	18918	0.20	270	0.11
43.650	355	0.00	23	0.01
58.467	36576	0.38	343	0.14
Totals	9580025	100.00	245481	100.00

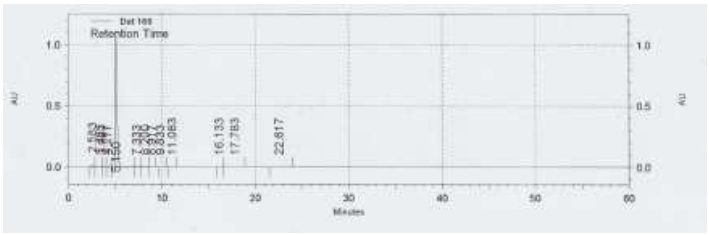
5-((3-Bromophenoxy)methyl)-1*H*-pyrazol-3(2*H*)-one (17)

HPLC method A (isocratic; MeCN:H_2O 40:60, 60 min; 0.1% TFA): r.t. = 5.83 min, purity = 95.2%.



Time	Area	Area %	Height	Height %
2.167	115980	3.12	4345	2.25
2.933	19644	0.53	937	0.48
3.600	3412	0.09	299	0.15
5.833	3536563	95.23	186646	96.54
7.983	1551	0.04	87	0.04
9.217	1264	0.03	67	0.03
9.750	619	0.02	35	0.02
10.317	271	0.01	38	0.02
10.567	684	0.02	43	0.02
13.050	274	0.01	23	0.01
15.200	7484	0.20	203	0.10
19.283	14404	0.39	335	0.17
21.950	2122	0.06	70	0.04
24.433	9409	0.25	213	0.11
Totals	3713681	100.00	193341	100.00

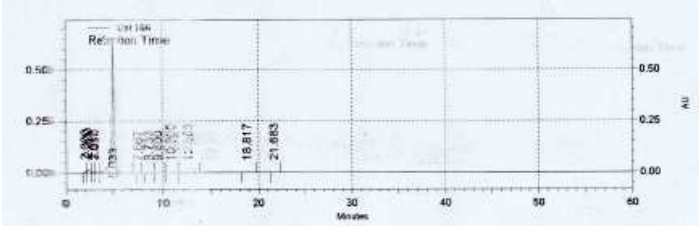
HPLC method B (isocratic; MeOH:H_2O 65:35, 60 min; 0.1% TFA): r.t. = 5.15 min, purity = 97.5%.



Time	Area	Area %	Height	Height %
2.583	134519	1.01	17397	1.53
3.483	40154	0.30	1314	0.12
3.767	30478	0.23	1631	0.14
4.217	28248	0.21	1219	0.11
5.150	12941686	97.47	1114577	97.87
7.333	7696	0.06	319	0.03
8.200	7630	0.06	361	0.03
8.917	2531	0.02	141	0.01
9.833	2030	0.02	91	0.01
11.083	1773	0.01	77	0.01
16.133	1844	0.01	67	0.01
17.783	28181	0.21	596	0.05
22.617	50348	0.38	1006	0.09
Totals	13277118	100.00	1138796	100.00

5-((Biphenyl-3-yloxy)methyl)-1*H*-pyrazol-3(2*H*)-one (18)

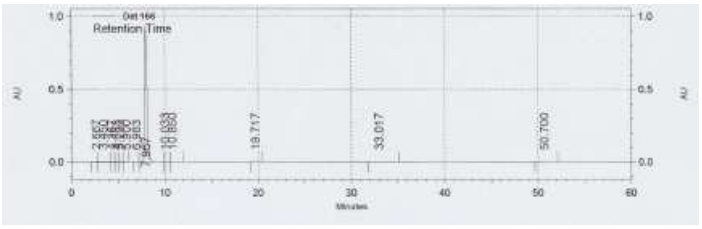
HPLC method A (isocratic; MeCN:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 5.03 min, purity = 95.0%.



Time	Area	Area %	Height	Height %
2.000	89655	0.80	6567	0.95
2.700	288616	2.58	14599	2.12
2.917	52518	0.47	3280	0.48
3.350	77390	0.69	3203	0.47
5.033	10620733	94.99	658931	95.74
7.667	906	0.01	60	0.01
8.733	4467	0.04	190	0.03
9.600	4529	0.04	176	0.03
10.800	9375	0.08	289	0.04
12.583	23165	0.21	691	0.10
18.817	8206	0.07	221	0.03
21.683	1694	0.02	56	0.01

Totals	11181254	100.00	688263	100.00

HPLC method B (isocratic; MeOH:H_2O 65:35, 60 min; 0.1% TFA): r.t. = 7.97 min, purity = 98.6%.

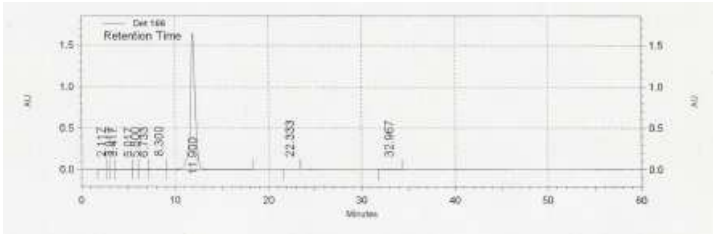


Time	Area	Area %	Height	Height %
2.667	25717	0.16	2555	0.27
3.450	64309	0.40	2059	0.22
4.367	15433	0.10	552	0.06
4.883	13726	0.09	607	0.06
5.233	28619	0.18	2292	0.24
5.900	6215	0.04	239	0.03
6.983	4199	0.03	270	0.03
7.967	15700724	98.56	935485	98.93
10.033	10784	0.07	360	0.04
10.850	19189	0.12	633	0.07
19.717	5867	0.04	173	0.02
33.017	22459	0.14	249	0.03
50.700	13088	0.08	164	0.02

Totals	15930329	100.00	945638	100.00

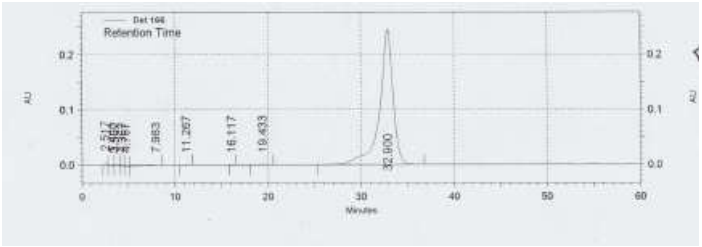
5-((5-Phenylbiphenyl-3-yloxy)methyl)-1*H*-pyrazol-3(2*H*)-one (19)

HPLC method A (isocratic; MeCN:H_2O 50:50, 60 min; 0.1% TFA): r.t. = 11.90 min, purity = 98.9%.



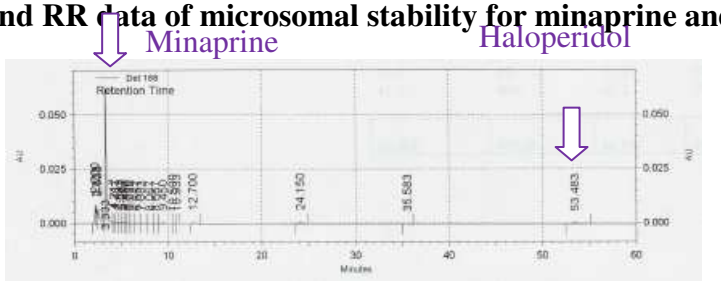
Time	Area	Area %	Height	Height %
2.117	77526	0.13	2231	0.13
3.017	6104	0.01	438	0.03
3.417	23275	0.04	1531	0.09
5.017	96751	0.16	1721	0.10
5.800	31518	0.05	1019	0.06
6.733	64396	0.11	1131	0.07
8.300	265502	0.45	8461	0.51
11.900	58861536	98.92	1645688	98.92
22.333	14830	0.02	325	0.02
32.967	60259	0.10	1041	0.06
Totals	59501697	100.00	1663586	100.00

HPLC method B (isocratic; MeOH:H_2O 65:35, 60 min; 0.1% TFA): r.t. = 32.90 min, purity = 98.1%.



Time	Area	Area %	Height	Height %
2.517	51730	0.21	3541	1.38
3.400	37023	0.15	1270	0.50
3.567	49933	0.20	1289	0.50
4.367	46164	0.18	1575	0.62
4.767	34610	0.14	1702	0.66
7.983	199297	0.79	508	0.20
11.267	3237	0.01	90	0.04
16.117	1029	0.00	48	0.02
19.433	55156	0.22	1093	0.43
32.900	24663517	98.10	244842	95.66
Totals	25141696	100.00	255958	100.00

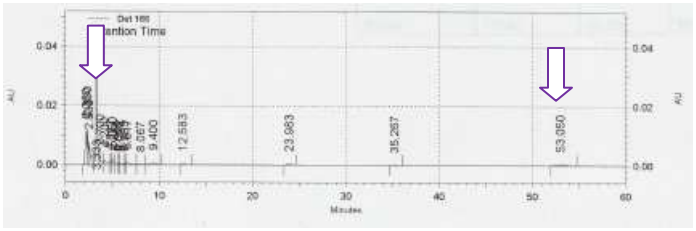
HPLC spectra and RR data of microsomal stability for minaprine and 1.



Time	Area	Area %	Height	Height %
2.200	66116	7.65	8408	9.30
2.333	46788	5.41	6522	7.22
2.533	77155	8.92	6962	7.70
3.333	541189	62.58	62572	69.23
4.217	11057	1.28	835	0.92
4.467	5732	0.66	388	0.43
4.833	5661	0.65	427	0.47
5.067	11226	1.30	811	0.90
5.450	3991	0.46	349	0.39
5.700	4509	0.52	295	0.33
5.933	4609	0.53	213	0.24
6.667	7222	0.84	294	0.33
7.083	5226	0.60	151	0.17
8.067	4691	0.54	128	0.14
8.667	2568	0.30	111	0.12
9.450	13043	1.51	466	0.52
10.500	584	0.07	37	0.04
10.933	520	0.06	28	0.03
12.700	8483	0.98	414	0.46
24.150	19903	2.30	599	0.66
35.583	2475	0.29	71	0.08
53.483	21983	2.54	308	0.34
Totals	864731	100.00	90389	100.00

Figure S1. Minaprine, 0 min #3, reconstituted in DMSO:H_2O = 1:9, which contains haloperidol (100 µL, 100 µM).

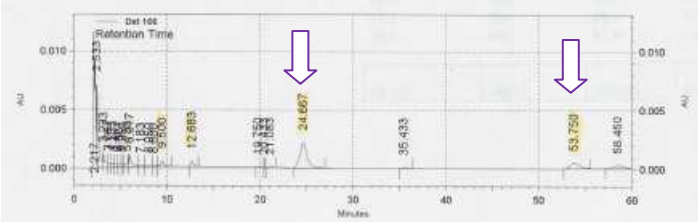
Minaprine Haloperidol



Time	Area	Area %	Height	Height %
2.200	115156	12.31	11422	12.84
2.350	85896	9.18	12053	13.55
2.533	87305	9.33	7679	8.63
3.333	424978	45.44	46025	51.73
3.700	37268	3.98	2655	2.98
4.200	27791	2.97	1706	1.92
4.817	8307	0.89	709	0.80
5.050	21863	2.34	1792	2.01
5.433	7699	0.82	606	0.68
5.667	3363	0.36	336	0.38
5.900	6945	0.74	319	0.36
6.383	4441	0.47	411	0.46
6.617	17440	1.86	913	1.03
8.067	5021	0.54	141	0.16
9.400	16758	1.79	719	0.81
12.583	9927	1.06	464	0.52
23.983	14905	1.59	437	0.49
35.267	3526	0.38	89	0.10
53.050	36749	3.93	497	0.56
Totals	935338	100.00	88973	100.00

Figure S2. Minaprine, 20 min #3, reconstituted in DMSO:H_2O = 1:9, which contains haloperidol (100 µL, 100 µM).

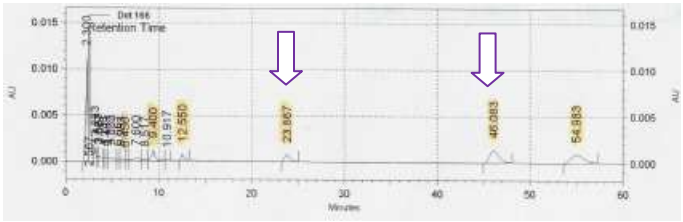
1 Haloperidol



Time	Area	Area %	Height	Height %
2.217	165198	30.74	11671	42.74
2.533	81161	15.10	7179	26.29
3.233	17706	3.30	1100	4.03
3.667	5777	1.08	397	1.45
4.133	7476	1.39	321	1.18
4.467	4617	0.86	266	0.97
4.867	7036	1.31	320	1.17
5.250	3442	0.64	246	0.90
5.733	7042	1.31	329	1.20
6.017	20906	3.89	1006	3.68
7.183	5872	1.09	148	0.54
8.050	4949	0.92	114	0.42
8.600	1705	0.32	84	0.31
9.500	12385	2.30	531	1.94
12.683	9940	1.85	471	1.72
19.750	1317	0.25	44	0.16
20.433	138	0.03	23	0.08
21.083	1401	0.26	46	0.17
24.667	114693	21.35	2151	7.88
35.433	4777	0.89	109	0.40
53.750	36753	6.84	490	1.79
58.450	23037	4.29	260	0.95
Totals	537328	100.00	27306	100.00

Figure S3. Compound **1**, 0 min #2, reconstituted in DMSO:H_2O = 1:9, which contains haloperidol (100 µL, 100 µM).

1 Haloperidol



Time	Area	Area %	Height	Height %
2.300	150915	21.77	11147	31.51
2.567	184310	26.59	14765	41.73
3.233	15203	2.19	1379	3.90
3.533	6248	0.90	514	1.45
3.667	11432	1.65	505	1.43
4.133	4032	0.58	305	0.86
4.483	3181	0.46	257	0.73
4.833	15332	2.21	369	1.04
5.667	5097	0.74	311	0.88
√5.983	6125	0.88	223	0.63
√6.450	3427	0.49	165	0.47
7.600	16300	2.35	340	0.96
8.517	6671	0.96	185	0.52
9.400	33840	4.88	1116	3.15
10.917	1332	0.19	63	0.18
12.550	15000	2.16	679	1.92
√23.867	28938	4.17	743	2.10
√46.083	97694	14.09	1385	3.91
54.983	88091	12.71	929	2.63
Totals	693168	100.00	35380	100.00

Figure S4. Compound **1**, 20 min #1, reconstituted in DMSO:H_2O = 1:9, which contains haloperidol (100 µL, 100 µM).

Table S1. Raw data for microsomal stability study with minaprine and **1**

Sample Name	Time (min)	No	Analyte retention time (min)	Analyte Peak Area	Haloperidol retention time (min)	Haloperidol Peak Area	RR
Minaprine	0	#1	3.367	450935	53.567	19994	22.55
		#2	3.350	536553	53.750	24992	21.47
		#3	3.333	541189	53.483	21983	24.62
	20	#1	3.333	512171	53.333	44233	11.58
		#2	3.333	565219	53.350	32329	17.48
		#3	3.333	424978	53.050	36749	11.56
1	0	#1	24.750	80053	53.850	44525	2.667
		#2	24.667	114693	53.750	36753	3.121
		#3	24.633	91344	46.233	34255	1.798
	20	#1	23.867	28938	46.083	97694	0.296
		#2	23.967	19676	46.100	64179	0.307
		#3	23.900	17720	46.150	52317	0.339

Table S2. Microsomal stability data processing of minaprine and **1**

Sample Name	RR Mean	% Mean ± S.E.
Mina 0 min	22.88	100 ± 7.11

Mina 20 min	13.54	59.2 ± 14.9
1 0 min	2.529	100 ± 26.4
1 20min	0.314	12.4 ± 0.89

Metabolite profiling of 1 and 3

Only one new peak/metabolite was observed from either test agent **1** or **3** from microsomal incubation (Figure S5). Comparing the total ion current spectra of microsomal incubation with test agents to the ones with blank at 30 min., no new peaks were identified beyond the peaks at retention time 1.7 min (the unmetabolized parent **1** or **3**), and retention time 1.4 min (the new peak/metabolite).

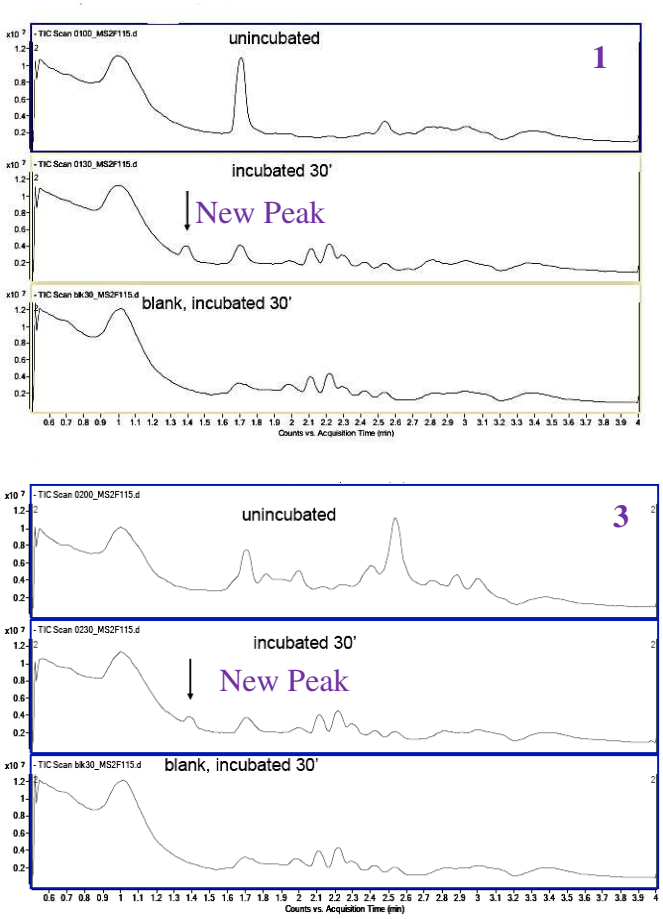


Figure S5. Total ion current chromatogram of MS2 full mass scan on samples incubated for 0 min (top) and 30 min (middle) with rat liver microsomes or blank microsomes (bottom). Microsomes were incubated with **1** (top panel) and **3** (bottom panel).

The complete parent molecular ion mass spectrum was then analyzed for the new peak that eluted at 1.4 min. The spectra are shown below. These spectra show that the new peak/metabolite shows a molecular ion $(M-H)^-$ of 255 (from **1**) and 257 (from **3**), which indicates the addition of oxygen to the parent compound (Figure S6).

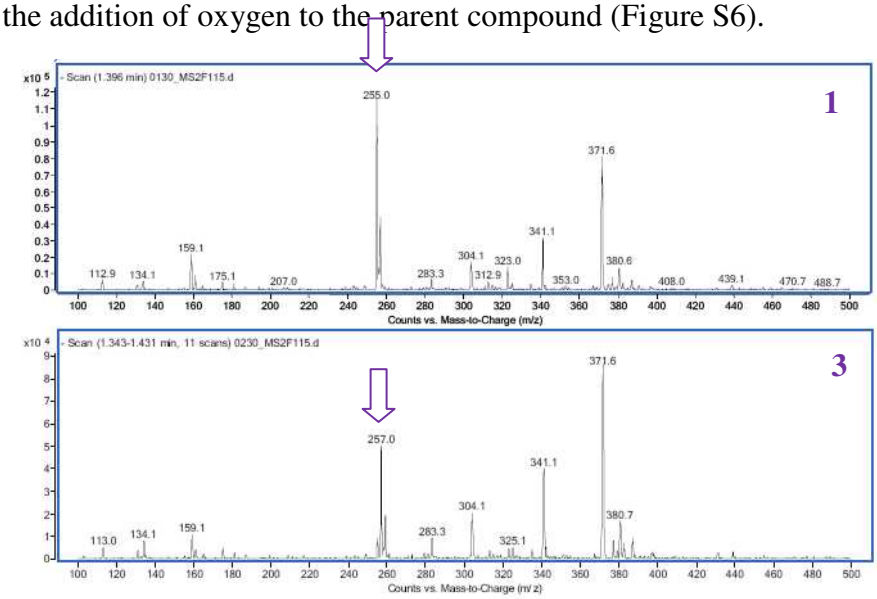


Figure S6. Mass spectra of new peak/metabolite from **1** (top) and **3** (bottom).

A product ion scan was run on the samples (peak m/z = 255 for **1**; peak m/z = 257 for **3**) to look for the generation of fragment ions in the mass spectrometer. The results of these scans show both test agents generated fragment ion of m/z = 159 (Figure S7).

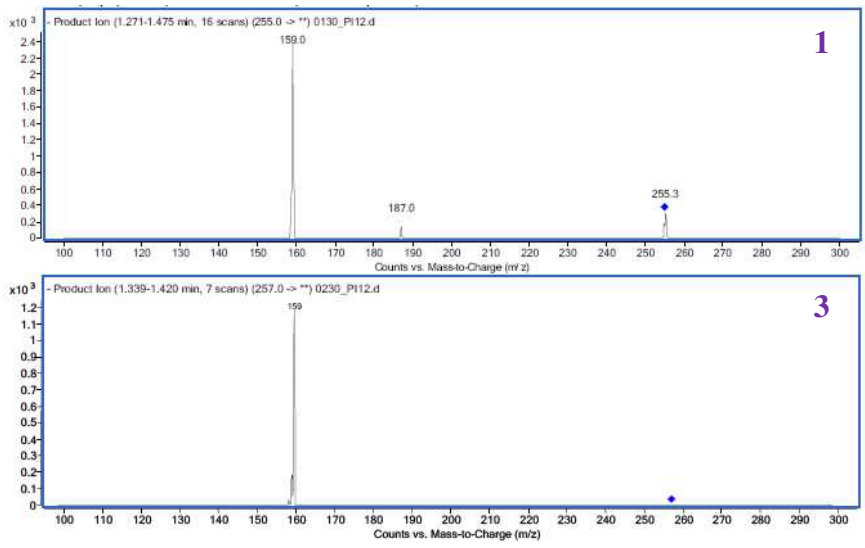


Figure S7. Production ion scan of new peak/metabolite (r.t. = 1.4 min) for **1** (top) and **3** (bottom) from microsomal incubation.

To confirm the identity of **4** as a direct metabolite of **1** from microsomal incubation, an authentic sample of **4** was tested under identical mass spectrometric and chromatographic conditions (Figure S8 and S9).

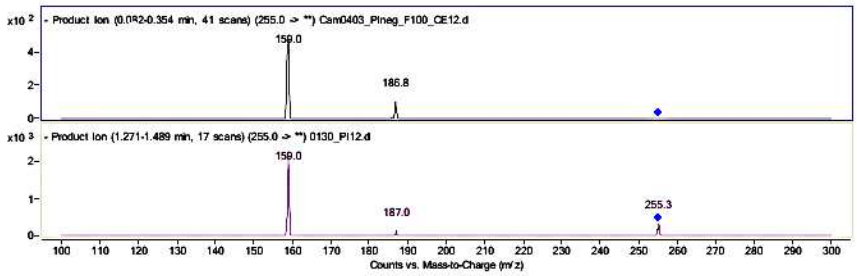


Figure S8. Product ion spectrum of authentic **4** (top) and of the new peak/metabolite of **1** (bottom) from microsomal incubation.

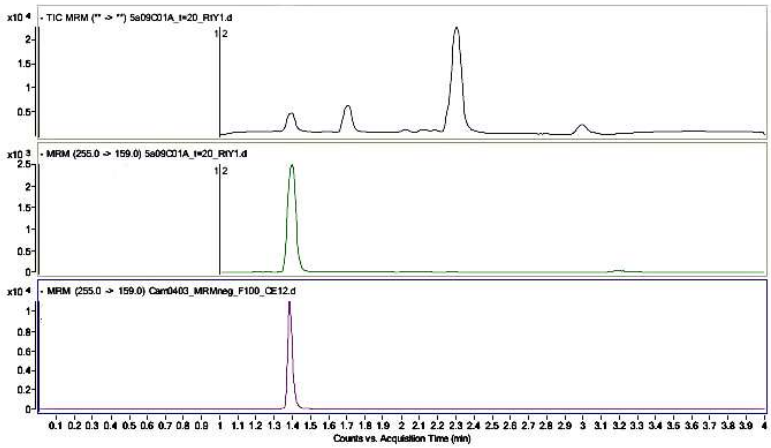


Figure S9. Total ion current chromatogram of microsomal incubation with **1** (top), of the new peak/metabolite, MRM, extracted from first chromatogram (middle), and of authentic **4** (bottom).

Rat liver microsomal stability data in the presence of NADPH with **1** and **3** are shown in Table S3. Compounds **1** and **3** showed poor rat liver microsomal stability, corresponding to the previous rat liver microsomal stability data for **1** by the HPLC detection method.

Table S3. Microsomal intrinsic clearance summary for **1** and **3** in the presence of NADPH

ID	Test concentration (μM)	Cl'_{int} [a] (mL/min/kg)	$T_{1/2}$ (min)[b]	comment
Verapamil	1	> 400	< 3.3	highly metabolized control
Warfarin	1	< 8	> 180	poorly metabolized control

1	5	192	7.3	
3	5	195	7.2	
1/3 mixture	5	169	8.3	

[a]The intrinsic clearance was calculated as follows: $CL'_{int} = (0.693/T_{1/2}) \times$ (mL incubation/mg microsomal protein) × (mg microsomal protein/g liver) × (g liver/kg bodyweight).[2] Reaction mixture contained 1 mg/mL microsomal protein. The scale-up factor for microsomes protein to g of liver is 45 mg/g of liver.[3] Liver weights used for rats were 45 g/kg body weight.[4] [b]Half-life.

In vivo mouse steady-state level study and in vivo blood brain barrier study of 13.
(All bioanalysis was done by Apredica, Watertown, MA)

To assess levels of drug present in blood and brain tissue specimens, a 50 mg/kg dose was peritoneal administered to wild-type B6SJL mice (n = 6). An untreated group (n = 6) was used a negative control. Blood and brain were removed 1 h after injection and rapidly quenched at -80 ^{o}C. Specimens were subsequently mixed with extraction buffer (100% ice-cold methanol), sonicated on ice for 20 s, and then spun at analyzed using a Ceas 16 channel EC-HPLC. Experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Blood samples were thawed on ice and kept at 4 ^{o}C. Blood samples from animals treated with test agent were dilute 50-fold in control blood prior to analysis. An aliquot of blood (25 µL) was mixed with an equal volume of water, and 150 µL methanol containing internal standard was added to precipitate the proteins. Brain samples were weighed, diluted with an equal volume of PBS (phosphate buffered saline, pH 7.2) and homogenized. Brain homogenates from test agent-treated animals were diluted 50-fold with commercial control brain homogenate prior to analysis.

A calibration curve was prepared in each media (blood and brain homogenate). Control blood and brain homogenate (from a commercial source) was used to prepare the calibration standards. Calibration samples were prepared by diluting 50x stock solution in DMSO with matrix to the appropriate concentration. Stock solutions were prepared by serial dilution according to the table S4.

Table S4. Stock solution preparation

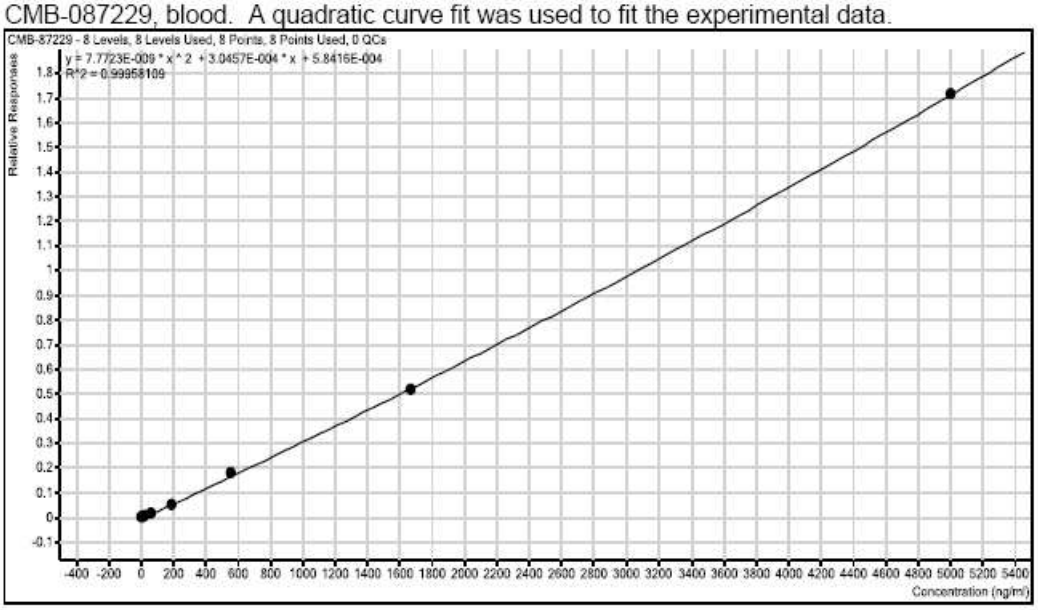
Nominal concentration (nM)	Stock concentration (µM)
0.254	0.0127

0.762	0.0381
2.29	0.114
6.86	0.343
20.6	1.03
61.7	3.09
185	9.26
556	28
1667	83
5000	250

Samples were analyzed by LC/MS/MS using an Agilent 6410 mass spectrometer coupled with an Agilent 1200 HPLC and a CTC PL chilled autosampler, all controlled by MassHunter software (Agilent). After separation on a C18 reverse phase HPLC column using an acetonitrile-water gradient system, peaks were analyzed by mass pectrometry (MS) using ESI ionization in MRM mode. MassHunter software was used to calculate sample concentration using the calibration standards of known concentration.

Two calibration curves were used for blood and brain samples, depending on the concentration (Figure S10).

CMB-087229, blood. A quadratic curve fit was used to fit the experimental data.



CMB-087229, brain. A quadratic curve fit was used to fit the experimental data.

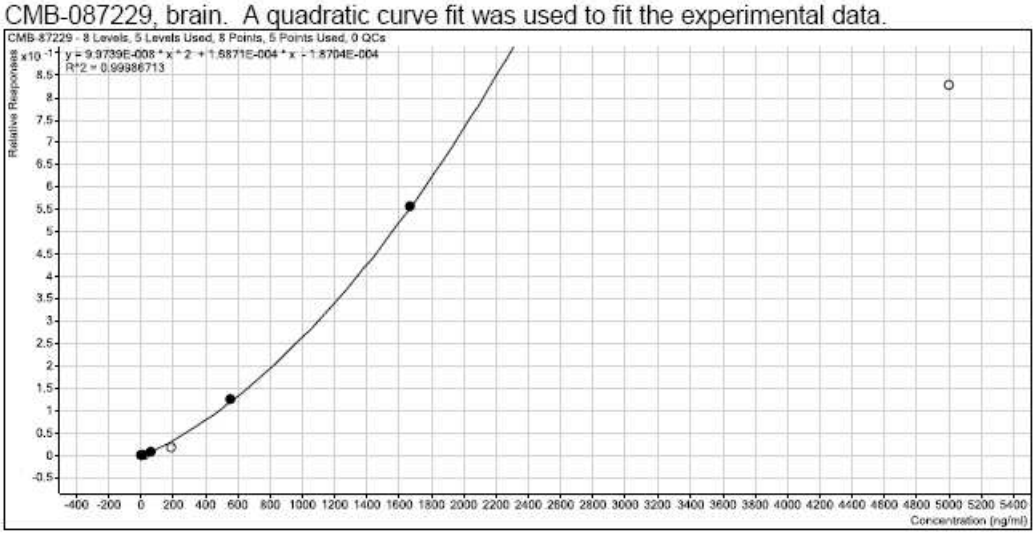


Figure S10. Quadratic curves for blood and brain samples.

Brain and blood sample analysis is given in Table S5. The lower limit of quantification (LLOQ) was 2.3 nM in the analytical samples. The upper limit of quantification for blood was 5000 nM in the analytical sample. The upper limit of quantification for brain homogenate was 1667 nM in the analytical sample.

Analyte	Blood (analytical sample) (nM)	Blood (after calibration) (µM)	Brain (analytical sample) (nM)	Brain (after calibration) (µM)
blank	< LLOQ		< LLOQ	
	24.3		8.3	
	5.2		10.1	
	< LLOQ		5.6	
	13.4		< LLOQ	
	108.7		5.5	
	< LLOQ		< LLOQ	
	71.3		6.4	
	176.7		27.0	
	< LLOQ		87.2	
	335.9		4.9	
	106.2		4.7	
	< LLOQ		4.7	
	< LLOQ		< LLOQ	
	75.4		< LLOQ	

	< LLOQ		< LLOQ	

Effect of 13 on hERG Potassium Channel

A summary of the results of each test article is shown in Table S5 below. The positive control (E-4031) confirms the sensitivity of the test system of hERG inhibition.

Table S6. Results of hERG inhibition

Test Article ID	Conc. (μM)	Mean % hERG Inhibition	Standard Deviation	Standard Error	n	Individual Data Points (% Inhibition)
13	10	0.6	2.2	1.5	2	2.1
						-0.9
E-4031	0.5	98.7	0.7	0.5	2	98.2
						99.1

Effect of 13 on Enzymes and Receptors

Radioligand binding assays for **13** (concentration = 10 μM, n = 2) were carried out by MDS Pharma Services (Taipei, Taiwan).

Table S7. Competitive binding assays of 13

Target (Species)	Inhibition (%)	Target (Species)	Inhibition (%)
Adenosine A_1 (human)	15	Histamine H_3 (human)	15
Adenosine A_2 (human)	-2	Imidazoline I_2, Central (rat)	12
Adenosine A_3 (human)	6	Interleukin IL-1 (mouse)	3
Adrenergic α_{1A} (rat)	-2	Leukotriene, Cysteinyl $CysLT_1$ (human)	0
Adrenergic α_{1B} (rat)	0	Melatonin MT_1 (human)	3
Adrenergic α_{1D} (human)	3	Muscarinic M_1 (human)	2
Adrenergic α_{2A} (human)	8	Muscarinic M_2 (human)	-2

Adrenergic β_1 (human)	1	Muscarinic M_3 (human)	-4
Adrenergic β_2 (human)	-2	Neuropeptide Y Y_1 (human)	-1
Androgen (Testosterone) AR (rat)	3	Neuropeptide Y Y_2 (human)	6
Bradykinin B1 (human)	5	Nicotinic Acetylcholine (human)	7
Bradykinin B2 (human)	-10	Nicotinic Acetylcholine D_1, Bungarotoxin (human)	6
Calcium Channel L-Type, Benzothiazepin (rat)	-5	Opiate δ (OP1, DOP) (human)	-15
Calcium Channel L-Type, Dihydropyridin (rat)	-2	Opiate κ (OP2, KOP) (human)	25
Calcium Channel N-Type (rat)	3	Opiate μ (OP3, MOP) (human)	-9
Dopamine D_1 (human)	3	Phorbol Ester (mouse)	1
Dopamine D_{25} (human)	14	Platelet Activating Factor (PAF)	-1
Dopamine D_3 (human)	-4	Potassium Channel [KATP] (hamster)	-17
Dopamine $D_{4.2}$ (human)	-3	Potassium Channel hERG (human)	-7
Endothelin ET_A (human)	3	Prostanoid EP_4 (human)	-8
Endothelin ET_B (human)	0	Purinergic P_{2x} (rabbit)	1
Epidermal Growth Factor (EGF) (human)	5	Purinergic P_{2y} (rat)	5
Estrogen ERα (human)	10	Rolipram (rat)	-2
G Protein-Coupled Receptor GPR103 (human)	3	Serotonin (5-Hydroxytryptamine) 5-HT_{1A} (human)	-6
$GABA_A$, Flunitrazepam, Central (rat)	12	Serotonin (5-Hydroxytryptamine) 5-HT_3 (human)	7
$GABA_A$, Muscimol, Central (rat)	1	Sigma δ_1 (human)	-18
$GABA_{B1A}$ (human)	-7	Sigma δ_2 (rat)	5
Glucocorticoid (human)	4	Sodium Channel, Site 2 (rat)	4

Glutamate, Kainate (rat)	24	Tachykinin NK_1 (human)	3
Glutamate, NMDA, Agonism (rat)	-3	Thyroid Hormone (rat)	10
Glutamate, NMDA, Glycine (rat)	5	Transporter, Dopamine (DAT) (human)	11
Glutamate, NMDA, Phencyclidine (rat)	-11	Transporter, GABA (rat)	-4
Histamine H_1 (human)	1	Transporter, Norepinephrine (NET) (human)	-10
Histamine H_2 (human)	3	Transporter, Serotonin (5-Hydroxytryptamine) (SERT) (human)	-6

References

[1] Hierner, S.; Pankin, O. Edefuhr, M.; Somfai, P. Synthesis of aryl glycinnes by the α arylation of wienreb amides, *Angew, Chem. Int. Ed.*, **2008**, *47*, 1907-1909.

[2] Obach, R. S. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes, *Drug Metab. Dispos.*, **1999**, *27*, 1350-1359.

[3] Houston, J. B. Utility of in vitro rug metabolism data in predicting in vivo metabolic clearance, *Biochem. Pharmacol.*, **1994**, *47*, 1469-1479.

[4] Lu C., Li P., Gallegos R., Uttamsingh V., Xia C. Q., Miwa G. T., Balani S.K., Gan L. Comparison of intrinsic clearance in liver microsomes and hepatocytes from rats and humans: evaluation of free fraction and uptake in hepatocytes, *Drug Metab. Dispos.*, **2006**, *34*, 1600-1605.