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Discovery of NBI-77860/GSK561679, a potent corticotropin-releasing factor (CRF_1) receptor antagonist with improved pharmacokinetic properties

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ABSTRACT

Antagonists of the corticotropin-releasing factor (CRF) neuropeptide may prove effective in treating stress and anxiety related disorders. In an effort to identify antagonists with improved physico-chemical properties a new series of CRF_1 antagonists were designed to substitute the propyl groups at the C7 position of the pyrazolo[1,5-*a*]pyrimidine core of **1** with heterocycles. Compound (*S*)-**8d** was identified as a high affinity ligand with a pK_i value of 8.2 and a functional CRF_1 antagonist with pIC_{50} value of 7.0 in the in vitro CRF ACTH production assay.

Corticotropin-releasing factor (CRF) is a 41 amino acid residue C-amidated peptide that is secreted by cells of the paraventricular nucleus of the hypothalamus in response to stressful stimuli and is the key mediator of an organism's response to stress. Secretion of CRF causes release of adrenocorticotropin-releasing hormone (ACTH) from corticotrophs in the anterior pituitary via binding to the CRF_1 receptor, a member of the class B family of G-protein coupled receptors. The stress-induced release of ACTH, in turn, acts at the adrenal gland and results in the secretion of glucocorticoids which feed back at the level of the pituitary and the hypothalamus to attenuate the further release of ACTH and CRF, respectively. This hormone loop is known as the hypothalamic-pituitary adrenal (HPA) axis and is tightly regulated in mediating the stress response.[1]

The CRF_1 receptor is localized to a number of tissues and brain sites, notably the hypothalamus, cerebral cortex, pituitary, adrenals, testes, placenta, and gastrointestinal tract.[2] In the years since the discovery of the CRF_1 receptor, a large body of evidence has accumulated that links hyperactivity of the CRF system to a number of disease states, in particular depression, anxiety, irritable bowel syndrome (IBS), premature labor, and addiction disorders. Consequently, the search for antagonists to the CRF_1 receptor has been an active area of investigation in drug discovery for more than two decades.[3]

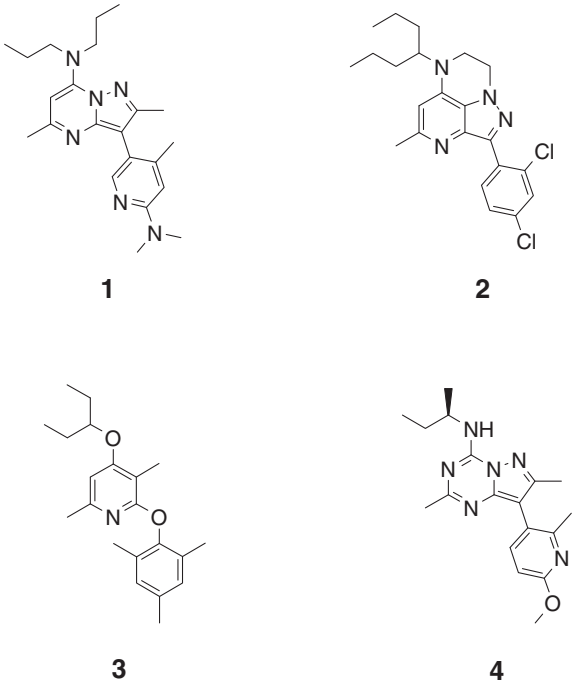

Figure 1. Clinically evaluated CRF_1 receptor antagonists.

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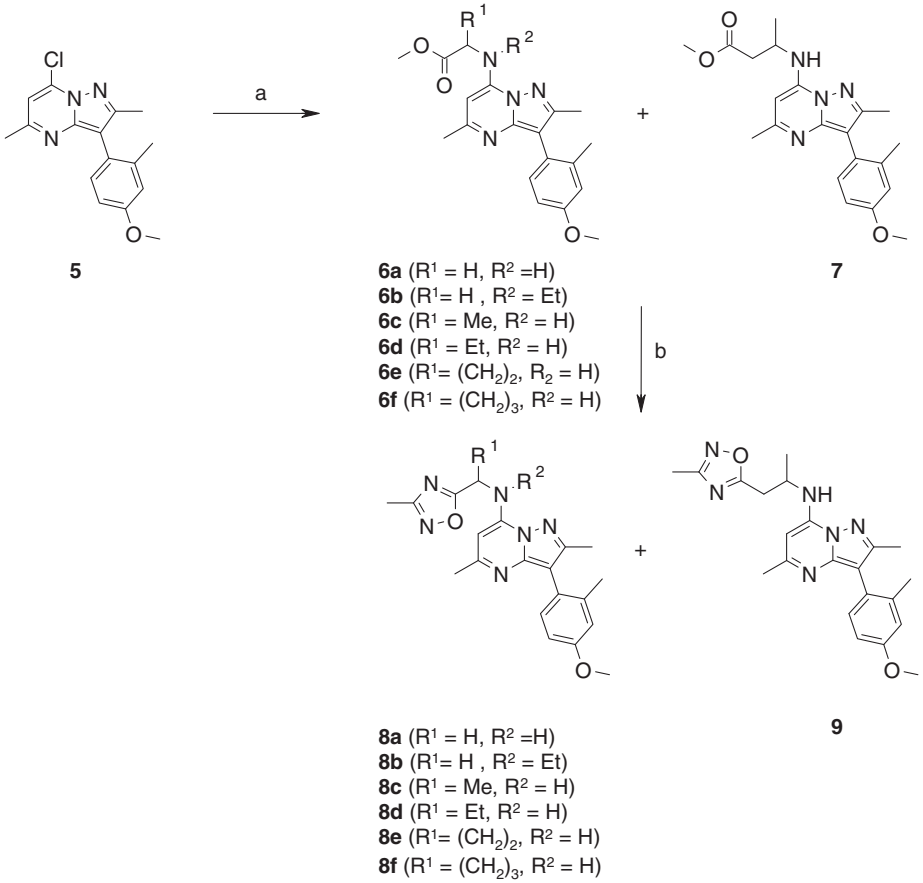
In the first published clinical study of a CRF_1 receptor antagonist, NBI-30775/R121919 (compound **1**, Fig. 1) demonstrated significant improvement in depression and anxiety scores in patients with severe depression without demonstrating any untoward effects in this population. While this trial was not considered proof of concept due to the design of an open label trial, the apparent improvement in both depression and anxiety scores over the 30-day treatment period provided a pharmacological rationale for blocking this system in the primary disease indication. Further clinical studies demonstrated that this particular compound caused a reversible increase in liver enzymes thus precluding any further development.[4] More recent reports of clinical trial results have been mixed, for example, it was reported that NBI-34041/SB-723620 (**2**) inhibited the stress-induced increase of ACTH and cortisol levels in healthy volunteers during a Trier Social Stress Test.[5] On the other hand, CP-316,311 (**3**) and pexacerfont (**4**) failed to show any evidence of efficacy in patients suffering from major depression or generalized anxiety disorder, respectively.[6,7] In spite of these seemingly disparate results, there is still a need for a CRF_1 receptor antagonist that can be used in later stage clinical trials using an extended dosing regimen to address the apparent ambiguities raised by the earlier reported clinical studies.

Typically CRF_1 receptor antagonists are thought to possess physico-chemical properties that are sub-optimal for drugs that target the central nervous system. Previously reported compounds have high hepatic clearance, large volumes of distribution and high plasma protein binding.[8] The main objective of this current work was to lower the lipophilicity of **1** ($\log P = 4.9$)[9] by the introduction of a heterocycle into one of the *N*-alkyl chains at the C7 position of the pyrazolo[1,5-*a*]pyrimidine core structure. Based on calculated $\log P$ values[10] it was anticipated that replacement one methylene unit of the starting propyl side chain with an oxadiazolyl heterocycle would lower the overall calculated $\log P$ by half of a log unit. By decreasing the $\log P$ (<4) it was hoped that the compounds would retain potent CRF_1 receptor functional inhibition while decreasing the volume of distribution (V_{dss} <10 L/kg) and clearance (<33 mL/min kg) in rat pharmacokinetic studies.

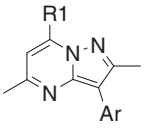
The synthesis of analogs where an 1,2,4-oxadiazolyl heterocycle is introduced into the top region side chain is summarized in Scheme 1. Key intermediate **5** has been previously described[11] and addition of the requisite amino esters provided the intermediates **6a–f** and **7**. Condensation with methylamidoxime and the ester intermediates, in the presence of sodium hydride, followed by thermal cyclization gave the compounds **8a–f** and **9**.

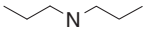
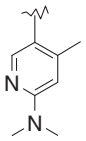
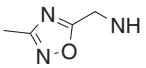
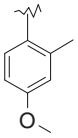
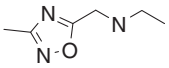
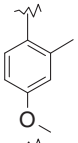
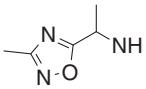
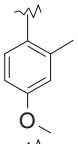
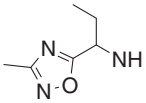
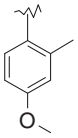
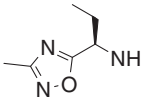
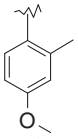
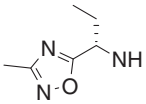
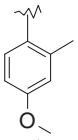
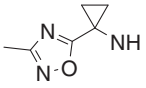
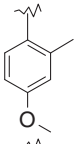
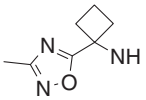
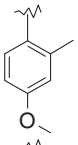
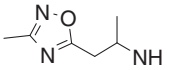
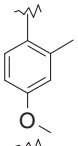
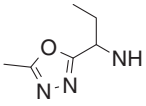
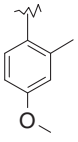
The 1,2,4-oxadiazolyl analogs **8a–f** and **9** were assayed for binding to the recombinant human CRF_1 receptor and selected high affinity compounds were subsequently tested for in vitro metabolic stability in human liver microsomes expressed as maximum estimated bioavailability (max *F*% values) (Table 1).[12] Compound **1** was utilized as a positive control and it has been demonstrated to be a high affinity CRF_1 receptor antagonist with a pK_i value of 8.4 and a calculated $\log P$ value of 4.9. This structure typifies CRF_1 receptor antagonists where most have a branched alkyl 'top region', a core heterocycle and an aromatic bottom region with substitution at the 2 and 4 positions on the aryl ring. It appears that the core heterocycle may play the role of a hydrogen bond acceptor when interacting with the receptor[13] and the substituent at the 2 position of the bottom aromatic ring acts as a steering group[14] for that moiety.



Scheme 1. (a) Amino esters, Et_3N, acetonitrile, 80 °C; (b) MeCNOHNH, NaH, THF, reflux.

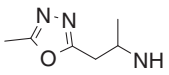
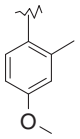
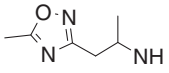
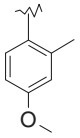
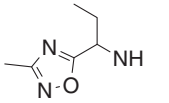
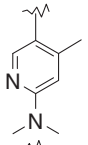
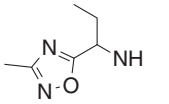
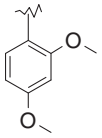
Table 1
In vitro CRF_1 receptor binding, metabolism and calculated log *P*



Compound	R^1	Ar	CRF_1 $pK_i \pm SD$[a]	HLM max $F\%$[b]	Calcd log P[c]
1			8.4 ± 0.2	17	4.9
8a			7.2 ± 0.1	ND	3.3
8b			8.3 ± 0.1	17	4.3
8c			7.6 ± 0.1	38	3.8
8d			8.1 ± 0.1	42	4.3
(*R*)-**8d**			8.0 ± 0.1	63	4.3
(*S*)-**8d**			8.2 ± 0.2	42	4.3
8e			7.5 ± 0.1	43	3.7
8f			7.3 ± 0.1	37	4.1
9			7.5 ± 0.1	32	3.9
14			7.0 ± 0.1	64	2.5

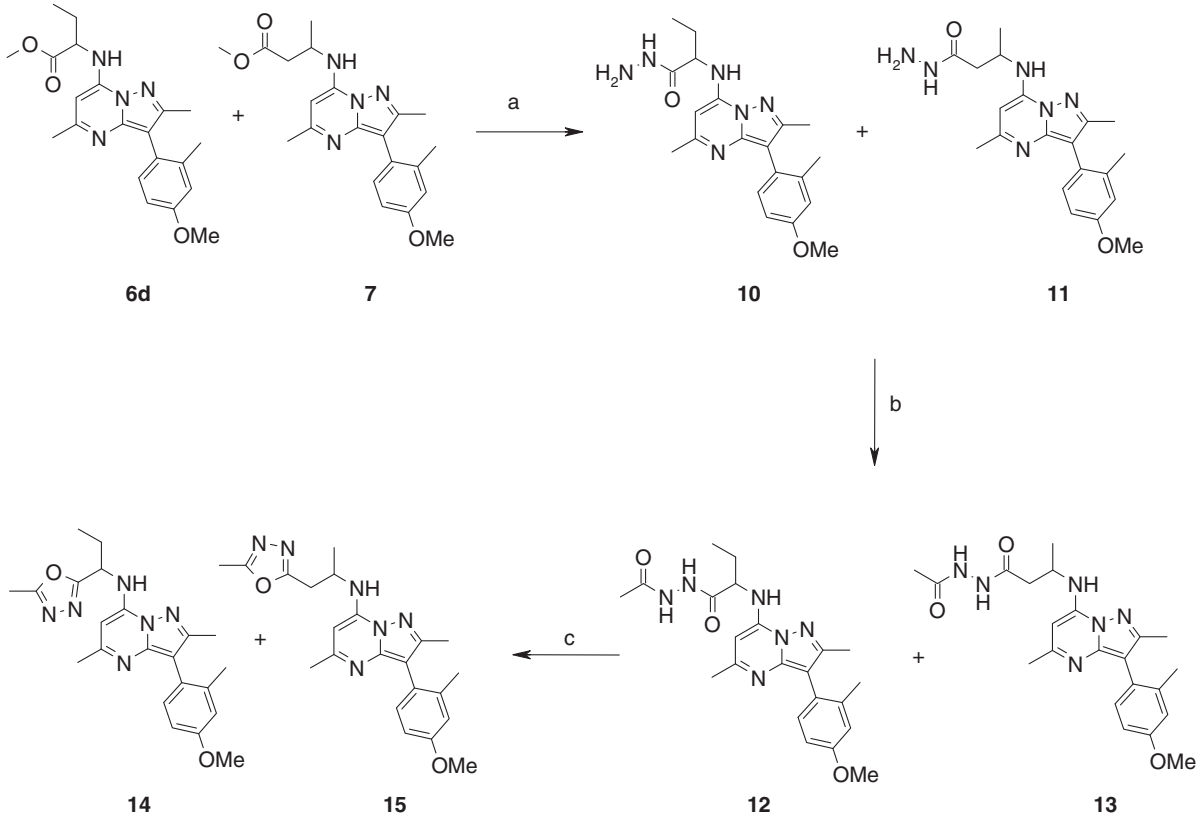
(*continued on next page*)

Table 1 (*continued*)

Compound	R^1	Ar	CRF_1 pK_i ± SD[a]	HLM max F%[b]	Calcd log P[c]
15			6.6 ± 0.1	ND	2.1
19			7.1 ± 0.1	26	3.9
20			7.3 ± 0.1	ND	3.7
21			7.3 ± 0.2	ND	3.6

[a] See Ref. 12.
[b] Maximum estimated bioavailability values were determined in human liver microsomes: ND = not determined.
[c] See Ref. 10.

Introduction of a single 1,2,4-oxadiazolyl methyl amino side chain at the C7 position of the pyrazolo[1,5-*a*]pyrimidine (**8a**) provided a baseline binding affinity (pK_i = 7.2) for the series. It was clear at this point that additional substitution would be required to achieve the objective of increasing the affinity by 10-fold and the ethyl derivative **8b** accomplished this task (pK_i = 8.3). Additional characterization of compound **8b** showed that the decrease in calculated log *P* did not result in a more stable compound by comparing human liver microsomal predicted clearance values. Branching at the α position of 1,2,4-oxadiazolyl methyl substituent (**8c**), which re-introduced a secondary amine NH, did result in a compound with improved metabolic stability (estimated max F% = 38) with comparable CRF_1 receptor binding affinity (pK_i = 7.6). Extending the α position substituent by one methylene



Scheme 2. (a) H_2NNH_2, EtOH, 80 °C, 16 h; (b) acetic anhydride, NEt_3, CH_2Cl_2; (c) *p*-TsCl, DBU, THF, 150 °C, 10 min, microwave.

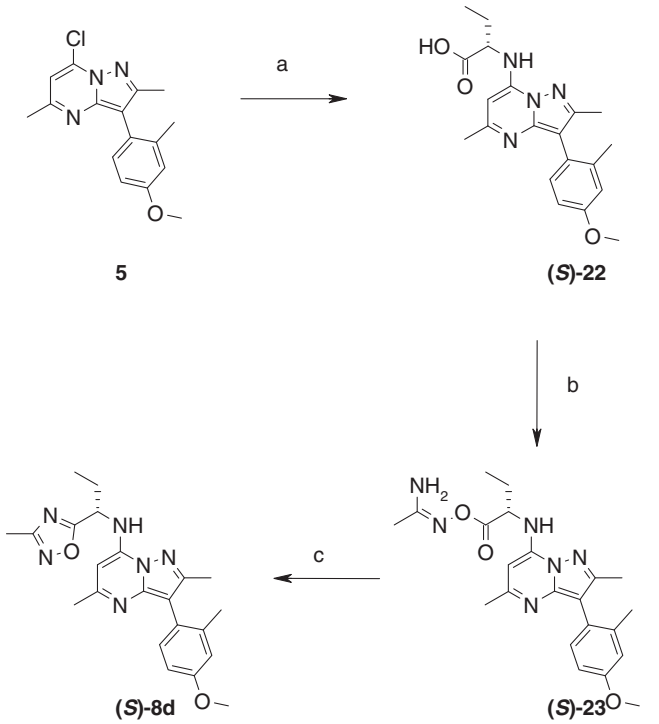
further increases the CRF_1 receptor activity (**8d**, $pK_i = 8.1$) while decreasing the overall lipophilicity as measured by calculated log *P* values as compared to compound **1**. In an effort to maintain this new level of reduced lipophilicity, the branching carbon atoms were also incorporated into cyclopropyl **8e** ($pK_i = 7.5$) and cyclobutyl **8f** ($pK_i = 7.5$) ring structures but it was clear that straight alkyl chains provided higher affinity analogs.

In an effort to explore the nature of the interaction of the heterocycles with the CRF_1 receptor, the orientation of the oxadiazole heterocycle was also varied. Scheme 2 outlines the synthesis 1,3,4-oxadiazole analogs **14** and **15**. Conversion of ester intermediates **6d** and **7** to the hydrazides **10** and **11** was followed by acetylation to provide the intermediates **12** and **13**. Cyclization of the acylhydrazides in the presence of *p*-TsCl and DBU under microwave conditions provided the final compounds.

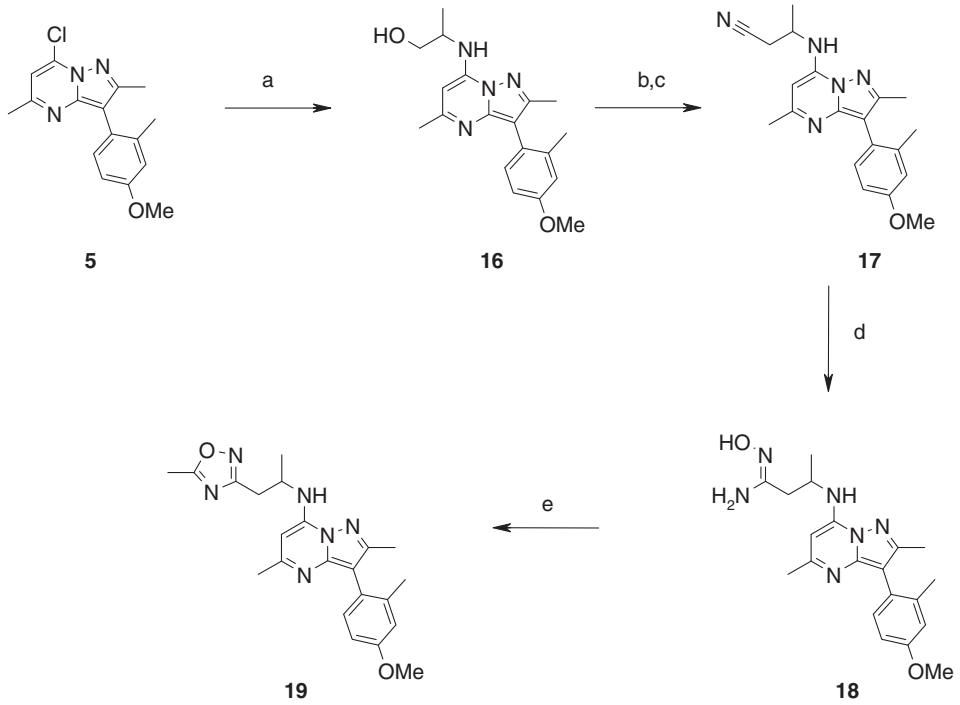
Scheme 3 outlines the synthesis of a 1,2,4-oxaxidiazole analog where the orientation of the ring has been reversed. Due to the required stability of the precursor nitrile (**17**) only the homologated analog **19** was prepared. Analogous to Scheme 1, 2-aminopropanol was used to displace the 7-chloro group of **5** which resulted in **16**. Activation of the hydroxyl group with methanesulfonyl chloride followed by displacement of the mesylate group with sodium cyanide provided **17**. Condensation with hydroxylamine followed by 1,2,4-oxadiazole formation by heating in the presence of dimethylformamide dimethyl diacetal (DMFDMA) provided compound **19**.

Biological evaluation of these additional analogs demonstrated that exchange of the nitrogen and oxygen atoms within the heterocycle, surprisingly, resulted in a 10-fold loss in affinity for the CRF_1 receptor as seen in the 1,3,4-oxadiazolyl analog **14** ($pK_i = 7.0$) compared to **8d**. A final avenue of top region investigation was to further probe the area of chemical space occupied by the newly identified heterocyclic side chain. The homologated analog **9** ($pK_i = 7.5$) was less active but it was appreciated that this new analog may significantly change how the heterocycle interacts with the receptor. As a result, the orientation of the 1,2,4-oxadiazole ring was reversed (compound **19**) and the homologated 1,3,4-oxadiazole analog **15** was also prepared but both proved less active with pK_i values of 7.1 and 6.6, respectively.

Additional analogs of compound **8d** were prepared using the procedure described in Scheme 1 with the objective of maintaining the CRF_1 receptor affinity but with lower lipophilicity. The bottom region aromatic pyridine compound (**20**) was prepared as a direct analog of compound **1** as well as the 2,4-dimethoxyphenyl derivative (**21**) to further drive down the lipophilicity but unfortunately both analogs did not maintain the desired level of CRF_1 receptor affinity ($pK_i = 7.3$).



Scheme 4. (a) (*S*)-2-Aminobutyric acid, $NaHCO_3$, dioxane/H_2O, 100 °C, 14 h; (b) methyl amidoxime, DIC, HOBt, CH_2Cl_2/DMF, −15 °C to room temperature; (c) pyridine, 100 °C.



Scheme 3. (a) 2-Aminopropanol, NEt_3, acetonitrile, 80 °C; (b) MsCl, NEt_3, CH_2Cl_2; (c) NaCN, K_2CO_3, DMF; (d) $NH_2OH{\cdot}HCl$, KOH, EtOH, reflux; (e) DMFDMA, neat, 110 °C.

Compound **8d** proved to be the best combination of CRF_1 affinity and metabolic stability; consequently, the individual enantiomers were prepared following the procedure outlined in Scheme 4. Although the procedure as illustrated in Scheme 1 is convenient, the protocol resulted in complete racemization of the final compound when optically pure amino esters were utilized. To avoid the troublesome ester hydrolysis step (*S*)- and (*R*)-2-aminobutyric acids were substituted for the 2-aminobutyric acid methyl ester in the addition to intermediate **5**. Coupling of the intermediates (*R*)-**22** and (*S*)-**22** with methyl amidoxime, followed by cyclization under mildly basic[15] conditions, provided final products (*R*)-**8d** and (*S*)-**8d** in high enantiomeric purity (>99% ee).[16]

Both enantiomers of **8d** proved to be high affinity CRF_1 receptor antagonists with pK_i values of 8.2 for (*S*)-**8d** and 8.0 for (*R*)-**8d**. Both compounds proved to be metabolically stable (estimated max *F*% = 42% and 63%, respectively) and an experimentally (shake flask) determined log $D_{7.4}$ of 3.8. The compounds were further assayed for in vitro suppression of sauvagine-induced ACTH release in rat anterior pituitary cells[11] and unexpectedly (*S*)-**8d** was 10-fold more active than its enantiomer with pIC_{50} values of 7.0 and 6.0, respectively. As a result of the functional activity, compound (*S*)-**8d** was evaluated in a 24 h rat pharmacokinetic study at an oral dose of 10 mg/kg. Compound (*S*)-**8d** demonstrated good oral bioavailability (66%) and good exposure in both plasma (AUC_{0-24} = 3130 ng h/mL) and brain (1 h brain/plasma ratio = 1.6). A 72 h iv (5 mg/kg) study established that (*S*)-**8d** had moderate clearance (14 mL/min kg) and a volume of distribution at steady state (V_{dss} = 7.5 L/kg) within our target range. In addition, the human plasma protein binding for the compound was 94% as determined by equilibrium dialysis.

In summary, a series of novel and potent CRF_1 receptor antagonists have been synthesized where analogs of **1** have heterocycles replacing alkyl chains in the top region of the molecule which resulted in analogs with reduced overall lipophilicity. The most promising compound, (*S*)-**8d** (NBI-77860/GSK561679), possesses a good pharmacokinetic profile and was selected as a candidate for further preclinical investigation. Results of further preclinical and clinical studies with this compound will be disclosed in future publications.

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References and notes

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16. Enantiomeric excess was determined by normal-phase chiral HPLC using Chiralcel OD columns with hexane/ethanol or hexane/isopropanol as eluant (in the presence of diethylamine as an additive in some cases).