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Synthesis, molecular modeling and biological activity of methyl and thiomethyl substituted pyrimidines as corticotropin releasing hormone type 1 antagonists †

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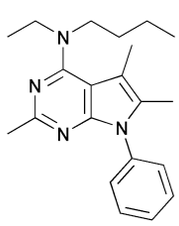
Four small, targeted libraries of differentially substituted amino pyrimidines were synthesized in moderate to good yields. Excellent regiochemistry was observed for substitution at C2/C4 with selectivity >50 : 1 noted. All analogues were screened for their ability to interact with CRH_1 and CRH_2 receptors. In all instances only poor agonistic and/or antagonistic behaviour was noted at CRH_2. However, several compounds were potent and selective CRH_1 antagonists, most notably **13a** $K_i = 39$ nM. Additionally we have utilized these data and that recently reported by others to refine our original CRH_1 pharmacophore (*J. Med. Chem.*, 1999, **42**, 2351–2357).

Introduction

Corticotropin releasing hormone (CRH) is a 41 amino acid peptide implicated in a number of physiological conditions, examples of which include (but are not limited to) anorexia nervosa, Alzheimer's disease, the mediation of stress responses, and being linked to a biological clock as the initiator of the onset of labour.[1] Our primary interest is in the interaction of CRH and the onset of human parturition.

While the hormonal mechanisms that control the onset of parturition and labour are largely unknown, a large body of data suggests that CRH plays a key role in this process. Placental CRH concentrations rise exponentially during pregnancy in maternal circulation. McLean *et al.* have demonstrated that the exponential rise in CRH is linked to a biological clock which determines the length of gestation.[2] Various groups have shown that women in preterm labour have higher CRH concentrations than gestationally matched controls.[3,4] Smith's group has also demonstrated similar results from different perspectives.[5–8] Firstly, women undergoing induction of labour are more likely to have a successful induction if CRH concentrations are high. Secondly, women who are in apparent preterm labour are significantly more likely to deliver prematurely if they have high CRH levels.



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CRH receptors are members of the 7-transmembrane family and are normally linked *via* a G-protein to adenylate cyclase.[9] Several types of receptor have been described,[10] CRH_1, $CRH_{2\alpha}$, $CRH_{2\beta}$ and more recently, $CRH_{2\psi}$, although the key CRH receptor involved in human parturition is of the type 1.[11] Therefore we might reasonably be able to predict that a type 1 receptor antagonist would inhibit the progression of labour and conversely, a type 1 receptor agonist would promote the onset of labour. Recently Smith *et al.* demonstrated the use of antalarmin (**1**) in delaying the onset of labour in a controlled study involving pregnant ewes.[12] We and others have recently reviewed the therapeutic implications and medicinal chemistry developments in corticotropin releasing hormone related research.[13]

Because of the key role of the CRH_1 receptor in human parturition, and the lack of an alternative suitable treatment, there is an urgent need for low molecular weight ligands that are active at this receptor either as agonists or antagonists. Such ligands would not only serve as potential therapeutics, but could lead to a greater understanding of the structure and function of the receptor itself. There are numerous peptidic and non-peptidic antagonists and agonists currently available, but the drive remains to produce a therapeutically acceptable substance, in combination with the apparent limited structural diversity of these known compounds.[10,14–18]

Over the past 5–10 years there has been an up-surge in the number, but unfortunately, not the diversity in the types of small molecules shown to interact specifically with CRH_1 receptors. However, such a situation can be advantageous in producing a detailed structure–activity relationship (SAR) study, and thus, we developed a pharmacophore for this receptor.[12] With this in mind, we have revisited the substituted pyrimidines developed by Whitten *et al.*[19] in producing additional analogues for investigations into pharmacophore development.[20] Herein we wish to report upon our recent results, in the development of CRH_1 antagonists and pharmacophore refinement.

Chemistry

In our efforts to further refine our published pharmacophore we sought simple and expedient routes to the synthesis of small targeted libraries of differentially substituted pyrimidines. We envisaged the development of four such libraries, exemplified by type A–D compounds shown in Fig. 1.

Type **A** analogues most closely resemble those reported by Whitten *et al.*, however types **B**–**D** allow for subtle changes in the spatial orientation of key substituents which should be

† Electronic supplementary information (ESI) available: detailed description of pharmacophore development using CATALYST©. See http://www.rsc.org/suppdata/ob/b3/b305458f/

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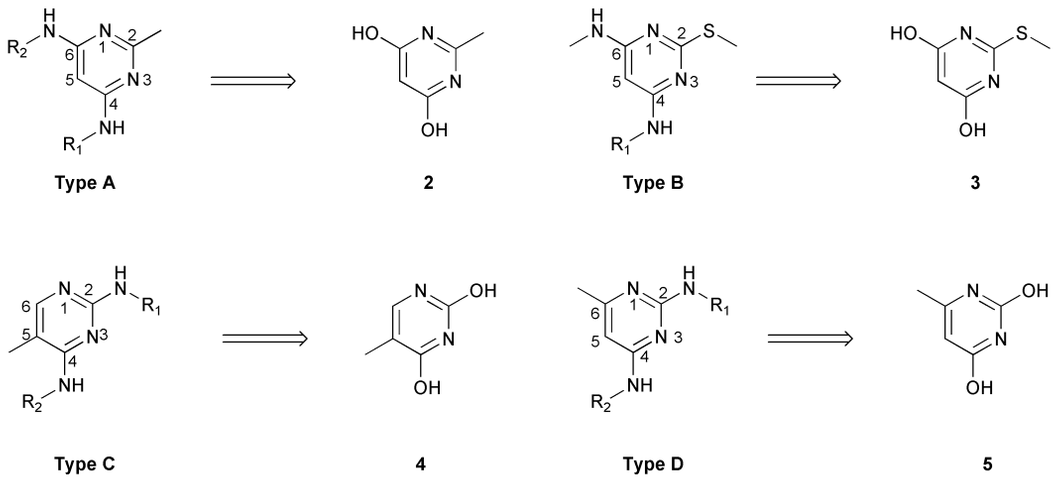
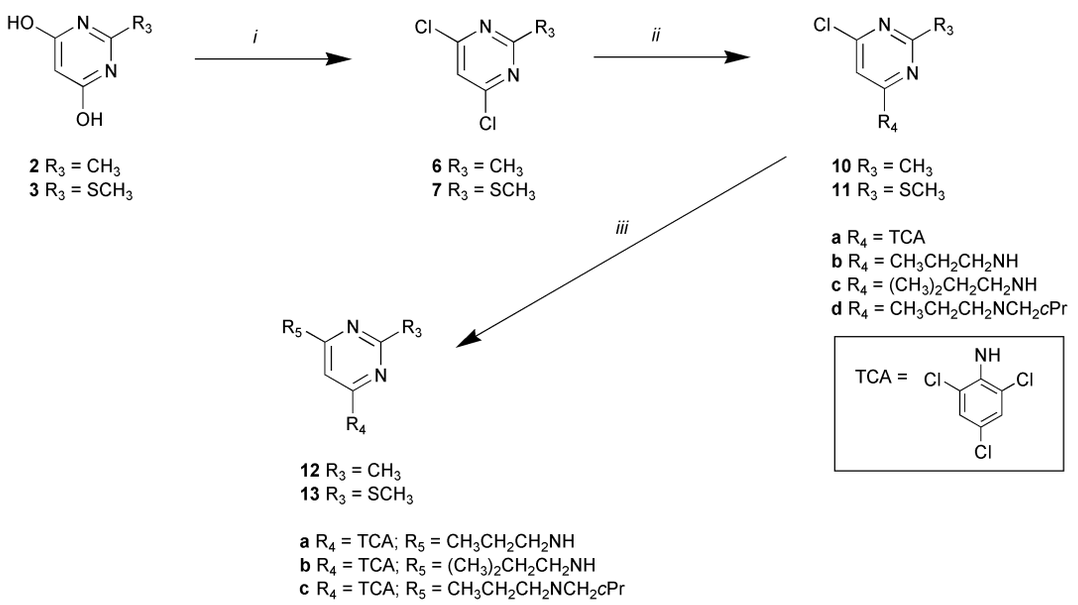


Fig. 1 Targeted libraries of differentially substituted pyrimidines as potential CRH antagonists for pharmacophore refinement.



Scheme 1 Synthesis of type A and B compounds (i) $POCl_3$ (15 equiv.), reflux 5 h; (ii) option 'a' NaH–THF, TCA, 17–24 h; (ii) option 'b' 5 M LPDE–amine, 12 h; (iii) amine, sealed tube, 160 °C.

reflected in differing activities at CRH_1 receptors, and consequently allow further refinement of our pharmacophore.[20]

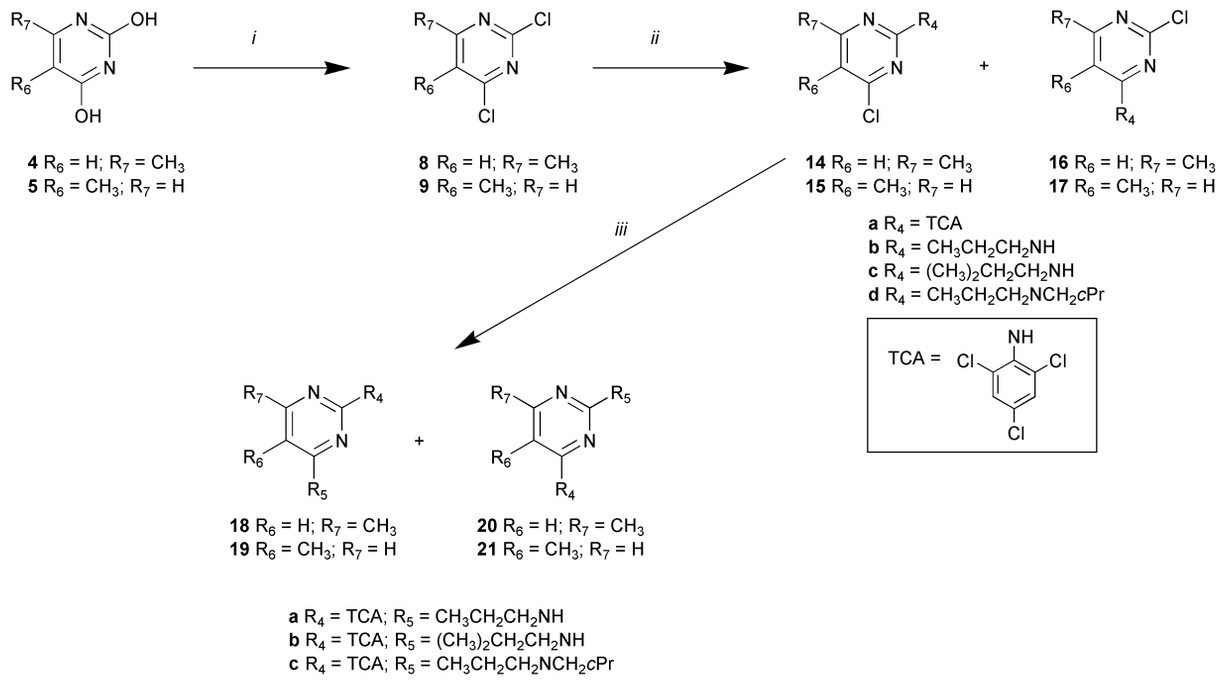
Previously efforts from our laboratory have shown that it is possible to selectively substitute at either the 4- or 6-chloro substituent in a range of 4,6-dichloropyrimidines where the C2 position is occupied by either a methyl or a thiomethyl moiety.[21] In order to use our in-house methodology we first synthesized the appropriate dichloropyrimidines, thus treatment of the parent dihydroxypyrimidines (**2**–**5**) with an excess of $POCl_3$ at reflux affords, cleanly, the desired dichloro species (**6**–**9**) in good to excellent yields (Schemes 1 and 2, Table 1 entries 1, 9, 17, 28).

We had originally envisaged that subsequent displacement of the thiomethyl moiety in **7**, would afford rapid access to appropriately substituted dichloroamino pyrimidines. Aminolysis reactions of this nature are known to be typical in pyrimidine chemistry. Subsequent efforts using typical conditions: sealed tube, 110 °C, 24–72 h, EtOH, NHR_2, as previously described by Chebib *et al.*[22] afforded none of the anticipated thiomethyl substitution product. In all instances, we detected products corresponding to nucleophilic substitution at C2 (minor isomer) and C4 (major isomer) (data not shown).

The selective displacement of the C4 chlorine led us to explore the possible use of alternate reaction media in an attempt to improve our observed selectivity. Accordingly, we examined the use of 5 M lithium perchlorate–diethyl ether (LPDE) solutions as a solvent system for the selective reaction at C4 in **6** and **7**; and at C2 and/or C6 in **8** and **9**. Gratifyingly good selectivity (>20 : 1) was observed, especially with symmetrically substituted dichloropyrimidines **6** and **7** (option 'a' in Schemes 1 and 2). Typically, a symmetrically substituted dichloropyrimidine stirred overnight at room temperature in scrupulously anhydrous LPDE along with an amine, followed by extractive work-up gave, in all instances, mono-substituted products in moderate to good yields (Table 1, entries: 3–5, 11–13). The second chlorine was then readily substituted with a range of amines by simply heating the amine and pyrimidine in a sealed tube at 160 °C, with moderate yields for type **A** or **B** compounds. A slight reduction in selectivity was observed with **4** and **5**, although in these latter cases one isomer dominated. Indeed, in some instances, the second isomer could not be detected.

Subsequent stepwise amination of **6** and **7** in the C4 position, followed by the C6 position, yielded two series of 2-methyl-4,6-diaminated pyrimidines (**12** and **13**) (Scheme 1); and amination of **8** and **9** in the equivalent C2 and C4 positions yields a series of 5/6-methyl-2,4-diaminated pyrimidines (**18** to **21**) (Scheme 2).

The synthesis of the C5 and C6 methylated pyrimidines, type **C** and **D** compounds, was undertaken using typical pyrimidine chemistry by amination of the corresponding dichloro derivatives (option 'a' in Schemes 1 and 2). Highlighting the



Scheme 2 Synthesis of type C and D compounds. (i) $POCl_3$ (15 equiv.), reflux 5 h; (ii) option 'a' NaH–THF, TCA, 17–24 h; (ii) option 'b' 5 M LPDE–amine, 12 h; (iii) amine, sealed tube, 160 °C.

effectiveness of selectivity shown by the synthesis for the type **A** and **B** compounds, these compounds showed little selectivity in amination and required chromatographic separation of the regioisomers.

The activities of these pyrimidine analogues against the CRH type 1 and 2 receptors are shown in Table 1. A negative value for CRH_2 indicates agonist activity whereas a positive value shows an antagonist activity. A dash indicates no clear outcome was resolved. All CRH_2 data, expressed as a %-inhibition of the suavagine mediated stimulation of cAMP, was obtained at 100 μM drug concentration. In our laboratory <40% inhibition at 100 μM is operationally defined as inactive and not worthy of further examination, hence none of the analogues shown in Table 1 showed sufficient promise to warrant K_i determination.

On examining the results for effectiveness against the CRH_1 receptor, it is clear the presence of a chloro substituent (**6**–**9**) attached directly to the pyrimidine ring shows no activity. Somewhat disappointingly, neither type **C** nor type **D** compounds showed any activity against CRH_1 (Table 1, entries 17–36; K_i > 2000 nM). With type **A** and **B** compounds, similar results were noted for the simple mono-aminated analogues (Table 1, entries 1–5 and 9–13; K_i > 2000 nM). This is in keeping with previous reports.[19] The introduction of a second amino group into type **A** compounds afforded the first indication of biological activity against CRH_1 (Table 1, entry 6, R^5 = *n*PrNH; K_i = 1429 nM). Increasing the bulk of this substituent significantly improves activity (Table 1, entries 7 and 8, R^5 = *i*BuNH and *n*PrNCH*c*Pr; K_i = 986 and 130 nM respectively). These data suggest that the presence of a bulky hydrophobic substituent distal to the TCA moiety increases activity, indeed the tertiary amine (**12c**) was the most active of the type **A** compounds examined. However, examination of type **B** compounds showed the opposite trend, that is a reduction in CRH_1 potency as a function of R^5 bulk (Table 1, entries 14–16; K_i = 39, 376 and >2000 nM respectively). This modification in potency must be a function of the thiomethyl moiety, however it is not apparent whether this is due to increased steric bulk or the introduction of the sulfur lone pairs, or a combination of both. These data do, however, suggest that there remains a small binding pocket, as yet largely unexplored. Additionally, these data suggest that the introduction of a thiomethyl moiety necessitates a reduction in steric bulk in the R^5 substituent. This combination affords greater water solubility, a considerable advantage in this field. Given the unexplored nature of this small binding pocket and the restriction in total steric bulk, we felt that these data warranted a reexamination of our preliminary pharmacophore.[20]

Pharmacophore development for the CRH_1 receptor

Since our 1st generation pharmacophore model of the CRH_1 receptor,[20] numerous publications have appeared containing novel antagonists of this receptor.[13,14] In an iterative process, we have refined our original pharmacophore to include these compounds and have also investigated the effects of the novel thiomethyl derivatives from this paper on these models. All pharmacophore modeling work has been done using CATALYST© 1991–1999 (for a more detailed description of pharmacophore development using CATALYST©, see the attached electronic supplementary information† and references cited therein).[23–25] Briefly, the refinement undertaken in this study involved, the inclusion of novel recently reported CRH_1 antagonists, the removal of redundant data and ambiguous data. Further refinement involved the inclusion of activity data spanning four orders of magnitude (nM–μM), avoiding bias towards highly active analogues. This 2nd generation CRH_1 receptor pharmacophore is shown in Fig. 2.

The 2nd generation hypothesis (Fig. 2) has a null − total cost difference of 144 bits suggesting this model has a greater than 90% probability of being a true representation of the data (refer to Table 2 for cost values). The model contained one hydrogen bond acceptor, one ring aromatic and three hydrophobic regions and accurately predicted the activity of a test set of data (correlation 0.989).[26]

The 3rd generation model (Fig. 3) involved the additional inclusion of the data from this paper into the previous training set, in particular the inclusion of compounds showing higher activity when containing the thiomethyl group (**13a** and **13b**). It was believed that this feature may be highlighted as being important for activity by the modeling program. However, this proved to not be the case with the addition of these compounds having very little effect on the appearance of the pharmacophore, containing the same features as the previous model (Fig. 2). It did, however, cause a change in the cost of the

Table 1 Antagonist activity against CRH type 1 and type 2 receptors

Entry	Compound	R groups				CRH_1 K_i/nM	CRH_2 (%)[a]	Yield (%)
Type A compounds								
1	**6**					>2000	3	79
		R^3	R^4					
2	**10a**	CH_3	TCA			>2000	−5	82
3	**10b**	CH_3	*n*PrNH			>2000	−3	57
4	**10c**	CH_3	*i*BuNH			>2000	17	38
5	**10d**	CH_3	*n*PrN-CH_2*c*Pr			>2000	—	25
				R^5				
6	**12a**	CH_3	TCA	*n*PrNH		1429	13	47
7	**12b**	CH_3	TCA	*i*BuNH		986	16	34
8	**12c**	CH_3	TCA	*n*PrN-CH_2*c*Pr		130	12	79
Type B compounds								
9	**7**					>2000	5	88
		R^3	R^4					
10	**11a**	SCH_3	TCA			>2000	—	60
11	**11b**	SCH_3	*n*PrNH			>2000	6	61
12	**11c**	SCH_3	*i*BuNH			>2000	6	61
13	**11d**	SCH_3	*n*PrN-CH_2*c*Pr			>2000	13	27
				R^5				
14	**13a**	SCH_3	TCA	*n*PrNH		39	24	55
15	**13b**	SCH_3	TCA	*i*BuNH		376	−2	78
16	**13c**	SCH_3	TCA	*n*PrN–CH_2*c*Pr		>2000	−2	47
Type C compounds								
17	**8**		H			>2000	13	64
		R^4	**R^5**	**R^6**	**R^7**			
18	**14a**	TCA	—	H	CH_3	>2000	13	56
19	**14b**	*n*PrNH	—	H	CH_3	>2000	13	7
20	**14c**	*i*BuNH	—	H	CH_3	>2000	8	41
21	**14d**	Pr-N-CH_2*c*Pr	—	H	CH_3	>2000	−10	12
22	**16a**	—	TCA	H	CH_3	>2000	8	29
23	**16b**	—	*n*PrNH	H	CH_3	>2000	—	50
24	**16c**	—	*i*BuNH	H	CH_3	>2000	17	23
25	**16d**	—	Pr-N-CH_2*c*Pr	H	CH_3	>2000	13	46
26	**18a**	TCA	Pr-N-CH_2*c*Pr	H	CH_3	>2000	—	53
27	**20a**	Pr-N-CH_2*c*Pr	TCA	H	CH_3	>2000	—	27
Type D compounds								
28	**9**		CH_3	H		>2000	−33	72
	R^4	R^5	R^6	R^7				
29	TCA	—	CH_3	H		>2000	−39	4[b]
30	*n*PrNH	—	CH_3	H				—[c]
31	*i*BuNH	—	CH_3	H		>2000	−10	1.6[b]
32	*n*PrN-CH_2*c*Pr	—	CH_3	H		—	−30	0.15[b]
33	—	TCA	CH_3	H		>2000	−38	68[b]
34	—	*n*PrNH	CH_3	H		>2000	−28	46[b]
35	—	*i*BuNH	CH_3	H		>2000	−8	43[b]
36	—	*n*PrN-CH_2*c*Pr	CH_3	H		>2000	−42	43[b]

[a] % Inhibition of the sauvagine-stimulated adenosine 3′,5′-phosphate (cAMP) at a drug concentration of 100 μM. [b] Selectivity **17a** : **15a** = 17 : 1; **17b** : **15b** = >50 : 1; **17c** : **15c** = 27 : 1; **17d** : **15d** = >50 : 1. [c] None detected in the reaction mixture.

Table 2 Cost values for the generation of all three pharmacophore models, where the fixed cost (cost of a perfect hypothesis), the null cost (cost of a hypothesis for which we assume there is no structural data) and the total cost (the cost for the hypothesis generation) are all measured in arbitrary units (bits)

	2nd generation model	3rd generation model
Fixed cost	433.85	474.05
Null cost	797.18	885.25
Total cost	653.52	713.85
Null − total cost difference	143.66	171.4

hypothesis model, significantly increasing the null − total cost difference and therefore increasing the statistical validity of the pharmacophore model. This also resulted in the highest scoring (lowest cost) hypothesis being selected as the best hypothesis model. This unexpected occurrence leads to this 'third generation model' being the most statistically valid and therefore presumably the most accurate model to date.

In order to test the possibility that the range of derivatives containing the thiomethyl substituents provided sufficient structure–activity information to generate a valid pharmacophore of equal quality to either/both our 2nd generation or 3rd generation models, a separate model was created using only compounds reported in this paper. One important question to ask was, given that the training set would contain a higher

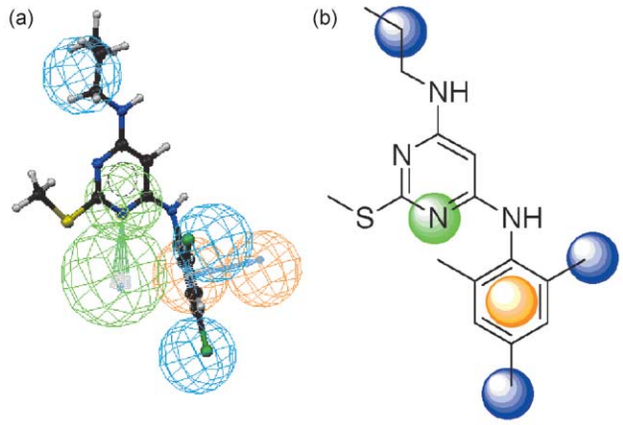


Fig. 2 The highest scoring hypothesis for the 'second generation model' generated by CATALYST. (a) Overlay of **13a** on this hypothesis, estimated activity of 2.7 nM (observed K_i = 39 nM). (b) Cartoon representation of **13a** mapped onto the hypothesis highlighting actual groups and atoms that correspond with the CATALYST image in panel (a); the regions of importance in the molecule: green: hydrogen bond acceptor, orange: ring aromatic, blue: hydrophobic aliphatic.

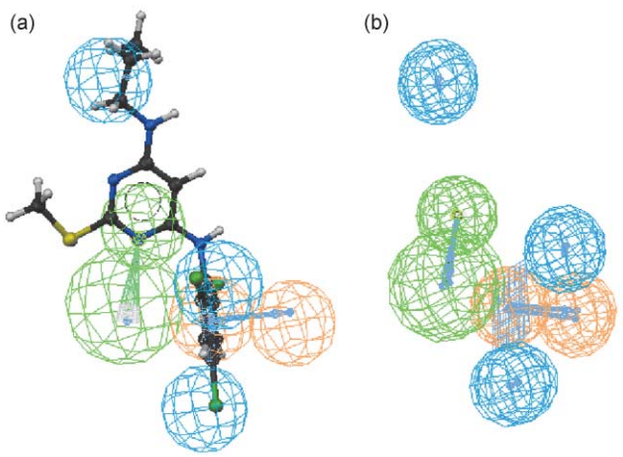


Fig. 3 (a) The highest scoring hypothesis for the 'generation three model' overlayed with **13a**, estimated activity of 2 nM (observed K_i = 39 nM). (b) An overlay of the 'generation two' and 'generation three' models showing their similarity.

proportion of derivatives with a thiomethyl substituent, would this feature show as being an important feature in the final model?

The training set for this model was small, containing only 13 compounds, and therefore was not expected to yield a statistically valid result. The returned highest scoring hypothesis is shown in Fig. 4, with compound **13a** overlaid. The highest scoring hypothesis contained 2 hydrophobic features, 1 ring aromatic and 1 hydrogen bond acceptor feature; none of these features however, mapped the thiomethyl substituent (Fig. 4). Therefore, even though the model possesses a low statistical validity (indicating that it should be treated with caution), and despite a heavy weighting in the training set of molecules containing a thiomethyl substituent, the result lends support to the conclusion that the improvement in our 3rd generation model is valid and the lack of the thiomethyl overlaying on top of a 'feature' of the pharmacophore is more than likely a true representation.

In conclusion, the inclusion of the thiomethyl containing compounds from this paper led to an increase in the statistical validity of the current pharmacophore model for the CRH_1 receptor without selecting this group as being important for activity. The significance of this analysis lies in the continual refinement of the model, with the SAR consideration that inclusion of specific substituents can increase the quality of the

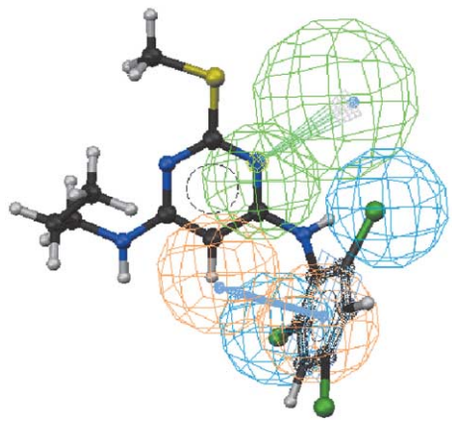


Fig. 4 Hypotheses from the thiomethyl model, generated using only compounds from this paper. The highest scoring hypothesis overlayed on **13a** does not map the thiomethyl group as an important feature, estimate activity of 26 nM (observed K_i = 39 nM).

pharmacophore without apparently being directly related to a proposed 'receptor' interaction. Rather, the presence of these substituents contributes to the electronic nature of the SAR, or the conformational analysis of the ligands giving rise to the pharmacophore model to yield a more accurate representation, both electronically and spatially, of the proposed binding site shape of the CRH_1 receptor antagonists. In these studies, however, the true importance of the thiomethyl moiety may have been concealed by the large size of the training set with so few compounds containing this functional group. We are currently aiming towards generating a larger array of thiomethyl based compounds for screening and incorporation into our pharmacophore model. These models are currently being utilized in further studies, both in database mining and *de novo* design experiments in an attempt to generate novel, structurally variable antagonists of CRH_1.

Experimental

General methods

THF and ether were freshly distilled from sodium–benzophenone. Flash chromatography was carried out using silica gel 200–400 mesh (60 Å). ^1H and ^{13}C NMR were recorded at 300 MHz and 75 MHz respectively using a Bruker Avance 300 MHz spectrometer in $CDCl_3$.

GCMS was performed using a Shimadzu GCMS-QP5050A. The instrument uses a quadrupole mass spectrometer and detects samples *via* electron impact ionization (EI). The University of Wollongong Biomolecular Mass Spectrometry Laboratory analysed samples for HRMS. The spectra were run on the VG Autospec-oa-tof tandem high resolution mass spectrometer using CI +ve (chemical ionization), with methane as the carrier gas and PFK (perfluorokerosene) as the reference.

Stimulation of cAMP production by sauvagine

Adenylate cyclase activity was performed using cells transfected with either the human CRF_1 or $CRF_{2\alpha}$ receptor. Cells were plated onto 24-well cell culture plates and grown to confluency. On the day of assay, the plates were removed from the incubator, the medium was aspirated, and the cells were washed once with PBS (phosphate buffered saline). Assays were carried out at 37 °C for 1 h in a final volume of 0.5 ml in assay buffer containing Dulbecco's modified Eagle's medium, 2 mM L-glutamine, 20 mM HEPES, and 1 mM 3-isobutyl-1-methylxanthine. In stimulation studies, various concentrations of CRF-related and -unrelated peptides were incubated with the cells to establish the pharmacological rankorder profile of this receptor subtype. For the functional assessment of antagonists [α-helical

CRF(9–41) or D-Phe-CRF(12–41)], 10 nM sauvagine (causing ~60–80% stimulation of cAMP production) was incubated along with various concentrations of competing peptides (10^{-12} to 10^{-6} M). After the incubations, the medium was aspirated, the wells were rinsed once gently with fresh medium and aspirated. Intracellular cAMP was extracted from the cells by the addition of 300 µl of a solution of 95% ethanol containing 20 mM HCl and incubated at −20 °C for 16–18 h. The solution was then transferred to 1.5-ml Eppendorf tubes, and the wells were washed with an additional 200 µl of 95% ethanol containing 20 mM HCl and pooled with the first fraction. The samples were lyophilized and resuspended with 500 µl of sodium acetate buffer. The measurement of cAMP in the samples was performed using a single antibody cAMP radioimmunoassay kit (Biomedical Technologies, Stoughton, MA).

^{125}I-tyr^0-sauvagine binding studies

Radioligand binding of ^{125}I-Tyr^0-sauvagine in transfected CHO cells was performed essentially as previously described in detail for the binding of either ^{125}I-r/hCRF or ^{125}I-oCRF to many different tissues. All drugs and reagents (*i.e.*, guanine nucleotides) were made up in assay buffer, which was the same as the tissue buffer described above with the addition of 0.15 mM bacitracin, pH 7.0, at 22 °C. Briefly, 1.5 ml Eppendorf tubes received (in order) 100 µl of buffer (with or without competing peptides, guanine nucleotides, and so on), 50 µl of ^{125}I-Tyr^0-sauvagine, and 150 µl of the membrane suspension as described above, for a total assay volume of 300 µl. The assay was incubated at equilibrium routinely for 2 h at 22 °C as determined by direct kinetic experiments described below. Non-specific binding was determined in the presence of 1 µM unlabeled peptide antagonist D-Phe-CRF. Reactions were terminated by centrifugation in a Beckman microfuge for 10 min at 12000 × *g*. The resulting pellets were washed gently with 1 ml of ice-cold PBS containing 0.01% Triton X-100 and centrifuged again for 10 min at 12000 × *g*. The supernatants were aspirated, and the tubes were cut just above the pellet, placed into 12 × 75 mm polystyrene tubes, and monitored for radioactivity in a Packard Cobra II γ-counter at ~80% efficiency.

Kinetic studies were performed to determine the association and dissociation rate constants for ^{125}I-Tyr^0-sauvagine and determine the optimal time for equilibrium binding. Association studies were initiated with the addition of the membrane suspension to triplicate tubes containing the radiolabel (100–200 pM final concentration) and buffer, with or without 1 µM D-Phe-CRF to define nonspecific binding. Tubes were allowed to incubate for various times before centrifugation and washing of the membranes. Specific binding was calculated for each time point, and the rate constant for the association was determined For dissociation experiments, tubes were set up as described for the association assays and allowed to incubate for 2 h at 22 °C After the 2 h incubation, 1 µM D-Phe-CRF (final concentration) was added to all tubes (total and nonspecific) to initiate dissociation of the label in a final volume of 10 µl to minimize the effects of dilution. The tubes again were centrifuged at various times, and the specific binding was used to calculate the dissociation rate constant as described below.

For competition studies, tubes received (in order) 50 µl of buffer, 50 µl of competing peptides (final concentration, 1 pM to 1 µM), 50 µl of ^{125}I-Tyr^0-sauvagine (final concentration, 100–200 pM), and 150 µl of membrane suspension as described above. Homogenates were typically allowed to incubate for 2 h at 22 °C, and the reaction was terminated by separation of the bound from free radioligand by centrifugation as described above.

For Scatchard saturation studies, tubes received (in order) 50 µl of buffer, 50 µl of ^{125}I-Tyr^0-sauvagine (final concentration, 10 pM to 2 nM), and 50 µl of buffer with or without D-Phe-CRF to define non-specific binding. All assays were carried out at equilibrium at 22 °C as determined by the association experiments described above. Specific binding was determined at each concentration of radioligand in the presence of 1 µM D-Phe-CRF(12–41). This concentration was chosen from direct competition studies demonstrating that at a concentration of 1 µM, D-Phe-CRF could displace >95% of ^{125}I-Tyr^0-sauvagine binding from CHO cells expressing the CRF_{2a}, receptor.

Data analysis

All data analyses, including Scatchard, competition and kinetic experiments, were analysed using the iterative nonlinear least-squares curve-fitting program Prism (GraphPAD, San Diego, CA). The saturation analyses of ^{125}I-Tyr^0-sauvagine binding yielded K_d values that were subsequently used in the calculation of the apparent K_i values for competing ligands in competition assays performed under identical conditions. For competition curves, the data were routinely fit to single- and multiple-site models, and the fits were compared statistically to determine whether a more-complex data model was justified.

Synthesis

4,6-Dichloro-2-methylpyrimidine (6). 4,6-Dihydroxy-2-methylpyrimidine (5.0 g, 40 mmol) was mixed with $POCl_3$ (30 mL, 15 eq) and refluxed for three hours, cooling to room temperature, the reaction mixture was added dropwise to vigorously stirred ice water (200 mL) in a salt–ice bath. The yellow solid formed was collected by vacuum filtration (2.82 g). The filtrate was extracted with ethyl acetate (2 × 70 mL). The extract was washed with brine (30 mL), dried over $MgSO_4$ and evaporated *in vacuo* to give an additional 0.86 g of light brown solid. Recrystallization with ethyl acetate gave a light brown solid in a total yield of 3.70 g, 58%.

Mp 48–49 °C, ^{1}H NMR: 2.75 (s, 3H), 7.25 (s, 1H); ^{13}C NMR: 26.25, 118.97, 162.17, 170.39. GCMS: 163.0; calcd 163.0.

4-Chloro-2-methyl-6-(2′,4′,6′-trichloroanilino)pyrimidine (10a). A solution of 2,4,6-trichloroaniline (400 mg, 2.0 mmol) in THF (8 mL) was treated with NaH (160 mg, 60% dispersion in oil, 4.0 mmol) at room temperature, under nitrogen. The yellow suspension was stirred at room temperature for 10 minutes before 4,6-dichloro-2-methylpyrimidine (**6**) (0.326 mg, 2.0 mmol) was added and the mixture heated at reflux under nitrogen for 24 hours. The reaction was quenched with ice water (5 mL) and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (25 mL), dried ($MgSO_4$), filtrated and the solvent removed *in vacuo* to give a brown solid. Recrystallization from ethyl acetate–hexanes gave a pale yellow solid, yield 0.530 g, 82%.

Mp 166–168 °C, ^{1}H NMR: 2.42 (s, 3H), 5.93 (s, 1H), 7.46 (s, 2H), 7.67 (br s, 1H); ^{13}C NMR: 26.16, 100.51, 129.72, 131.64, 134.94, 135.85, 161.30, 162.60, 169.13.

4-Chloro-2-methyl-6-(*N*-propylamino)pyrimidine (10b). 4,6-Dichloro-2-methylpyrimidine (**10a**) (250 mg, 1.5 mmol) was dissolved in 5 mL of 5 M LPDE. Propylamine (1.26 mL, 10 eq) was added and the sealed container was stirred for 24 hours. Water (10 mL) was added and the solution was extracted with ether (3 × 30 mL). The combined organic layers were dried ($MgSO_4$) and the solvent removed *in vacuo*. The yellow solid obtained was purified by flash chromatography using 15 : 85 ethyl acetate–hexanes yielding **10b** as an off-white solid, yield 160 mg, 57%.

Mp 69–70 °C, ^{1}H NMR: 0.82 (t, 3H), 1.48 (q, 2H), 2.31 (s, 3H), 3.08 (br s, 2H, -CH_2-NH-), 5.72 (br s, 1H), 6.10 (s, 1H); ^{13}C NMR: 11.79, 22.77, 26.06, 43.69, 97.92, 159.95, 164.02, 168.42. HRMS: $(M + H)^+$ = 186.07979; calcd, 186.07987 (^{35}Cl).

4-Chloro-6-(*N*-isobutylamino)-2-methylpyrimidine (10c). Synthesized using the general procedure as for (**10b**). The yellow

solid obtained was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes and gave the product as a yellow oil, yield 116 mg, 38%.

^{1}H NMR: 0.84 (d, 6H), 1.76 (sept, 1H), 2.34 (s, 3H), 2.95 (br s, 2H, -CH_2-NH-), 5.64 (br s, 1H), 6.08 (s, 1H); ^{13}C NMR: 20.61, 26.06, 28.63, 97.81, 160, 164.17, 168.40. HRMS: $(M + H)^+$ = 200.09522; calcd, 200.09552 (^{35}Cl).

4-Chloro-6-(*N*-propyl-*N*-cyclopropylmethylamino)-2-methylpyrimidine (10d). Synthesized using the general procedure as for (**10b**). The brown solid obtained was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes and gave the product as a yellow oil; yield 36 mg, 25%.

^{1}H NMR: 0.17 (dd, 2H), 0.40 (dd, 2H), 0.80 (t, 3H), 0.90 (m, 1H), 1.5 (sext, 2H), 2.3 (s, 3H), 3.23 (br s, 2H + 2H), 6.1 (s, 1H); ^{13}C NMR: 4.17, 10.02, 11.77, 20.92, 26.40, 50.46, 52.70, 98.32, 159.74, 162.87, 168.03. HRMS: $(M + H)^+$ = 240.12657; calcd, 240.12682 (^{35}Cl).

2-Methyl-4-(*N*-propylamino)-6-(2′,4′,6′-trichloroanilino)-pyrimidine (12a). A solution of 4-chloro-2-methyl-6-(2′,4′,6′-trichloroanilino)pyrimidine (**10a**) (150 mg, 0.46 mmol) in dry THF (10 mL) was added to a pressure vessel with propylamine (0.39 mL, 10 eq). The vessel was flushed with nitrogen, sealed and stirred at 160 ºC for 24 hours. The solvent was removed *in vacuo* to give a brown solid which was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes yielding a pale yellow solid, yield 75 mg, 47%.

Mp 156–158 °C, ^{1}H NMR: 0.85 (t, 3H), 1.48 (sext, 2H), 1.95 (q, 2H), 2.12 (s, 3H), 4.73 (s, 1H), 5.29 (br s, 1H), 7.38 (s, 2H); ^{13}C NMR: 12.06, 22.95, 25.73, 43.90, 78.75, 129.33, 133.44, 133.57, 136.02, 161.97, 164.07, 167.12. HRMS: $(M + H)^+$ = 345.04272; calcd, 345.04413 (^{35}Cl).

2-Methyl-4-(*N*-isobutylamino)-6-(2′,4′,6′-trichloroanilino)-pyrimidine (12b). Synthesized using the general procedure as for (**12a**). The brown solid product was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes to give a cream solid, yield 56 mg, 34%.

Mp 159–162 °C, ^{1}H NMR: 0.887 (d, 6H), 1.76 (sept, 1H), 2.23 (s, 3H), 2.84 (t, 2H), 4.78 (s, 1H), 5.11 (br s, 1H), 7.42 (s, 2H); ^{13}C NMR: 20.20, 25.10, 28.16, 49.17, 78.40, 128.73, 132.59, 132.84, 134.98. 160.99, 163.20, 166.41.

2-Methyl-4-(*N*-propyl-*N*-cyclopropylmethylamino)-6-(2′,4′,6′-trichloroanilino)pyrimidine (12c). Synthesized using the general procedure as for (**12a**). The brown oil product was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes to give an orange solid, yield 97.3 mg, 79%.

Mp 126–128 °C, ^{1}H NMR: 0.10 (dt, 2H), 0.29 (dt, 2H), 0.64 (t, 3H), 0.79 (m, 1H), 1.38 (sext, 2H), 2.23 (s, 3H), 3.25 (br d, 2H + 2H), 4.87 (s, 1H), 7.40 (s, 2H); ^{13}C NMR: 4.37, 10.47, 12.05, 21.30, 26.44, 50.43, 52.58, 77.28, 77.71, 78.13, 79.88, 129.30, 132.96, 134.03, 135.48, 161.26, 163.20, 167.12.

4,6-Dichloro-2-methylmercaptopyrimidine (7). Synthesized using the general procedure as for (**6**). Recrystallization with MeOH yielded a white solid, yield 3.45 g, 88%.

Mp 43–44 °C, ^{1}H NMR: 2.6 (s, 3H), 7.08 (s, 1H); ^{13}C NMR: 15.15, 116.47, 162.03, 175.17. GCMS: 195.1; calcd 195.1.

4-Chloro-2-methylmercapto-6-(2′,4′,6′-trichloroanilino)-pyrimidine (11a). Synthesized using the general procedure as for (**10a**). The brown solid was recrystallized from ethyl acetate–hexanes to give a white solid, yield 360 mg, 60%.

^{1}H NMR: 2.48 (s, 3H), 5.90 (s, 1H), 7.21 (br s, 1H), 7.48 (s, 2H); ^{13}C NMR: 14.78, 99.04, 129.51, 131.57, 134.73, 135.74, 160.90, 161.85, 173.31. HRMS: $(M + H)^+$ = 353.91987; calcd, 353.91937 (^{35}Cl).

4-Chloro-6-(*N*-propylamino)-2-methylmercaptopyrimidine (11b). Synthesized using the general procedure as for (**10b**). The pale yellow solid was recrystallized in hexane to give white crystals, yield 336 mg, 61%.

Mp 115–116 °C, ^{1}H NMR: 0.94 (t, 3H), 1.60 (sext, 2H), 2.46 (s, 3H), 3.21 (br s, 2H, -CH_2-NH-), 5.17 (br s, 1H), 5.99 (s, 1H); ^{13}C NMR: 11.92, 14.58, 22.96, 43.77, 96.31, 99.79, 163.33, 172.51. HRMS: $(M + H)^+$ = 218.05182; calcd, 218.05194 (^{35}Cl).

4-Chloro-6-(*N*-isobutylamino)-2-methylmercaptopyrimidine (11c). Synthesized using the general procedure as for (**10b**). The yellow solid was purified by flash chromatography using 15 : 85 ethyl acetate–hexanes leaving the product as a cream solid, yield 181 mg, 61%.

Mp 72–75 °C, ^{1}H NMR: 0.89 (d, 6H), 1.82 (non, 1H), 2.43 (s, 3H), 3.05 (br s, 2H, -CH_2-NH-), 5.42 (br s, 1H), 5.99 (s, 1H); ^{13}C NMR: 14.59, 20.70, 28.81, 49.55, 96.25, 99.75, 163.48, 192.51. HRMS: $(M + H)^+$ = 232.06722; calcd, 232.06757 (^{35}Cl).

4-Chloro-6-(*N*-propyl-*N*-cyclopropylmethylamino)-2-methylmercaptopyrimidine (11d). Synthesized using the general procedure as for (**10b**). The brown solid was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes to give a yellow oil, yield 38 mg, 27%.

^{1}H NMR: 0.18 (dd, 2H), 0.42 (dd, 2H), 0.81 (t, 3H), 0.93 (m, 1H), 1.52 (sext, 2H), 2.35 (s, 1H), 3.30 (br s, 2H + 2H), 6.00 (s, 1H); ^{13}C NMR: 4.11, 9.90, 11.69, 14.38, 20.93, 50.62, 52.86, 96.75, 159.34, 161.93, 171.79. HRMS: $(M + H)^+$ = 272.09861; calcd, 272.09889 (^{35}Cl).

2-Methylmercapto-4-(*N*-propylamino)-6-(2′,4′,6′-trichloroanilino)pyrimidine (13a). Synthesized using the general procedure as for (**12a**). The brown solid product was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes to give a yellow solid, yield 105 mg, 55%.

Mp 119–121°C, ^{1}H NMR: 0.90 (t, 3H), 1.51 (sext, 2H), 2.93 (s, 3H), 3.07 (q, 2H), 4.79 (s, 1H), 4.85 (br s, 1H), 6.58 (br s, 1H), 7.38 (s, 2H); ^{13}C NMR: 12.06, 14.40, 23.19, 43.93, 79.02, 129.27, 133.22, 133.39, 135.35, 161.02, 163.79, 171.24. HRMS: $(M + H)^+$ = 377.01395; calcd, 377.01620 (^{35}Cl).

2-Methylmercapto-4-(*N*-isobutylamino)-6-(2′,4′,6′-trichloroanilino)pyrimidine (13b). Synthesized using the general procedure as for (**12a**). The brown product was purified by flash chromatography using 1 : 4 ethyl acetate–hexanes to give a yellow solid, yield 129 mg, 78%.

Mp 144–146 °C, ^{1}H NMR: 0.87 (d, 6H), 1.78 (m, 1H), 2.94 (br t, 2H), 4.80 (br s, 1H+1H), 4.83 (s, 3H), 6.34 (br s, 1H, NH), 7.40 (s, 2H); ^{13}C NMR: 14.47, 20.93, 29.04, 49.80, 79.18, 129.33, 133.25, 133.36, 135.24, 160.96, 163.94, 171.33. HRMS: $(M + H)^+$ = 391.03025; calcd, 391.03185 (^{35}Cl).

2-Methylmercapto-4-(*N*-propyl-*N*-cyclopropylmethylamino)-6-(2′,4′,6′-trichloroanilino)pyrimidine (13c). Synthesized using the general procedure as for (**12a**). The brown oil was purified by speedy column using 1 : 4 ethyl acetate–hexanes to give a white crystalline solid, yield 115 mg, 47%.

Mp 150–151°C, ^{1}H NMR: 0.18 (dt, 2H), 0.46 (dt, 2H), 0.81 (t, 3H), 0.98 (m, 1H), 1.55 (sext, 2H), 2.42 (s, 3H), 3.28 (br t, 2H + 2H), 4.88 (s, 1H), 6.33 (br s, 1H NH), 7.41 (s, 2H); ^{13}C NMR: 4.38, 10.42, 12.07, 14.53, 21.43, 50.80, 52.95, 79.14, 129.28, 132.96, 133.69, 135.09, 160.79, 162.55, 170.71. HRMS: $(M + H)^+$ = 431.06122; calcd, 431.06315 (^{35}Cl).

2,4-Dichloro-6-methylpyrimidine (8). Synthesized using the general procedure as for (**6**). Recrystallization with MeOH gave white crystals, yield 4.18 g, 64%.

Mp 46–47 °C, ^{1}H NMR: 2.57 (s, 3H), 7.20 (s, 1H); ^{13}C NMR: 24.50, 112.29, 120.15, 163.01, 172.49. GCMS: 163.0; calcd 163.0.

2-Chloro-6-methyl-4-(2′,4′,6′-trichloroanilino)pyrimidine (16a) and 4-chloro-6-methyl-2-(2′,4′,6′-trichloroanilino)pyrimidine (14a). Synthesized using the general procedure as for (**10a**). The yellow solid was recrystallized from ethyl acetate–hexanes to give a pale yellow solid. Separation of the isomers using flash chromatography with 1 : 4 ethyl acetate–hexanes gave pale yellow solids, yield **14a** 187 mg, 29%; **16a** 363 mg, 56%.

14a ^{1}H NMR: 2.38 (s, 3H), 5.92 (s, 1H), 7.53 (2H), 7.87 (br s, 1H); ^{13}C NMR: 24.68, 101.57, 129.64, 131.69, 134.94, 136.00, 160.56, 163.35, 170.07. HRMS: $(M + H)^+$ = 186.07941; calcd, 186.07987 (^{35}Cl).

16a ^{1}H NMR: 2.29 (s, 3H), 6.63 (s, 1H), 7.25 (br s, 1H, NH), 7.39 (s, 2H); ^{13}C NMR 24.42, 112.70, 129.12, 133.08, 133.35, 135.48, 160.61, 162.08, 170.69. HRMS: $(M + H)^+$ = 186.07946; calcd, 186.07987 (^{35}Cl).

2-Chloro-6-methyl-4-(*N*-propylamino)pyrimidine (16b) and 4-chloro-6-methyl-2-(*N*-propylamino)pyrimidine (14b). Synthesized using the general procedure as for (**10b**). The yellow solid was purified by flash chromatography using 15 : 85 ethyl acetate–hexanes gave the products as yellow oils, yield **14b** 23 mg, 50%; **16b** 25 mg, 7%. Ratio of isomers **14b** : **16b** is 7.5 : 1.

16b ^{1}H NMR: 0.85 (t, 3H), 1.56 (sex, 2H), 2.27 (s, 3H), 3.24 (br s, 2H, -CH_2-NH-), 5.77 (br s, 1H), 6.06 (s, 1H); ^{13}C NMR: 11.16, 22.23, 23.61, 43.19, 99.10, 159.92, 164.10, 167.53; HRMS: $(M + H)^+$ = 202.09245; calcd, 202.09552 (^{37}Cl).

14b ^{1}H NMR: 0.89 (t, 3H), 1.71 (sex, 2H), 2.27 (s, 3H), 3.07 (d, 2H), 5.63 (br s, 1H), 6.10 (s, 1H); ^{13}C NMR: 11.63, 20.4, 24.08, 53.69, 110.9, 158.0, 169.5, 170.8. HRMS: $(M + H)^+$ = 200.09576; calcd, 200.09552 (^{35}Cl).

2-Chloro-6-methyl-4-(*N*-isobutylamino)pyrimidine (16c) and 4-chloro-6-methyl-2-(*N*-isobutylamino)pyrimidine (14c). Synthesized using the general procedure as for (**10b**). The product was recrystallized from hexane and the isomers separated by flash chromatography using 1 : 3 ethyl acetate–hexanes to give off-white crystals, yield **14c** 101 mg, 41%; **16c** 57 mg, 23%. Ratio of isomers **14c** : **16c** is 1.8 : 1.

14c ^{1}H NMR: 0.88 (d, 6H), 1.81 (m, 1H), 2.23 (s, 3H), 3.18 (t, 2H), 5.32 (br s, 1H, NH), 6.35 (s, 1H); ^{13}C NMR: 20.08, 23.77, 28.30, 48.82, 108.85, 160.97, 162.34, 169.37. HRMS: $(M + H)^+$ = 240.12670; calcd, 240.12682 (^{35}Cl).

16c ^{1}H NMR: 0.90 (d, 6H), 1.81 (m, 1H), 2.27 (s, 3H), 3.04 (br s, 2H), 5.32 (br s, 1H, NH), 6.03 (s, 1H); ^{13}C NMR: 20.07, 23.79, 28.17, 49.14, 98.82, 160.25, 164.23, 167.89. HRMS: $(M + H)^+$ = 240.12504; calcd, 240.12682 (^{35}Cl).

2-Chloro-6-methyl-4-(*N*-propyl-*N*-cyclopropylmethylamino)-pyrimidine (16d) and 4-chloro-6-methyl-2-(*N*-propyl-*N*-cyclopropylmethylamino)pyrimidine (14d). Synthesized using the general procedure as for (**10b**). The brown oil was purified using a speedy column using 1 : 3 ethyl acetate–hexanes to give the isomers as yellow oils, yield **16d** 135 mg, 46%; **14d** 35 mg, 12%. Ratio of isomers **16d** : **14d** is 3.8 : 1.

16d ^{1}H NMR: 0.28 (dd, 2H), 0.46 (dd, 2H), 0.90 (t, 3H), 1.06 (m, 1H), 1.62 (sext, 2H), 2.25 (s, 3H), 3.48 (d, 2H), 3.58 (t, 2H), 6.30 (s, 1H); ^{13}C NMR: 3.55, 9.93, 11.37, 20.79, 23.97, 49.34, 51.81, 107.27, 160.68, 161.71, 168.71.

14d ^{1}H NMR: 0.21 (dd, 2H), 0.46 (dd, 2H), 0.87 (t, 3H), 0.97 (m, 1H), 1.56 (sext, 2H), 2.29 (s, 3H), 3.34 (d, 2H), 3.40 (t, 2H), 6.10 (s, 1H); ^{13}C NMR: 3.58, 9.36, 11.19, 20.29, 23.82, 49.91, 52.17, 99.37, 159.99, 162.80, 166.67.

6-Methyl-2-(*N*-propyl-*N*-cyclopropylmethylamino)-4-(2′,4′,6′-trichloroanilino)pyrimidine (20c) and 6-methyl-4-(*N*-propyl-*N*-cyclopropylmethylamino)-2-(2′,4′,6′-trichloroanilino)pyrimidine (18c). Synthesized using the general procedure as for (**12a**). The pale yellow solid was purified by flash column using 1 : 3 ethyl acetate–hexanes. Yield **20c** 100 mg, 53% as a yellow oil; **18c** 30 mg, 27% as a white solid, mp 130–131 °C.

20c: ^{1}H NMR: 0.21 (m, 2H), 0.44 (m, 2H), 0.82 (t, 3H), 1.06 (m, 1H), 1.56 (m, 2H), 2.19 (s, 3H), 3.38–3.42 (m, 4H), 5.48 (2, 1H), 5.99 (s, 1H), 7.40 (s, 2H); ^{13}C NMR: 3.49, 10.14, 11.47, 20.93, 24.44, 49.18, 51.43, 92.10, 128.30, 132.05, 133.34, 134.58, 161.07, 166.98.

18c : ^{1}H NMR: 0.1 (br s, 2H), 0.45 (br s, 2H), 0.75–1.02 (m, 4H), 1.50 (m, 2H), 2.26 (s, 3H), 3.14–3.26 (m, 4H), 5.82 (s, 1H), 6.60 (br s, 1H), 7.37 (s, 2H); ^{13}C NMR: 3.52, 9.57, 11.29, 20.23, 24.29, 49.75, 51.79, 100, 127.61, 130.65, 134.85, 135.08, 162.16, 165.18.

2,4-Dichloro-5-methylpyrimidine (9). Synthesized using the general procedure as for (**6**) with the exception that the extraction solvent used was dichloromethane. The product is was a white solid or pale yellow oil (at RT), yield 4.69 g, 72%.

Mp 25°C; ^{1}H NMR: 2.32 (s, 3H), 8.4 (s, 1H); ^{13}C NMR: 16.32, 129.63, 158.71, 160.59, 163.05. GCMS: 163.0; calcd 163.0.

2-Chloro-5-methyl-4-(2′,4′,6′-trichloroanilino)pyrimidine (17a). Synthesized using the general procedure as for (**10a**). The product was obtained as an off-white gum. The product was stirred with a hot 1 : 1 ethyl acetate–tetrahydrofuran solution and was filtered giving a cream solid, with only one isomer detected by TLC. The product was not purified by chromatography or recrystallization due to its low solubility in all solvents. Yield 168 mg, 68%.

^{1}H NMR: 2.29 (s, 3H), 7.65 (s, 2H), 8.09 (s, 1H); ^{13}C NMR: 13.09, 114.21, 125.77, 129.02, 133.83, 136.31, 157.06, 157.44, 162.10.

2-Chloro-5-methyl-6-(*N*-propylamino)pyrimidine (17b). Synthesized using the general procedure as for (**10a**). The second isomer was not present by TLC after the reaction. The brown solid was purified using a speedy column using 1 : 3 ethyl acetate–hexanes to give a cream solid, yield **17b** 105 mg, 46%.

^{1}H NMR: 0.96 (t, 3H), 1.64 (m, 2H), 1.96 (s, 3H), 3.46 (dt, 2H), 4.68 (br s, 1H, NH), 7.76 (s, 1H); ^{13}C NMR: 11.33, 12.94, 22.59, 42.86, 111.66, 154.51, 158.77, 162.33. HRMS: $(M + H)^+$ = 186.07984; calcd, 186.07987 (^{35}Cl).

2-Chloro-5-methyl-6-(*N*-isobutylamino)pyrimidine (17c) and 6-chloro-5-methyl-2-(*N*-isobutylamino)pyrimidine (15c). Synthesized using the general procedure as for (**9**). The brown oil was purified using a speedy column using 1 : 3 ethyl acetate–hexanes to give the isomers as yellow oils, yield **17c** 185 mg, 50%; **15c** 25 mg, 6.8%. Ratio of isomers **17c** : **15c** is 7.4 : 1.

17c ^{1}H NMR ($CDCl_3$): 0.82 (d, 6H), 1.80 (m, 1H), 1.88 (s, 3H), 3.20 (t, 2H), 5.18 (br s, 1H, -NH-), 7.61 (s, 1H); ^{13}C NMR: 13.93, 21.00, 28.95, 49.20, 112.92, 154.98, 159.30, 163.39. HRMS: $(M + H)^+$ = 200.09578; calcd, 200.09552 (^{35}Cl).

15c ^{1}H NMR ($CDCl_3$): 0.87 (d, 6H), 1.78 (m, 1H), 2.13 (s, 3H), 3.25 (t, 2H), 5.10 (br s, 1H, –NH–), 8.05 (s, 1H); ^{13}C NMR: 13.95, 20.95, 29.08, 49.86, 104.88, 155.43, 157.54, 164.59. GCMS: 200.1; calcd, 200.1.

2-Chloro-5-methyl-6-(*N*-propyl-*N*-cyclopropylmethylamino)-pyrimidine (17d) and 4-chloro-5-methyl-2-(*N*-propyl-*N*-cyclopropylmethylamino)pyrimidine (15d). Synthesized using the general procedure as for (**10b**). The brown oil product was purified by a speedy column using 10 : 10 : 80 ethyl acetate–chloroform–hexanes, resulting in two yellow oils, yield **17d** 185 mg, 50%. **15d** 25 mg, 7%. Ratio of isomers **17d** : **15d** is 7.5 : 1.

17d ^{1}H NMR: 0.12 (dt, 2H), 0.40 (dt, 2H), 0.78 (t, 3H), 0.90 (m, 1H), 1.50 (sext, 2H), 2.10 (s, 3H), 3.23 (d, 2H), 3.37 (t, 2H), 7.63 (s, 1H); ^{13}C NMR: 4.11, 10.43, 11.51, 18.60, 22.01, 51.56, 54.49, 113.28, 157.97, 159.41, 164.08. GCMS: 239.1; calcd 239.1.

15d ^{1}H NMR: 0.25 (dt, 2H), 0.44 (dt, 2H), 0.88 (t, 3H), 1.03 (m, 1H), 1.59 (sext, 2H), 2.06 (s, 3H), 3.43 (d, 2H), 3.52 (t, 2H), 8.00 (s, 1H); ^{13}C NMR: 4.21, 10.52, 12.08, 15.85, 21.36, 50.10, 52.57, 115.73, 159.37, 161.19, 161.40. GCMS: 239.1; calcd 239.1.

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References

1 P. J. Gilligan, P. R. Hartig, D. W. Robertson and R. Zaczek, Corticotropin-releasing hormone (CRH) receptors and the discovery of selective non-peptide CRH1 antagonists, *Annu. Rep. Med. Chem.*, 1997, **32**, 41–50.
2 M. McLean, A. Bisits, J. Davies, R. Woods, P. Lowry and R. Smith, A placental clock controlling the length of human pregnancy, *Nature Med. (N. Y.)*, 1995, **1**, 460–463.
3 R. Smith, The timing of birth, *Sci. Am.*, 1999, **280**, 68–75.
4 K. Erickson, P. Thorsen, G. Chrousos, D. E. Grigoriadis, O. N. Khongsaly, J. McGregor and J. Schulkin, Preterm birth: associated neuroendocrine, medical, and behavioral risk factors, *J. Clin. Endocrinol. Metab.*, 2001, **86**, 2544–2552.
5 R. Smith, J. Cubis, M. Brinsmead, T. Lewin, B. Singh, P. Owens, E. C. Chan, C. Hall, R. Adler and M. Lovelock, Mood changes, obstetric experience and alterations in plasma cortisol, beta-endorphin and corticotrophin releasing hormone during pregnancy and the puerperium, *J. Psychosom. Res.*, 1990, **34**, 53–69.
6 R. Smith, E. C. Chan, M. Lovelock, T. Lewin, D. Hurt, K. Thornton, Plasma corticotropin releasing hormone immunoreactivity in human pregnancy, *Proceedings of the 30th Annual Meeting of The Endocrine Society of Australia*, Sydney, Australia, 1987.
7 R. Smith, The timing of birth, *Sci. Am.*, 1999, **280**, 68–75.
8 R. Smith, Corticotropin-releasing hormone and the fetoplacental clock: an Australian perspective, *Am. J. Obstet. Gynecol.*, 1999, **180**, S269–271.
9 M. H. Perrin, Y. Haas, J. E. Rivier and W. W. Vale, Corticotropin-releasing factor binding to the anterior pituitary receptor is modulated by divalent cations and guanyl nucleotides, *Endocrinology*, 1986, **118**, 1171–1179.
10 E. B. De Souza, T. W. Lovenberg, D. T. Chalmers, D. E. Grigoriadis, C. W. Liaw, D. P. Behan and J. R. McCarthy, Heterogeneity of corticotropin releasing factor receptors: multiple targets for the treatment of CNS and inflammatory disorders, *Annu. Rep. Med. Chem.*, 1995, **30**, 21–30.
11 H. Asakura, I. H. Zwain and S. S. C. Yen, Expression of genes encoding corticotropin-releasing factor (CRF), type 1 CRF receptor, and CRF-binding protein and localization of the gene products in the human ovary, *J. Clin. Endocrinol. Metab.*, 1997, **82**, 2720–2725.
12 E. C. Chan, J. Falconer, G. Madsen, K. C. Rice, E. L. Webster, G. P. Chrousos and R. Smith, A corticotropin-releasing hormone type I receptor antagonist delays parturition in sheep, *Endocrinology*, 1998, **139**, 3357–3360.
13 P. A. Keller, L. Elfick, J. Garner, J. Morgan and A. McCluskey, Corticotropin releasing hormone: therapeutic implications and medicinal chemistry developments, *Bioorg. Med. Chem.*, 2000, **8**, 1213–1223.
14 P. J. Gilligan, D. W. Robertson and R. Zaczek, Corticotropin releasing factor (CRF) receptor modulators: progress and opportunities for new therapeutic agents, *J. Med. Chem.*, 2000, **43**, 1641–1660.
15 T. W. Lovenberg, D. E. Grigoriadis, D. T. Chalmers, J. R. McCarthy and E. B. De Souza, Corticotropin-releasing factor receptors: inhibitors, subtypes, pharmacology, localization, and their role in central nervous system function, *Curr. Pharm. Des.*, 1995, **1**, 305–316.
16 J. F. Hernandez, W. Kornreich, C. Rivier, A. Miranda, G. Yamamoto, J. Andrews, Y. Tache, W. Vale and J. Rivier, Synthesis and relative potencies of new constrained CRF antagonists, *J. Med. Chem.*, 1993, **36**, 2860–2867.
17 D. W. Schulz, R. S. Mansbach, J. Sprouse, J. P. Braselton, J. Collins, M. Corman, A. Duaiskis, S. Faraci, A. W. Schmidt, T. Seeger, P. Seymour, J. D. Tingley III, E. N. Winston and Y. L. Chen, Heym, CP-154,526 - A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 10477–10482.
18 A. McCluskey, M. Finn, M. Bowman, P. A. Keller and R. Smith, 3,5-Dimethyl-7-hydrazinothiazolo[4,5-*d*]pyradazine-7-thiol: A corticotrophin releasing hormone type 1 receptor agonist, *Aus. J. Chem.*, 2000, **53**, 905–912.
19 J. P. Whitten, Y. F. Xie, P. E. Erickson, T. R. Webb, E. B. D. Souza, D. E. Grigoriadis and J. R. McCarthy, Rapid microscale synthesis, a new method for lead optimization using robotics and solution phase chemistry: Application to the synthesis and optimization of corticotropin releasing factor 1 receptor antagonists, *J. Med. Chem.*, 1996, **39**, 4354–4357.
20 P. A. Keller, M. Bowman, K.-H. Dang, S. P. Leach, J. Garner, R. Smith and A. McCluskey, Pharmacophore development for corticotrophin releasing hormone; new insights into inhibitor activity, *J. Med. Chem.*, 1999, **42**, 2351–2357.
21 J. Garner and A. McCluskey, Regiocontrolled amination of dichloropyrimidines in $LiClO_4$–Et_2O solutions, *Heterocycl. Commun.*, 1999, **5**, 503–508.
22 M. Chebib, D. McKeveney and R. J. Quinn, 1-Phenylpyrazolo[3,4-*d*]pyrimidines; structure-activity relationships for C6 substituents at A1 and A2A adenosine receptors, *Bioorg. Med. Chem.*, 2000, **8**, 2581–2590.
23 H. Li, J. Sutter, R. Hoffmann, In *Pharmacophore Perception, Development, and Use in Drug Design*, O. F. Guner, Ed., International University Line, California, 2000, p. 173.
24 P. Sprague, *Hypothesis Generation in Catalyst*, www.accelrys.com/catalyst/cathypo.html, 30/04/1999.
25 P. W. Sprague, *Perspect. Drug Discovery Des.*, 1995, **3**, 1.
26 The cost analysis of any pharmacophore is a statistical representation of the probability of the pharmacophore being a true representation of the data. This value is the difference (in bits) between the null cost (cost of generating a hypothesis where the error cost is high) and the total cost (actual cost of hypothesis generation) of the hypothesis generated. If the difference between the total cost and the null hypothesis cost is more than 60 bits, there is greater than 90% probability that the model is a true representation of the data. If the difference is 40–60 bits, there is a 75–90% chance that it represents a true correlation of the data. When the difference becomes less than 40 bits, the probability of the hypothesis being a true representation rapidly falls below 50% and if the total − null cost difference is less than 20 bits there is little chance of it being accurate and the training set should be reconsidered.[23] These analyses are the underpinning statistical validation of the visual output as represented in Fig. 2, and is the Catalyst programs' direct measure of the validity of a hypothesis generated. Therefore, when comparing similar pharmacophores (*e.g.* by refinement), it is the null − total cost difference that yields the direct statistical comparison of which is the better pharmacophore, where the greater the difference, the more accurate the model. The greater the difference between the null cost and the total cost the more statistically valid the hypothesis is, and the greater the probability of this model being a true representation of the data. Note, that an analysis such as this does not necessarily produce the lowest cost hypothesis as the best pharmacophore model. This refinement and analysis process was used for the all the pharmacophore models generated.