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# Design, synthesis and evaluation of constrained tetrahydroimidazopyrimidine derivatives as antagonists of corticotropin-releasing factor type 1 receptor ($CRF_1R$)

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ABSTRACT

Several tetrahydroimidazopyrimidines were prepared using silver assisted cyclization as the key step. The binding affinities of compounds thus prepared were evaluated in vitro toward $hCRF_1R$. Initial lead compound **16** ($K_i$ = 32 nM) demonstrated modest putative anxiolytic effects in the mouse canopy test. Further optimization using parallel synthesis provided compounds with $K_i$'s <50 nM.



The hypothalamus, which is located at the base of the brain, functions as a control center for the neuroendocrine system.[1] In response to stress the hypothalamus increases the production of a 41 amino acid neurohormone, Corticotropin Releasing Factor (CRF).[2] CRF regulates the hypothalamic-pituitary-adrenal axis (HPA axis) and is involved in coordinating the endocrine as well as autonomic and behavioral responses of the organism to stress.[3] CRF exerts its effects by binding to and activating class 2 G protein-coupled receptors. There are two subtypes of CRF receptors, namely, $CRF_1$ and $CRF_2$, with three splice variants $CRF_2\alpha$, $CRF_2\beta$ and $CRF_2\gamma$ for the $CRF_2$ receptors. These receptors exhibit differential expression and pharmacology.[4] The potential for $CRF_1R$ as a target for the development of therapeutics for anxiety and depression has been reviewed extensively in literature[5a–f] and stems from several observations. For example, intracavernous administration of CRF resulted in stimulation of ACTH, release of corticosterone, and an increase in anxiety-like behavior in rats and primates.[6] Furthermore, analysis of cerebrospinal fluid (CSF) of untreated patients suffering from depression indicated elevated levels of CRF. Normalization of CRF levels was achieved following the treatment with antidepressants.[7a,b] Finally, mice over-expressing CRF display anxious and depressive-like behavior[8] while $CRF_1R$ gene knock out mice demonstrate reduced anxiety.[9]

Several research groups have successfully demonstrated the efficacy of $CRF_1R$ antagonists in in vivo models of anxiety and depression.[5a–f] Examination of a number of such non-peptide chemotypes (Fig. 1) led to pharmacophoric models and identification of certain structural requirements for antagonistic activity as depicted in Figure 1.[5c,10] Some key features of these mono or bicyclic systems containing an $sp^2$ nitrogen include: (a) an orthogonal ring system capable of interacting with a lipophilic pocket of the receptor; (b) a lipophilic side-chain at the top; and (c) a short alkyl group on the core as illustrated in Figure 1.

We sought to design and synthesize novel chemotypes incorporating such recognition elements and examine their activity as antagonists of $CRF_1R$. In this communication we describe the synthesis and evaluation of constrained tetrahydroimidazopyrimidines and dihydroimidazopyrimidinones as $CRF_1R$ antagonists. Within the scope of these two chemotypes, the synthesis and evaluation of three classes of compounds viz., 'bis-amides', C3-amides and C3-amines as antagonists of $CRF_1R$ is described.

The three general targets for synthesis are shown in Scheme 1. These are C-3 tertiary amides containing Me, Et and $CF_3$ substituents at the 2-position on the imidazole ring, C-3 tertiary amines containing the electron-withdrawing CF3 substituent at C-2 of the imidazole ring and finally bis-amides bearing a C-3 amide side-chain and a 7-oxo group in the core. The amine targets in Scheme 1 were expected to be susceptible to elimination of the amine side-chain and therefore were prepared with an electron-with-

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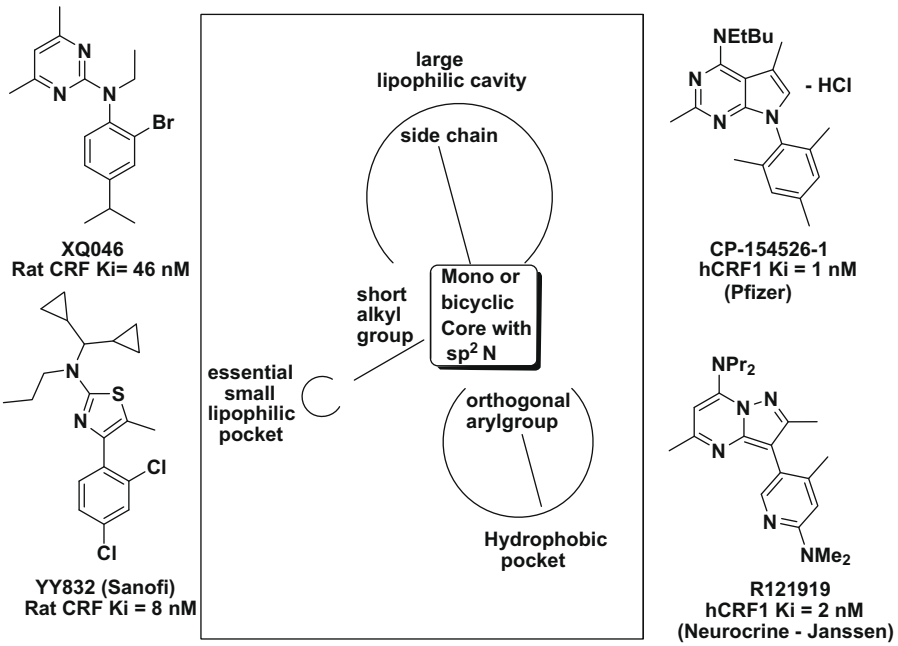
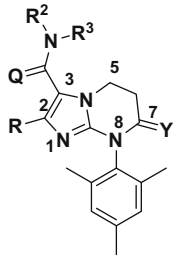
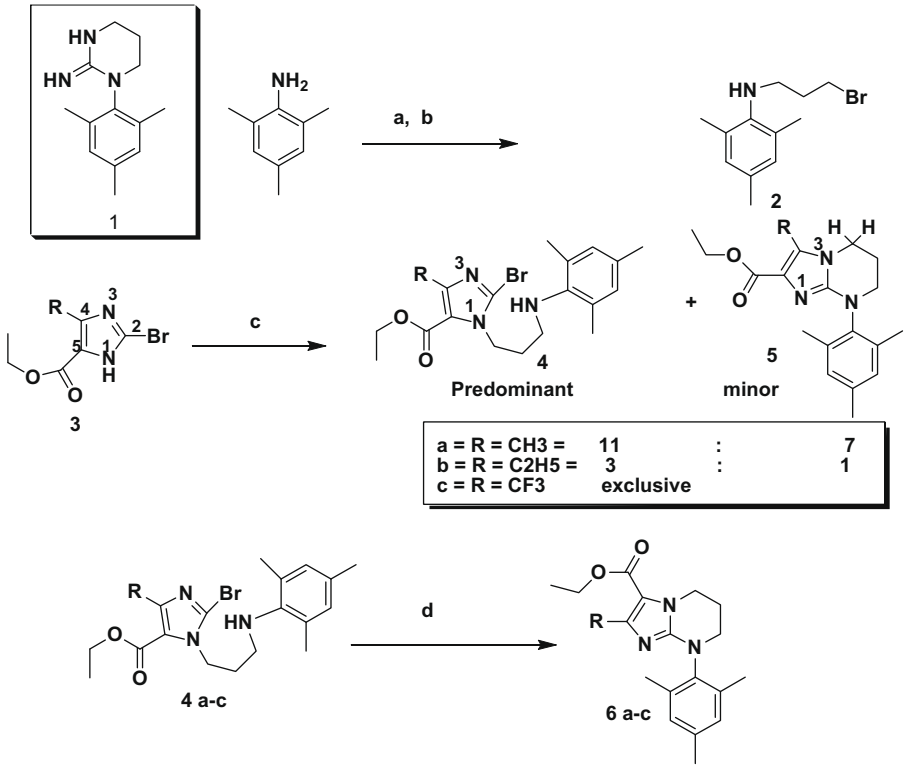



**Figure 1.** Key structural features of non-peptide CRF antagonists.

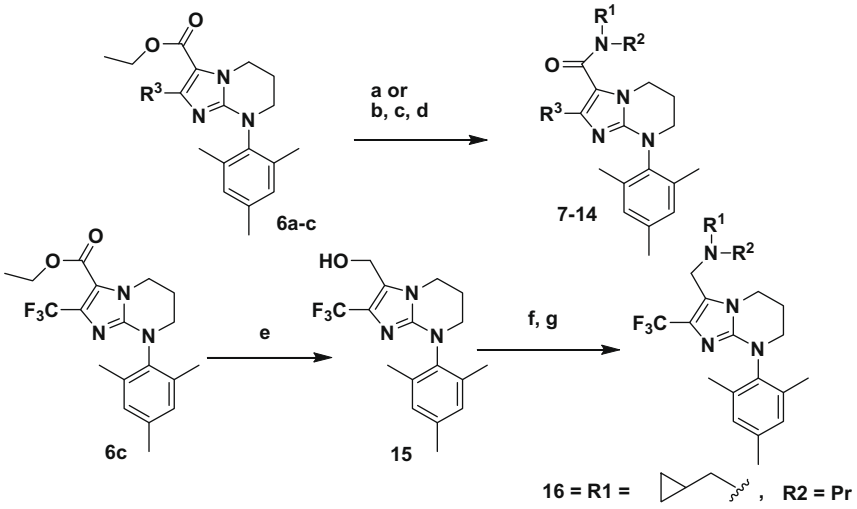


**Tetrahydroimidazopyrimidine Amide Targets**
$R^2 = R^3$ = Alkyl groups, Q = O, Y = $H_2$, R = Me, Et, $CF_3$
**Tetrahydroimidazopyrimidine Amine Targets**
$R^2 = R^3$ = Alkyl groups Q = Y = $H_2$ R = $CF_3$
**Dihydroimidazopyrimidinone Bis-amide Targets**
$R^2 = R^3$ = Alkyl groups, Q = O, Y = O, R = Me, Et

**Scheme 1.** Proposed targets for synthesis for evaluation as antagonists of $CRF_1R$.



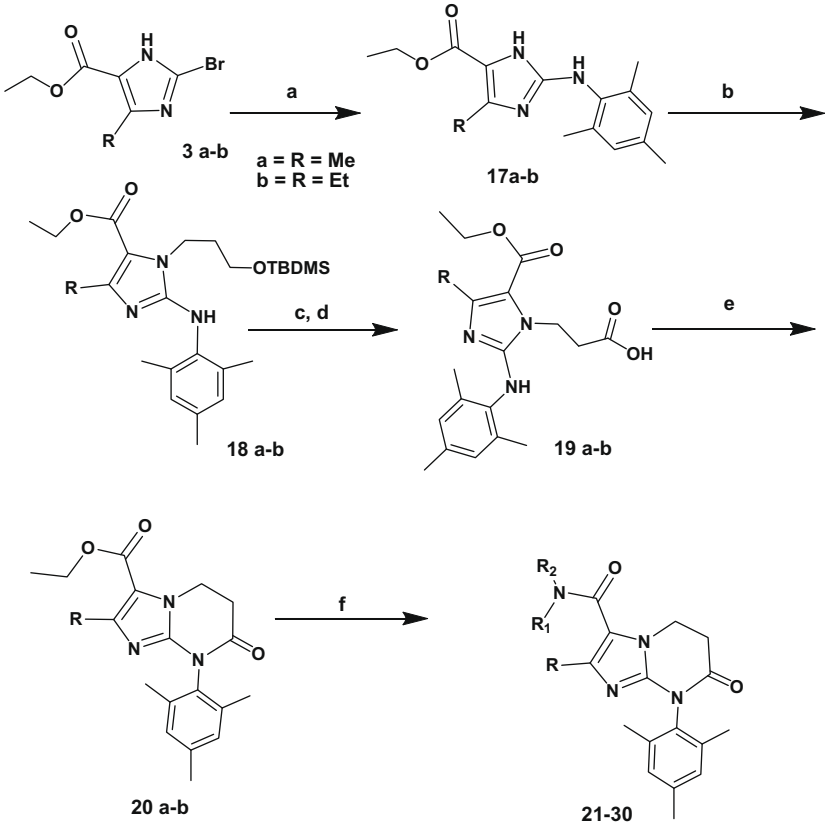

**Scheme 2.** Reagents and conditions: (a) *n*-BuLi/dioxane–hexane/0 °C; (b) excess $Br(CH_2)_3Br$, 22% overall yield; (c) **2**/DBU/acetone or $Cs_2CO_3$/DMF, 21–65% yield; (d) $Ag_2CO_3$ or AgOTf/sulfolane/150 °C/20–60% yield.




**Scheme 3.** Reagents and conditions: (a) $R^1NHR^2/AlMe_3$/toluene/80 °C or; (b) LiOH/DME–$H_2O$; (c) $CF_3COOC_6F_5$/DMF, 60% yield of ester; (d) $R^1NHR^2/Cs_2CO_3$/DMF, 0–83% yield; (e) LAH/THF, 97% yield; (f) $SOCl_2$/DCE; (g) $R^1NHR^2$/MeCN.

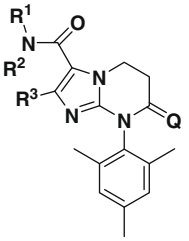
drawing group at C-2. The dialkylamine reagents necessary for the preparation of these targets are from a focused library previously reported from these laboratories.[11] Our initial approach to the synthesis of the 5,6-fused ring system was similar to the synthesis of 5,5-fused ring system from cyclic guanidines reported from our laboratories.[12] Even though a cyclic guanidine such as **1** could be prepared from mesitylamine, subsequent elaboration to final target was unsuccessful. It became necessary to devise an alternative methodology for building such a fused system from mesitylamine as shown in Scheme 2.

Intramolecular cyclization of suitably substituted bromoimidazoles such as **4a–c** appeared to be a viable approach to prepare the desired 5,6-fused system. Alkylation of unsymmetrically substituted bromoimidazole **3a–c** can lead to the formation of two N-alkylated regioisomers. The ratio of the two regioisomers formed during the alkylation of an unsymmetrically substituted




**Scheme 4.** Reagents and conditions: (a) Mesitylamine/AgOTf/diglyme/150 °C, yield = 81% (R = Et) and 89% (R = Me); (b) $Br(CH_2)_3OTBDMS/Cs_2CO_3$/DMF, yield = 52% (R = Me) and 65% (R = Et); (c) TBAF/THF, yield = 63% (R = Me) and 86% (R = Et); (d) Jones oxidation, yield = 28% (R = Me) and 62% (R = Et); (e) EDC·HCl/DIEA/DCM, yield = 22% (R = Me) and 53% (R = Et); (f) $R^1NHR^2/AlMe_3$/toluene/80 °C, 6–24% yield.

**Table 1**
Binding affinities of amides and bis-amides toward hCRF$_1$R



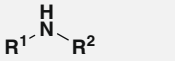
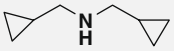
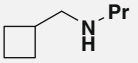
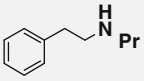
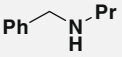
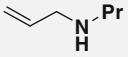
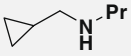
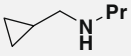
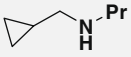
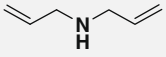
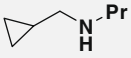
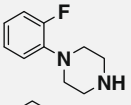
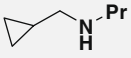
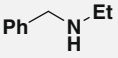
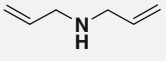
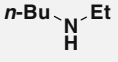
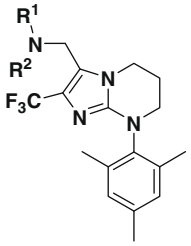
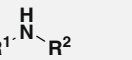
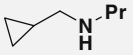
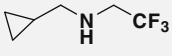
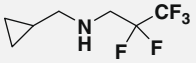
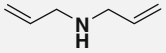
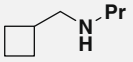
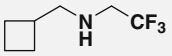
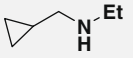
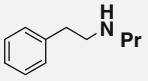
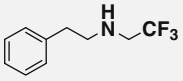
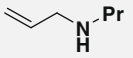
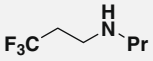
| Compd |  | $R^3$ | Q | $K_i$ (nM) |
|---|---|---|---|---|
| **7** |  | Me | $H_2$ | 3400 |
| **8** |  | Me | $H_2$ | 3300 |
| **9** |  | Me | $H_2$ | 1700 |
| **10** |  | Me | $H_2$ | >5000 |
| **11** |  | Me | $H_2$ | >5000 |
| **12** |  | Me | $H_2$ | >5000 |
| **13** |  | Et | $H_2$ | 2300 |
| **14** |  | $CF_3$ | $H_2$ | 1200 |
| **21** |  | Me | O | >5000 |
| **22** |  | Me | O | >5000 |
| **23** |  | Me | O | >5000 |
| **24** |  | Me | O | >5000 |
| **25** |  | Me | O | >5000 |
| **26** |  | Et | O | >5000 |
| **27** |  | Et | O | >5000 |
| **28** |  | Et | O | >5000 |
| **29** |  | Et | O | >5000 |
| **30** |  | Et | O | >5000 |

**Table 2**
Binding affinities of amines toward hCRF$_1$R



| Compd |  | $K_i$ (nM) |
|---|---|---|
| **16** |  | 32 |
| **31** |  | 8 |
| **32** |  | 14 |
| **33** |  | 19 |
| **34** |  | 21 |
| **35** |  | 27 |
| **36** |  | 31 |
| **37** |  | 67 |
| **38** |  | 38 |
| **39** |  | 48 |
| **40** |  | 63 |
| **41** |  | 245 |
| **42** |  | 364 |
| **43** |  | 1510 |
| **44** |  | 441 |
| **45** |  | >5000 |

imidazole can be influenced by the conditions of alkylation,[13a,b] and the nature of the alkyl group on the imidazole ring. For alkylation of **3**, the N$^1$-isomer was isolated from the reaction mixture, while the N$^3$-isomer under the conditions of alkylation underwent cyclization to form **5**. During the alkylation of **3a–c** with **2**, the composition of the two regioisomers formed was found to be influenced by the nature of the alkyl group on the imidazole ring. Electron-donating groups such as Me and Et led to the formation of N$^1$-alkylated isomer as the major product and N$^3$-alkylation derived cyclization product as the minor. When the alkyl group on the imidazole was an electron-withdrawing $CF_3$ group, alkylation led to exclusive formation of the N$^1$-alkylated isomer. While the cyclization of the minor N$^3$-alkylated material occurred readily under the conditions of alkylation itself, the cyclization of N$^1$-alkylated material did not. After exploring several conditions, silver ion assisted cyclization in sulfolane at 150 °C led to successful formation of the desired tetrahydroimidazopyrimidine ring system. Conversion of the vinylogous urethane to the C-3 amide derivatives was carried out either by Weinreb amidation[14] or via coupling of the amines with the activated pentafluorophenyl ester (Scheme 3). Sterically hindered amines such as di-sec-butylamine and amines containing electron-withdrawing groups such as $PhCH_2NHCH_2CF_3$ failed to couple in this activated ester coupling.

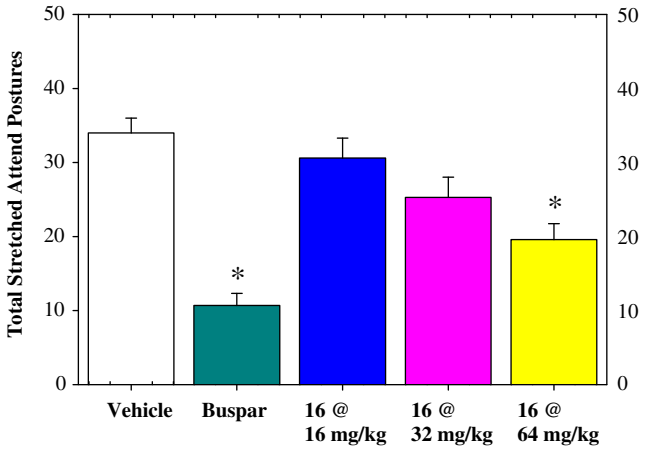



**Figure 2.** Dose-dependent decrease in total stretched attend postures with ip administration of **16** in mouse canopy test as a measure of putative anxiolytic activity. Vehicle is DMSO/cremophor/water = 1:1:80. Asterisk indicates significant difference from vehicle ($p$ <0.05).

The C-2 $CF_3$, C-3-dialkylaminomethyl targets were prepared from the vinylogous urethane by first reducing it to alcohol and converting the alcohol to the chloromethyl derivative followed by displacement of chlorine with the appropriate dialkylamine as shown in Scheme 3.

Synthesis of the bis-amide targets required a different route as shown in Scheme 4. Silver assisted displacement of bromide from **3a–b** by mesitylamine to afford **17a–b** was performed as the first step. Subsequently, alkylation of the imidazole with TBDMS protected bromoethanol, followed by TBAF-mediated removal of the protecting TBDMS group and Jones oxidation gave the carboxylic acids **19a–b** which were converted to the dihydroimidazopyrimidinones **20a–b**. The cyclic amides were then converted to *N,N*-dialkylated bis-amide targets **21**–**30** via Weinreb amidation.

The binding affinities of compounds thus prepared were determined by displacement of [$^{125}$I]-Tyr-*o*-CRF from *h*$CRF_1$R endogenously expressed on IMR-32 human neuroblastoma cells following the protocol described previously.[12]

Initial results with several examples (**21**–**30**) of bis-amide derivatives containing methyl or ethyl substituent on the imidazole ring demonstrated poor binding affinity with $K_i$ values of >10,000 nM (Table 1).

In the C-3 amide series which lacked one carbonyl function compared to the bis-amide series (i.e., Q = $H_2$, Table 1), *N*-cyclopropylmethyl-*N*-propyl amides (compounds **12**–**14**) showed a trend of improved binding affinity when the C-2 substituent was varied from electron-donating alkyl groups to electron- withdrawing $CF_3$ group (i.e., **14** vs **12**).

Removing the C3 amide carbonyl afforded the dialkylaminomethyl analogs lacking a carbonyl group. In this series (Table 2) the corresponding derivative **16** containing the *N*-cyclopropylmethyl-*N*-propyl aminomethyl moiety had a $K_i$ value of 32 nM indicating a significant improvement in affinity. Hence our attention was turned to these tetrahydroimidazo pyrimidine derivatives.

In an effort to improve upon the activity in this series, we utilized a focused library of sixty amines. Many of the dialkylamines necessary for this purpose were prepared as described in literature from our laboratories.[11] The results obtained with these compounds are shown in Table 2. Hydrophobic secondary amine residues containing small rings such as cyclopropyl and cyclobutyl as alkyl groups (e.g., **16**, **34** and **36**) demonstrated low nanomolar range binding affinity. Amines containing fluorinated alkyl groups as one of the alkyl groups also demonstrated pronounced binding affinity as illustrated by examples such as **31** and **32**. Cyclic tertiary amines such as **41** and **42** or cyclic tertiary amines containing a second hetero atom such as **43** and **44** displayed diminished activity. Introduction of a second basic site in the amine residue as in **45** led to a significant loss of binding affinity.

Compound **16** was evaluated in vivo as a prototype of the C-3 amine series. Intraperetoneal (ip) administration of **16** at a dose of 32 mg/kg in a vehicle containing cremophor/DMSO/water (1:1:8) in mice showed that the compound achieved highest brain penetration 30 min post administration (352 ng of **16**/g of brain with a brain/plasma ratio of 1.2). One hour post administration the brain/plasma ratio for **16** was found to be 1.0 (217 ng of **16**/g of brain). The putative anxiolytic effects of **16** were evaluated in a mouse canopy test as described previously.[12,15] In this experiment ip administration of **16** in a vehicle containing cremophor/DMSO/water (1:1:8) demonstrated a dose-dependent reduction of stretched attenuated postures, demonstrating anxiolytic effects (Fig. 2).

In summary, in this Letter we described the synthesis of novel tetrahydroimidazopyrimidines and dihydroimidazopyrimidinones as targets for evaluation as $CRF_1$R antagonists. Silver assisted cyclization was used as a methodology to prepare these constrained imidazoles. Dihydromidazopyrimidinones (the bis-amides) were inactive, and the tetrahydroimidazopyrimidine amides (C3-amides) were only modestly active. Tetrahydroimidazopyrimidine amines demonstrated significant $CRF_1$R affinity. Numerous tetrahydroimidazopyrimidine amines demonstrated $K_i$'s <50 nM. In vivo activity for **16** was demonstrated in the mouse canopy model, demonstrating $CRF_1$R antagonism in vivo. Further efforts to improve the physiochemical properties of this series (i.e., reduce the hydrophobic nature of these constrained imidazoles) while maintaining their potency will be described in a future report.

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