Pharmacophore Development for Corticotropin-Releasing Hormone: New Insights into Inhibitor Activity

Paul A. Keller,*,† Maria Bowman,§ Kor Hin Dang,† James Garner,‡ Scott P. Leach,† Roger Smith,§ and Adam McCluskey*,‡

Department of Chemistry, The University of Wollongong, Wollongong, NSW 2522, Australia, Medicinal Chemistry Group, Mothers & Babies Health Research Centre, The Department of Chemistry, The University of Newcastle, Callaghan, NSW 2308, Australia, and Department of Endocrinology, Mothers & Babies Health Research Centre, John Hunter Hospital, The University of Newcastle, Newcastle, NSW 2310, Australia

Received January 7, 1999

Corticotropin-releasing hormone (CRH) is an endogenous 41-amino acid peptide involved in a wide ranging series of systems including the brain, the coordination of the body's overall response to stress, and more recently as a crucial initiator in the onset of labor, also known as the placental clock. Although more physiological data on CRH is emerging shedding more light on the processes involved and their integration, the mode of action of the hormone and the postulated binding site(s) remain unknown. Recently, a number of small-molecular-weight ligands have emerged as potent antagonists but, as therapeutics, suffer from a lack of solubility. Additionally, despite a number of exhaustively large patents, the lack of structural diversity with these antagonists has enabled little scope for comprehensive and wide ranging studies into the structure of the binding sites of this hormone. As part of a program investigating new, structurally diverse antagonists and agonists of CRH, we have developed a preliminary pharmacophore based on the known small-molecular-weight ligands as an initial step in our program. This pharmacophore was validated by comparison with some of the compounds we postulated to be active.

Introduction

Corticotropin-releasing hormone (CRH, also known as CRF) is a 41-amino acid hormone[1] of increasing biological significance. CRH has been linked to various conditions including neurological disorders, ranging from anxiety to depression, and a number of physical ailments, encompassing Alzheimer's to anorexia nervosa.[2] However, our interest in this area stems from CRH's observed effects during the onset of human parturition.

The hormonal mechanisms which control the onset of human parturition and labor are presently unknown. Nevertheless, a large body of data, produced to a large extent in our laboratory, suggests that placental production of CRH plays a key role in this process.[3] Placental CRH synthesis and secretion into the maternal circulation increase exponentially as gestation advances.[4,5] Several groups have shown that women in preterm labor have high plasma CRH concentrations compared to gestationally matched controls.[6−9] We have shown that the exponential increase in maternal plasma CRH is linked to a biological clock which determines the length of gestation.[3] Our group has also demonstrated that women undergoing induction of labor are more likely to have successful induction if the levels of CRH are high. From a different perspective, we have shown that women who are present in apparent preterm labor are significantly more likely to deliver preterm if they have high plasma CRH levels.[10]

CRH receptors are members of the 7-transmembrane family and are normally linked via a G-protein to adenylate cyclase.[11] Although several types of receptors have been described[2] (CRH_1, $CRH_{2\alpha}$, $CRH_{2\beta}$, and more recently $CRH_{2\psi}$), the key CRH receptor involved in human parturition is of the type 1.[12] A type 1 receptor antagonist might therefore reasonably be predicted to inhibit the progression of labor; conversely, a type 1 receptor agonist might therefore reasonably be predicted to promote the onset of labor.

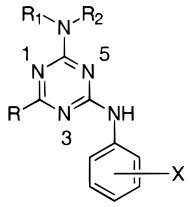
Because of the key role of the type 1 CRH receptor in human parturition, there is an urgent need for low-molecular-weight ligands that are active at this receptor as either agonists or antagonists. Such ligands could not only serve as potential therapeutics but also lead to a greater understanding of the structure and function of the receptor itself. There are numerous peptidic and nonpeptidic antagonists and agonists currently available.[2,14−17] However, several major shortcomings exist with these CRH active ligands: the lack of water solubility registers them as not being suitable as therapeutics,[18] and further, the nonpeptidic ligands could be mostly conceived as belonging to the same basic class of compounds. This lack of structural diversity in active compounds imposes severe limitations on the search for novel leads for antagonists and agonists of CRH, as well as restricting studies on the receptor site structure.

Despite these limitations, we present here a preliminary pharmacophore based on published structures of CRH antagonists, as part of an extensive program aimed at producing structurally diverse therapeutics targeting CRH receptor(s). The validation of the phar-

* Corresponding authors. E-mail: paul_keller@uow.edu.au or amcclusk@mail.newcastle.edu.au.
† The University of Wollongong.
‡ Medicinal Chemistry Group, The University of Newcastle.
§ Department of Endocrinology, The University of Newcastle.

10.1021/jm9900117 CCC: $18.00
Published on Web 06/08/1999

Table 1. Training Set Used in the Generation of the CRH Pharmacophore[20]

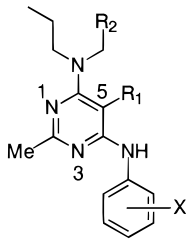


1-12

compd	R	R_1	R_2	X	K_i (nM)[a]
1	Me	$PhCH_2CH_2$	Me	2,4,6-triMe	2100
2	Me			2,4,6-triMe	1050
3	Me	*n*-Bu	*n*-Bu	2,4,6-triMe	490
4	Me	$PhCH_2$	Bu	2,4,6-triMe	1050
5	Me	*n*-Pr	*n*-Pr	2,4,6-triMe	130
6	Me	*n*-Pr	CH_2*c*-Pr	2,4,6-$(OMe)_2$	8000
7	Et	*n*-Pr	CH_2*c*-Pr	2,4,6-triMe	>10000
8	Me	allyl	allyl	2,4,6-triMe	115
9	Me	CH_2*c*-Pr	*n*-Pr	2,4,6-triMe	57
10	Me	Et	CH_2Ph	2,4,6-triMe	1470
11	Me	*n*-Pr	CH_2Ph	2,4,6-triMe	490
12	Me	H	CH_2Ph-4-CF_3	2,4,6-triMe	1690

[a] Inhibition (K_i) values are tested as their ability to inhibit [^{125}I]CRH binding as reported.

Table 2. Training Set Used in the Generation of the CRH Pharmacophore[19]



13-20

compd	R_1	R_2	X	K_i (nM)[a]
13	H	*c*-C_3H_5	2,4,6-triCl	30
14	Me	*c*-C_3H_5	2,4,6-triCl	2.3
15	Et	*c*-C_3H_5	2,4,6-triCl	3.8
16	Me	Et	2,4,6-triCl	2.5
17	H	*c*-C_3H_5	2,4,6-triCl	253
18	H	*c*-C_3H_5	2,4,6-triMe	1390
19	Cl	*c*-C_3H_5	2,4,6-triCl	1.7
20	Br	*c*-C_3H_5	2,4,6-triCl	2.0

[a] Inhibition (K_i) values are tested as their ability to inhibit [^{125}I]CRH binding as reported.

macophore using some of our own compound data suggests consistencies in required structural features.

Pharmacophore Generation

The training set for the pharmacophore development for nonpeptide CRH_1 receptor antagonists is summarized in Tables 1 and 2. There are two major structural classes: the pyrimidine-based structures[19] and the triazine structures.[20] Although additional compounds are available for analysis,[21] the vast majority of these are inactive. So as not to bias the pharmacophore too heavily in any direction, only a selection of the inactive compounds were included in the training set.

The underlying operation of the Catalyst software has already been described in detail.[22−24] Standard parameters were utilized, and the training set was analyzed to generate hypotheses comprising of all/any of the following descriptors: hydrogen bond donors, hydrogen bond acceptors, hydrophobic aliphatic groups, ring aromatic interactions, and positively ionizable groups. This combination of descriptors results in aliphatic groups being treated as normal whereas aromatic rings are mapped as vector (2-point) features instead of the usual centroid single-point feature. Activity data used was as reported using as K_i values for the antagonists as a measure of their ability to inhibit [^{125}I]CRH binding.[19−20]

Table 3. Correlation Coefficient (Predicted Activity versus Actual Activity) for the Three Highest Scoring Hypotheses[a]

hypothesis	correlation coefficient	hypothesis features
1	0.925149	3 hydrophobic groups 1 π-stacking interaction 1 hydrogen bond acceptor
2	0.908083	4 hydrophobic groups 1 hydrogen bond donor
3	0.924639	4 hydrophobic groups 1 hydrogen bond donor

[a] A value of 1.0 denotes precise prediction of activity. Features listed are the interactions defined by Catalyst as necessary for activity.

Results

Previous pharmacophore studies have tended to focus exclusively on the highest scoring hypothesis. While it is generally acknowledged that this hypothesis is the most likely to yield relevant information, there is no reason to exclude nearby hypotheses which are statistically close to the highest scoring result and could also yield useful and relevant information. Therefore, in an attempt to extract maximum information from the pharmacophore, we analyzed the three highest scoring hypotheses. The correlation coefficients (predicted activity versus actual activity) for these pharmacophores are listed in Table 3 as well as the features defined by Catalyst as being necessary for activity. The hypotheses themselves are illustrated in Figure 1.

Analysis of Pharmacophores. Hypothesis 1: Features of this pharmacophore include a π-stacking interaction associated with the aromatic ring and three hydrophobic regions which correspond to an *ortho* and a *para* substituent on this ring (aligned with the chloro groups in the example illustrated in Figure 1) and one *N*-alkyl side. The substitution patterns on the aryl ring are thought to contribute significantly to activity with trichloro substitutions being reported as better than trimethyl substitutions.[19] Our model predicts the necessity for two of these substitutions to be present. Further studies have shown that there is a finite size requirement for the alkyl side chains attached to nitrogen at position 6 of the heterocyclic aromatic ring with the introduction of bulkier groups (e.g., butyl or benzyl groups) resulting in a loss of activity.[20] Our model only maps one of these hydrophobic groups at 2−3 sp^3 bond lengths from the nitrogen atom.

The final feature illustrated is a hydrogen bond acceptor arising from the nitrogen at position 3 of the heterocyclic ring. Previous SAR studies have noted the importance of this feature with examples showing a complete loss of antagonist activity with the absence of this hydrogen-bonding site.[19]

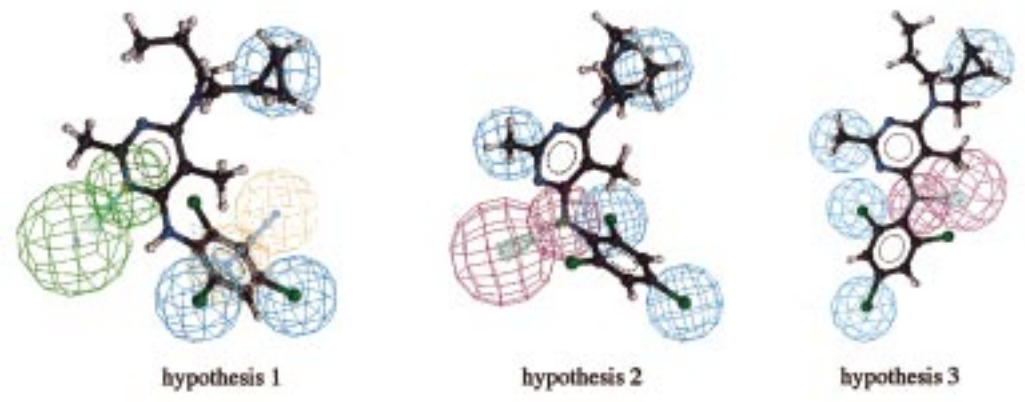


Figure 1. Three highest scoring hypotheses generated for the CRH_1 pharmacophore. The example overlaid on all three hypotheses is compound **14** (see Table 2). Hydrophobic regions are highlighted by blue spheres; orange spheres represent π-stacking interactions including the projected point of interaction; hydrogen bond acceptors are represented by green spheres with the projected interaction illustrated by a cone; similarly, projected interactions of hydrogen bond donors are shown by a cone within magenta spheres.

Hypothesis 2: This model also defines the same hydrophobic features as in hypothesis 1 (two aryl substitutions and an *N*-alkyl side chain) but predicts an additional factor attached to the 2 position of the heterocyclic ring. Although previous SAR studies have not detailed the importance of this feature, it appears that this region cannot tolerate groups much larger than a methyl. For example, the presence of an ethyl group in this position results in a significant loss of activity.[20]

Also noteworthy with this hypothesis is the inclusion of the anilino nitrogen as a hydrogen bond donor. This feature has already been noted as significant as *N*-methyl compounds have been tested and shown to lose over 100-fold in activity.[19]

Hypothesis 3: This hypothesis generates a different combination of the features discussed above. They include the anilino nitrogen as a hydrogen-bonding site, two hydrophobic sites, *ortho* and *para*, attached to the aryl ring, a hydrophobic substituent at position 2 of the heterocyclic ring, and a hydrophobic *N*-alkyl chain of 2−3 sp^3 bond lengths from the nitrogen atom.

Comparison of Hypotheses. There are significant similarities between all three hypotheses. The aryl ring in all three cases has the *ortho* and *para* substituents present. Noteworthy is that this *ortho* substituent is always orientated in the opposite direction to the linking anilino NH. A further common feature is the presence of the *N*-alkyl side chains; in all cases only one side chain was noted as being significant, and this was calculated to be optimal at 2−3 sp^3 bond lengths from the nitrogen. It is clear that variations in this hydrophobic region can be significant, with the previously mentioned SAR studies showing a maximal size possible before observable losses in activity. However, a qualitative observation of the different mappings of compounds from the training set onto the pharmacophores shows significant variations in the conformation of these side chains with no apparent correlation to, or effect on, the predicted activity.

Another feature of the hypotheses illustrated is the regularity of the relative conformation the aryl ring compared to the pyrimidinal ring in all three cases. Despite the availability of any conformation within a 20-kcal/mol radius of the calculated lowest energy conformation, if the same training set compound is overlaid on the pharmacophores from hypotheses 1−3, the angles between the aromatic rings are 104.5°, 104.5°, and 111°, respectively.

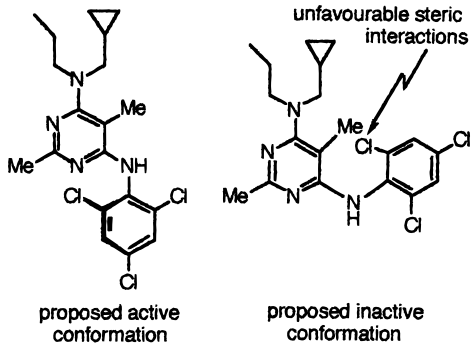


Figure 2. Compound **14** (Table 2) showing the conformation of ligand proposed as being necessary for activity.[19] This conformation has the aryl ring underneath and orthogonal to the heterocyclic ring.

The relative conformation of the two aromatic rings in the different hypotheses is noteworthy. Previous studies have predicted an active conformation with the anilino nitrogen orthogonal to and below the heterocyclic aromatic ring (Figure 2).[19] This prediction arose from SAR studies that showed the absence of the heterocyclic 5-substitution giving rise to a loss of activity, and this was attributed to the substituent forcing a conformation such that the aryl ring swung down more toward the pyrimidinal nitrogen at position 3. An analysis of our models shows hypothesis 1 and 2 with the orientation of the aryl ring swinging up such that there is interaction between the substituent at position 5 and the aromatic ring. Further, hypothesis 1 has the projected π-stacking interaction from the aryl ring pointing toward this substituent, although it should be noted that this projected π-interaction could equally point in the opposite direction. However, hypothesis 3 predicts a relative conformation of the two aromatics closer to the predicted active conformation in Figure 2. The precise orientation necessary for activity cannot be distinguished from these pharmacophore models and would require further refinement in future pharmacophore and SAR studies.

A further significant similarity between all three hypotheses is the concentration of features at one end

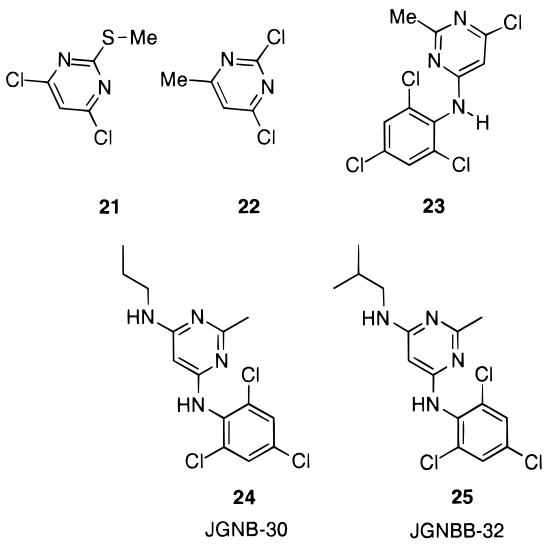


Figure 3. New CRH_1 antagonists generated and tested in our laboratory.

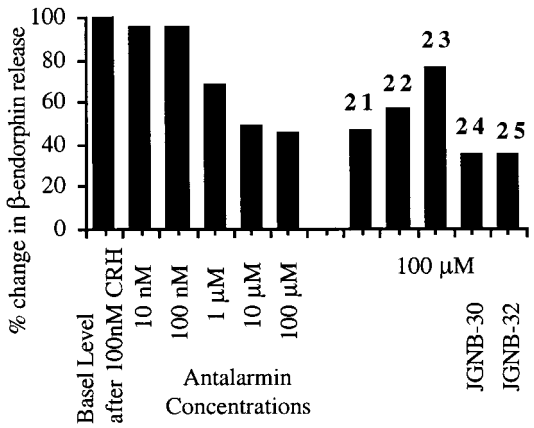


Figure 4. CRH type 1 antagonist activity: left, effects of antalarmin on CRH (10 nM) stimulation of β-endorphin in cultured cells at different concentrations; right, effects upon addition of the synthesized compounds. The antagonistic effects of compounds **21**−**25** were recorded at 100 μM.

of the pharmacophore. This corresponds to the region occupied by the aryl ring, anilino nitrogen, and pyrimidine nitrogen in our training set compounds. This suggests a predominance of the probable interactions necessary for biological effect is clustered in this region and highlights the significance of having the correct conformation of these features for maximal activity.

Combined Pharmacophore Model. As part of the process of validating our generated pharmacophores, we have utilized published SAR results in direct comparison to features predicted to be necessary for activity within our pharmacophore models. We have not restricted ourselves to a single model but have extended our analyses to encompass the three highest scoring hypotheses. Significant duplication of features between these models has been illustrated. However, some molecular components have not appeared in all of the pharmacophore models analyzed, although studies have shown them to be probably necessary for activity. For example, no single model predicted the three features, i.e., an *ortho* aryl substituent, the anilino nitrogen, and the pyrimidinal nitrogen, as concurrently being significant for activity. Additionally, the question concerning the precise conformation needed for activity has not been fully resolved. Therefore, to maximize the predictive possibilities of our model, we decided to utilize all three models in a 'combined pharmacophore' for all future studies.

New CRH Antagonists. A series of compounds were synthesized (see Figure 3) and tested in our laboratory for CRH_1 activity (Figure 4). The agonist/antagonist activity of these molecules was assessed with a β-endorphin (ACTH) stimulation assay in AtT20 cells. These cells are cultured and then stimulated with 10 nM CRH. The increase in β-endorphin levels above the basal level is recorded; then test compounds are added to the stimulated cells, which are incubated, and the level of β-endorphin is recorded. A decrease in β-endorphin levels (<100%) is indicative of antagonist activity, and an increase in β-endorphin levels (>100%) is indicative of agonist activity. Antalarmin, a known CRH type 1 antagonist, is used as the control. It should be noted that the CRH-stimulated β-endorphin release reported below has been normalized to 100%, with the decrease reported relative to the stimulated level.

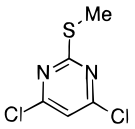
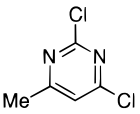
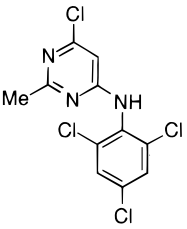
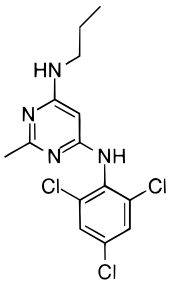
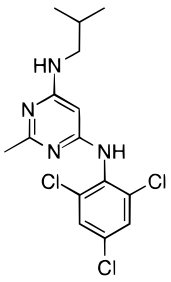
Figure 4 shows that a 100 μM concentration of antalarmin results in a 54% decrease in CRH-stimulated β-endorphin release, typical of its antagonistic effects. Similarly the CRH type 1 antagonistic effects of compounds **21**−**25** are clearly illustrated with decreases by 54%, 43%, 23%, 64%, and 65%, respectively, for each compound.

Table 4 summarizes the results from the superposition of these new antagonists with the three highest scoring hypotheses generated. The conformational energy calculated for each is given, along with the calculated fit[25] and estimated activity. The features which map onto the hypotheses are listed, and Figure 5 illustrates an example of each compound mapped onto one of the hypotheses.

For both **21** and **22**, the fit calculated is only moderate, and as a consequence, a poor estimate of activity is obtained. This arises from the size of these molecules which are approximately one-half that compared to the compounds used to generate the pharmacophores. Consequently, there are features of the pharmacophores which are completely missed by overlaying these compounds. However, a qualitative analysis of overlays shows a good alignment of three of the features in the area in that **21** and **22** occupy. Compound **21** has a tested antagonistic activity 11% better than compound **22**. Although it is only hypothesis 3 that predicts a slightly better activity between these two compounds, the effects of the small molecular size relative to the pharmacophore size probably has too large an effect for the precise prediction of relative activity. However, the strong antagonist effects of these small compounds suggest that they possess many of the molecular features required for activity. This supports the finding that a cluster of pharmacophore features in the region occupied by these compounds possibly accounts for the major interactions between antagonists and receptor.

Compounds **24** and **25** have essentially the same antagonistic activity with 64% and 65% decreases in simulated β-endorphin release, respectively. Both examples map all the features for all three pharmaco-

Table 4. Summary of Results for Compounds **21**−**25** and Their Interactions with the Pharmacophore Hypotheses Generated[a]

compd	conformational energy (kcal·mol^{-1})	fit	estimated activity K_i (nM)	matching mapping features
	hypo 1: 0.00	6.99	8.1×10^5	chloro substituent (aryl ring), methyl substituent (hydrophobic region), π-stacking
	hypo 2: 1.30	3.41	3.4×10^7	*ortho* and *para* chloro substituents (aryl ring), methyl substituent (hydrophobic region)
	hypo 3: 0.01	5.45	7.7×10^6	*ortho* and *para* chloro substituents (aryl ring), methyl substituent (hydrophobic region)
21				
	hypo 1: 0.01	7.42	3.0×10^5	π-stacking, *ortho* and *para* chloro substituents (aryl ring)
	hypo 2: 0.01	4.24	5.0×10^6	*ortho* and *para* chloro substituents (aryl ring)
	hypo 3: 0.01	4.98	2.3×10^7	*ortho* and *para* chloro substituents (aryl ring)
22				
	hypo 1: 6.52	9.89	1000	*ortho* and *para* chloro substituents (aryl ring), π-stacking, pyrimidine nitrogen H-bond donor
	hypo 2: 8.19	8.44	310	*ortho* and *para* chloro substituents (aryl ring) map, anilino nitrogen present as H-bond donor, hydrophobic region (methyl substituent)
	hypo 3: 8.19	9.72	410	*ortho* and *para* chloro substituents (aryl ring), π-stacking interaction, anilino nitrogen present as H-bond donor
23				
	hypo 1: 1.158	12.35	3.6	maps all features of the pharmacophore
	hypo 2: 13.00	10.52	2.6	maps all features of the pharmacophore
	hypo 3: 13.82	12.28	1.1	maps all features of the pharmacophore
24				
	hypo 1: 6.65	12.30	4.0	maps all features of the pharmacophore
	hypo 2: 8.62	10.36	3.8	maps all features of the pharmacophore
	hypo 3: 15.95	12.09	1.8	maps all features of the pharmacophore
25				

[a] Values reported for the 'fit' function are generated by Catalyst and reflect directly the number of features of the test compounds and how well these features map onto the pharmacophores. The values reported for the 'estimated activity' are given as K_i, reflecting the input data of the training set. Comparisons of activity between **21**−**25** and the estimated fit generated by Catalyst are relative only, due to the different assay used for **21**−**25** compared to that used for the compounds in the training set.

phores and consequently give rise to excellent fit and estimated activity values, as would be expected for good antagonists. It should be noted that the predicted activity is in the same order of magnitude for each hypothesis.

The fit and estimated activity for these compounds are approximately the same for all three hypotheses, and this suggests that although some features of these hypotheses are different, they probably all contribute as important features of the combined pharmacophore. This result is confirmed by **23** which shows a similarity between its fit and estimated activities for all three hypotheses.

Compound **23** has a 23% decrease in simulated β-endorphin release, and this decrease in antagonist activity relative to **24** and **25** is reflected in the fit and estimated K_i values when it is overlaid on all three pharmacophores. This decrease in expected activity arises from **23** missing a hydrophobic group on all pharmacophores. This would normally correspond to the *N*-alkyl side chain present in the training set compounds. The compound maps three features for each of the hypoth-

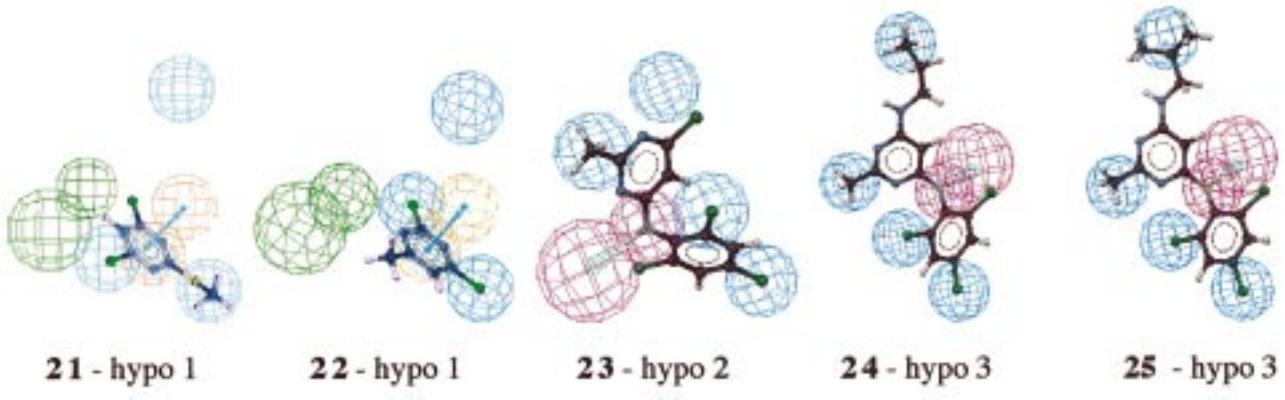


Figure 5. Compounds **21**−**25** overlaid on selected hypotheses. Features illustrated are coded as defined in Figure 1.

eses, and therefore, any differences in fit arise from how well the molecule overlays onto each of the pharmacophore features.

A comparison of the estimated K_i values of **23** with **24** and **25** shows a relative correlation between predicted K_i values and actual activity. Although these activities are 2 orders of magnitude different, a direct comparison of absolute antagonist effects is not possible due to the difference in assays between the data used to generate the pharmacophores and the data generated in our laboratory.

Conclusions

A preliminary pharmacophore for CRH_1 antagonists has been generated and validated based upon new antagonists developed in our laboratories and by comparison with published SAR studies. For our studies, we have used the three highest scoring hypotheses generated, and although many of the features predicted necessary for activity are comman in all three, the features which only mapped to one or two of these hypotheses were shown to still be significant. This gave rise to a combined hypothesis model, which was substantiated by comparison with both published SAR studies and five new antagonists developed in our laboratory. The combined pharmacophore suggested that for substantial activity, a clustering of features in one region is required. The conformation of these features within the pharmacophore required for optimal activity requires further investigation, and these pharmacophore studies are currently underway.

Experimental Section

AtT20 Cell Cultures. AtT20 mouse anterior pituitary tumor cells were grown and subcultured in Dulbecco's modified Eagle's medium (DMEM) (4500 mg of glucose/L, with glutamine) (Gibco, Grand Island, NY) buffered with Hepes and $NaHCO_3$ (BDH Chemicals) at pH 7.4, supplemented with 10% fetal bovine serum (Cytosystems, NSW, Australia), 5% horse serum (Gibco), and penicillin−streptomycin (100 IU/mL, 100 mg/mL). Cells were plated in 24-well plates (nunclon-22, falcon-23) at an initial density of 50 000 cells/well and were used 3 days after subculturing (~60% confluency).

ACTH Release Experiments. The cells were washed with incubation medium (DMEM containing bovine serum albumin (0.2%, w/v) (Sigma Chemical Co. Ltd., St. Louis, MO) and then incubated for 90 min at 37 °C in a humidified atmosphere of 5% CO_2 in air. The supernatant was removed, and cells were treated with 0.5 mL of incubation medium containing the specified drug together with 10 nM hCRH (Peninsula Laboratories, Belmont, CA). Following a 60-min incubation the supernatant was collected and frozen at −80 °C until assayed.

Pharmacophore Generation. Standard parameters were utilized and the training set was analyzed to generate hypotheses comprising of all/any of the following descriptors: hydrogen bond donors, hydrogen bond acceptors, hydrophobic aliphatic groups, ring aromatic interactions, and positively ionizable groups. Activity data used was as reported using K_i values for the antagonists as a measure of their ability to inhibit [^{125}I]CRH binding.[19,20] Overlays of most compounds with pharmacophores was completed using the Best Fit command within Catalyst, utilizing a 20-kcal·mol^{-1} limit for conformational energy. Overlays using **22** utilized the Fast Fit command within Catalyst due to a known program bug which does not allow the Best Fit command to be used with conformationally inflexible compounds.

Acknowledgment. We gratefully acknowledge the generous financial support from the ARC, NH&MRC, and Clive and Vera Ramaciotti Foundation.

References

(1) Vale, W.; Spiess, C.; Rivier, C. Characterization of a 41-Residue Ovine Hypothalic Peptide that Stimulates Secretion of Corticotropin and β-Endorphin. *Science* **1981**, *213*, 1394−1397.
(2) De Souza, E. B.; Lovenberg, T. W.; Chalmers, D. T.; Grigoriadis, D. E.; Liaw, C. W.; Behan, D. P.; McCarthy, J. R. Heterogeneity of Corticotropin Releasing Factor Receptors; Multiple Targets for the Treatment of CNS and Inflammatory Disorders. *Annu. Rep. Med. Chem.* **1995**, *30*, 21−30.
(3) McClean, M.; Bisits, A.; Davies, J.; Lowry, P.; Smith, R. A Placental Clock Controlling the Length of Human Pregnancy. *Nature Med.* **1995**, *1*, 460−463.
(4) McClean, M.; Walters, W. A.; Smith, R. *Obstet. Gynecol. Surv.* **1993**, *48* (4), 209−225.
(5) Smith, R.; Chan, E.-C.; Bowman, M. E.; Harewood, W. J.; Phippard, A. F. Corticotropin Releasing Hormone in Baboon Pregnancy. *J. Clin. Endocrinol. Metab.* **1993**, *76* (4), 1063−1067.
(6) Stalla, G. K.; Bost, H.; Stalla, J.; Kaliebe, T.; Dorr, H. G.; Pfeiffer, D. Human Corticotropin Releasing Hormone During Pregnacy. *Gynecol. Endocrinol.* **1989**, *3*, 1−10.
(7) Wolfe, C. D. A.; Patel, S. P.; Campbell, E. A.; Linton, E. A.; Anderson, J.; Lowry, P. J. Plasma Corticotropin Releasing Factor in Normal Pregnacy. *Br. J. Obstet. Gynaecol.* **1988**, *95*, 997−1002.
(8) Petraglia, F.; Giardino, L.; Coukos, G.; Calza, L.; Vale, W.; Genazzani, A. R. Corticotropin Releasing Hormone and Parturition: Plasma and Amniotic Fluid Levels and Placental Binding Sites. *Obstet. Gynecol.* **1990**, *349*, 423−426.
(9) Sasaki, S.; Shinkawa, O.; Margioris, A. N.; Liotta, A. S.; Sato, S.; Murakami, O. Immunoreactive Corticotropin Releasing Hormone in Human Plasma During Pregnacy, Labour and Delivery. *J. Clin. Endocrinol. Metab.* **1987**, *64*, 224−229.
(10) McClean, M.; Thompson, D.; Zhang, H. P.; Brinsmead, M.; Smith, R. Corticotropin Releasing Hormone and β-Endorphin in Labour. *Eur. J. Endocrinol.* **1994**, *131*, 167−172.
(11) Chen, R.; Lewis, K. A.; Perrin, M. H.; Vale, W. Expression Cloning of a Human Corticotropin-Releasing-Factor Receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8967−8971.

(12) Asakura, H.; Zwain, I. H.; Yen, S. S. C. 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* (8), 2720−2725.
(13) Koerber, S. C.; Gulyas, J.; Lahrichi, S. L.; Corrigan, A.; Craig, A. C.; Rivier, C.; Vale, W.; Rivier, J. Constrained Corticotropin-Releasing Factor (CRF) Agonists and Antagonists with *i*-(*i*+3) G*lu-Xaa-DXbb-Ly*s Bridges. *J. Med. Chem.* **1998**, *41*, 5002−5011.
(14) Lovenberg, T. W.; Grigoriadis, D. E.; Chalmers, D. T.; McCarthy, J. R.; De Souza, E. B. *Curr. Pharm. Des.* **1995**, *1*, 305.
(15) Schulz, D. W.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Duaiskis, A.; Faraci, S.; Schmidt, A. W.; Seeger, T.; Seymour, P.; Tingley, J. D., III; Winston, E. N.; Chen, Y. L.; Heym. CP-154,526 − A Potent and Selective Nonpeptide Antagonist of Corticotropin Releasing Factor Receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10477−10482.
(16) Hernandez, J.-F.; Kornreich, W.; Rivier, C.; Miranda, A.; Yamamoto, G.; Andrews, J.; Tache, Y.; Vale, W.; Rivier, J. Synthesis and Relative Potencies of New Constrained CRF Antagonists. *J. Med. Chem.* **1993**, *36*, 2860−2867.
(17) McCluskey, A.; Finn, M.; Smith, R.; Bowman, M.; Keller, P. A. Development of a CRH Type 1 Receptor Agonist: MFNA-48A. Unpublished results.
(18) As an example, in sheep trials, the known CRH_1 antagonist antalarmin was administered as a 1:1 polyethoxylated castor oil/ethanol solution; see: Chan, E. C.; Falconer, J.; Madsen, G.; Rice, K. C.; Webster, E. L.; Chrousos, G. P.; Smith, R. A Corticotropin-Releasing Hormone Type 1 Receptor Antagonist Delays Parturition in Sheep. *Endocrinology* **1998**, *139* (7), 3357−3360.
(19) Chen, C.; Dagnino, R., Jr.; De Souza, E. B.; Grigoriadas, D. E.; Huang, C. Q.; Kim, K.-I.; Lui, Z.; Moran, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. Design and Synthesis of a Series of Non-Peptide High-Affinity Human Corticotropin-Releasing $Factor_1$ Receptor Antagonists. *J. Med. Chem.* **1996**, *39*, 4358−4360.
(20) Whitten, J. P.; Xie, Y. F.; Erickson, P. E.; Webb, T. R.; De Souza, E. B.; Grigoriadas, D. E.; McCarthy, J. R. Rapid Microscale Synthesis, a New Method for Lead Optimisation Using Robotics and Solution Phase Chemistry: Application to the Synthesis of Corticotropin-Releasing $Factor_1$ Receptor Antagonists. *J. Med. Chem.* **1996**, *39*, 4354−4357.
(21) Reference 18, Supporting Information.
(22) Catalyst v3.0; Molecular Simulations, Inc., Burlington, MA.
(23) Sprague, P. W. Automated Chemical Hypothesis Generation and Database Searching with Catalyst. *Perspect. Drug Discovery Des.* **1995**, *3*, 1−20.
(24) Kaminski, J. J.; Rane, D. F.; Snow, M. E.; Weber, L.; Rothofsky, M. L.; Anderson, S. D.; Lin, S. L. Identification of Novel Farnesyl Protein Transferase Inhibitors Using Three-Dimensioanl Database Searching Methods. *J. Med. Chem.* **1997**, *40*, 4103−4112.
(25) The calculated fit function within the Catalyst program is a weighted value based on the number of features generated for each pharmacophore. Essentially, the higher the value the better the fit. Since our three hypotheses all contain five features, direct comparisons can be made.

JM9900117