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Docking Study of Corticotropin-Releasing Factor-1 Receptor with Its Antagonists

Sathya Babu†

Abstract

CRFR is involved in the pathophysiology of various disorders including depression, stress, anxiety, post-traumatic stress disorder, and addiction. The discovery of novel and structurally diverse CRF1 receptor inhibitors becomes essential. In this study, we have performed molecular docking of CRF1R with the derivatives of 8-substituted-2-aryl-5-alkylaminoquinolines as CRF1R inhibitors. The antagonist molecules were optimized and docked into the binding site of the receptor. On analysing the docked complexes we have identified that the residues HIS214, THR215, ARG227, ARG1008, LYS1060 and ASP1061 are important in forming hydrogen bond with the inhibitors. Further studies on these residues could reveal important structural features required for the formation of CRF1R-inhibitor complex and thus in the discovery of novel and potent inhibitors.

Keywords: CRF1R, Corticotrophin, Molecular Docking.

1. Introduction

Corticotropin - releasing hormone (CRH) also known as corticotropin - releasing factor (CRF) is a 41 amino acid peptide hormone acts as a neurotransmitter involved in the stress response[1]. It is secreted in the paraventricular nucleus (PVN) of the hypothalamus in response to stress, in peripheral tissues, such as T lymphocytes, and is highly expressed in the placenta[1]. Corticotropin -releasing factor receptor (CRFR) which belongs to G protein-coupled receptors, binds with the corticotropin-releasing hormone[2]. There are two receptors in the family, type 1 and 2, each encoded by a separate gene (CRHR1 and CRHR2 respectively)[3]. CRF1 receptor is abundantly found in the pituitary and is involved in the regulation of ACTH, a key mediator of stress response. Corticotropin-releasing factor receptors (CRFRs) activates the hypothalamic pituitary adrenal axis (HPA axis), which is one of the 2 parts of the fight or flight response to stress[4]. Increased CRH level has been observed in Alzheimer's disease and major depression,[5] and autosomal recessive hypothalamic corticotropin deficiency fatal metabolic consequences including hypoglycemia[1]. Also, chronic activation of CRHR1s by CRH induced by early life stress results in memory deficits and learning impairments and anxiety in adulthood.

CRF in Central nervous system (CNS) plays major role in the pathophysiology variety of disorders including depression, stress, anxiety, post-traumatic stress disorder, and addiction. The involvement of CRF in stress-induced phosphorylation of tau implies a potential link between stress and Alzheimer's disease pathology[6]. In the periphery, CRF is involved in inflammation and cancer and considered to be one of the links between stress and cancer. CRF plays an important role in the development and maintenance of bone cancer pain via activation of neurons.

The discovery of CRF1 receptor antagonists for the treatment of depression or other stress-related disorders has become an important topic in pharmaceutical research. However, the benefits of blocking the CRF2 receptor remain uncertain. Pexacerfont, Antalarmin, CP-316311 and CP-154,526 are the available antagonists for CRF1. Pexacerfont is currently in clinical trials for the treatment of anxiety disorders[7] whereas, in the

Molecular modeling lab, Department of Genetic Engineering, School of Bioengineering, SRM University, SRM Nagar, Kattankulathur, Chennai 603203, India

†Corresponding author : sathyainfo26@gmail.com
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case of Antalaramin only animal studies for the treatment of anxiety, depression and other conditions, but no human trials have been carried out. The results so far have yielded only limited success, and failed to produce an effect comparable with conventional antidepressant drugs[8]. The drug CP-316311 was unsuccessful in a double-blind study for depression[9] and CP-154,526 is under investigation for the potential treatment of alcoholism[10]. Hence, it is apparent that the discovery of structurally diverse CRF1 receptor antagonists and the accumulation of clinical studies for clarifying the role of CRF in humans are essential. In previous studies, we have performed 3D QSAR of CRF1R, which revealed the important physiochemical features required for the formation of CRF1R-inhibitor complex[11,12]. In this study, we have performed molecular docking of corticotropin-releasing factor-1 receptor with its antagonists.

2. Material and Methods

2.1. Preparation of Protein Structure

The crystal structure of human CRF1R (PDB ID – 4K5Y) was downloaded from Protein Data Bank. The structure was prepared using protein preparation tool in biopolymer module of SYBYL. The co crystallized ligand and water molecules were removed. Energy minimization was performed for 100 iterations using Tripos force field, Gasteiger Huckel charge and Powell method.

2.2. Preparation of Ligand Molecules

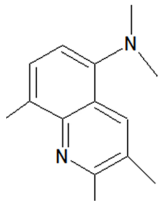
In a study by Takeda et al., 8-substituted-2-aryl-5-alkylaminoquinolines were reported as antagonists for corticotropin-releasing factor-1 receptor (Table 1). The chemical structures of the 23 antagonists were taken from the literature[13] and were sketched using sketch molecule function in SYBYL software[14]. The energy minimization of all the molecules was performed using Tripos force field and atomic charges were assigned using Gasteiger Huckel method.

2.3. Molecular Docking

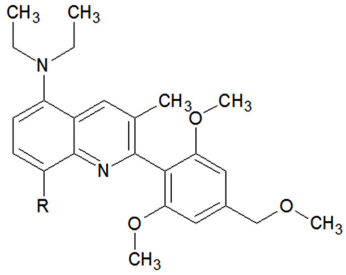
Molecular docking was performed utilizing Surflex dock module of SYBYL. 23 antagonists were docked with CRF1R receptor. The docking algorithm in Surflex dock uses an idealized active site called protomol[15]. The protomol is the representation of intended binding

Table 1. Structures and biological activities (pIC_{50}) of CRFR inhibitors

The indole/azaindole CRFR inhibitor scaffold

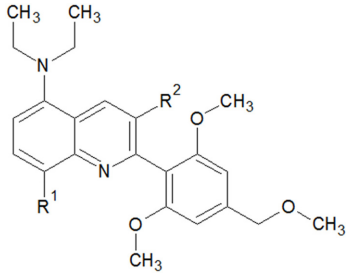


a) Compound 1-8



Compound	R	pIC50 values
1	Methyl	7.102
2	F	6.646
3	Cl	7.208
4	CF_2H	6.383
5	CF_3	6.541
6	CN	6.991
7	Methoxymethyl	6.000
8	OMe	6.959

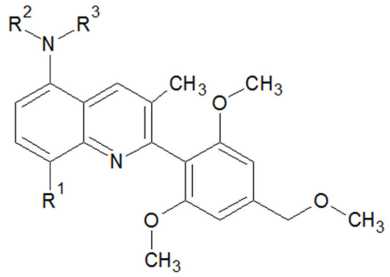
b) Compound 9-15



Compound	R^1	R^2	pIC50 values
9	OMe	H	6.695
10	OMe	F	6.928
11	OMe	Cl	6.842
12	OMe	Ethyl	6.967
13	Me	H	6.735
14	Me	F	6.842
15	Me	Cl	7.091

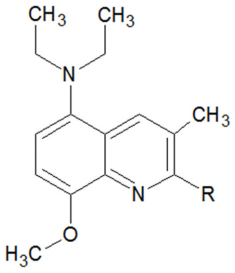
Table 1. Continued

c) Compound 16-20



Compound	R^1	R^2	R^3	pIC50 values
16	OMe	nPr	nPr	6.979
17	OMe	Ethyl	Methoxyethyl	6.407
18	OMe	Isobutyl	Methoxyethyl	6.807
19	Me	nPr	nPr	7.055
20	Me	Ethyl	Methoxyethyl	6.963

c) Compound 21-23



Compound	R^1	pIC50 values
21	2-chloro-4-methoxymethyl-6-methoxyphenyl	7.174
22	2,6-dimethoxy-4-cyanophenyl	6.880
23	2,6-dimethoxy-4-methylphenyl	7.004

site to which the ligand molecules were docked. Two parameters, such as threshold and bloat, determine the extent of a protomol. The protomol was generated using automated mode. Surflex dock uses an empirical scoring function to score the docked ligand conformation which takes into account several terms, including hydrophobic, polar, repulsive, entropic and solvation[16]. To evaluate the docking results, the docking scores are expressed in terms of $-\log_{10}K_d$ units, where K_d represents a dissociation constant of a ligand.

3. Results and Discussion

3.1. Molecular Docking

Molecular docking of CRF1R antagonists with the CRF1R structure was performed. 20 different conformations were generated for each molecule and the best conformation was chosen based on Surflex score and interaction with the residues. The docking score and H-bond forming residues for all the molecules are tabulated in Table 2. The interaction of the antagonists with

Table 2. Docking scores and H-bond interaction of the antagonists

Compound No	Sybyl score	No. of H-bonds	H – Bond Residues
01	5.69	0	-
02	5.61	4	LYS1060
03	5.01	2	ARG227, LYS1060
04	4.90	1	HIS214
05	5.59	0	-
06	6.74	3	ARG1008, LYS1060
07	6.01	2	ARG1008
08	6.35	2	ARG1008
09	5.96	2	ARG1008, LYS1060
10	6.34	3	ARG1008, LYS1060
11	4.51	1	HIS214
12	4.87	1	LYS1060
13	5.39	1	LYS1060
14	5.66	3	ARG1008, LYS1060
15	5.22	1	ASP1061
16	5.72	2	THR215, ARG1008
17	7.46	5	HIS214, LYS1060
18	6.62	2	ARG1008
19	6.95	2	ARG1008, LYS1060
20	7.67	2	ARG1008
21	7.03	1	LYS1060
22	6.39	2	LYS1060
23	7.65	1	LYS1060

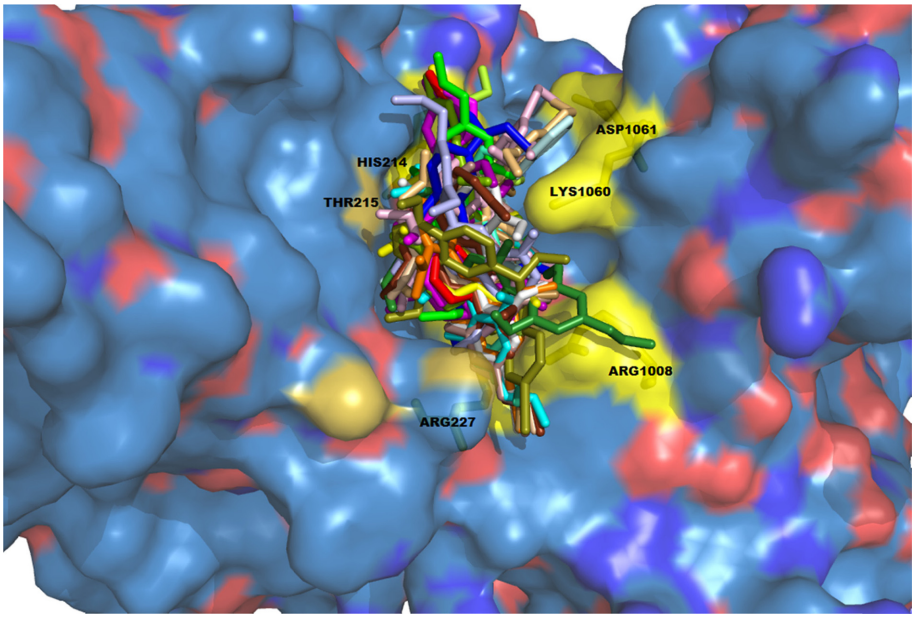


Fig. 1. Docking mode and interaction of CP-199330 with the CysLT1 receptor.

the receptor was represented in Fig. 1. On analyzing the docked complexes, residues HIS214, THR215, ARG227, ARG1008, LYS1060 and ASP1061 were identified to be involving in forming H-bond interactions with the antagonists.

4. Conclusion

Molecular docking of CRF1R with 8-substituted-2-aryl-5-alkylaminoquinolines as its antagonists was performed. The analysis of docking results revealed that the antagonists docked well within the receptor and the crucial residues forming H-bond interaction with the binding site of the receptor were identified. Further research concentrating on these residues could throw light on the important structural features involved in the formation of CRF1R-inhibitor complex.

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