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Synthesis and Structure–Activity Relationships of 1-Arylmethyl-3-(1-methyl-2-amino)ethyl-5-aryl-6-methyluracils as Antagonists of the Human GnRH Receptor

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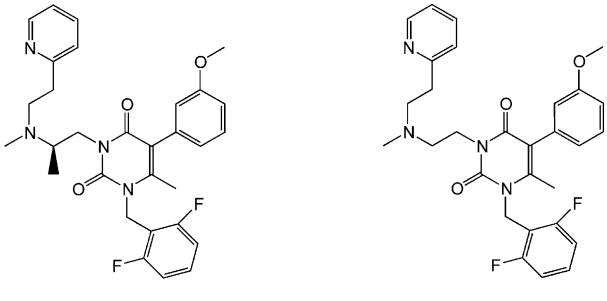
Abstract—A new class of small molecule GnRH antagonists, the 1-arylmethyl-3-(1-methyl-2-amino)ethyl-5-aryl-6-methyluracils, was designed and a novel stereoselective synthesis for these compounds was developed. The stereochemical integrities of key intermediates (***S***)-**6** and (***R***)-**6** were confirmed by a combination of X-ray crystallography and chiral HPLC determinations. SAR studies were performed, which allowed the identification of derivatives (***R***)-**9f**, (***R***)-**9h** and (***R***)-**12** as potent *h*GnRH antagonists ($K_i = 20$ nM).

In the previous letter,[1] we described the initial SAR of the 1-arylmethyl-5-aryl-6-methyluracils containing a substituted 2-aminopropyl side-chain at the *N*-3 position as gonadotropin-releasing hormone (GnRH) antagonists. In that study, compound **1** was identified as a potent antagonist of the human GnRH receptor (*h*GnRH) ($K_i = 5$ nM), and it represented an improvement over our previous lead compound **2**[2] (Fig. 1). Compound **1** differs from **2** by a methyl group of (*R*)-configuration at the β-carbon of the *N*-3 side-chain, which we speculate may contribute to rotational restriction to the molecule as well as orienting the basic amine towards the respective acidic residue in the receptor. Based on this premise, we decided to investigate the effects of the introduction of a methyl group at the α-position of the *N*-3 side-chain, and the results are presented herein.

5-Bromo-1-(2,6-difluorobenzyl)- 6-methyluracil (**3**) was utilized as our starting point in this study, and was prepared according to the procedure developed by our group.[1] (*R*)- and (*S*)-1-amino-2-propanol [(***R***)-**4**, (***S***)-**4**] were separately protected as their *tert*-butyl carbamate derivates and used in the Mitsunobu alkylation of uracil **3** (Scheme 1). The Mitsunobu reactions on the secondary alcohols (***R***)-**5** and (***S***)-**5** occurred with complete inversion of configuration, leading to the corresponding alkylated uracils (***S***)-**6** and (***R***)-**6**, with (*S*)- and (*R*)-configurations, respectively. The stereochemical integrities of (***S***)-**6** and (***R***)-**6** were confirmed by chiral HPLC[3] analysis, and the absolute (*R*)- stereochemistry of (***R***)-**6** was determined by X-ray crystallography (Fig. 2), which was possible due to the anomalous contributions of the heavy atom (bromine).[4] The 5-(3-methoxy)-phenyl group was introduced through Suzuki couplings



1 - *h*GnRH K_i = 5 nM

2 - *h*GnRH K_i = 34 nM

Figure 1. First-generation uracil-based GnRH antagonists.

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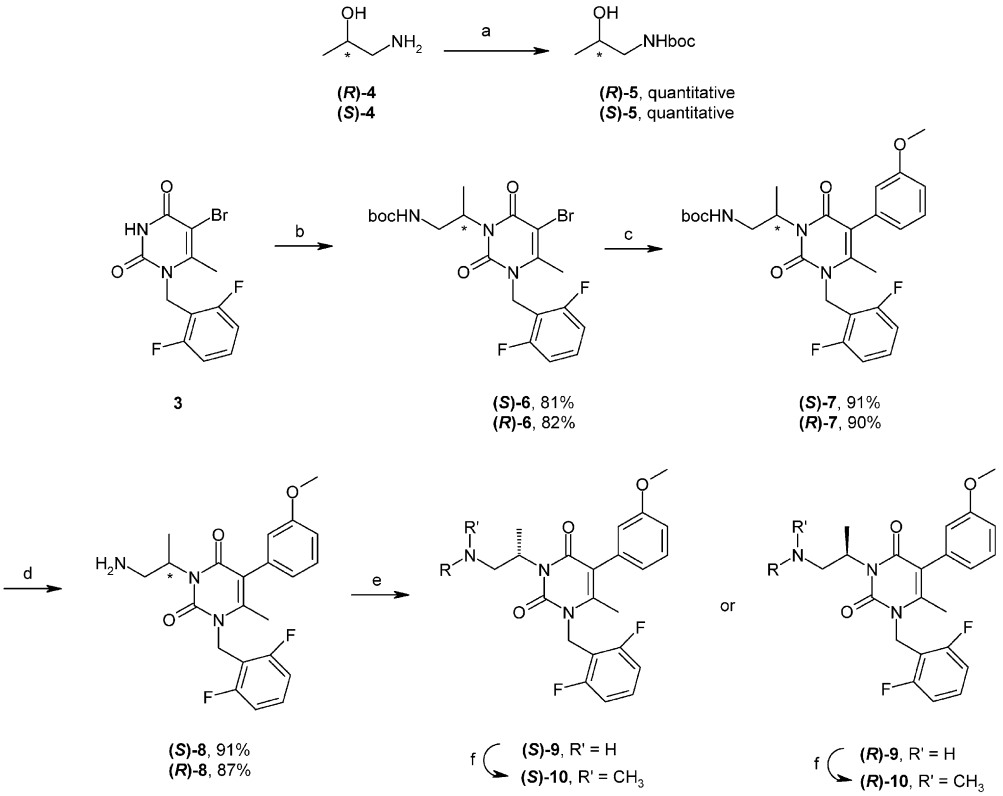


Scheme 1. (a) $(Boc)_2O$, Et_3N, CH_2Cl_2, 0 °C to rt, 2 h; (b) (***R***)-**5** or (***S***)-**5**, DEAD, Ph_3P, THF, rt, 17 h; (c) 3-methoxyphenyl boronic acid, $Pd[Ph_3P]_4$, K_2CO_3, toluene/EtOH/H_2O, 110 °C, 18 h; (d) TFA/CH_2Cl_2 1:1 v/v, rt, 1 h; (e) carbonyl compound, MeOH, $NaBH_4$, rt, 1 h; (f) CH_2O aq, MeOH, $NaBH_4$, rt, 15 min.

of the appropriate boronic acid with bromides (***S***)-**6** and (***R***)-**6**. The products (***S***)-**7** and (***R***)-**7** were treated with trifluoroacetic acid in CH_2Cl_2 to furnish the corresponding amines (***S***)-**8** and (***R***)-**8** in excellent yields. Each compound was then reacted with a variety of aldehydes and ketones, under reductive conditions, to give the corresponding secondary amines (***S***)-**9** and (***R***)-**9**. Some of the secondary amines were further alkylated with formaldehyde and $NaBH_4$ in MeOH to give the tertiary amines (***S***)-**10** and (***R***)-**10**.

The derivatives containing the 2-pyridylethyl side-chain on the basic nitrogen were synthesized according to Scheme 2. Both amines (***S***)-**8** and (***R***)-**8** were reacted with 2-vinylpyridine in EtOH at reflux to give the secondary amines (***S***)-**11** and (***R***)-**11**. These compounds were then methylated with aqueous formaldehyde and $NaBH_4$ in MeOH to provide (***S***)-**12** and (***R***)-**12**.

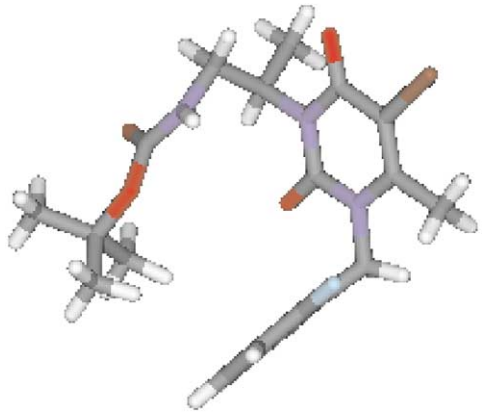
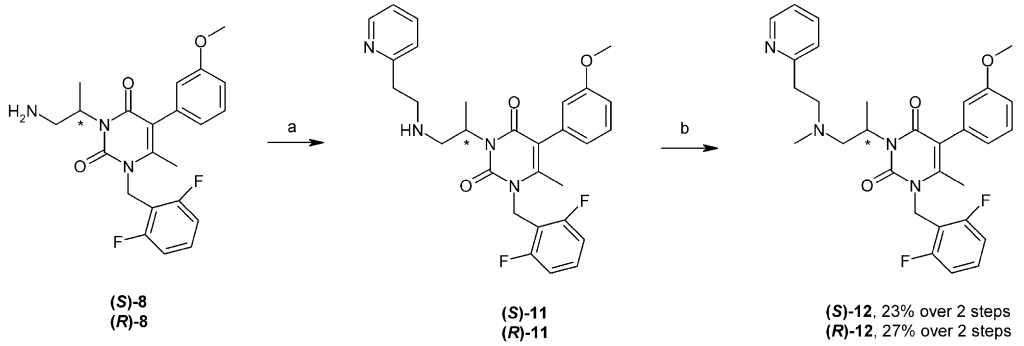


Figure 2. X-ray structure of (***R***)-**6**.

All compounds were then evaluated for their competitive binding to the cloned human GnRH receptor expressed in HEK293 cells, using a 96-well filtration setup[5,6] and des-Gly^{10}[^{125}I-Tyr^5, DLeu^6, NMeLeu^7, Pro^9-NEt]GnRH as the radiolabel. The results of the binding assay are presented in Tables 1 and 2. The functional antagonism of the compounds herein synthesized was confirmed by their ability to inhibit GnRH stimulated Ca^{2+} flux in the transfected cells, in a dose-dependent manner.[7] For instance, compounds (***R***)-**9f** and (***R***)-**9h** were found to be functional antagonists with IC_{50}'s = 12 and 26 nM, respectively. Analogously to our previous findings,[1,8a] the compounds described in this letter were specific for the human variant of the receptor. Compared to the *h*GnRH receptor, compound (***R***)-**9f** is 8-fold less potent (K_i = 160 nM) in the monkey GnRH receptor and 600-fold less potent (K_i = 12,700 nM) in the rat receptor.

Previous knowledge from past SAR studies of our bicyclic and monocyclic series[1,2,8] indicated that the best binding affinity towards the *h*-GnRH receptor was achieved when the basic nitrogen was alkylated with both methyl and 2-pyridylethyl side-chains. We postulated that the 'bidentated' basic nature of this motif, in



Scheme 2. (a) 2-Vinylpyridine, Et_3N, EtOH, reflux, 3 days; (b) CH_2O aq, MeOH, $NaBH_4$, rt, 15 min.

Table 1. Binding affinities of 1-(2,6-difluorobenzyl)-3-(1-methyl-2-amino)ethyl-5-(3-methoxyphenyl)-6-methyluracils [(***S***)- or (***R***)-**9a**–**d**, (***S***)- or (***R***)-**10a**–**b**, (***S***)-**12** and (***R***)-**12**] towards the *h*GnRH receptor

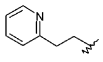
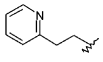
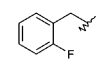
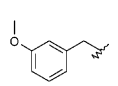
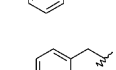
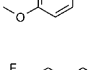
Compd	R	R′	K_i (nM)
(***S***)-**12**		CH^3	1800
(***R***)-**12**		CH_3	20
(***S***)-**9a**		H	370
(***R***)-**9a**		H	75
(***S***)-**10a**		CH_3	2000
(***R***)-**10a**		CH_3	150
(***S***)-**9b**		H	1600
(***R***)-**9b**		H	780
(***S***)-**10b**		CH_3	7000
(***R***)-**10b**		CH_3	590
(***S***)-**9c**		H	950
(***R***)-**9c**		H	430
(***S***)-**9d**		H	1200
(***R***)-**9d**		H	190

Table 2. Binding affinities of 1-(2,6-difluorobenzyl)-3-(1-methyl-2-amino)ethyl-5-(3-methoxyphenyl)-6-methyluracils [(***S***)- or (***R***)-**9e**–**j**] towards the *h*GnRH receptor

Compd	R	R′	K_i (nM)
(***S***)-**9e**		H	400
(***R***)-**9e**		H	70
(***S***)-**9f**		H	320
(***R***)-**9f**		H	20
(***S***)-**9g**		H	550
(***R***)-**9g**		H	90
(***R***)-**9 h**		H	20
(***R***)-**9i**		H	720
(***R***)-**9j**		H	85
(***R***)-**9k**		H	250

addition to the π interaction provided by the pyridine ring, could account for the enhanced affinity towards the receptor.[8f] Compound (***R***)-**12**, which represents a direct comparison to both **1** and **2** bound with $K_i = 20$ nM, whereas its (*S*)-counterpart (***S***)-**12** was 90 times less potent ($K_i = 1800$ nM). Therefore, the introduction of a methyl group of (*R*)-configuration on the α-carbon of the *N*-3 side-chain contributes to enhance the binding affinity, albeit only marginally, over the unsubstituted **2**. However, the gain in energy is not as large as that realized by the introduction of a methyl group on the β-carbon, as in **1**.

The (*R*)-enantiomeric preference for the receptor was further confirmed by alkylation of amines (***S***)-**8** and (***R***)-**8** with various carbonyl compounds. For instance, the *N*-benzylated compound (***S***)-**9a** ($K_i = 370$ nM) was

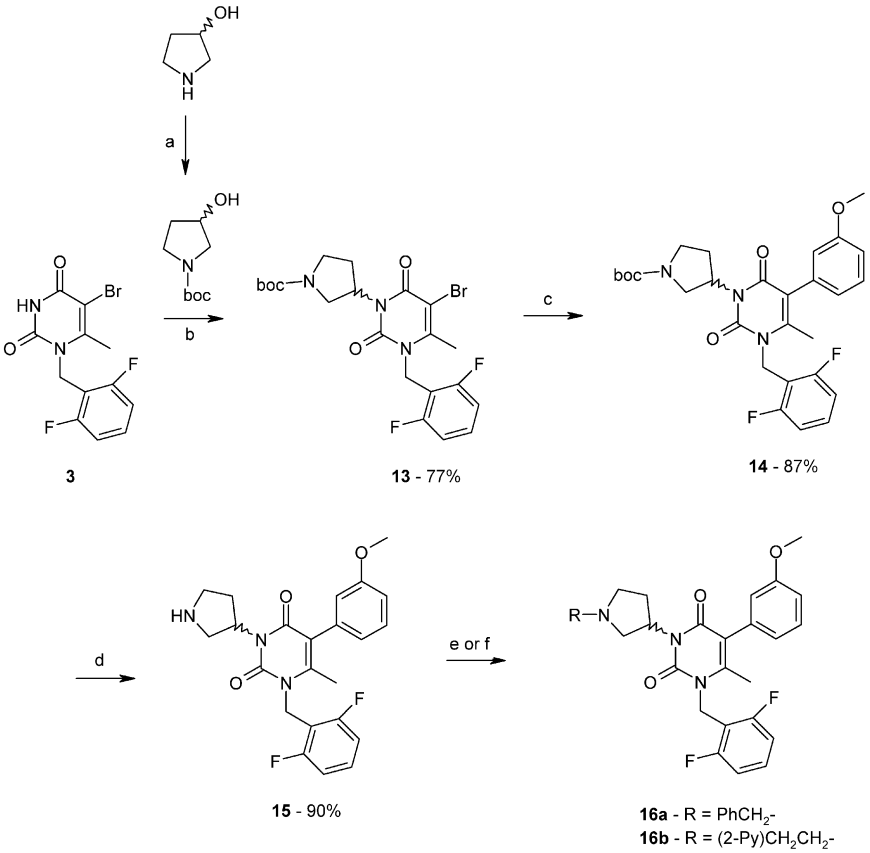
about 5 times less potent than its counterpart (***R***)-**9a** ($K_i = 75$ nM). Interestingly, when both compounds were methylated, the corresponding tertiary amines (***S***)-**10a** and (***R***)-**10a** displayed much lower affinity for the receptor [(***S***)-**10a** $K_i = 2000$ nM and (***R***)-**10a** $K_i = 150$ nM]. This observation contrasts with the 2-(pyridylethyl) side-chain compound series, where the *N*-methylated tertiary amines are the more active species.[1,2] According to the data presented in Table 1, it is clear that benzylic side-chains were preferred over aliphatic ones. For example, when compared to (***R***)-**9a**, compound (***R***)-**9b**, which is the cyclopropylmethyl derivative, is about 10-fold less potent. In addition, the isolipophilic isobutyl derivative (***R***)-**9d** and the cyclopentyl analogue (***R***)-**9c** are 3- and 6-fold less active, respectively. Further *N*-methylation of these aliphatic secondary amines did not translate into a significant improvement in potency. For example, compound (***R***)-**9b** had similar binding to (***R***)-**10b**.

The substitution pattern of the benzylic side-chain was investigated next (Table 2). Introduction of an *ortho*-methyl group [(***R***)-**9e**] caused little change in affinity when compared to (***R***)-**9a**. When an *ortho*- or *meta*-methoxy groups were introduced, however, the corresponding compounds (***R***)-**9f** and (***R***)-**9h** were about 4-fold more potent than (***R***)-**9a**, and matched the potency of the pyridyl derivative (***R***)-**12** ($K_i = 20$ nM). Substitution at the *para*-position [(***R***)-**9i**], on the other hand, led to a dramatic decrease in affinity. The same trend was observed for the fluoro-substituted series. Both the *ortho*-[(***R***)-**9g**] and the *meta*-[(***R***)-**9j**] fluorinated analogues are very similar in potency (K_i's = 90 and 85 nM, respectively), whereas the *para*-compound (***R***)-**9k** has a K_i value of 250 nM.

In addition to the compounds described in Tables 1 and 2, we were interested in further restraining the rotational freedom of the *N*-3 side-chain. Thus, by formally connecting the methyl groups at both the basic nitrogen and the α- position of compound **(*R*)-12**, we arrived at 3-pyrrolidinyl substituted derivatives (Scheme 3).

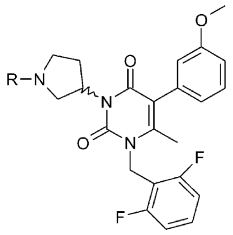
The synthesis of these compounds started with *N*-boc protection of the racemic 3-pyrrolidinol in quantitative yield. The resulting protected alcohol was coupled to 5-bromouracil **3** under Mitsunobu conditions. The product **13** was obtained in 77% yield and was subsequently subjected to Suzuki coupling with 3-methoxyphenyl boronic acid, in the presence of catalytic amounts of $Pd[Ph_3P]_4$, to give **14** in excellent yield. Deprotection, followed by alkylation with either benzaldehyde or 2-vinylpyridine gave **16a** and **16b**, respectively.

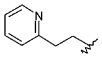
Disappointingly, neither compound showed improved binding over their acyclic counterparts (Table 3). In fact, the binding affinity of **16b** was dramatically inferior to the one of (***R***)-**12** ($K_i = 8{,}300$ nM vs 20 nM,



Scheme 3. (a) $(Boc)_2O$, Et_3N, CH_2Cl_2, 0 °C to rt, 4 h; (b) DEAD, Ph_3P, THF, rt, 17 h; (c) 3-methoxyphenyl boronic acid, $Pd[Ph_3P]_4$, K_2CO_3, toluene/EtOH/H_2O, 110 °C, 15 h; (d) TFA/CH_2Cl_2 1:1 v/v, rt, 1 h; (e) benzaldehyde, MeOH, $NaBH_4$, rt, 1 h; (f) 2-vinylpyridine, EtOH, Et_3N, reflux, 48 h.

Table 3. Binding affinities of 1-(2,6-difluorobenzyl)-3-(3-pyrrolidinyl)-5-(3-methoxyphenyl)-6-methyluracils **15**, **16a** and **16b** towards the *h*GnRH receptor



Compd	R	K_i (nM)
15	H	15,000
16a		280
16b		8300

respectively), which may reflect the extent to which the contacts between the pyridine and the receptor residues were disrupted. On the other hand, the difference between **16a** and (***R***)-**10a** is much smaller ($K_i = 280$ nM vs 150 nM, respectively), especially if one considers that **16a** is a racemate. The main difference between these two pairs of compounds is the group attached to the basic nitrogen (pyridylethyl vs benzyl), and this clearly points to two distinct binding modes. The constraint imposed by the pyrrolidine ring in **16b**, may disrupt the interaction between the pyridylethyl-substituent and, possibly, a polar residue in the receptor, whereas in the case of **16a**, the predominantly aromatic/lipophilic interaction is maintained. Based on the results obtained thus far, we speculate that the benzyl group most probably interacts with Phe 309 on TM7 of the *h*GnRH receptor.

In summary, we have developed a novel synthesis of a new class of small-molecule antagonists to the human GnRH receptor, the 1-arylmethyl-3-(1-methyl-2-amino)ethyl-5-aryl-6-methyluracils. We have also demonstrated that the introduction of a methyl group of (*R*)-configuration at the α-carbon of the *N*-3 side-chain gave a modest improvement in activity when compared to the unsubstituted ethylene analogues.[2] The SAR of this class of compounds was explored and three potent antagonists were identified, (***R***)-**9f**, (***R***)-**9h** and (***R***)-**12** ($K_i = 20$ nM). In addition, we have demonstrated for the first time in the uracil series[1,2] that compounds lacking the 2-pyridylethyl motif on the basic nitrogen can still display excellent potency against the *h*GnRH receptor [e.g., (***R***)-**9f**, (***R***)-**9h**], as long as the α-methyl group of (*R*)-configuration is present.

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3. The enantiomeric excesses (% e.e.) of compounds (***S***)-**6** and (***R***)-**6** were determined by HPLC, using a long Pirkle Covalent (R,R) WHELK column (25 cm×4.6 mm), manufactured by REGIS. The samples were eluted with an isocratic 80:20 (v/v) mixture of hexanes and ethanol, respectively. For (***S***)-**6**, rt = 11.10 min, e.e. = > 99%. For (***R***)-**6**, rt = 9.93 min, e.e. = > 99%.
4. (***R***)-**6** was crystallized from slow diffusion of diethyl ether into a dichloromethane solution. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 204509. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).
5. Perrin, M. H.; Haas, Y.; Rivier, J. E.; Vale, W. W. *Mol. Pharmacol*. **1983**, *23*, 44.
6. On each assay plate, a standard antagonist of comparable affinity to those being tested was included as a control for plate-to-plate variability. Overall, K_i values were highly reproducible with an average standard deviation of 45% for replicate K_i determinations. Key compounds were assayed in 3–8 independent experiments.
7. Inhibition of GnRH stimulatd Ca^{2+} flux: functional activity of compounds for the *h*GnRH receptor was determined by inhibition of GnRH stimulated Ca^{2+} flux. RBL cells stably expressing the full-length human GnRH receptor were seeded into 96-well, black wall clear bottom plates (Corning) at a density of 50,000 cells/well and the cells allowed to attach overnight. Cells were then loaded with the Ca^{2+} sensitive dye, Fluo-4 (Molecular Probes), by incubation in loading medium [(DMEM with 20 mM Hepes, 10% FBS, 2 μM Fluo-4, 0.02% pluronic acid (Molecular Probes) and 2.5 mM probenecid (Sigma)] for 1 h at 37 °C. Cells were then washed three times with assay buffer (Hanks balanced salt, 20 mM Hepes, 2.5 mM probenecid). Compounds at varying concentrations in assay buffer were pre-incubated with cells for 1 min prior to stimulation with GnRH (5 nM). Measurement of fluorescence due to GnRH stimulated Ca^{2+} flux was performed according to manufacturer's instructions on a FLIPR system (Molecular Devices, FLIPR^{384} system). IC_{50} values for the inhibition of GnRH-stimulated Ca^{2+} flux were calculated using the Prism software package (GraphPad Software) with a 'sigmoidal dose–response (variable slope)' option for curve fitting.
8. (a) Zhu, Y.-F.; Struthers, R. S.; Gao, Y.-H.; Connors, P. J.; Gross, T. D.; Saunders, J.; Wilcoxen, K.; Reinhart, G. J.; Ling, N.; Chen, C. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 399. (b) Zhu, Y.-F.; Wilcoxen, K.; Saunders, J.; Guo, Z.; Gao, Y.-H.; Connors, P. J.; Gross, T. D.; Tucci, F. C.; Struthers, R. S.; Reinhart, G. J.; Chen, C. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 403. (c) Wilcoxen, K.; Zhu, Y.-F.; Connors, P. J., Jr.; Saunders, J.; Gross, T. D.; Gao, Y.; Reinhart, G. J.; Struthers,

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