Bioorganic & Medicinal Chemistry Letters 19 (2009) 365–368

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Redefining the structure–activity relationships of 2,6-methano-3-benzazocines. Part 7: Syntheses and opioid receptor properties of cyclic variants of cyclazocine ☆

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ARTICLE INFO

Article history:
Received 18 September 2008
Revised 18 November 2008
Accepted 20 November 2008
Available online 24 November 2008

Keywords:
Opioid
SAR

ABSTRACT

A series of 7,8- and 8,9-fused triazole and imidazole analogues of cyclazocine have been made and characterized in opioid receptor binding and [^{35}S]GTPγS assays. Target compounds were designed to explore the SAR surrounding our lead molecule for this study, namely the 8,9-fused pyrrolo analogue **2** of cyclazocine. Compared to **2**, many of the new compounds in this study displayed very high affinity for opioid receptors.

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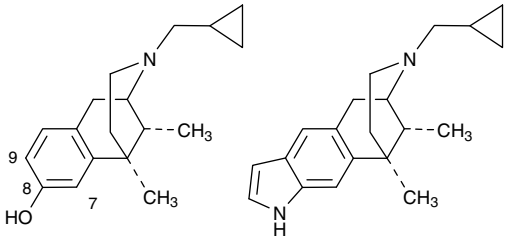
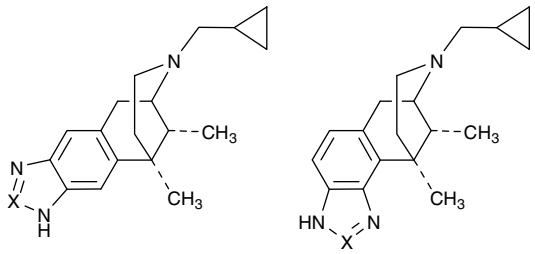
In hopes of identifying long acting orally available analogues of cyclazocine (**1**)[1] as potential anti-cocaine medications, we reported in 2003 the syntheses and pharmacological evaluation of a group of analogues of cyclazocine where the phenolic 8-OH was replaced by traditional bioisosteres.[2] Cyclazocine has high affinity for μ and κ opioid receptors (Table 1) and was evaluated in humans in the 1960s and early1970s as an analgesic and as a possible treatment for preventing relapse in post-addicts of heroin.[1] Among this group was the known indole derivative **2**[3] which had 56- and 39-fold lower binding affinity to μ and κ opioid receptors, respectively, than cyclazocine.[2] While the activity of **2** did not meet our needs with regard to the overall goals of the project, its interesting structure and reasonably good binding affinity prompted us to use it as a lead for further analogue design and SAR study. From our studies[2] as well as published SAR data,[4] a H-bond donating group (i.e., OH, NH) at position-8 is a vital part of the pharmacophore and plays a major role in the design of new analogues of **2**.



1 (Cyclazocine) **2**



3 **4**

We now wish to report the syntheses and pharmacological evaluation of novel aza-substituted analogues of **2** where one or both CH's of the fused-pyrrolo ring are replaced by nitrogen. Specifically we

☆ Presented, in part, at Ganorkar, R. R.; Lu, Q.; Wentland, M. P.; Bidlack, J. M. Syntheses and opioid receptor binding affinities of conformationally restricted analogs of cyclazocine. Abstracts of Papers, 234th ACS National Meeting, Boston, MA, United States, August 19–23, 2007; MEDI-140.

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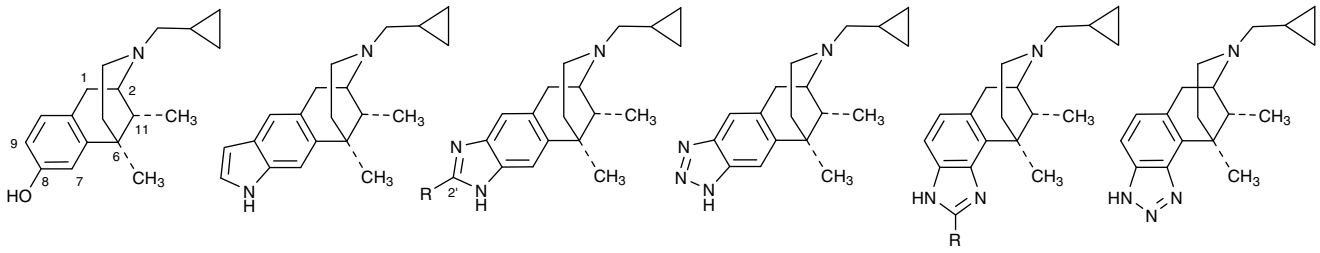
doi:10.1016/j.bmcl.2008.11.076

Table 1
Opioid receptor binding data for cyclic variants of cyclazocine



1 **2** **13**: R = H; **14**: R = CH_3; **15**: R = OH **16** **17**: R = H; **18**: R = CH_3 **19**

Compound	K_i (nM)[a]			κ:μ[b]	κ:δ[c]
	[^{3}H]DAMGO (μ)	[^{3}H]Naltrindole (δ)	[^{3}H]U69,593 (κ)		
1[d]	0.16 ± 0.01	2.0 ± 0.22	0.07 ± 0.01	2	30
2[e,f]	18 ± 1.9	250 ± 14	7.0 ± 0.22	3	36
13[f]	0.31 ± 0.050	5.1 ± 0.65	0.063 ± 0.0016	5	81
14[f]	0.77 ± 0.051	18 ± 0.90	0.050 ± 0.002	15	360
15[f]	60 ± 7.9	730 ± 8.3	12 ± 1.3	5	61
16[f]	10 ± 1.0	51 ± 7.8	0.81 ± 0.19	12	63
17[f]	9.5 ± 0.58	680 ± 89	0.92 ± 0.040	10	740
18[f]	2.5 ± 0.23	160 ± 15	0.51 ± 0.023	5	310
19[f]	1.4 ± 0.043	46 ± 2.6	0.23 ± 0.009	6	200

[a] Binding assays used to screen compounds are similar to those previously reported (see Ref. 10). Membrane protein from CHO cells that stably expressed one type of the human opioid receptor were incubated with 12 different concentrations of the compound in the presence of either 1 nM [^{3}H]U69,593 (μ), 0.25 nM [^{3}H]DAMGO (δ) or 0.2 nM [^{3}H]naltrindole (κ) in a final volume of 1 mL of 50 mM Tris–HCl, pH 7.5 at 25 °C. Incubation times of 60 min were used for [^{3}H]U69,593 and [^{3}H]DAMGO. Because of a slower association of [^{3}H]naltrindole with the receptor, a 3-h incubation was used with this radioligand. Samples incubated with [^{3}H]naltrindole also contained 10 mM $MgCl_2$ and 0.5 mM phenylmethylsulfonyl fluoride. Nonspecific binding was measured by inclusion of 10 μM naloxone. The binding was terminated by filtering the samples through Schleicher & Schuell No. 32 glass fiber filters using a Brandel 48-well cell harvester. The filters were subsequently washed three times with 3 mL of cold 50 mM Tris–HCl, pH 7.5, and were counted in 2 mL Ecoscint A scintillation fluid. For [^{3}H]naltrindole and [^{3}H]U69,593 binding, the filters were soaked in 0.1% polyethylenimine for at least 60 min before use. IC_{50} values will be calculated by least squares fit to a logarithm-probit analysis. K_i values of unlabeled compounds were calculated from the equation $K_i = (IC_{50})/1 + S$ where S = (concentration of radioligand)/(K_d of radioligand)—see Ref. 13 The K_d values for [^{3}H]DAMGO, [^{3}H]U69,593, and [^{3}H]naltrindole were 0.56, 0.34, and 0.10 nM, respectively. Data are the mean ± SEM from at least three experiments performed in triplicate.

[b] $κ:μ = K_i(μ)/K_i(κ)$.

[c] $κ:δ = K_i(δ)/K_i(κ)$.

[d] See Ref. 1.

[e] See Refs. 2 and 3.

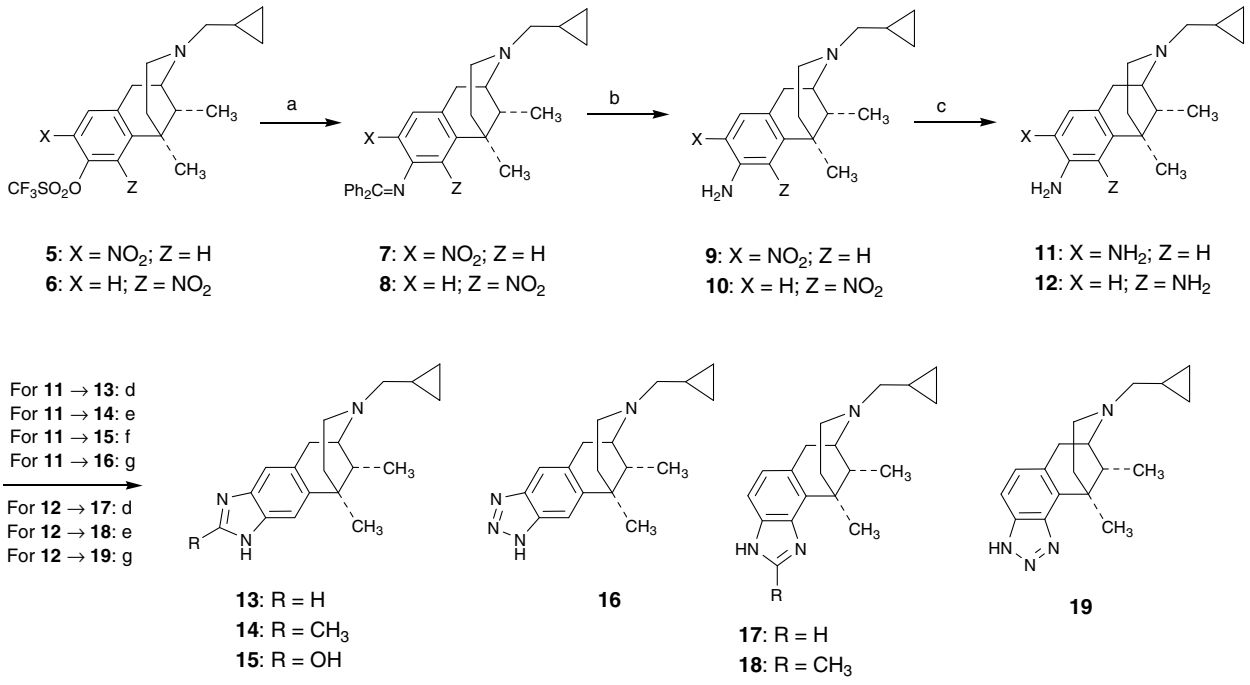
[f] Proton NMR, IR, and MS were consistent with the assigned structures of all new compounds. C, H, and N elemental analyses were obtained for all new target compounds and most intermediates and were within ±0.4% of theoretical values.

made the 8,9- and 7,8-fused imidazole (**3**/**4**: X = CH) and triazole (**3**/**4**: X = N) derivatives, respectively, as well as the 2′-methyl imidazole analogues (**3**/**4**: X = CCH_3). To the best of our knowledge, there are no reports in the literature of such imidazole and triazole ring fusions to opioid core structures. Reports of 8,9-fused (cyclazocine numbering) thiazole[5] and oxazole[6] derivatives have appeared.

Target compounds **13**–**19** were prepared as shown in Scheme 1. Amination[7,8] of the known pair[9] of regioisomeric nitro triflate derivatives **5** and **6** with benzophenone imine provided **7** and **8** in yields of 88% and 56%, respectively. Hydrolysis of **7** and **8** proceeded smoothly in 3 N HCl to provide amine derivatives **9** and **10**, respectively, in yields of 88% and 98%, respectively. Reduction of **9** and **10** using standard conditions provided the somewhat unstable diamine derivatives **11** and **12**, respectively, in quantitative yield. An alternative process for making diamine **11** involved reaction of triflate ester **5** with benzylamine in acetonitrile at reflux for 18 h to give the corresponding 8-benzylamino-9-nitro derivative in 77% yield which was subsequently reduced (10% Pd/C, HCO_2NH_4, CH_3OH, 65 °C, 20 h) to give diamine **11** in 67% yield.

Compound **11** was treated with formic acid, acetic acid, or phosgene to give 8,9-fused imidazole compounds **13**, **14**, and **15**, respectively, in yields of 69%, 62%, and 78%, respectively. In similar fashion, reaction of diamine intermediate **12** with formic acid or acetic acid provided 7,8-fused imidazole derivatives **17** and **18**, respectively, in yields of 80% and 97%, respectively. In $CDCl_3$ solution, proton NMR data show that imidazoles **14**, **17**, and **18** exist as a mixture of NH tautomers (i.e., **3**/**3a** and **4**/**4a**); compound **13** was isolated and characterized as a dihydrochloride salt and thus cannot exhibit this type of tautomerism. Diamines **11** and **12** were also treated with $NaNO_2$ in acetic acid to provide triazole compounds **16** and **19**, respectively, in yields of 68% and 62%, respectively. In $CDCl_3$ solution, both triazole analogues show the NH as a broad singlet suggesting that their respective tautomers rapidly equilibrate on the NMR timescale.

Target compounds were screened for their affinity and selectivity for μ, δ, and κ opioid receptors stably expressed in Chinese hamster ovary (CHO) cell membranes (Table 1).[10] In addition, several high affinity compounds were evaluated for functional activity in [^{35}S]GTPγS assays (Table 2).[11] Details of these assays are found in footnotes to the appropriate tables. Data for cyclazocine (**1**) and lead compound **2** are also included. All compounds in the Tables are racemic. Against the δ receptor, binding affinity for all new compounds **13**-**19** in Table 1 is low (K_i = 5.1–730 nM) relative to their corresponding affinities for μ (K_i = 0.31–60 nM) and κ (K_i = 0.050–12 nM) opioid receptors. Therefore, we focused our analysis of data against the μ and κ receptors. Imidazole target compounds **13** and **14** have very high binding affinity for the μ opioid receptor (K_i = 0.31 and 0.77 nM, respectively) and exceptionally high affinity for κ (K_i = 0.063 and 0.050 nM, respec-



Scheme 1. Reagents and conditions: (a) $Pd(OAc)_2$, BINAP, Cs_2CO_3, $HN{=}C(Ph)_2$, tol, 150 °C, 15 min, microwaves; (b) 3 N HCl, THF, 25 °C, 30 min; (c) 10% Pd/C, MeOH, 42 psi H_2, 25 °C, 8 h; (d) HCO_2H, 101 °C, 20 h; (e) CH_3CO_2H, 130 °C, 30 min, microwaves; (f) $COCl_2$ in tol, THF, 25 °, 16 h; (g) $NaNO_2$, CH_3CO_2H, 25 °C, 1 h.

Table 2
EC_{50} and E_{max} values for the stimulation of [^{35}S]GTPγS binding and IC_{50} and I_{max} values for the inhibition of agonist-stimulated [^{35}S]GTPγS binding to the human μ and κ opioid receptors[a]

Compound	Functional description	EC_{50} (nM)	E_{max} (% maximal stimulation)	IC_{50} (nM)	I_{max} (% maximal inhibition)
Mu opioid receptor					
DAMGO	Agonist	55 ± 7	116 ± 4	NI[b]	NI
1	Agonist/antagonist	4.0 ± 1.3	24 ± 2.7	13 ± 2.2	67 ± 3.1
13	Weak agonist/antagonist	9.8 ± 3.3	22 ± 2.5	170 ± 54	89 ± 1.9
14	Agonist/weak antagonist	18 ± 1.6	55 ± 2.8	11000 ± 1000	100 ± 0.20
16	Antagonist	NA[c]	<20%	NA	73 ± 3.6[d]
19	Antagonist	NA	0.58 ± 1.6	99 ± 5.1	98 ± 1.8
Kappa opioid receptor					
U50,488	Agonist	36 ± 5.0	77 ± 11	NI	NI
1	Agonist	1.3 ± 0.20	57 ± 3.8	NI	NI
13	Agonist	4.7 ± 0.93	69 ± 4.3	NI	NI
14	Agonist	5.8 ± 0.42	73 ± 2.5	NI	NI
16	Agonist	77 ± 2.3	110 ± 8.8	NI	NI
19	Agonist	8.6 ± 1.6	65 ± 2.7	NI	NI

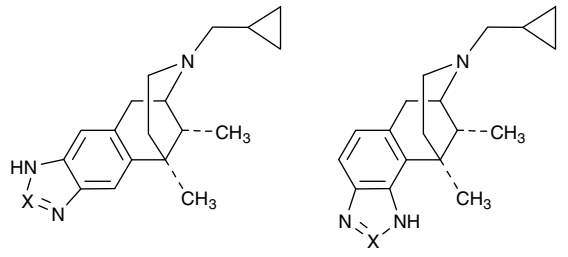
[a] See Ref. 11 for experimental details. Data are the mean values ± SEM from at least three separate experiments, performed in triplicate. For calculation of the E_{max} values, the basal [^{35}S]GTPγS binding was set at 0%. For inhibition studies, 200 nM DAMGO was used as the agonist for the μ receptor and U50,488 at final concentration of 100 nM was used for the κ receptor.
[b] NI, no inhibition.
[c] NA, not applicable.
[d] This compound produced this inhibition at 10 μM, but did not reach a plateau in inhibiting DAMGO-stimulated [^{35}S]GTPγS binding. An IC_{50} value could not be determined because the inhibition of DAMGO-stimulated [^{35}S]GTPγS binding did not reach a plateau at concentrations up to 10 μM.

tively). When the 2′-substuent on the imidazole ring is OH (**15**), binding affinity is very low [K_i = 60 nM (μ) and 12 nM (κ)]. 8,9-Fused triazole **16** displays K_i values of 10 nM (μ) and 0.81 nM (κ). We also prepared target compounds to probe the effect of fusing imidazole or triazole rings to the 7,8-positions of cyclazocine. The 2′-H and 2′-methyl imidazole analogues **17** and **18**, respectively, display moderate affinity for μ (K_i = 9.5 and 2.5 nM, respectively) and high affinity for the κ receptor (K_i = 0.92 and 0.51 nM, respectively). The 7,8-fused triazole analogue **19** displays very good affinity for both μ and κ receptors with K_i values of 1.4 and 0.23 nM, respectively.

From a SAR perspective, it is apparent that replacing the pyrrolo CH closest to the benzenoid ring of lead **2** with the classical[12] bioisosteric replacement N (to give **13**) results in a significant increase in binding affinity for μ and κ of ca. 60- and 110-fold, respectively. Replacing the 2′-H of **13** with methyl (**14**) has little effect on μ and κ affinity, however, compound **15** with a 2′-OH group has ca. 200-fold lower affinity for the receptors than **13**/**14**. Compared to lead compound **2**, triazole **16**, where both pyrrolo CH's are replaced by N, binding affinity is approximately the same for μ, however, affinity for the κ receptor is increased nearly 7-fold. When the lone pyrrolo CH of 2′-H imidazole **13** is replaced by N, however, binding affinity of the same resulting triazole **16** is significantly decreased for μ (32-fold) and κ (13-fold). Transposing the 2′-H imidazole ring from the 8,9- to 7,8-positions (compare **13** to **17**) results in

significantly decreased binding affinity for both μ (30-fold) and κ (15-fold). However, when the imidazole ring bears a 2′-methyl group (compare **14** to **18**), binding affinity decreases only 3-fold for μ and 10-fold for κ. Upon rearrangement of the triazole ring in similar fashion (compare **16** to **19**), a divergent SAR is observed, namely, the 7,8-isomer **16** has significantly higher affinity for μ (7-fold) and κ (4-fold) than the 8,9-fused isomer **19**.

Unlike lead compound **2**, the imidazole and triazole compounds **13**–**19** of this study can exist in two different tautomeric states (e.g., **3**/**3a** and **4**/**4a**). Since historical SAR data indicates that an H-bond donor group (e.g., OH or NHR) at the 8-position is an important part of the pharmacophore,[2,4] we assume that the bioactive form (i.e., **3** and **4**) of each potent target compound in this study is that where the putative NH donor resides at the same site (position-8) as the OH of cyclazocine. In the unbound state, however, proton NMR data indicate that several target compounds exist as tautomeric mixtures in $CDCl_3$ solution. Similarly, interpretation of the binding data (i.e., poor affinity) for **15** is made difficult since the 2′-OH imidazole motif (as drawn) may well exist as the cyclic urea tautomer at the active site of the protein.



High affinity opioid ligands **13** and **14** (both 8,9-fused imidazoles) as well as both isomeric triazole derivatives **16** and **19** were evaluated for functional activity in [^{35}S]GTPγS assays (Table 2). Rather than using the relatively low affinity lead compound **2** as a comparator, cyclazocine (**1**) was used. At the μ receptor, imidazole compounds **13** and **14** display mixed agonist/antagonist properties although they have somewhat different potencies. Triazoles **16** and **19** are both very low affinity antagonists at the μ receptor. Cyclazocine in these assays is a high potency mixed agonist/antagonist. Against the κ receptor, all four target compounds as well as cyclazocine are agonists and potency correlates reasonably well with binding affinity.

In summary, using the known 8,9-fused pyrrolo cyclazocine analogue **2** as a lead structure, design of initial structures for this study was based on a classical bioisosterism strategy whereby we replaced the pyrrolo CH group(s) of **2** with N, a group of similar size. Such a CH ↔ N strategy might be expected to produce broadly similar biological properties, however, unexpected results were observed for novel compounds **13** and **16**. Compared to **2**, imidazole **13** displayed very high affinity for μ and exceptionally high affinity for the κ receptor, while triazole **16** had impressive affinity for κ. From these data it is reasonable to conclude that the role of the added aza substitution(s) in the heterocyclic ring of **2** is not well-understood and goes beyond the classical bioisosterism concept. In addition, a divergent SAR was noted upon transposing the fused imidazole and triazole rings from the 8,9- to the 7,8-positions. Studies to determine if/how tautomerization, electronics, H-bond donor/acceptor or other physicochemical properties affect binding affinity are ongoing in our laboratories and will be the subject of future communications.

Acknowledgments

We gratefully acknowledge the contributions of Rensselaer's mass spectroscopist Dr. Dmitri Zagorevski and the technical assistance provided by Brian I. Knapp of the University of Rochester. Funding of this research was from NIDA (DA12180 and KO5-DA00360) and the NSF (Agilent 1100 series LC/MSD system).

References and notes

1. Archer, S.; Glick, S. D.; Bidlack, J. M. *Neurochem. Res.* **1996**, *21*, 1369.
2. Wentland, M. P.; Ye, Y.; Cioffi, C. L.; Lou, R.; Zhou, Q.; Xu, G.; Duan, W.; Dehnhardt, C. M.; Sun, X.; Cohen, D. J.; Bidlack, J. M. *J. Med. Chem.* **2003**, *46*, 838.
3. Allen, R. C.; Wettlaufer, D. G. U.S. Patent 4929622, 1990.
4. Aldrich, J. V.. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M. E., Ed.; John Wiley & Sons: New York, 1996; Vol. 3, pp 321–441.
5. Zhang, A.; Xiong, W.; Hilbert, J. E.; DeVita, E. K.; Bidlack, J. M.; Neumeyer, J. L. *J. Med. Chem.* **2004**, *47*, 1886.
6. Peng, X.; Knapp, Brian I.; Bidlack, J. M.; Neumeyer, J. L. *Bioorg. Med. Chem.* **2007**, *17*, 4106.
7. Wolfe, J. P.; Buchwald, S. L. *J. Org. Chem.* **1997**, *62*, 1264.
8. Louie, J.; Driver, M. S.; Hamann, B. C.; Hartwig, J. F. *J. Org. Chem.* **1997**, *62*, 1268.
9. Wentland, M. P.; Sun, X.; Cohen, D. J.; Bidlack, J. M. *Bioorg. Med. Chem.* **2008**, *16*, 5653.
10. Neumeyer, J. L.; Zhang, A.; Xiong, W.; Gu, X.; Hilbert, J. E.; Knapp, B. I.; Negus, S. S.; Mello, N. K.; Bidlack, J. M. *J. Med. Chem.* **2003**, *46*, 5162.
11. Wentland, M. P.; VanAlstine, M.; Kucejko, R.; Lou, R.; Cohen, D. J.; Parkhill, A. L.; Bidlack, J. M. *J. Med. Chem.* **2006**, *49*, 5635.
12. Thronber, C. W. *Chem. Soc. Rev.* **1979**, *8*, 563.
13. Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.