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Furo[3,4-*d*]pyrimidine-2,4-dione derivatives with antihypertensive activity. Analogues of thienopyrimidine-2,4-diones

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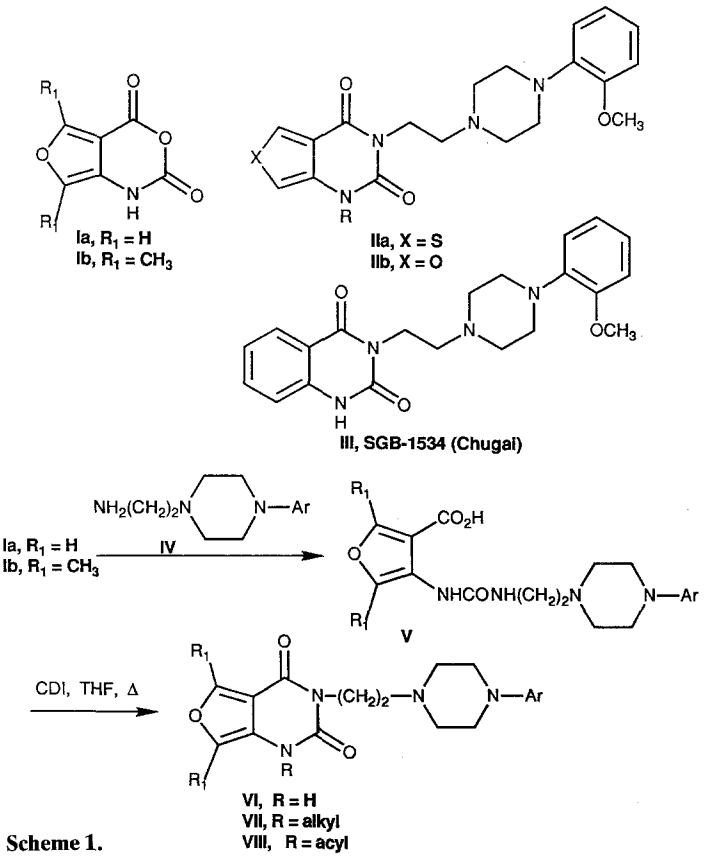
Introduction

Our laboratory has been interested for some time in the isosteric replacement of benzene in pharmacologically active agents. The thiophene moiety has usually served as the replacement of choice [1]. In general, furan derivatives have not been investigated in such detail as a consequence of their reduced stability and lessened aromatic character vis-à-vis their benzene or thiophene counterparts. The lack of necessary amine-substituted furan starting materials (both commercial and as a result of inherent instability of such intermediates) has also impeded research in this area. In a previous study [2], we had reported some investigative chemistry for the preparation of **Ia**, the furan isotere of isatoic anhydride. In contrast to the chemistry of isatoic anhydride, nucleophiles react only at the C-2 carbonyl of **Ia** to give urea acid derivatives with no competition from attack at C-4. This material appears to be an excellent precursor to derivatives of amine-substituted furans.

We recently reported the synthesis and antihypertensive activity of a series of thienopyrimidinedione derivatives including thieno[3,4-*d*]pyrimidine-2,4-dione system **IIa** [3]. These compounds were modelled on the isosteric potent α-adrenergic blocking agent 3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]quinazoline-2,4-dione (**III**, SGB-1534) [4]. These thiophene derivatives with hydrogen at N-1 and an *o*-methoxyphenylpiperazine substituent attached to the ethylene side chain were potent, orally active antihypertensive agents in spontaneously hypertensive rats (SHR) and were potent α-adrenergic blocking agents as evidenced by their antagonism of the phenylephrine-pressor response in dogs. As part of our ongoing studies directed toward novel antihypertensive agents and based on the chemistry of **Ia**, we felt furan isosteres **IIb** might be achievable synthesis targets and would be especially interesting since the thieno[3,4-*d*] derivative **IIa** was the most interesting derivative in our earlier study. The goal of this study was then to determine if furan might effectively replace benzene and thiophene in this series of antihypertensive agents.

Chemistry

The target furo[3,4-*d*]pyrimidine-2,4-diones were prepared as outlined in Scheme 1. Previously unknown dimethyl 4H-furo[3,4-*d*]oxazine-2(1H),4-dione **Ib** was prepared from diethyl 2,5-dimethyl-furan-3,4-dicarboxylate [5] using essentially the published procedures for the preparation of **Ia** [2]. Reaction of **I** with the appropriately substi-



Scheme 1.

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tuted aryl piperazinyl alkyl amines **IV** produced the expected urea acid derivatives **V**. Ring closure to target compounds **VI** was accomplished by treatment of **V** with *N,N*-carbonyldiimidazole. Several N-1 alkyl derivatives **(VII)** and N-1 acyl derivatives **(VIII)** were prepared by treating **VI** with sodium hydride and treating the resulting anion with alkyl iodides or acyl chlorides, respectively. The acyl derivatives **VIII** were also prepared by reaction of **VI** with acid anhydrides. As was the case for the isosteric thienopyrimidinediones [3], substitution at N-1 (as opposed to reaction at the carbonyl oxygen of this potential ambident system) resulted as evidenced by ^1H NMR and NOE studies.

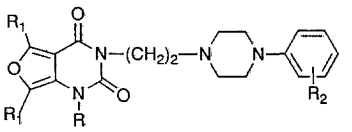
Results and Discussion

The biological activity of the furo[3,4-*d*]pyrimidine-2,4-dione derivatives in spontaneously hypertensive rats (SHR) is summarized in Table I and compared with **III** (SGB-1534) [4] and selected thiophene isosteres [3]. A brief summary of the SAR of this series of furo[3,4-*d*]pyrimidine-2,4-diones in the SHR is quite similar to that of the thienopyrimidinediones [3]. The effects of substitution on the arylpiperazine portion of the molecule show that 2-methoxy substituted **(1)** as well as the unsubstituted **(2)** derivatives have the greatest potency. Substitution with similar electronic **(4, 5)** or steric **(3, 6, 7)** moieties reduces potency. Clearly both the electronic and steric effects of the 2-methoxy substitution are important to activity; an electron-rich aromatic ring and a twisting of the aryl-piperazine rings out of plane probably is important to a receptor fit of the molecule.

Several compounds were prepared to examine the effects of N-1 substitution. With the 2-methoxyphenylpiperazine substituent held fixed, *N*-alkylation **(8, 9, 10)** and *N*-acylation **(11, 12, 14)** did not dramatically alter potency of the series, with the exception that larger groups such as pivaloyl **(12)** and butyrate **(10)** were less interesting. This is in contrast to our findings for the thiophene isosteres wherein such substitution did attenuate potency [3]. Two compounds (**13** and **14**) were prepared to examine the effects of alkyl substitution on furan and both were quite potent in the SHR.

In this series, unsubstituted **1** as well as N1-methyl **(8)** and N1-acetyl **(11)** derivatives were interesting and were examined in somewhat more detail to evaluate their potency and mechanism of action (Table II). ED_{30} determinations (the dose that reduces mean arterial blood pressure (MABP) by 30%) were calculated from dose response experiments in SHR using direct determinations in animals with cannulae implanted in the carotid artery and these values are shown in Table II. As a consequence of the direct determination, ED_{30} values from this assay are

Table I. SHR results for furo[3,4-*d*]pyrimidine-2,4-diones **VI**, **VII** and **VIII**.



Compound	R_1	R	R_2	Dose (mg/kg, *p.o.*)	SHR results[a]	
					ΔSBP[b] (mm Hg)	Time to peak (h)
1	H	H	2-OCH_3	5.0	−86±5	0.5
2	H	H	H	2.5	−56±5	1.0
3	H	H	2-CH_3	5.0	−43±7	1.0
4	H	H	3-OCH_3	5.0	−12±9	4.0
5	H	H	4-OCH_3	5.0	−37±10	0.5
6	H	H	2-Cl	5.0	−26±12	1.0
7	H	H	2,6-$(CH_3)_2$	5.0	−15±14	4.0
8	H	CH_3	2-OCH_3	5.0	−62±2	0.5
9	H	*n*-C_4H_9	2-OCH_3	2.5	−49±12	3.0
10	H	$(CH_2)_3CO_2Et$	2-OCH_3	2.5	−26±16	4.0
11	H	$COCH_3$	2-OCH_3	5.0	−52±5	1.0
12	H	$COC(CH_3)_3$	2-OCH_3	5.0	−12±7	2.0
13	CH_3	H	2-OCH_3	2.5	−48±8	0.5
14	CH_3	$COCH_3$	2-OCH_3	2.5	−54±9	0.5
III (SGB 1534)				1.25	−60±2	0.5
Thieno[3,4-*d*]pyrimidine-2,4-dione[c]			2-OCH_3	0.5	−88±10	0.5
Thieno[3,2-*d*]pyrimidine-2,4-dione[d]			2-OCH_3	1.25	−74±19	0.5
Thieno[2,3-*d*]pyrimidine-2,4-dione[e]			2-OCH_3	1.25	−50±4	0.5

[a]Spontaneously hypertensive rat (SHR) results from groups of 4−6 animals.
[b]SBP measured indirectly using an inflatable tailcuff. Vehicle ΔSBP was 15±5 mm Hg.
[c]3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]thieno[3,4-*d*]pyrimidine-2,4-dione [3].
[d]3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]thieno[3,2-*d*]pyrimidine-2,4-dione [3].
[e]3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]thieno[2,3-*d*]pyrimidine-2,4-dione [3].

not directly comparable to the measurements using a tail cuff procedure. All derivatives studied are similar in potency.

The compounds were further evaluated for *in vivo* and *in vitro* α_1-antagonist activity (Table II). The *in vivo* activity was determined by antagonism of phenylephrine pressor response in dogs. As was the case for the thiophene isosteres [3], compounds **1**, **8** and **11** had α_1-antagonist activity with potency similar to **III**. While the thieno[3,4-*d*]- and thieno[3,2-*d*] isosteres are more potent than the furan derivatives in this assay, the thieno[2,3-*d*] isostere was significantly less potent. Finally, compounds **1**, **8**, **11** and the standards were evaluated for their affinity for α_1-adrenergic binding sites (Table II). These compounds had high affinity for [^{3}H] prazosin labelled binding sites with *ki* values ranging from 6–18 nM. The affinity for the α_1-adrenoceptor was essentially the same for **1** and **III**, while compounds **8** and **11** were less interesting. These results differ from the inhibition potency (ED_{50}) of the phenylephrine-induced pressor response *in vivo* wherein the furan derivatives **(1, 8, 11)** and **III** are essentially equipotent. In this assay, the furans as well as **III** had very significantly lower affinity for α_1 binding sites as compared to the thiophene isosteres. While these data support α-blockade as the possible antihypertensive mechanism of the furan derivatives, it is clear from the substantial differences in binding affinity as well as *in vivo* activity for compounds of quite similar structure that subtle differences in metabolism, bioavailability and elimination play a significant role.

In summary, all the furan[3,4-*d*]pyrimidine-2,4-dione derivatives prepared in this brief study had antihypertensive properties. The best compounds **(1, 8, 11)** were potent agents with activity similar to that of the previously reported thiophene isosteres [3] or the benzene isostere **III** (SGB-1534). The SAR for the furan series is greatly similar to that of the thiophenes with the exception that N-1 alkyl or acetyl substitution only slightly attenuates potency. Methyl substitution on the furan ring also does not seem to affect the potency of these furan derivatives while such substitution in the comparable thiophene series did lower potency. Although the syntheses would be far more difficult than those reported herein, it would be interesting to investigate the other isomeric furan systems to determine the full SAR of these potent α_1-adrenergic blocking agents.

Experimental protocols

Chemistry

Melting points were determined on a Mel-temp® capillary block apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Brucker WP-100 FT spectrometer with chemical shifts reported in δ downfield for tetramethylsilane as an internal standard. All characterized compounds were homogeneous by TLC analysis and had combustion analyses within ± 0.4% for C, H, N. Standard flash chromatography utilized 230–400 mesh E. Merck silica gel under positive nitrogen pressure. Compounds listed in Table III are prepared using the procedures as described below for the preparation of the dimethyl substituted furan derivatives.

Table III. Physical properties of furo[3,4-*d*]pyrimidine-2,4-dione derivatives.

Compound	Formula	Yield[a]	mp(°C)[b]	Analysis
1	$C_{19}H_{22}N_4O_4 \cdot 0.25H_2O$	42	194–196 dec	C, H, N
2	$C_{18}H_{20}N_4O_3$	49	209–210 dec	C, H, N
3	$C_{19}H_{22}N_4O_3$	43	195–196 dec	C, H, N
4	$C_{19}H_{22}N_4O_4 \cdot 0.25H_2O$	64	174–175 dec	C, H, N
5	$C_{19}H_{22}N_4O_4$	59	215–216 dec	C, H, N
6	$C_{18}H_{19}ClN_4O_3$	69	215–217 dec	C, H, N, Cl
7	$C_{20}H_{24}N_4O_3$	27	184–188 dec	C, H, N
8	$C_{20}H_{24}N_4O_4$	37	150–152	C, H, N
9	$C_{23}H_{30}N_4O_4$	59	112–114	C, H, N
10	$C_{25}H_{32}N_4O_6$	20	88–90	C, H, N
11	$C_{21}H_{24}N_4O_5$	83	128–131	C, H, N
12	$C_{24}H_{30}N_4O_5$	44	86–88	C, H, N
13	$C_{21}H_{28}N_4O_4$	76	224–225	C, H, N
14	$C_{23}H_{28}N_4O_5$	83	100–102	C, H, N

[a]Yield represents either: 1) the combined yield of ring-opening of **IV** by amine and subsequent ring closure to compounds **1–7** and **13**; or 2) yield of alkylation or acylation.
[b]Melting point, dec = decomposition.

Table II. Potency of selected furo[3,4-*d*]pyrimidine-2,4-dione derivatives and standards as antihypertensive agents and as α_1-adrenergic antagonists.

Compound	SHR ED_{30}[a]	α-blockade ED_{50}[b]	*ki* (nM)[c]
1	4.2(2.5, 8.5)	5.4(3.1, 11.1)	5.8±0.7
8	4.8(2.9, 12.2)	7.1(5.4, 9.5)	15.4±3.4
11	5.6(2.9, 20.0)	6.5(4.0, 12.2)	18.1±3.4
III (SGB 1534)	2.9(2.2, 4.0)	3.3(1.6, 10.6)	7.7±1.7
Thieno[3,4-*d*]pyrimidine-2,4-dione[d]	3.5(2.3, 5.8)	1.7(0.9, 3.4)	0.64±0.15
Thieno[3,2-*d*]pyrimidine-2,4-dione[e]	5.8(3.7, 13.1)	2.1(1.2, 3.5)	0.89±0.18
Thieno[2,3-*d*]pyrimidine-2,4-dione[f]	4.4(2.6, 10.3)	15.4(10.3, 23.1)	1.83±0.72

[a]The dose (mg/kg, *p.o.*) of the test compound required to produce a 30% reduction in mean arterial blood pressure (MABP) using direct measurement in the spontaneously hypertensive rat (SHR, n= 8–12/group). The fiducial limits are shown in parentheses.
[b]The dose (μg/kg, i.v.) of the test compound required to produce a 50% inhibition of the phenylephrine pressor response in the spontaneously hypertensive rat (SHR). Fiducial limits are shown in parentheses.
[c]Affinity for α_1-adrenoceptors; each value represents the mean ± SEM of 3 experiments performed in triplicate. *Ki* values were calculated from the IC_{50} values by the equation of Cheng and Prusoff [9].
[d]3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]thieno[3,4-*d*]pyrimidine-2,4-dione [3].
[e]3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]thieno[3,2-*d*]pyrimidine-2,4-dione [3].
[f]3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]thieno[2,3-*d*]pyrimidine-2,4-dione [3].

2,5-Dimethyl-4H-furo[3,4-d][1,3]oxazine-2, (1H), 4-dione **Ib**
A solution of 3,4-dicarboethoxy-2,5-dimethylfuran [5] was treated with sodium hydroxide using essentially the procedure described for the preparation of **Ia** [2] to give 2,5-dimethyl-3,4-furandicarboxylic acid monoethyl ester in 51% yield, mp 79–81°C. Anal. ($C_{10}H_{12}O_5$): C, H.

This ester was treated with an excess of hydrazine in ethanol at reflux to form 2,5-dimethyl-3,4-furandicarboxylic acid monohydrazide in 73% yield, mp > 300°C. Anal. ($C_8H_{10}O_4N_2$): C, H, N. A suspension of the monohydrazide in 3 N HCl and $CHCl_3$ was treated with 1.1 equivalents of sodium nitrite in water at 5°C. Upon thermolysis of the intermediate azide in $CHCl_3$, **Ib** crystallized from solution as an off-white solid, mp 191–193°C; IR (KBr) 1600, 1694, 1727, 1792, cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 11.15 (br, s, 1H, N*H*), 2.52 (s, 3H, CH_3), 2.28 (s, 3H, CH_3). Anal. ($C_8H_7O_4N$): C, H, N.

5,7-Dimethyl-3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]furo[3,4-d]pyrimidine-2,4-dione **13**
1-[1-(2-Aminoethyl)]-4-(2-methoxyphenyl)piperazine [6, 7] (2.8 g, 11.8 mmol) was added to a suspension of **Ib** (1.65 g, 9.11 mmol) in THF (80 ml). The mixture was stirred at room temperature for 16 h and the resultant precipitate was collected by filtration, washed with THF and air-dried to give the urea intermediate (3.5 g, 92%) as a white solid, mp 193–196°C. IR (KBr) 1686, 1670 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.22–6.61 (m, 4H, Ar-*H*), 3.42–2.30 (m, 15 H, NCH_2, CH_3), 2.44 (s, 3H, OCH_3), 2.13 (s, 3H, CH_3); MS (m/z) 417 (MH^+).

The urea (without further purification, 3.32 g, 7.97 mmol) and 1,1′-carbonyldiimidazole (CDI, 1.68 g, 10.4 mmol) were combined in THF (100 ml) and the mixture was heated to reflux for 1.5 h. The solvent was removed *in vacuo* and the residue was triturated with hot water to provide a brown solid. The pure product (**13**, 2.65 g, 83%) was obtained as a white solid by trituration with hot ethanol; mp 224–225°C dec; IR (KBr) 2823, 1717, 1695, 1667 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 10.82 (br, s, 1H, N*H*), 7.16–6.76 (m, 4H, Ar-*H*), 4.14 (t, 2H, CH_2), 3.87 (s, 3H, OCH_3), 2.59 (s, 3H, CH_3), 2.34 (s, 3H, CH_3), 3.31–2.09 (m, 10H, NCH_2); MS (m/z) 399 (MH^+). Anal. ($C_{21}H_{26}N_4O_4$): C, H, N.

For most of our work, the urea was never isolated. In these cases, CDI was added directly to the reaction mixture in THF wherein the urea formed and ring closure occurred uneventfully.

1-Acetyl-3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]furo[3,4-d]pyrimidine-2,4-dione **11**
3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]furo[3,4-*d*]pyrimidine-2,4-dione (**1**, 3.0 g, 8.10 mmol) was dissolved in acetic anhydride (50 ml) and heated to reflux for 16 h. The solvent was removed *in vacuo* and the residue was dissolved in toluene which was removed *in vacuo* and the process was repeated. The resultant oil was crystallized from Et_2O to give pure **11** as a off-white solid, 2.77 g (83%), mp 128–131°C; IR (KBr) 2820, 1742, 1717, 1702, 1621 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.21 (d, 1H, furan-*H*), 8.08 (d, 1H, furan-*H*), 7.02–6.67 (m, 4H, Ar-*H*), 4.18 (t, 2H, CH_2), 3.82 (s, 3H, OCH_3), 3.18–2.92 (m, 4H, NCH_2), 2.72 (s, 3H, $COCH_3$), 2.87–2.50 (m 6H, NCH_2); MS (m/z) 413 (MH^+). Anal. ($C_{21}H_{24}N_4O_5$): C, H, N.

1-Methyl-3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]furo[3,4-d]-pyrimidine-2,4-dione **8**
3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]furo[3,4-*d*]pyrimidine-2,4-dione (**1**, 2.03 g, 5.48 mmol) was added to a suspension of sodium hydride (60% suspension in mineral oil, 0.24 g, 6.03 mmol, prewashed with pentane) in DMF (50 ml) and the mixture was stirred for 1 h. Iodomethane (0.42 g, 6.75 mmol) was added and the mixture was stirred for 2.5 d. The mixture was poured into ice water (200 ml) and the precipitate was collected by filtration. The solid was dissolved in 2% MeOH in CH_2Cl_2 and eluted through a short column of magnesium silicate. After concentration, the resultant solid was triturated with Et_2O to give **8** as a colorless solid, 0.775 g (37%), mp 150–152°C; IR (KBr) 1712, 1673, 1648, 1501 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.02 (d, 1H, furan-*H*), 7.28 (d, 1H, furan-*H*), 7.00–6.73 (m, 4H, Ar-*H*), 4.18 (t, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.33 (s, 3H, NCH_3), 3.22–2.95 (m, 4H, NCH_2), 2.91–2.53 (m, 6H, NCH_2); MS (m/z) 385 (MH^+). Anal. ($C_{20}H_{24}N_4O_4$): C, H, N.

Pharmacology

Antihypertensive activity. This was initially evaluated in spontaneously hypertensive rats (SHR) as described previously [3] and the data is reported in Table I.

Subsequent evaluation of the antihypertensive effects of compounds **1**, **8** and **11** was conducted in conscious SHR using a direct measurement of mean arterial blood pressure. On the day of the experiment, catheters were implanted into the left carotid artery and the left jugular vein of rats under light ether anesthesia. The catheters were exteriorized at the nape of the neck and secured with an adhesive wrap. Animals were then placed in stainless steel restraint cages in a quiet room and allowed at least 90 min post-surgical recovery before recordings were collected. Recordings of arterial pressure were obtained using a Statham pressure transducer connected to a Gould 2800 chart recorder. The oral dose of drug required to produce a 30% reduction in systolic blood pressure (ED_{30}) was calculated by regression analysis and is reported in Table II. The data in Table II are presented as ED_{30} (mg/kg, *p.o.*) with 95% fiducial limits.

α_1-Adrenergic antagonist activity. This was determined in SHR as previously described and the data is summarized in Table II.

[^{3}H] Prazosin binding affinity. This was determined using homogenates of the frontal cortex of rats [8]. A crude membrane fraction was incubated with a test compound at various concentrations in borosilicate glass tubes on a shaking metabolic incubator at 37°C for 10 min. The assay medium, in order of addition, consisted of: 50 mM Tris (pH 7.4); 2.5 mM $MgCl_2$ and 2 nM [^{3}H] prazosin (specific activity = 82 Ci/mmol; New England Nuclear, Boston, MA). The binding reaction was initiated by the addition of 350–450 μg of protein. The final incubation volume was 250 μl and nonspecific binding was defined with 10 μM phentolamine. The reaction was terminated by the addition of 3.0 ml ice-cold rinse buffer (20 mM Tris, pH 7.4) and the contents were rapidly filtered through glass fiber filters. Thirteen to 15 concentrations of each drug were used in the competition experiments and the data were analyzed by nonlinear regression using LUNDON. All binding data are reported as *ki* values calculated by the equation of Cheng and Prusoff [9].

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