Dihydropyridopyrazinones and Dihydropteridinones as Corticotropin-Releasing Factor-1 Receptor Antagonists: Structure−Activity Relationships and Computational Modeling

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The CRF antagonist pharmacophore is a heterocyclic ring bearing a critical hydrogen-bond acceptor nitrogen and an orthogonal aromatic ring. CRFR1 antagonists have shown a 40-fold and 200-fold loss in potency against the CRFR1 H199V and M276I mutant receptors, suggesting key interactions with these residues. We have derived a two component computational model that correlates CRFR1 binding affinity within the reported series to antagoinst/H199 complexation energy and M276 hydrophobic contacts.

Introduction

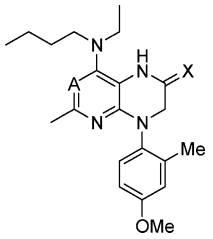
Corticotropin releasing factor (CRF[a]), a 41 amino acid peptide first isolated in 1981,[1] is the primary regulator of the hypothalamus-pituitary-adrenal (HPA) axis, coordinating the endocrine, behavioral, and autonomic responses to stress.[2−5] It has been postulated that hypersecretion of CRF may be involved in affective disorders including depression and anxiety.[6−13] Patients suffering from depression have elevated levels of CRF in the cerebral spinal fluid (CSF). These levels are reduced when the depression symptoms have been successfully treated.[10,14−16] Additionally, a small molecule CRFR1 antagonist, CP-154,526, has been shown to block the behavioral effects of exogenously administered CRF in rat models of anxiety.[17−19] Moreover, an open-labeled clinical trial studying the effects of CRFR1 antagonist R121919 in depressed patients demonstrated reductions in depression and anxiety scores.[20] This evidence suggests that small molecule antagonists of CRFR1 would serve as a treatment for anxiety-related disorders and/or depression.

We recently reported the synthesis, structure−activity relationships (SARs), and in vivo properties of 3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones as CRFR1 antagonists.[21] During the course of this investigation, we discovered some interesting trends in the binding affinity of various analogs within this and closely related series. In this paper we demonstrate the use of molecular modeling to help explain the differences in binding affinity that were observed and develop this as a predictive tool to help guide further SAR studies.

Structure−Activity Relationships

To develop the SARs for this series of small molecules, we evaluated the compounds in a CRFR1 binding assay by measuring displacement of [^{125}I]Tyr-*o*-CRF from rat frontal cortex homogenate. As seen in Table 1, pyridopyrazinone analog **1a** is a very potent CRFR1 antagonist with an IC_{50} of 0.92 nM. Reduction of the amide carbonyl (X= O → X = H_2) in the right-hand ring led to a 4.6-fold loss in potency (**1b**, IC_{50} = 4.2 nM). Even more dramatically, when the pteridinone analog (A = CH_2 → A = N) of **1a** was evaluated, we observed a 100-fold loss in activity (**1c**, IC_{50} = 95 nM). However, when the amide carbonyl of the pteridinone analog was reduced, some of the potency was regained (**1d**, IC_{50} = 11.7 nM).

Table 1. Binding Affinities for Pyridopyrazinone/Pyridopyrazine vs Pteridinone/Pteridine



cmpd	A	X	mean rCRF$_1$ IC_{50}[a] (nM)
1a	CH	O	0.92 ± 0.29
1b	CH	H_2	4.2 ± 0.76
1c	N	O	95 ± 5
1d	N	H_2	11.7 ± 2.0

[a] IC_{50} values (n = 2 or 3) ± SEM.

To determine if this trend holds true for other analogs in this series, we examined several other sets of compounds for binding potency (Table 2), varying the 8-amino side chain as well as the 4-aryl groups. When a pyridyl substituent was attached to the 4-position of the pyridopyrazinone, the potency of the analog was maintained (**2a**, IC_{50} = 0.82 nM). Reduction of the pyrazinone carbonyl lead to a 2.7-fold loss in potency (**2b**, IC_{50} = 2.2 nM). Again, a more drastic loss of potency was observed for the pteridinone analog (**2c**, IC_{50} = 60 nM), while the reduced pteridine analog was more potent (**2d**, IC_{50} = 9.9 nM) than the pteridinone.

When a less lipophilic methoxy group is added to the 8-amino side chain, as in compounds **3a**−**d**, the overall potency is much

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[a] Abbreviations: CRFR1, corticotropin releasing factor-1 receptor; HPA, hypothalamus-pituitary-adrenal; CRF, corticotropin releasing factor; CSF, cerebral spinal fluid; SAR, structure−activity relationship; CE, complexation energy; CP-154,526, *N*-butyl-*N*-ethyl-7-mesityl-2,5-dimethyl-7*H*-pyrrolo-[2,3-d]pyrimidin-4-amine hydrochloride; R121919, 3-(6-methoxy-4-methyl-pyridin-3-yl)-2,5-dimethyl-*N*,*N*-dipropylpyrazolo[1,5-a]pyrimidin-7-amine.

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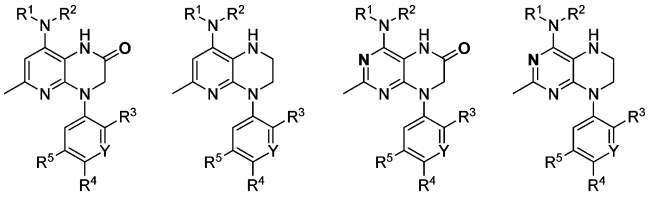
Table 2. Binding Affinities for Pyridopyrazinone/Pyridopyrazine vs Pteridinone/Pteridine



a b c d

cmpd	R^1NR^2	R^3	Y	R^4	R^5	mean $rCRF_1$ IC_{50}[a] (nM)
2a	EtNBu	OMe	N	OMe	H	0.82 ± 0.20
2b	EtNBu	OMe	N	OMe	H	2.2 ± 0.11
2c	EtNBu	OMe	N	OMe	H	60 ± 10
2d	EtNBu	OMe	N	OMe	H	9.9 ± 2.6
3a	$MeOCH_2CH_2NPr$	Me	CH	OMe	OMe	43 ± 2
3b	$MeOCH_2CH_2NPr$	Me	CH	OMe	OMe	87 ± 6
3c	$MeOCH_2CH_2NPr$	Me	CH	OMe	OMe	3113 ± 384
3d	$MeOCH_2CH_2NPr$	Me	CH	OMe	OMe	210 ± 34
4a	EtNBu	Me	CH	CN	H	3.3 ± 1.0
4b	EtNBu	Me	CH	CN	H	3.5 ± 0.4
4c	EtNBu	Me	CH	CN	H	573 ± 56
4d	EtNBu	Me	CH	CN	H	102 ± 10
5a	EtNBu	Me	CH	COMe	H	5.8 ± 0.6
5b	EtNBu	Me	CH	COMe	H	8.2 ± 0.5
5c	EtNBu	Me	CH	COMe	H	713 ± 145
5d	EtNBu	Me	CH	COMe	H	172 ± 28

[a] IC_{50} values (n = 2 or 3) ± SEM.

worse compared to the more lipophilic **1a**−**d** analogs, but the trend above still holds. Pyridopyrazinone **3a** (IC_{50} = 43 nM) is 2-fold more potent than pyridopyrazine **3b** (IC_{50} = 87 nM) and 72-fold more potent than pteridinone **3c** (IC_{50} = 3113 nM). Pteridinone **3c** is 15-fold less potent than the pteridinone **2d** (IC_{50} = 210 nM).

To reduce the electron density of the lower aryl ring of these analogs, an electron withdrawing group was attached to the 4-aryl ring *para* to the anilinic nitrogen (−CN for analogs **4a**−**d** and −COMe for analogs **5a**−**b**). In these cases the potency of the pyridopyrazinones was similar to that of the pyridopyrazines (compare **4a**, IC_{50} = 3.3 nM, to **4b**, IC_{50} = 3.5 nM; and compare **5a**, IC_{50} = 5.8 nM, to **5b**, IC_{50} = 8.2 nM). However, the difference in potency between the pyridopyrazine analogs and the pteridinone analogs was even more pronounced (compare **4a**, IC_{50} = 3.3 nM, to **4c**, IC_{50} = 573 nM; and compare **5a**, IC_{50} = 5.8 nM, to **5c**, IC_{50} = 713 nM). This trend in potency between analogs **a**−**d** was fairly consistent within a variety of sets of analogs in this series. We next set out to develop a computational model to help explain the observed binding trends and to potentially serve as a predictive tool for other analogs in this series.

Several pharmacophore models of CRFR1 antagonists have been reported.[22−24] The dihydropyridopyrazinone and dihydropteridinone based compounds fit within the established model, but it was apparent that subtle SAR effects could not be predicted with pharmacophoric methods. The SAR suggests that the electronics of the bicyclic core and the pendant aryl ring play a critical role in their interaction with the CRF1 receptor. There are two immutable features of the CRFR1 pharmacophore that could potentially be modulated by the electronic character of the antagonist; a hydrogen bond acceptor that is generally a nitrogen in a heteroaromatic ring and a closely spaced aromatic moiety. The proximity and chemical nature of these two features led us to the hypothesis that a critical interaction was formed with either a charged residue (lysine, arginine) or an aromatic residue with a hydrogen bond donor (tryptophan, histidine, tyrosine). Mutagenesis studies have identified two residues in the transmembrane region of CRFR1 that interact with the amino pyrimidine based CRF antagonists.[25] Mutation of H199 and M276 to their corresponding residues in the related CRF-2 receptor subtype, valine and isoleucine, reduced the potency of known CRFR1 anatagonist NBI 27914 40-fold and 200-fold, respectively. Based on these results, we postulated that the binding energy of the dihydropyridopyrazinone and dihydropteridinone series of CRFR1 antagonists is derived from a direct interaction with H199 and additional contributions from contact with M276. The H199 interaction consists of a hydrogen bond between the sp2 nitrogen in the core scaffold and the protonated histidine nitrogen in addition to a $\pi-\pi$ stack with the pendant aromatic ring (Figure 1).

Figure 1. Overlay of the B3LYP/6-31G* minimized inhibitor/imidazole complexes. Compounds **1a**−**d** are in green, **2a**−**d** are in cyan, **3a**−**d** are in magenta, **4a**−**d** are in yellow, and **5a**−**d** are in salmon.

If the antagonists are forming a direct interaction with the imidazole side chain from H199, one might expect a correlation between the interaction energy of the antagonist/histidine complex and the experimental IC_{50} values. The interaction energies between the imidazole moiety of H199 and the series of antagonists reported here were evaluated with ab initio methods as described in the Experimental Section. The model system used in this study consisted of the compounds listed in Table 2 in complex with 4-methyl imidazole. There are four possible orientations of the 4-methyl group of the imidazole ring moiety relative to the core scaffold of the antagonist. At the outset it was not possible to predict which was preferred, so four complexes were evaluated for each antagonist. The initial geometries of the antagonist/imidazole complexes were generated starting from the B3LYP/6-31G* minimized antagonist and 4-methylimidazole structures. The complexes were then built by placement of the 4-methylimidazole with one of its nitrogens within 2.5 Å of the antagonist sp2 nitrogen closest to the pendant aryl ring. The centroids of 4-methylimidazole ring and the pendant aryl ring were aligned, then the planes of the two rings were made parallel and separated by 3 Å. These initial geometries were minimized with MMFF94s and used as the starting geometries for the ab initio minimizations. Each complex was fully minimized with no constraints.

From the minimized complexes, the complexation energy (CE) was calculated using

$$CE = E_{complex} - (E_{antagonist} + E_{imidazole})$$

where $E_{complex}$ is the B3LYP/6-31G** energy of the complex and $E_{antagonist}$ and $E_{imidazole}$ are the energies of the antagonist and 4-methylimidazole, respectively. There appeared to be no relationship between the complexation energies and IC_{50} values

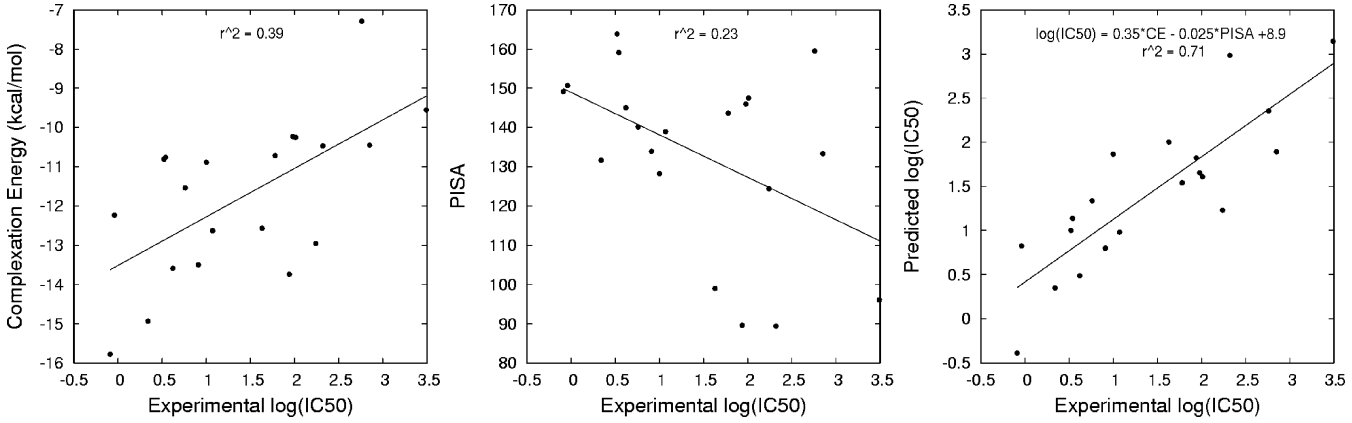


Figure 2. (A) Correlation between the calculated complexation energy of the antagonist/4-methylimidazole complex and experimental IC_{50}. (B) Experimental IC_{50} versus PISA. (C) Experimental IC_{50} versus predicted IC_{50} calculated from the model.

for three out of four of the possible orientations, however, there did appear to be a modest correlation from the 4-methylimidazole orientation shown in Figure 1, resulting in the equation with an R^2 of 0.16 (data not shown). Because the predicted interaction between the antagonists and imidazole involves both a hydrogen bond and a $\pi-\pi$ interaction, it is likely that B3LYP does not accurately model the energetics of the system. A higher level of theory that incorporates electron correlation and diffuse functions may serve as a better model for the hydrogen bonding and $\pi-\pi$ stacking in this system.[26] Thus, energies were calculated at the LMP2/6-311G**++ level of theory. This did in fact result in a more robust model (Figure 2A), giving the equation with an R^2 of 0.39.

Given that the mutagenesis work had suggested that M276 was involved in antagonist binding, it seemed reasonable to add a hydrophobic component to our model to capture this interaction. The Qikprop[27] set of surface area derived descriptors (SASA, FOSA, FISA, PISA, WPSA) were selected to describe this aspect of compound binding. Model building with these descriptors in combination with CE resulted in a substantially more predictive two component PISA and CE containing model with an R^2 of 0.71 (Figure 2C). The correlation between PISA alone and log(IC_{50}) was weak (Figure 2B) and PISA was found to be uncorrelated with CE ($R^2 = 0.083$, data not shown). Because the PISA descriptor is a measure of the π component of the solvent accessible surface area, it is consistent with a predicted hydrophobic interaction with M276 with an aromatic portion of the compound. The combined model is interpreted to capture both the interaction with H199 (CE) and M276 (PISA).

Conclusions

The dihydropyridopyrazinone and dihydropteridinone derived compounds reported here possess the two key pharmacophoric elements of CRFR1 antagonists; a hydrogen bond acceptor and pendant aryl ring. As such, they all bind to CRFR1, but with affinities that range from subnanomolar to low micromolar. The SAR observed within the two series suggests a sensitivity to the electronic nature of the pendant aryl ring as those compounds with electron withdrawing groups tended to be less potent. The electronics of the core also play a role, presumably though modulation of the hydrogen bond acceptor. This is demonstrated by the loss of potency upon reduction of the carbonyl of the pyridopyrazinone series and a further loss of potency in the equivalent pteridinone analogs. These observations coupled with reported effects of the H199V and M267I mutations on NBI 27914 led to the hypothesis that specific interactions between H199, M276, and the antagonists may exist. In an attempt to test this hypothesis, a computational model was built to evaluate the interaction between the imidazole side chain of H199 as well as the putative hydrophobic contribution of M276. An initial single component model containing only the interaction energy between 4-methyl-imidazole and the antagonist showed a trend between increasing energy and weaker binding affinity, but the correlation was modest. The predictive ability of the model was significantly enhanced by the inclusion of the PISA descriptor, which captures the hydrophobic character of the aromatic portions of the antagonist. Our interpretation of these results is that the combination of the energetic and PISA descriptors results in a reasonable model of the antagonist interaction with H199 and M276 within the dihydropyridopyrazinone and dihydropteridinone series. We are currently exploring whether this model can be extrapolated to a more diverse set of CRFR1 antagonists.

Experimental Section

Biology. Rat CRF Receptor Binding Assay. Frozen rat frontal cortex (source of CRFR1) or frozen porcine choroid plexus (source of CRFR2) were thawed rapidly in assay buffer containing 50 mM Hepes (pH 7.0 at 23 °C), 10 mM $MgCl_2$, 2 mM EGTA, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, 0.005% Triton X-100, 10U/mL bacitracin, and 0.1% ovalbumin and homogenized. The suspension was centrifuged at 32 000 *g* for 30 min. The resulting supernatant was discarded and the pellet resuspended by homogenization in assay buffer and centrifuged again. The supernatant was discarded, and the pellet was resuspended by homogenization in assay buffer and frozen at −70 °C. On the day of the experiment, aliquots of the homogenate were thawed quickly, and the homogenate (25 μg/well rat frontal cortex or 10 μg/well porcine choroid plexus) was added to the ligand (150 pM ^{125}I-ovine-CRF for CRFR1 binding or 100 pM ^{125}I-sauvagine for CRFR2 binding) and drugs in a total volume of 100 μL assay buffer. The assay mixture was incubated for 2 h at 21 °C. Bound and free radioligand were then separated by rapid filtration using glass fiber filters (Whatman GF/B, pretreated with 0.3% PEI) on a Brandel Cell Harvester. Filters were then washed multiple times with ice cold wash buffer (PBS w/o Ca^{2+} and Mg^{2+}, 0.01% Triton X-100 (pH 7.0 at 23 °C)). Nonspecific binding was defined using 1 μM DMP696 in the CRFR1 binding assay and 1 μM α-helical CRF (9−41) in the CRFR2 binding assay. Filters were then counted in a Wallac Wizard gamma counter.

Computational. Starting geometries of the antagonist structures and 4-methyl imidazole were generated from 2D structures using the ligprep protocol implemented in the Maestro[29] modeling package. These structures were then minimized in Macromodel using the MMFF94s force field with a constant dielectric of 1.0. The minimized geometries were used for the construction of the

antagonist/imidazole complexes. The centroids of the antagonist pendant aromatic ring and 4-methyl imidazole were aligned, and the plane of the two rings was made parallel and separated by 3 Å. The imidazole ring was then rotated on the axis defined by the two ring centroids until the methyl group was in the desired location and one of the two nitrogens within hydrogen-bonding distance to the antagonist acceptor nitrogen. The imidazole tautomer was adjusted so the protonated nitrogen hydrogen bonded to the antagonist acceptor nitrogen. These complexes were then minimized as above. The minimized geometries were used as the input structures for optimization at the B3LYP/6-31G** level of theory in vacuum using the Jaguar[30] program. Single point energies were calculated from the B3LYP/6-31G** optimized geometries at the LMP2/6-311G**++ level of theory.

The SASA, FOSA, FISA, and PISA descriptors were calculated from the optimized antagonist geometries using the Qikprop(27) program. QSAR models were generated using the Strike module within Maestro with $\log(IC_{50})$ as the dependent variable and CE, SASA, FOSA, FISA, and PISA as independent variables. Multiple linear regression was used to generate an optimal two component model, which is shown in Figure 2C.

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Supporting Information Available: Characterization data for all compounds. This information is available free of charge via the Internet at http://pubs.acs.org.

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