Supporting Information

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Pyrimidine-2,4,6-trione Derivatives and Their Inhibition of Mutant SOD1-dependent Protein Aggregation. Toward a Treatment for Amyotrophic Lateral Sclerosis

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1. X-ray analysis of 3

Data Collection

A colorless block crystal of $C_{10}H_{16}N_2O_3$ having approximate dimensions of 0.59 x 0.44 x 0.20 mm was mounted using oil (Infineum V8512) on a glass fiber. All measurements were made on a CCD area detector with graphite monochromated CuK\α radiation.
Cell constants and an orientation matrix for data collection corresponded to a monoclinic cell with dimensions:

a = 10.6592(2) Å
b = 15.3170(3) Å β = 108.1620(10)º
c = 6.90890(10) Å
V = 1071.80(3) Å^3

For Z = 4 and F.W. = 212.25, the calculated density is 1.315 g/cm3. Based on a systematic absence, and the successful solution and refinement of the structure, the space group was determined to be:

P2(1)/c

The data were collected at a temperature of 100(2)K with a theta range for data collection of 5.78 to 67.00º. Data were collected in 0.5º oscillations with 5 second exposures. The crystal-to-detector distance was 40.00 mm.

Data Reduction

Of the 12853 reflections which were collected, 1867 were unique (Rint = 0.0435). Data were collected using APEX2 V2.1-4 (Bruker, 2007) detector and processed using SAINTPLUS from Bruker.

The linear absorption coefficient, mu, for CuK\a radiation is 0.809 mm^{-1}. A numerical absorption correction was applied. Minimum and maximum transmission factors were: 0.6447 and 0.8562, respectively. The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement

The structure was solved by direct methods and expanded using Fourier techniques3. The non-hydrogen atoms were refined anisotropically.
Hydrogen atoms were included in idealized positions, but not refined. The final cycle of full-matrix least-squares refinemene4 on F2 was based on 1867 reflections and 138 variable parameters and converged (largest parameter shift was 0.001 times its esd) with unweighted and weighted agreement factors of:

$$R1 = \Sigma| |F_o|-|F_c| |/\Sigma|F_o| = 0.0396$$

$$wR^2 = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2} = 0.1259$$

The weighting scheme was calc.
calc $w=1/[\sigma^2(F_o^2)+(0.0760P)^2 + 0.3382P]$ where $P=(F_o^2+2F_c^2)/3$

The standard deviation of an observation of unit weight5 was 1.259. The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. Plots of $\Sigma\ w\ (|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, sin θ/λ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.481 and -0.541 e-/Å3, respectively.

Neutral atom scattering factors were taken from Cromer and Waber[1]. Anomalous dispersion effects were included in Fcalc[2]; the values for Df' and Df'' were those of Creagh and McAuley[3]. The values for the mass attenuation coefficients are those of Creagh and Hubbell[4]. All calculations were performed using the Bruker SHELXTL3 crystallographic software package.

Table 1. Crystal data and structure refinement for **3.**

Identification code	s27z
Empirical formula	C10 H16 N2 O3
Formula weight	212.25

Temperature	100(2) K
Wavelength	1.54178 Å
Crystal system, space group	Monoclinic, P2(1)/c
Unit cell dimensions	a = 10.6592(2) Å b = 15.3170(3) Å β = 108.1620(10) º c = 6.90890(10) Å
Volume	1071.80(3) Å3
Z, Calculated density	4, 1.315 Mg/m3
Absorption coefficient	0.809 mm-1
F(000)	456
Crystal size	0.59 x 0.44 x 0.20 mm
Theta range for data collection	5.78 to 67.00 º
Limiting indices	-12<=h<=12, -17<=k<=8, -8<=l<=8
Reflections collected / unique	12853 / 1867 [R(int) = 0.0435]
Completeness to theta = 67.00	97.3 %
Absorption correction	Numerical
Max. and min. transmission	0.8562 and 0.6447
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	1867 / 0 / 138
Goodness-of-fit on F^2	1.259
Final R indices [I>2sigma(I)]	R1 = 0.0396, wR2 = 0.1259
R indices (all data)	R1 = 0.0462, wR2 = 0.1390
Largest diff. peak and hole	0.481 and -0.541 e-/Å-3

Table 2. Atomic coordinates and equivalent isotropic displacement parameters (A^2 x 10^3) for **3**.

U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(1)	0.89610(11)	0.60389(8)	0.24442(17)	25(1)
O(2)	0.43109(11)	0.60383(7)	0.00561(16)	25(1)
O(3)	0.65552(10)	0.69371(7)	0.64497(15)	24(1)
N(1)	0.77756(12)	0.64772(8)	0.44782(18)	17(1)
N(2)	0.54154(12)	0.64570(8)	0.32815(19)	18(1)
C(1)	0.78916(15)	0.61538(9)	0.2664(2)	18(1)
C(2)	0.66425(15)	0.59429(10)	0.1016(2)	20(1)
C(3)	0.53589(15)	0.61494(9)	0.1375(2)	18(1)
C(4)	0.65831(14)	0.66405(9)	0.4826(2)	18(1)
C(5)	0.90023(14)	0.66882(10)	0.6132(2)	20(1)
C(6)	0.94837(15)	0.59346(10)	0.7594(2)	22(1)
C(7)	1.07411(15)	0.61758(11)	0.9279(2)	24(1)
C(8)	0.41616(14)	0.66490(10)	0.3681(2)	20(1)
C(9)	0.36691(14)	0.58879(10)	0.4651(2)	21(1)
C(10)	0.24161(15)	0.61390(11)	0.5139(2)	23(1)

Table 3. Bond lengths [A] and angles [deg] for **3**.

O(1)-C(1)	1.2089(19)
O(2)-C(3)	1.2136(19)
O(3)-C(4)	1.2189(19)
N(1)-C(4)	1.3881(19)
N(1)-C(1)	1.3884(19)
N(1)-C(5)	1.4794(17)
N(2)-C(3)	1.382(2)
N(2)-C(4)	1.3926(19)
N(2)-C(8)	1.4760(18)
C(1)-C(2)	1.493(2)
C(2)-C(3)	1.498(2)
C(2)-H(2A)	0.9900
C(2)-H(2B)	0.9900
C(5)-C(6)	1.514(2)
C(5)-H(5A)	0.9900
C(5)-H(5B)	0.9900
C(6)-C(7)	1.521(2)
C(6)-H(6A)	0.9900
C(6)-H(6B)	0.9900
C(7)-H(7A)	0.9800
C(7)-H(7B)	0.9800
C(7)-H(7C)	0.9800
C(8)-C(9)	1.517(2)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
C(9)-C(10)	1.525(2)
C(9)-H(9A)	0.9900
C(9)-H(9B)	0.9900
C(10)-H(10A)	0.9800
C(10)-H(10B)	0.9800
C(10)-H(10C)	0.9800
C(4)-N(1)-C(1)	124.38(12)
C(4)-N(1)-C(5)	117.60(12)
C(1)-N(1)-C(5)	117.99(12)
C(3)-N(2)-C(4)	124.20(12)
C(3)-N(2)-C(8)	118.23(12)
C(4)-N(2)-C(8)	117.48(12)
O(1)-C(1)-N(1)	121.21(13)
O(1)-C(1)-C(2)	121.62(13)
N(1)-C(1)-C(2)	117.17(13)
C(1)-C(2)-C(3)	118.12(13)
C(1)-C(2)-H(2A)	107.8
C(3)-C(2)-H(2A)	107.8
C(1)-C(2)-H(2B)	107.8

C(3)-C(2)-H(2B)	107.8
H(2A)-C(2)-H(2B)	107.1
O(2)-C(3)-N(2)	121.32(14)
O(2)-C(3)-C(2)	121.36(13)
N(2)-C(3)-C(2)	117.32(13)
O(3)-C(4)-N(1)	120.86(13)
O(3)-C(4)-N(2)	120.54(13)
N(1)-C(4)-N(2)	118.60(12)
N(1)-C(5)-C(6)	112.51(12)
N(1)-C(5)-H(5A)	109.1
C(6)-C(5)-H(5A)	109.1
N(1)-C(5)-H(5B)	109.1
C(6)-C(5)-H(5B)	109.1
H(5A)-C(5)-H(5B)	107.8
C(5)-C(6)-C(7)	111.08(13)
C(5)-C(6)-H(6A)	109.4
C(7)-C(6)-H(6A)	109.4
C(5)-C(6)-H(6B)	109.4
C(7)-C(6)-H(6B)	109.4
H(6A)-C(6)-H(6B)	108.0
C(6)-C(7)-H(7A)	109.5
C(6)-C(7)-H(7B)	109.5
H(7A)-C(7)-H(7B)	109.5
C(6)-C(7)-H(7C)	109.5
H(7A)-C(7)-H(7C)	109.5
H(7B)-C(7)-H(7C)	109.5
N(2)-C(8)-C(9)	112.80(12)
N(2)-C(8)-H(8A)	109.0
C(9)-C(8)-H(8A)	109.0
N(2)-C(8)-H(8B)	109.0
C(9)-C(8)-H(8B)	109.0
H(8A)-C(8)-H(8B)	107.8
C(8)-C(9)-C(10)	110.56(13)
C(8)-C(9)-H(9A)	109.5
C(10)-C(9)-H(9A)	109.5
C(8)-C(9)-H(9B)	109.5
C(10)-C(9)-H(9B)	109.5
H(9A)-C(9)-H(9B)	108.1
C(9)-C(10)-H(10A)	109.5
C(9)-C(10)-H(10B)	109.5
H(10A)-C(10)-H(10B)	109.5
C(9)-C(10)-H(10C)	109.5
H(10A)-C(10)-H(10C)	109.5
H(10B)-C(10)-H(10C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters (A^2 x 10^3) for **3**.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
O(1)	18(1)	35(1)	26(1)	0(1)	9(1)	1(1)
O(2)	18(1)	30(1)	21(1)	-2(1)	-1(1)	-1(1)
O(3)	26(1)	29(1)	17(1)	-6(1)	7(1)	2(1)
N(1)	14(1)	19(1)	16(1)	0(1)	2(1)	-1(1)
N(2)	14(1)	20(1)	18(1)	1(1)	5(1)	1(1)
C(1)	19(1)	16(1)	18(1)	3(1)	6(1)	0(1)
C(2)	20(1)	23(1)	16(1)	-2(1)	5(1)	0(1)
C(3)	19(1)	16(1)	17(1)	2(1)	3(1)	-1(1)
C(4)	18(1)	17(1)	17(1)	2(1)	4(1)	1(1)
C(5)	15(1)	22(1)	18(1)	-1(1)	1(1)	-2(1)
C(6)	19(1)	25(1)	19(1)	2(1)	3(1)	1(1)
C(7)	19(1)	33(1)	18(1)	1(1)	3(1)	2(1)
C(8)	15(1)	24(1)	22(1)	1(1)	6(1)	2(1)
C(9)	18(1)	24(1)	21(1)	1(1)	7(1)	1(1)
C(10)	18(1)	32(1)	20(1)	-2(1)	7(1)	-1(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **3**.

	x	y	z	U(eq)
H(2A)	6650	5310	725	24
H(2B)	6658	6256	-229	24
H(5A)	8842	7200	6897	24
H(5B)	9699	6848	5528	24
H(6A)	9655	5422	6839	26
H(6B)	8790	5772	8203	26
H(7A)	11427	6340	8676	35
H(7B)	11042	5674	10185	35
H(7C)	10564	6669	10060	35
H(8A)	3482	6801	2381	24
H(8B)	4288	7162	4593	24
H(9A)	4360	5713	5919	25
H(9B)	3485	5383	3708	25
H(10A)	2608	6623	6116	35
H(10B)	2099	5636	5728	35
H(10C)	1736	6320	3885	35

Table 6. Torsion angles [deg] for **3**.

C(4)-N(1)-C(1)-O(1)	178.76(13)
C(5)-N(1)-C(1)-O(1)	0.7(2)
C(4)-N(1)-C(1)-C(2)	-2.1(2)
C(5)-N(1)-C(1)-C(2)	179.85(12)
O(1)-C(1)-C(2)-C(3)	-176.83(13)
N(1)-C(1)-C(2)-C(3)	4.1(2)
C(4)-N(2)-C(3)-O(2)	-176.60(13)
C(8)-N(2)-C(3)-O(2)	0.0(2)
C(4)-N(2)-C(3)-C(2)	4.5(2)
C(8)-N(2)-C(3)-C(2)	-178.87(12)
C(1)-C(2)-C(3)-O(2)	175.93(13)
C(1)-C(2)-C(3)-N(2)	-5.2(2)
C(1)-N(1)-C(4)-O(3)	-178.92(13)
C(5)-N(1)-C(4)-O(3)	-0.9(2)
C(1)-N(1)-C(4)-N(2)	1.2(2)
C(5)-N(1)-C(4)-N(2)	179.22(12)
C(3)-N(2)-C(4)-O(3)	177.63(13)
C(8)-N(2)-C(4)-O(3)	1.0(2)
C(3)-N(2)-C(4)-N(1)	-2.5(2)
C(8)-N(2)-C(4)-N(1)	-179.12(12)
C(4)-N(1)-C(5)-C(6)	88.79(16)
C(1)-N(1)-C(5)-C(6)	-93.06(15)
N(1)-C(5)-C(6)-C(7)	-179.72(12)
C(3)-N(2)-C(8)-C(9)	94.04(16)
C(4)-N(2)-C(8)-C(9)	-89.12(16)
N(2)-C(8)-C(9)-C(10)	176.81(12)

2. X-ray analysis of 43
Data Collection

A yellow plate crystal of C_{25} H_{36} N_2 O_5 having approximate dimensions of 0.29 x 0.25 x 0.08 mm was mounted using oil (Infineum V8512) on a glass fiber. All measurements were made on a CCD area detector with graphite monochromated CuK\α radiation.

Cell constants and an orientation matrix for data collection corresponded to a monoclinic cell with dimensions:

a = 6.15270(10) Å
b = 37.1816(9) Å β = 101.1810(10)º
c = 10.0416(2) Å
V = 2253.59(8) Å^3

For Z = 4 and F.W. = 444.56, the calculated density is 1.310 g/cm3. Based on a statistical analysis of intensity distribution, and the successful solution and refinement of the structure, the space group was determined to be:

Cc

The data were collected at a temperature of 100(2)K with a theta range for data collection of 5.08 to 66.69º. Data were collected in 0.5º oscillations with 10 second exposures. The crystal-to-detector distance was 40.00 mm.

Data Reduction

Of the 8493 reflections which were collected, 2189 were unique (Rint = 0.0604). Data were collected using APEX2 V2.1-4 (Bruker, 2007) detector and processed using SAINTPLUS from Bruker.

The linear absorption coefficient, mu, for CuK\a radiation is 0.734 mm-1. A numerical absorption correction was applied. Minimum and maximum transmission factors were: 0.8153 and 0.9436, respectively. The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement

The structure was solved by direct methods and expanded using Fourier techniques3. Group anisotropic displacement parameters were refined for the disordered carbon atoms C6a-C7b. It refined to a 57% disorder. The remaining non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinemene4 on F2 was based on 2189 reflections and 292 variable parameters and converged (largest parameter shift was 0.000 times its esd) with unweighted and weighted agreement factors of:

$R1 = \Sigma| |F_o|-|F_c| |/\Sigma|F_o| = 0.0708$

$wR^2 = \{\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]\}^{1/2} = 0.2018$

The weighting scheme was calc.
calc $w=1/[\sigma^2(F_o^2)+(0.1846P)^2 + 0.3393P]$ where $P=(F_o^2+2F_c^2)/3$

The standard deviation of an observation of unit weight5 was 1.109.
The weighting scheme was based on counting statistics and included a factor to downweight the intense reflections. Plots of Σ w $(|F_o| - |F_c|)^2$ versus $|F_o|$, reflection order in data collection, sin θ/λ and various classes of indices showed no unusual trends. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.606 and -0.596 e-/Å3, respectively.

Neutral atom scattering factors were taken from Cromer and Waber[1]. Anomalous dispersion effects were included in Fcalc[2]; the values for Df' and Df'' were those of Creagh and McAuley[3]. The values for the mass attenuation coefficients are those of Creagh and Hubbell[4]. All calculations were performed using the Bruker SHELXTL3 crystallographic software package.

Table 7. Crystal structure data and structure refinement for **43**.

Identification code	s28z
Empirical formula	C25 H36 N2 O5
Formula weight	444.56
Temperature	100(2) K
Wavelength	1.54178 Å
Crystal system, space group	Monoclinic, Cc
Unit cell dimensions	a = 6.15270(10) Å b = 37.1816(9) Å β = 101.1810(10) º c = 10.0416(2) Å
Volume	2253.59(8) Å3
Z, Calculated density	4, 1.310 Mg/m3
Absorption coefficient	0.734 mm-1
F(000)	960
Crystal size	0.29 x 0.25 x 0.08 mm
Theta range for data collection	5.08 to 66.69 º
Limiting indices	-1<=h<=7, -43<=k<=39, -11<=l<=11
Reflections collected / unique	8493 / 2189 [R(int) = 0.0604]
Completeness to theta = 66.69	96.6 %
Absorption correction	Numerical
Max. and min. transmission	0.9436 and 0.8153
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	2189 / 2 / 292
Goodness-of-fit on F^2	1.109
Final R indices [I>2sigma(I)]	R1 = 0.0708, wR2 = 0.2018
R indices (all data)	R1 = 0.0816, wR2 = 0.2232
Absolute structure parameter	0.2(5)
Largest diff. peak and hole	0.606 and -0.596 e-/Å-3

Table 8. Atomic coordinates and equivalent isotropic displacement parameters (A^2 x 10^3) for **43**.

U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

	x	y	z	U(eq)
O(1)	0.8781(10)	0.32762(13)	0.5083(6)	74(2)
O(2)	0.2463(7)	0.36989(11)	0.2519(5)	47(1)
O(3)	0.7613(6)	0.45177(10)	0.4337(4)	34(1)
O(4)	1.4379(6)	0.42809(10)	0.6603(3)	33(1)
O(5)	1.6205(7)	0.33840(12)	1.0141(4)	44(1)
N(1)	0.5491(8)	0.34851(14)	0.3942(5)	40(1)
N(2)	0.5194(7)	0.41006(12)	0.3276(4)	30(1)
C(1)	0.8382(8)	0.39054(15)	0.4974(5)	33(1)
C(2)	0.7640(10)	0.35374(16)	0.4714(5)	40(1)
C(3)	0.4274(9)	0.37570(17)	0.3204(5)	36(1)
C(4)	0.7125(8)	0.42041(14)	0.4203(5)	29(1)
C(5)	0.4470(12)	0.3125(2)	0.3856(8)	58(2)
C(6A)	0.438(3)	0.2978(3)	0.5302(14)	57(2)
C(7A)	0.250(3)	0.2613(3)	0.4614(17)	57(2)
C(6B)	0.321(4)	0.2993(4)	0.4520(19)	57(2)
C(7B)	0.317(4)	0.2637(5)	0.529(2)	57(2)
C(8)	0.395(2)	0.2358(3)	0.4173(15)	99(4)
C(9)	0.4108(18)	0.2521(2)	0.2722(11)	76(2)
C(10)	0.529(2)	0.2884(2)	0.2904(10)	85(3)
C(11)	0.3984(8)	0.43934(14)	0.2415(5)	29(1)
C(12)	0.1816(8)	0.44929(15)	0.2837(5)	33(1)
C(13)	0.0692(9)	0.47963(16)	0.1954(5)	38(1)
C(14)	0.0347(9)	0.47026(16)	0.0446(5)	38(1)
C(15)	0.2536(11)	0.46154(17)	0.0045(5)	42(1)
C(16)	0.3736(10)	0.43055(16)	0.0913(5)	37(1)
C(17)	1.0302(8)	0.40105(15)	0.5827(5)	32(1)
C(18)	1.1851(8)	0.38247(14)	0.6849(5)	31(1)
C(19)	1.3950(8)	0.39842(14)	0.7291(5)	31(1)
C(20)	1.5472(8)	0.38393(15)	0.8373(5)	32(1)
C(21)	1.4915(9)	0.35410(16)	0.9051(5)	35(1)
C(22)	1.2811(9)	0.33800(15)	0.8630(6)	38(1)
C(23)	1.1364(9)	0.35213(16)	0.7581(6)	38(1)
C(24)	1.6171(9)	0.45124(15)	0.7213(5)	36(1)
C(25)	1.8313(10)	0.35461(16)	1.0701(6)	42(1)

Table 9. Bond lengths [A] and angles [deg] for **43**.

O(1)-C(2)	1.213(8)
O(2)-C(3)	1.210(6)
O(3)-C(4)	1.205(7)
O(4)-C(19)	1.355(7)
O(4)-C(24)	1.439(6)
O(5)-C(21)	1.354(7)
O(5)-C(25)	1.442(7)
N(1)-C(3)	1.385(7)
N(1)-C(2)	1.410(7)
N(1)-C(5)	1.473(8)
N(2)-C(3)	1.393(7)
N(2)-C(4)	1.414(6)
N(2)-C(11)	1.496(6)
C(1)-C(17)	1.374(7)
C(1)-C(2)	1.450(8)
C(1)-C(4)	1.483(7)
C(5)-C(6B)	1.220(17)
C(5)-C(10)	1.468(12)
C(5)-C(6A)	1.564(14)
C(5)-H(5)	1.0000
C(6A)-C(7A)	1.83(2)
C(6A)-H(6A)	0.9900
C(6A)-H(6B)	0.9900
C(7A)-C(8)	1.432(18)
C(7A)-H(7A)	0.9900
C(7A)-H(7B)	0.9900
C(6B)-C(7B)	1.53(2)
C(6B)-H(6C)	0.9900
C(6B)-H(6D)	0.9900
C(7B)-C(8)	1.66(2)
C(7B)-H(7C)	0.9900
C(7B)-H(7D)	0.9900
C(8)-C(9)	1.597(16)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
C(9)-C(10)	1.529(10)
C(9)-H(9A)	0.9900
C(9)-H(9B)	0.9900
C(10)-H(10A)	0.9900
C(10)-H(10B)	0.9900
C(11)-C(16)	1.521(7)
C(11)-C(12)	1.522(7)
C(11)-H(11)	1.0000

C(12)-C(13)	1.516(7)
C(12)-H(12A)	0.9900
C(12)-H(12B)	0.9900
C(13)-C(14)	1.528(7)
C(13)-H(13A)	0.9900
C(13)-H(13B)	0.9900
C(14)-C(15)	1.513(8)
C(14)-H(14A)	0.9900
C(14)-H(14B)	0.9900
C(15)-C(16)	1.544(8)
C(15)-H(15A)	0.9900
C(15)-H(15B)	0.9900
C(16)-H(16A)	0.9900
C(16)-H(16B)	0.9900
C(17)-C(18)	1.436(7)
C(17)-H(17)	0.9500
C(18)-C(23)	1.410(8)
C(18)-C(19)	1.412(7)
C(19)-C(20)	1.398(7)
C(20)-C(21)	1.379(8)
C(20)-H(20)	0.9500
C(21)-C(22)	1.415(8)
C(22)-C(23)	1.347(9)
C(22)-H(22)	0.9500
C(23)-H(23)	0.9500
C(24)-H(24A)	0.9800
C(24)-H(24B)	0.9800
C(24)-H(24C)	0.9800
C(25)-H(25A)	0.9800
C(25)-H(25B)	0.9800
C(25)-H(25C)	0.9800

C(19)-O(4)-C(24)	118.6(4)
C(21)-O(5)-C(25)	118.5(5)
C(3)-N(1)-C(2)	123.1(5)
C(3)-N(1)-C(5)	116.8(5)
C(2)-N(1)-C(5)	120.1(5)
C(3)-N(2)-C(4)	124.4(4)
C(3)-N(2)-C(11)	119.1(4)
C(4)-N(2)-C(11)	116.3(4)
C(17)-C(1)-C(2)	125.7(5)
C(17)-C(1)-C(4)	114.2(5)
C(2)-C(1)-C(4)	119.9(4)
O(1)-C(2)-N(1)	118.9(6)
O(1)-C(2)-C(1)	123.8(5)
N(1)-C(2)-C(1)	117.2(5)

O(2)-C(3)-N(1)	121.1(5)
O(2)-C(3)-N(2)	120.9(5)
N(1)-C(3)-N(2)	118.0(5)
O(3)-C(4)-N(2)	119.7(4)
O(3)-C(4)-C(1)	125.0(5)
N(2)-C(4)-C(1)	115.3(4)
C(6B)-C(5)-C(10)	116.4(10)
C(6B)-C(5)-N(1)	129.9(11)
C(10)-C(5)-N(1)	113.4(6)
C(6B)-C(5)-C(6A)	37.9(11)
C(10)-C(5)-C(6A)	117.9(8)
N(1)-C(5)-C(6A)	110.8(8)
C(6B)-C(5)-H(5)	68.2
C(10)-C(5)-H(5)	104.4
N(1)-C(5)-H(5)	104.4
C(6A)-C(5)-H(5)	104.4
C(5)-C(6A)-C(7A)	92.4(9)
C(5)-C(6A)-H(6A)	113.2
C(7A)-C(6A)-H(6A)	113.2
C(5)-C(6A)-H(6B)	113.2
C(7A)-C(6A)-H(6B)	113.2
H(6A)-C(6A)-H(6B)	110.6
C(8)-C(7A)-C(6A)	102.7(12)
C(8)-C(7A)-H(7A)	111.2
C(6A)-C(7A)-H(7A)	111.2
C(8)-C(7A)-H(7B)	111.2
C(6A)-C(7A)-H(7B)	111.2
H(7A)-C(7A)-H(7B)	109.1
C(5)-C(6B)-C(7B)	133.9(16)
C(5)-C(6B)-H(6C)	103.7
C(7B)-C(6B)-H(6C)	103.7
C(5)-C(6B)-H(6D)	103.7
C(7B)-C(6B)-H(6D)	103.7
H(6C)-C(6B)-H(6D)	105.4
C(6B)-C(7B)-C(8)	99.6(13)
C(6B)-C(7B)-H(7C)	111.8
C(8)-C(7B)-H(7C)	111.8
C(6B)-C(7B)-H(7D)	111.8
C(8)-C(7B)-H(7D)	111.8
H(7C)-C(7B)-H(7D)	109.6
C(7A)-C(8)-C(9)	100.5(10)
C(7A)-C(8)-C(7B)	25.8(9)
C(9)-C(8)-C(7B)	117.0(9)
C(7A)-C(8)-H(8A)	111.7
C(9)-C(8)-H(8A)	111.7
C(7B)-C(8)-H(8A)	117.6

C(7A)-C(8)-H(8B)	111.7
C(9)-C(8)-H(8B)	111.7
C(7B)-C(8)-H(8B)	86.6
H(8A)-C(8)-H(8B)	109.4
C(10)-C(9)-C(8)	109.6(9)
C(10)-C(9)-H(9A)	109.7
C(8)-C(9)-H(9A)	109.7
C(10)-C(9)-H(9B)	109.7
C(8)-C(9)-H(9B)	109.7
H(9A)-C(9)-H(9B)	108.2
C(5)-C(10)-C(9)	113.8(7)
C(5)-C(10)-H(10A)	108.8
C(9)-C(10)-H(10A)	108.8
C(5)-C(10)-H(10B)	108.8
C(9)-C(10)-H(10B)	108.8
H(10A)-C(10)-H(10B)	107.7
N(2)-C(11)-C(16)	111.1(4)
N(2)-C(11)-C(12)	112.4(4)
C(16)-C(11)-C(12)	113.8(4)
N(2)-C(11)-H(11)	106.3
C(16)-C(11)-H(11)	106.3
C(12)-C(11)-H(11)	106.3
C(13)-C(12)-C(11)	110.1(4)
C(13)-C(12)-H(12A)	109.6
C(11)-C(12)-H(12A)	109.6
C(13)-C(12)-H(12B)	109.6
C(11)-C(12)-H(12B)	109.6
H(12A)-C(12)-H(12B)	108.2
C(12)-C(13)-C(14)	111.7(4)
C(12)-C(13)-H(13A)	109.3
C(14)-C(13)-H(13A)	109.3
C(12)-C(13)-H(13B)	109.3
C(14)-C(13)-H(13B)	109.3
H(13A)-C(13)-H(13B)	107.9
C(15)-C(14)-C(13)	110.7(4)
C(15)-C(14)-H(14A)	109.5
C(13)-C(14)-H(14A)	109.5
C(15)-C(14)-H(14B)	109.5
C(13)-C(14)-H(14B)	109.5
H(14A)-C(14)-H(14B)	108.1
C(14)-C(15)-C(16)	111.3(5)
C(14)-C(15)-H(15A)	109.4
C(16)-C(15)-H(15A)	109.4
C(14)-C(15)-H(15B)	109.4
C(16)-C(15)-H(15B)	109.4
H(15A)-C(15)-H(15B)	108.0

C(11)-C(16)-C(15)	110.1(4)
C(11)-C(16)-H(16A)	109.6
C(15)-C(16)-H(16A)	109.6
C(11)-C(16)-H(16B)	109.6
C(15)-C(16)-H(16B)	109.6
H(16A)-C(16)-H(16B)	108.1
C(1)-C(17)-C(18)	132.8(5)
C(1)-C(17)-H(17)	113.6
C(18)-C(17)-H(17)	113.6
C(23)-C(18)-C(19)	116.5(5)
C(23)-C(18)-C(17)	125.4(5)
C(19)-C(18)-C(17)	117.5(5)
O(4)-C(19)-C(20)	122.9(5)
O(4)-C(19)-C(18)	116.0(4)
C(20)-C(19)-C(18)	121.1(5)
C(21)-C(20)-C(19)	120.0(5)
C(21)-C(20)-H(20)	120.0
C(19)-C(20)-H(20)	120.0
O(5)-C(21)-C(20)	125.7(5)
O(5)-C(21)-C(22)	114.7(5)
C(20)-C(21)-C(22)	119.6(5)
C(23)-C(22)-C(21)	119.7(5)
C(23)-C(22)-H(22)	120.1
C(21)-C(22)-H(22)	120.1
C(22)-C(23)-C(18)	123.0(5)
C(22)-C(23)-H(23)	118.5
C(18)-C(23)-H(23)	118.5
O(4)-C(24)-H(24A)	109.5
O(4)-C(24)-H(24B)	109.5
H(24A)-C(24)-H(24B)	109.5
O(4)-C(24)-H(24C)	109.5
H(24A)-C(24)-H(24C)	109.5
H(24B)-C(24)-H(24C)	109.5
O(5)-C(25)-H(25A)	109.5
O(5)-C(25)-H(25B)	109.5
H(25A)-C(25)-H(25B)	109.5
O(5)-C(25)-H(25C)	109.5
H(25A)-C(25)-H(25C)	109.5
H(25B)-C(25)-H(25C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 10. Anisotropic displacement parameters (A^2 x 10^3) for **43**.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
O(1)	72(3)	41(3)	86(4)	-17(2)	-39(3)	17(2)
O(2)	34(2)	41(2)	58(2)	6(2)	-12(2)	-7(2)
O(3)	27(2)	36(2)	35(2)	1(1)	-3(1)	-3(1)
O(4)	26(2)	41(2)	28(2)	3(1)	-4(1)	-5(1)
O(5)	41(2)	43(2)	40(2)	9(2)	-11(2)	-4(2)
N(1)	37(2)	39(3)	39(2)	5(2)	-7(2)	-3(2)
N(2)	22(2)	37(2)	26(2)	4(2)	-6(2)	-1(2)
C(1)	22(2)	48(3)	26(2)	1(2)	-3(2)	0(2)
C(2)	38(3)	43(3)	33(3)	1(2)	-13(2)	9(2)
C(3)	28(3)	48(3)	28(2)	2(2)	-5(2)	1(2)
C(4)	21(2)	40(3)	23(2)	1(2)	1(2)	4(2)
C(5)	50(4)	52(4)	63(4)	13(3)	-10(3)	-12(3)
C(6A)	77(6)	45(3)	55(5)	13(4)	31(4)	7(4)
C(7A)	77(6)	45(3)	55(5)	13(4)	31(4)	7(4)
C(6B)	77(6)	45(3)	55(5)	13(4)	31(4)	7(4)
C(7B)	77(6)	45(3)	55(5)	13(4)	31(4)	7(4)
C(8)	111(8)	54(5)	143(11)	20(6)	53(8)	0(5)
C(9)	84(6)	48(4)	94(6)	0(4)	7(5)	-25(4)
C(10)	136(9)	50(5)	84(6)	-17(4)	58(6)	-37(5)
C(11)	24(2)	37(3)	24(2)	1(2)	1(2)	4(2)
C(12)	28(3)	44(3)	25(2)	3(2)	4(2)	3(2)
C(13)	35(3)	48(3)	32(3)	-3(2)	5(2)	9(2)
C(14)	39(3)	36(3)	34(3)	2(2)	-5(2)	6(2)
C(15)	55(4)	44(3)	27(2)	5(2)	8(2)	9(3)
C(16)	43(3)	38(3)	29(2)	-2(2)	4(2)	7(2)
C(17)	20(2)	41(3)	35(3)	0(2)	1(2)	3(2)
C(18)	24(2)	35(3)	30(2)	-5(2)	-1(2)	0(2)
C(19)	26(2)	39(3)	26(2)	1(2)	2(2)	3(2)
C(20)	22(2)	38(3)	33(3)	-2(2)	1(2)	-2(2)
C(21)	28(3)	44(3)	31(2)	-1(2)	-2(2)	1(2)
C(22)	37(3)	36(3)	40(3)	4(2)	4(2)	-11(2)
C(23)	28(3)	47(3)	38(3)	-3(2)	0(2)	-9(2)
C(24)	31(3)	45(3)	29(2)	-2(2)	3(2)	-8(2)
C(25)	36(3)	44(3)	40(3)	4(2)	-10(2)	-2(2)

Table 11. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for **43**.

	x	y	z	U(eq)
H(5)	2885	3169	3428	69
H(6A)	3720	3150	5863	68
H(6B)	5834	2892	5803	68
H(7A)	1766	2509	5320	68
H(7B)	1354	2698	3845	68
H(6C)	1753	2999	3893	68
H(6D)	3114	3178	5216	68
H(7C)	1673	2579	5444	68
H(7D)	4238	2638	6163	68
H(8A)	3312	2113	4095	118
H(8B)	5421	2353	4788	118
H(9A)	4935	2353	2237	92
H(9B)	2600	2551	2171	92
H(10A)	6892	2843	3234	102
H(10B)	5106	3004	2010	102
H(11)	4950	4612	2580	35
H(12A)	827	4281	2744	39
H(12B)	2111	4569	3800	39
H(13A)	1610	5016	2130	46
H(13B)	-761	4848	2197	46
H(14A)	-658	4493	254	46
H(14B)	-356	4908	-99	46
H(15A)	3490	4832	163	50
H(15B)	2270	4546	-925	50
H(16A)	2879	4080	707	44
H(16B)	5217	4268	689	44
H(17)	10680	4255	5717	39
H(20)	16892	3946	8642	38
H(22)	12420	3173	9086	46
H(23)	9950	3412	7323	46
H(24A)	16000	4576	8134	53
H(24B)	16149	4732	6669	53
H(24C)	17583	4387	7252	53
H(25A)	19258	3543	10020	63
H(25B)	19032	3410	11503	63
H(25C)	18082	3795	10963	63

Table 12. Torsion angles [deg] for **43**.

C(3)-N(1)-C(2)-O(1)	-163.9(6)
C(5)-N(1)-C(2)-O(1)	13.9(9)
C(3)-N(1)-C(2)-C(1)	13.7(8)
C(5)-N(1)-C(2)-C(1)	-168.4(6)
C(17)-C(1)-C(2)-O(1)	-10.3(10)
C(4)-C(1)-C(2)-O(1)	164.4(6)
C(17)-C(1)-C(2)-N(1)	172.1(5)
C(4)-C(1)-C(2)-N(1)	-13.1(7)
C(2)-N(1)-C(3)-O(2)	177.6(5)
C(5)-N(1)-C(3)-O(2)	-0.3(8)
C(2)-N(1)-C(3)-N(2)	-2.6(8)
C(5)-N(1)-C(3)-N(2)	179.5(5)
C(4)-N(2)-C(3)-O(2)	169.8(5)
C(11)-N(2)-C(3)-O(2)	-4.4(7)
C(4)-N(2)-C(3)-N(1)	-10.0(7)
C(11)-N(2)-C(3)-N(1)	175.8(4)
C(3)-N(2)-C(4)-O(3)	-168.7(5)
C(11)-N(2)-C(4)-O(3)	5.6(6)
C(3)-N(2)-C(4)-C(1)	9.9(6)
C(11)-N(2)-C(4)-C(1)	-175.8(4)
C(17)-C(1)-C(4)-O(3)	-4.1(7)
C(2)-C(1)-C(4)-O(3)	-179.4(5)
C(17)-C(1)-C(4)-N(2)	177.4(4)
C(2)-C(1)-C(4)-N(2)	2.1(7)
C(3)-N(1)-C(5)-C(6B)	-86.8(16)
C(2)-N(1)-C(5)-C(6B)	95.3(16)
C(3)-N(1)-C(5)-C(10)	99.6(8)
C(2)-N(1)-C(5)-C(10)	-78.4(9)
C(3)-N(1)-C(5)-C(6A)	-125.2(8)
C(2)-N(1)-C(5)-C(6A)	56.9(9)
C(6B)-C(5)-C(6A)-C(7A)	38.3(14)
C(10)-C(5)-C(6A)-C(7A)	-59.6(11)
N(1)-C(5)-C(6A)-C(7A)	167.4(7)
C(5)-C(6A)-C(7A)-C(8)	78.1(11)
C(10)-C(5)-C(6B)-C(7B)	43(3)
N(1)-C(5)-C(6B)-C(7B)	-130(2)
C(6A)-C(5)-C(6B)-C(7B)	-59(3)
C(5)-C(6B)-C(7B)-C(8)	-42(3)
C(6A)-C(7A)-C(8)-C(9)	-82.8(12)
C(6A)-C(7A)-C(8)-C(7B)	50(2)
C(6B)-C(7B)-C(8)-C(7A)	-63(2)
C(6B)-C(7B)-C(8)-C(9)	-8(2)
C(7A)-C(8)-C(9)-C(10)	66.2(14)

C(7B)-C(8)-C(9)-C(10)	45.1(17)
C(6B)-C(5)-C(10)-C(9)	10.0(17)
N(1)-C(5)-C(10)-C(9)	-175.4(8)
C(6A)-C(5)-C(10)-C(9)	52.8(14)
C(8)-C(9)-C(10)-C(5)	-47.1(13)
C(3)-N(2)-C(11)-C(16)	-61.9(6)
C(4)-N(2)-C(11)-C(16)	123.5(5)
C(3)-N(2)-C(11)-C(12)	66.9(6)
C(4)-N(2)-C(11)-C(12)	-107.7(5)
N(2)-C(11)-C(12)-C(13)	178.6(4)
C(16)-C(11)-C(12)-C(13)	-54.0(6)
C(11)-C(12)-C(13)-C(14)	55.2(6)
C(12)-C(13)-C(14)-C(15)	-57.8(7)
C(13)-C(14)-C(15)-C(16)	56.9(7)
N(2)-C(11)-C(16)-C(15)	-178.6(5)
C(12)-C(11)-C(16)-C(15)	53.4(6)
C(14)-C(15)-C(16)-C(11)	-54.3(7)
C(2)-C(1)-C(17)-C(18)	-13.4(9)
C(4)-C(1)-C(17)-C(18)	171.7(5)
C(1)-C(17)-C(18)-C(23)	-25.3(9)
C(1)-C(17)-C(18)-C(19)	163.7(5)
C(24)-O(4)-C(19)-C(20)	-17.4(7)
C(24)-O(4)-C(19)-C(18)	162.8(4)
C(23)-C(18)-C(19)-O(4)	-178.4(4)
C(17)-C(18)-C(19)-O(4)	-6.5(7)
C(23)-C(18)-C(19)-C(20)	1.9(7)
C(17)-C(18)-C(19)-C(20)	173.7(5)
O(4)-C(19)-C(20)-C(21)	178.6(5)
C(18)-C(19)-C(20)-C(21)	-1.6(8)
C(25)-O(5)-C(21)-C(20)	3.0(8)
C(25)-O(5)-C(21)-C(22)	-175.5(5)
C(19)-C(20)-C(21)-O(5)	-177.4(5)
C(19)-C(20)-C(21)-C(22)	1.0(8)
O(5)-C(21)-C(22)-C(23)	177.9(5)
C(20)-C(21)-C(22)-C(23)	-0.6(9)
C(21)-C(22)-C(23)-C(18)	1.0(9)
C(19)-C(18)-C(23)-C(22)	-1.6(8)
C(17)-C(18)-C(23)-C(22)	-172.7(5)

3. In vitro pharmacokinetic studies of 7

All of these studies were performed at Apredica, Inc. in Watertown, MA

3.1. Chromatography and mass spectrometry

Samples were analyzed by LC-MS/MS using an Agilent 6410 mass spectrometer coupled with an Agilent 1200 HPLC and a CTC PAL chilled auto sampler, all controlled by MassHunter software (Agilent). After separation on a C18 reverse phase HPLC column (Agilent, Waters, or equivalent) using a 4 min acetonitrile-water gradient system, peaks were analyze by mass spectrometry (MS) using ESI ionization in the MRM mode. The mass spectrometer gas flows and voltages were individually tuned to provide optimal signal for each compound. Trial spectra were obtained to determine the best conditions for data collection. The transition(s) that gave the best signal/noise ratio were used for data analysis.

3.2. Microsomal stability

Test compounds were incubated in duplicate at 5 μM concentration with human or mouse microsomes at 37 ^{o}C. The reaction mixture contained 0.275 mg/mL microsomal protein in 100 mM potassium phosphate, 2 mM NADPH, 3 mM $MgCl_2$, pH 7.4. A control was run for each test agent omitting NADPH to detect NADPH-independent degradation. After 15 sec, 10 min, 20 min, 40 min, and 60 min incubation, aliquots were removed from each experimental and control reaction and mixed with an equal volume of ice-cold Stop Solution (0.3% acetic acid in acetonitrile containing 0.3 μM haloperidol as an internal standard). Stopped reactions were incubated at least 10 min at -20 ^{o}C, and an additional volume of water was added. The samples were centrifuged to remove precipitated protein, and the supernatants were analyzed by LC-MS/MS to quantify the remaining parent. Excel was used for the calculations. The Response Ratio (RR) was calculated by the Mass Hunter software by dividing the peak area of the analyte by the peak area of the internal standard. The relative ratio was calculated by dividing the RR with NADPH by the RR from the –NADPH control at the same time point.

($Relative\ Ratio = PA_{+NADPH} / PA_{-NADPH}$)

The % remaining (RE) compound was calculated by dividing the relative ratio of the sample in question by A0,

$RE = RR / A0$

where A0 is a correction factor for small differences in the starting concentration, extraction efficiency, etc. Curve fitting was used to determine A0, k (first-order rate constant) using an exponential fit with 1/Y weighing. The half-life was calculated as: $T_{1/2} = In(2) / k$. The intrinsic clearance was calculated as follows: CL'_{int} = (0.693/$T_{1/2}$) × (mL incubation/mg microsomal protein) × (mg microsomal protein/g liver) × (g liver/kg bodyweight).[5] The scale-up factor for microsomes protein to g of liver is 45 mg/g of liver.[6] Liver weights used for human and mice were 20 g/kg body weight and 34 g/kg body weight, respectively.[7,8] The units on the $CL;_{int}$ are mL/min/kg.

3.3. Caco-2 monolayer permeability

Caco-2 permeability is measured by detecting the amount of compound that permeates through a confluent monolayer of Caco-2 cells. Caco-2 cells grown in tissue culture flasks were trypsinized, suspended in medium, and the suspensions were applied to wells of a collagen-coated BioCoat Cell Environment in a 24-well format (BD Biosciences). The cells were allowed to grow and differentiate for three weeks, feeding at 2-day intervals.

Stock solutions of 10 mM test compound or control prepared in DMSO were used to prepare solutions for the Caco-2 permeability studies. For apical to basolateral (A → B), the apical (A-side) buffer contained 50 μM test compound or control and 100 μM Lucifer yellow dye in the

transport buffer (1.98 g/L glucose in 10 mM HEPES, 1 × Hank's Balanced Slat Solution) at pH 6.5; the basolateral (B-side) buffer was the transport buffer at pH 7.4. For B → A studies, the A-side buffer contained 100 μM Lucifer yellow in the transport buffer, pH 6.5, and the B-side buffer contained 50 μM test compound or control in the transport buffer, pH 7.4. The Caco-2 cells were incubated with these buffers for 2 h, and the cell buffers and dosing solutions were removed for analysis.

To verify that the Caco-2 cell monolayer was properly formed, an aliquot of the cell buffer from each well was analyzed by fluorescence to determine the transport of the impermeable dye Lucifer Yellow. In all cases, acceptable transport of Lucifer Yellow was observed (< 1% transport in 2 h).

Caco-2 results are reported as apparent permeability, P_{app}, expressed in units of 10^{-6} cm/sec: $P_{app} = \frac{dQ/dt}{c_0 A}$,

where dQ/dt is the rate of permeation (mol test compound transported per unit time), c_0 is the initial concentration of test compound, and A is the area of the cell monolayer

3.4. Solubility screen

The solubility of the test compounds in PBS was estimated by absorbance. Three-fold serial dilutions were prepared in DMSO at 100 times the final concentration. This DMSO stock was diluted to the final concentration in PBS, the plate was incubated at room temperature for 1 h, then it was examined visually for the formation of precipitate, and the absorbance at 450 nm was measured. For non-colored compounds, the absorbance value was used to detect solubility, while for highly colored compounds, visual inspection was used. The highest concentration that was not visibly cloudy and whose absorbance was not different from background was judged to be the solubility limit.

3.5. Plasma and PBS stability

The test compounds, with warfarin and diltiazem as controls, were incubated in mouse plasma and phosphate buffered saline in duplicate at 5 μM. The time-zero samples were taken immediately (15 sec) after adding the compounds. The plate containing the plasma and PBS samples was incubated at 37 °C, and then time points were taken at 10, 20, 40, 60 min in the same way. The plasma samples and PBS samples were precipitated with acetonitrile containing haloperidol as an internal standard and either centrifuged or filtered through a Varian Captiva plate (following the manufacturer's directions) to remove precipitated protein. The samples were then analyzed on LC-MS/MS.

The response ratio (RR = area of analyte peak divided by the area of the internal standard peak), as calculated by the LC-MS software, was used for determining the plasma stability. The relative ratio was calculated by dividing the RR from plasma by the RR from the PBS control at the same time point.

($Relative\ Ratio = RR_{plasma} / RR_{PBS}$)

The % remaining (RE) compound was calculated by dividing the relative ratio of the sample in question by A0,

$RE = RR / A0$

where A0 is a correction factor for small differences in starting concentration, extraction efficiency, etc. Curve fitting was used to determine A0, k (first-order rate constant) using an exponential fit with 1/Y weighting. The half-life was calculated as: $T_{1/2} = In(2) / k$.

To determine PBS half-life, the RR was fit to a first-order exponential curve to determine A_{PBS} and k_{PBS}: $RR = A_{PBS} e^{-k_{PBS} t}$ The half-life was calculated as above: $T_{1/2} = In(2) / k_{PBS}$.

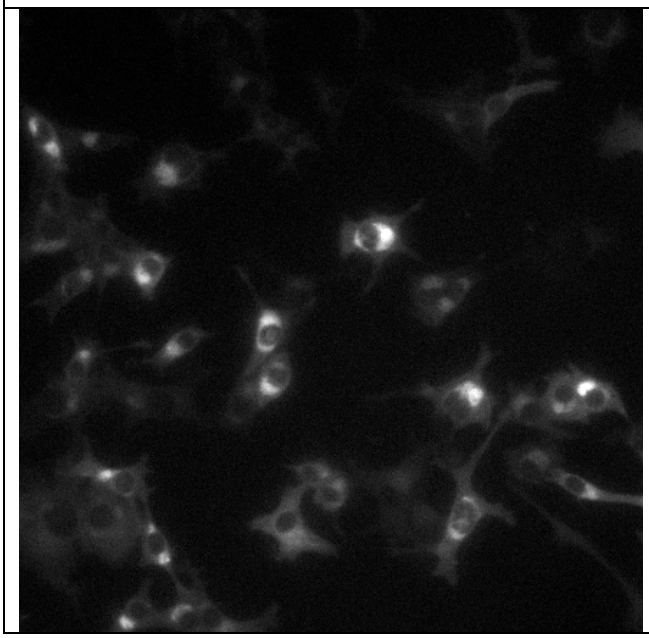
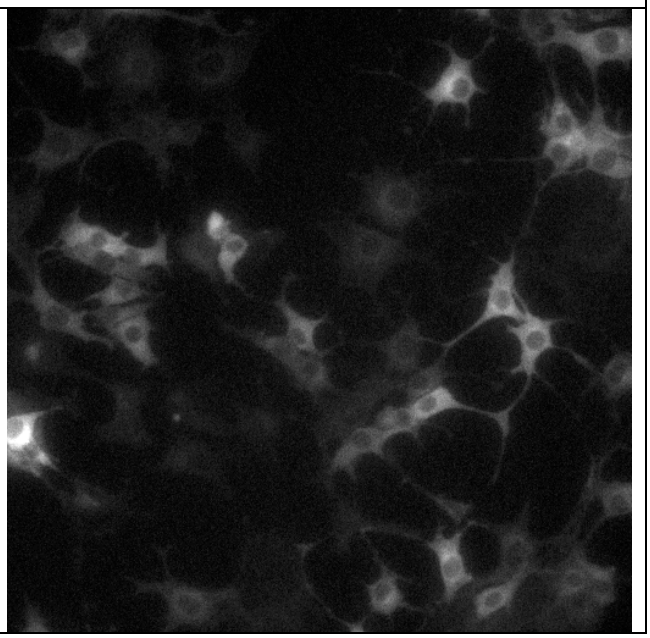
4. Radioligand Binding Assays. Radioligand binding assays for **7** (10 μM) were carried out by MDS Pharma Services (Taipei, Taiwan)

Table 13. Competitive binding assays of **7** (10 μM)

Target	Inhibition (%)	Target	Inhibition (%)
Adenosine A_1	15	Glutamate, NMDA, Phencyclidine	-3
Adenosine A_{2A}	-14	Histamine H_1	-20
Adenosine A_3	0	Histamine H_2	4
Adrenergic α_{1A}	-9	Histamine H_3	-1
Adrenergic α_{1B}	-1	Imidazoline I_2, Central	0
Adrenergic α_{1D}	-13	Interleukin IL-1	1
Adrenergic α_{2A}	-2	Leukotriene, Cysteinyl $CysLT_1$	17
Adrenergic $ß_1$	11	Melatonin MT_1	5
Adrenergic $ß_2$	-3	Muscarinic M_1	1
Androgen (Testosterone) AR	4	Muscarinic M_2	-1
Bradykinin B_1	3	Muscarinic M_3	7
Bradykinin B_2	4	Neuropeptide Y Y_1	11
Calcium Channel L-Type, Benzothiazepin	11	Neuropeptide Y Y_2	1
Calcium Channel L-Type, Dihydropyridin	0	Nicotinic Acetylcholine	0
Calcium Channel N-Typ	-3	Nicotinic Acetylcholine D_1, Bungarotoxin	2
Dopamine D_1	-11	Opiate δ (OP1, DOP)	-7
Dopamine D_{25}	6	Opiate κ (OP2, KOP)	0
Dopamine D_3	41	Opiate μ (OP3, MOP)	-5

Dopamine $D_{4.2}$	-7	Phorbol Ester	-5
Endothelin ET_A	15	Platelet Activating Factor (PAF)	-1
Endothelin ET_B	-7	Potassium Channel [KATP]	-5
Epidermal Growth Factor (EGF)	-8	Potassium Channel hERG	-2
Estrogen ERα	20	Prostanoid EP_4	-8
G Protein-Coupled Recepto GPR103	-9	Purinergic P_{2x}	3
GABAA, Flunitrazepam, Central	5	Purinergic P_{2y}	8
GABAA, Muscimol, Central	-2	Rolipram	9
$GABAB_{1A}$	-7	Serotonin (5-Hydroxytryptamine) 5-HT_{1A}	16
Glucocorticoid	-2	Serotonin (5-Hydroxytryptamine) 5-HT_3	1
Glutamate, Kainate	14	Sigma δ_1	-4
Glutamate, NMDA, Agonism	17	Sigma δ_2	4
Glutamate, NMDA, Glycine	7	Sodium Channel, Site 2	21
Tachykinin NK□	-2	Thyroid Hormone	12
Transporter, Dopamine (DAT)	1	Transporter, GABA	19
Transporter, Norepinephrine (NET)	-8	Transporter, Serotonin (5-Hydroxytryptamine) (SERT)	-4

G85R-SOD1 High Content Aggregation Assay. Reduction in SOD1 aggregates in cells treated with **7**

G85R-SOD1 cells treated with 1 μM MG132	**G85R-SOD1 cells treated with 1 μM MG132 + 10 μM compound 7**
	

References

(1) Cromer, D. T. & Waber, J. T.; *International Tables for X-ray Crystallograph* Vol. IV, The Kynoch Press, Birmingham, England, Table 2.2 A **1974**.
(2) Ibers, J. A. & Hamilton, W. C.; *Acta Crystallogr*, **1974**, *17*, 781.
(3) Creagh, D. C. & McAuley, W.J .; *International Tables for Crystallography*, Vol C, Table 4.2.6.8, **1992**, 219-222.
(4) Creagh, D. C. & Hubbell, J.H..; *International Tables for Crystallography,* Vol C, Table 4.2.4.3, **1992**, 200-206.
(5) Obach, R. S. *Drug Metab. Dispos.*, **1999**, *27*, 1360-1359.
(6) Houston, J. B. *Biochem. Pharmacol.*, **1994**, *47*, 1469-1479.
(7) Obach, R. S.; Baxter, J. G.; Liston, T. E.; Silber, B. M.; Jones, B. C.; Maclntyre, R.; Rance, D. J.; Wastall, P. *J. Pharmacol. Exp. Ther.*, **1997**, *283*, 46-58.
(8)Brown, R. P.; Delp, M. D.; Lindstedt, S. L.; Rhomberg, L. R.; Belilesi, R. P. *Toxicology and Industrial Health*, **1997**, *13*, 407-484.