Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4901–4905
Bioorganic & Medicinal Chemistry Letters

Discovery and optimization of novel, non-steroidal glucocorticoid receptor modulators

Nicholas C. Ray,[a,*] Robin D. Clark,[b] David E. Clark,[a] Karen Williams,[a] H. G. Hickin,[a] Peter H. Crackett,[a] Hazel J. Dyke,[a] Peter M. Lockey,[a] Melanie Wong,[a] René Devos,[a] Anne White[a] and Joseph K. Belanoff[b]

[a]*Argenta Discovery Ltd, 8/9 Spire Green Centre, Flex Meadow, Harlow, Essex CM19 5TR, UK*
[b]*Corcept Therapeutics, Inc., 149 Commonwealth Drive, Menlo Park, CA 94025, USA*

Received 20 April 2007; revised 8 June 2007; accepted 10 June 2007
Available online 13 June 2007

Abstract—A virtual screening approach comprising a 3-D similarity search based on known GR modulators was used to identify a novel series of non-steroidal glucocorticoid receptor (GR) antagonists. Optimization of the initial hit to provide potent compounds which exhibit good selectivity against other steroidal nuclear hormone receptors is described.

In the US, major depression is second only to ischemic heart disease as a cause of disease burden, affecting 15 million Americans each year. Major depressive disorder[1] (unipolar depression) is characterized by depressed mood, sleep disturbances (sleeping too much or too little), attenuated concentration and energy levels, and suicidal thoughts. Patients presenting with psychotic major depression (PMD) show the above symptoms and, additionally, psychotic symptoms such as delusions and hallucinations, which are typically auditory. They are also approximately 70 times more likely to commit suicide than the general population. There has been uncertainty in the psychiatric community over whether PMD constitutes a distinct syndrome,[2] although more recent evidence[3] indicates that patients with PMD comprise an endocrinologically distinct subset that is hypercortisolemic. It is hypothesized that excessive activation of the hypothalamic–pituitary–adrenal (HPA) axis leads to the observed psychosis, and that antagonism of glucocorticoid receptors (GRs) in the brain may be beneficial. GR antagonism causes a rapid rise in cortisol by blocking the feedback mechanism that regulates cortisol production, possibly leading to a downregulation of the mineralocorticoid receptor (MR). This may lead to a perturbation of the HPA axis and a subsequent 'resetting' of the HPA's normal rhythm. Mifepristone (RU-486) is an antagonist of both the progesterone receptor (PR) and the GR, and has been demonstrated to improve the psychotic and depressive symptoms observed in PMD.[4] We were therefore interested in identifying a GR-selective antagonist that might show a cleaner profile in vivo.

Virtual screening. 3-D similarity searches were carried out based on three known GR antagonists (Fig. 1) using

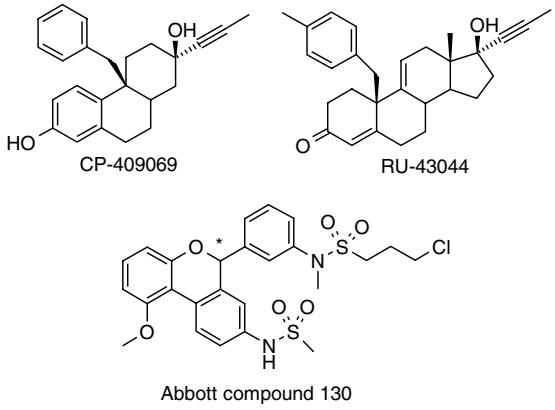


Figure 1. Query structures used for 3-D similarity searches (both enantiomers of the Abbott compound were used and for expediency, the chloroalkyl chain was truncated to a methyl group).

Keywords: Glucocorticoid; Depression; Psychotic; Antagonist.
* Corresponding author. Tel.: +44 (0)1279 645628; fax: +44 (0)1279 645646; e-mail: nick.ray@argentadiscovery.com

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.06.036

the FlexS program.[5] Owing to the computational time required by FlexS, it was necessary to create a focused database of compounds for searching using the properties of the known antagonists as a guide. A number of filtering and clustering steps were used to reduce a collection of ca. 718,000 commercially available screening compounds to a set of 862, which comprised the FlexS database for the searches.

Low-energy reference conformers of the compounds in Figure 1 were identified using conformational searches carried out in Macromodel[6] (Low Mode, MMFFs forcefield, GB/SA solvent model). FlexS was then used in its flexible superposition mode to overlay the database compounds on each of the reference structures. The highest scoring alignments from each of the searches were visually assessed and promising compounds selected for further consideration.

From the four searches, 123 compounds of interest were identified. Following the removal of duplicates and any compounds containing undesirable features, 91 remained. Clustering (0.85 Tanimoto similarity threshold, Daylight fingerprints[7]) to remove redundant compounds provided a set of 78 compounds. This list was refined further by more detailed examination and consideration of supplier reliability to give a final list of 18 compounds from four suppliers. These compounds were obtained and screened in the GR binding assay described below, providing **1** (Fig. 2) with a K_i value of 4.5 μM.

Affinity for GR was determined by ligand binding measuring displacement of [^{3}H]dexamethasone from recombinant baculovirus derived human GR.[8] Functional activity at human GR was determined in SW1353/MMTV-5 cells transfected with a plasmid encoding firefly luciferase located behind a glucocorticoid response element (GRE).[8] GR antagonist activity was measured as inhibition of dexamethasone induced luciferase expression. Selected compounds were tested for GR agonist activity in the same functional screen by performing the assay in the absence of dexamethasone—no compounds tested demonstrated any agonism. Selectivity over the estrogen (ERα), androgen (AR), mineralocorticoid (MR), and progesterone (PR) receptors was determined by ligand binding assays.[9]

2-D substructure and similarity searches were carried out around **1** using the full 718,000-compound database and a further 11 compounds were selected for purchase. The screening of these led to the discovery of **2** (Fig. 2),

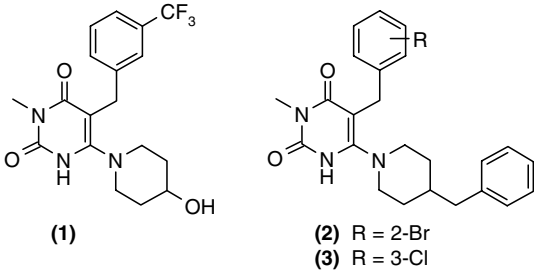


Figure 2. Initial hit from virtual screening.

displaying an activity of 33 and 300 nM in the binding and the functional GR assays, respectively. In selectivity screens, **2** showed 100-fold selectivity over the mineralocorticoid receptor (MR) and 21-fold selectivity over the progesterone receptor (PR).

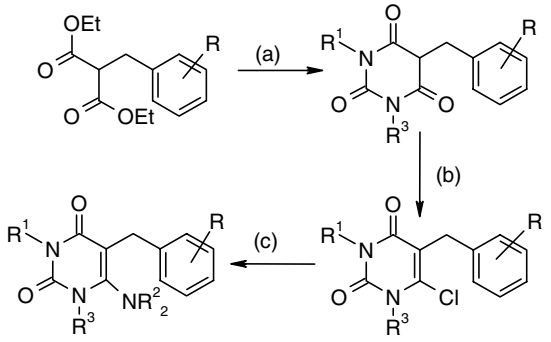
A final round of follow-up searching was carried out around **2** and 27 additional compounds were obtained for screening. This led to the identification of **3** (Fig. 2), the most potent compound found by virtual screening, with a K_i value of 16 nM in the GR binding assay and 135 nM in the GR functional assay.

Synthetic chemistry efforts commenced with examination of the 6-substituent using the route outlined in Scheme 1.

Reaction of the appropriate benzyl malonate with a suitably substituted urea gave the pyrimidinetrione in moderate (R^1 = H) or good (R^1 = Me) yields. Chlorination under forcing conditions gave the 6-chloropyrimidinediones in approximately 50% yields. These were functionalized by treatment with a range of amines, optionally in a microwave synthesizer.

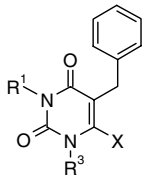
Data for a range of compounds thus prepared are shown in Table 1. All initial investigations were made around the 5-unsubstituted benzyl scaffold, since **4** was shown to have a similar level of potency to **3**. A large lipophilic group such as phenyl or benzyl at the 4-piperidyl position was preferred, since a 4-methyl piperidine analogue was shown to be inactive (**13**). The nature of attachment of the phenyl ring to the piperidyl group was also important: ring fusion (**8**), or chains longer than a methylene spacer (**14**, **15**) resulted in attenuated potencies. Replacement of the methylene with an oxygen (**6**) or a carbonyl (**16**) also reduced potency, indicating that the receptor tolerates only lipophilic moieties at this position. Removal of the methylene spacer completely to give **5** yielded an equipotent compound. A 3-methyl group was not required as illustrated by **11** and **12**, and methylation of the N-1 position was shown to be deleterious (**7**).

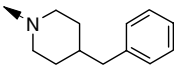
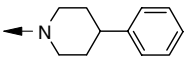
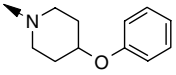
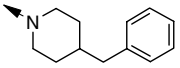
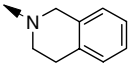
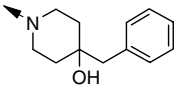
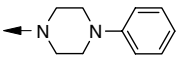
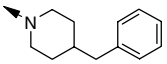
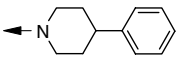
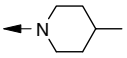
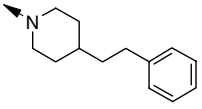
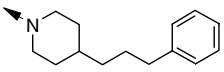
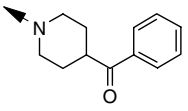
Exploration of the effects of modifying the 5-benzyl substitution pattern showed that once again (Table 2), more polar groups were not tolerated, with 2- and 3-substitution favored over 4-substitution. It is interesting to note



Scheme 1. Reagents and conditions: (a) $R^3HNC(=O)NHR^1$, NaOEt, 85 °C, 18 h; (b) $POCl_3$, 87% H_3PO_4, 100 °C, 3h; (c) $HNR^2{}_2$, $MeOCH_2CH_2OH$, 140 °C, 48 h.

Table 1. GR binding affinities and functional potencies for a range of 6-amino substituted pyrimidinediones



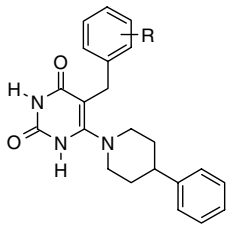
Compound	R^1	R^3	X	GR Binding K_i[a] (nM)	GR Funct. K_i[b] (nM)
4	Me	H		24	100
5	Me	H		20	190
6	Me	H		78	nt
7	H	Me		11%	nt
8	Me	H		23%	nt
9	Me	H		360	nt
10	Me	H		470	nt
11	H	H		19	150
12	H	H		8.1	93
13	Me	H		2%	nt
14	Me	H		154	nt
15	Me	H		18%	nt
16	Me	H		227	nt

[a] Values are means of two experiments. % values are % inhibition at 1 μM.
[b] nt, not tested.

the poorer potency of **23** compared to **22** especially as **20** shows that a large group can be tolerated at the 3-position. Gratifyingly, the more potent compounds also showed good levels of potency in the GR functional assay.

Similar probing of the substitution on the 5-((4-phenyl)-1-piperidyl) ring showed little tolerance for 4-phenyl substitution and no substitution pattern gave improved binding or functional potency over the non-substituted analogue, Table 3. Addition of small (Cl- or MeO-) substituents at the 2- or 3-positions is tolerated, but there appears to be less ability to substitute at the 4-position. Replacement of the 5-((4-phenyl)-1-piperidyl) group by a 5-((3-phenyl)-1-piperidyl) group (**33**) gave a potent compound with poorer functional potency, and by a 5-(3-phenyl)-1-pyrrolidinyl group (**34**) led to attenuated potency, Figure 3.

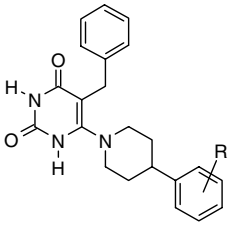
Table 2. Effect of substitution in the 5-benzyl ring on GR binding and functional potencies



Compound	R	GR binding K_i[a] (nM)	GR funct. K_i[b] (nM)
12	H	8.1	93
17	2-Cl	8.7	50
18	3-Cl	9.1	96
19	4-Cl	31	260
20	3-Br	14	65
21	4-CN	37%	nt
22	3-CN	37	nt
23	3-OMe	31%	nt

[a] Values are means of two experiments. % values are % inhibition at 1 μM.
[b] nt, not tested.

Table 3. Effect of substitution in the 5-benzyl ring on GR binding and functional potencies



Compound	R	GR Binding K_i[a] (nM)	GR funct. K_i[b] (nM)
12	H	8.1	93
24	4-OMe	45	nt
25	2-OMe	7.1	88
26	3-Indolyl	40	nt
27	3-Pyridyl	14%	nt
28	3-$NHCOCH_3$	11%	nt
29	4-Cl	103	nt
30	3-Cl	4.9	97
31	4-$NHCOCH_3$	46	nt
32	3-OMe	9.2	158

[a] Values are means of two experiments. % values are % inhibition at 1 μM.
[b] nt, not tested.

The selectivities of key compounds against the other nuclear hormone receptors PR, MR, ER (estrogen receptor) and AR (androgen receptor) were examined, and data for the most potent GR antagonists are shown in Table 4. For all compounds examined, little or no activity against ER or AR was observed. Selectivity of >500-fold against PR was obtained, and of >40-fold against MR.

Compound **12** was examined in a CYP panel and gave no significant cause for concern (25% inhibition of CYP3A4 at 1 μM). Examination of metabolism in a human S9 system showed 94% parent remaining after 30 min. In a p.o. PK study in rats, **12** demonstrated an AUC of 0.6 μg h/ml and C_{max} of 143 ng/ml following a 5 mg/kg dose. Following a 1 mg/kg iv dosing into the rat tail vein, **12** was detected at 45 ng/g in brain tissue and 178 ng/ml in plasma.

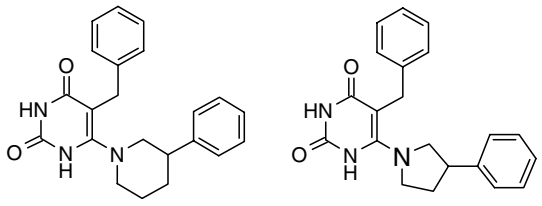


Figure 3. Replacement of (4-phenyl)-1-piperidyl group.

Table 4. Nuclear hormone receptor selectivity data for selected compounds

Compound	Receptor binding K_i[a] (nM)				
	GR	MR	PR	ER[b]	AR[b]
11	19	20%	35%	ia	24%
12	8.1	930	35%	ia	17%
17	8.7	3490	34%	ia	ia
18	9.1	585	7050	ia	ia
25	7.1	298	47%	ia	30%
30	4.9	527	24%	ia	13%
32	9.2	1270	15%	ia	9%

[a] Values are means of two experiments. % values are % inhibition at 10 μM.
[b] ia, inactive.

In summary, GR antagonists have been identified that show significant potencies in both binding and functional GR assays, as well as selectivity over the other nuclear hormone receptors. Of note is that these small molecules are not steroid-like (no fused rings, chiral centers) and structurally distinct from other classes currently described.[10] Work is ongoing to further optimize the pharmacokinetic profile of these leads and will be reported in due course.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Soraya Porres in running some of the biological assays, Colin Bright and Rob Jenkins for the eADME analysis, and Mike Podmore and Russell Scammell for analytical support.

References and notes

1. For descriptions of the classification and etiology of depressive diseases, see (a) *Diagnostic and Statistical Manual of Mental Disorders, 4*th *Edn. (DSM-IV)*, American Psychiatric Association, 2000; (b) Sadock, B.;

Sadock, V. In *Kaplan and Sadock's Synopsis of Psychiatry*; Williams and Wilkins: Lippincott, 2002.
2. Schatzberg, A. F.; Rothschild, A. J. *Am. J. Psychiatry* **1992**, *149*, 733.
3. Belanoff, J. K.; Kalehzan, M.; Sund, B.; Fleming Ficek, S. K.; Schatzberg, A. F. *Am. J. Psychiatry* **2001**, *158*, 1612.
4. Belanoff, J. K.; Rothschild, A. J.; Cassidy, F.; DeBattista, C.; Baulieu, E.-E.; Schold, C.; Schatzberg, A. F. *Biol. Psychiatry* **2002**, *52*, 386; DeBattista, C.; Belanoff, J.; Glass, S.; Kahn, A.; Horne, R. L.; Blasey, C.; Carpenter, L. L.; Alva, G. *Biol. Psychiatry* **2006**, *60*, 1343.
5. Lemmen, C.; Lengauer, T.; Klebe, G. *J. Med. Chem.* **1998**, *41*, 4502, FlexS is developed and distributed by BioSolveIT GmbH. http://www.biosolveit.de.
6. Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
7. Developed and distributed by Daylight Chemical Information Systems, Inc. http://www.daylight.com.
8. Morgan, B. P.; Swick, A. G.; Hargrove, D. M.; LaFlamme, J. A.; Moynihan, M. S.; Carroll, R. S.; Martin, K. A.; Lee, E.; Decosta, D.; Bordner, J. *J. Med. Chem.* **2002**, *45*, 2417.
9. Estrogen receptor: [^{3}H]estradiol, Pan Vera 26467A ERα; androgen receptor: [^{3}H]dihydrotesterone, Pan Vera 24938 AR; mineralocorticoid receptor: [^{3}H]aldosterone, Sf9 cells/recombinant MR; progesterone receptor: [^{3}H]progesterone, Pan Vera 24900 PR.
10. For a recent review of non-steroidal GR antagonists, see Mohler, M. L.; He, Y.; Wu, Z.; Hong, S. S.; Miller, D. D. *Expert Opin. Ther. Patents* **2007**, *17*, 59.