J. Chosun Natural Sci.
Vol. 8, No. 3 (2015) pp. 176 – 183
http://dx.doi.org/10.13160/ricns.2015.8.3.176

3D-QSAR Studies of 8-Substituted-2-aryl-5-alkylaminoquinolines as Corticotropin-releasing Factor-1 Receptor Antagonists

Santhosh Kumar Nagarajan and Thirumurthy Madhavan†

Abstract

Corticotropin-releasing actor receptors (CRFRs) activates the hypothalamic pituitary adrenal axis, one of the 2 parts of the fight or flight response to stress. Increased CRH production has is associated with Alzheimer's disease and major depression and hypoglycemia. In this study, we report the important structural and chemical parameters for CRFR inhibitors using the derivatives of 8-substituted-2-aryl-5-alkylaminoquinolines. A 3D QSAR study, Comparative molecular field analysis (CoMFA) was performed. The best predictions were obtained for the best CoMFA model with a q^2 of 0.607 with 6 components and r^2 of 0.991. The statistical parameters from the generated CoMFA models indicated that the data are well fitted and have high predictive ability. The contour map resulted from the CoMFA models might be helpful in the future designing of novel and more potent CRFR derivatives.

Keywords: 3D-QSAR, CoMFA, CRFR, CRHR

1. Introduction

Corticotropin-releasing hormone (CRH) also known as corticotropin-releasing factor (CRF) is a 41 amino acid peptide hormone[1]. CRH is a neurotransmitter involved in the stress response. CRF is secreted by the paraventricular nucleus (PVN) of the hypothalamus in response to stress. CRH is also produced in peripheral tissues, such as T lymphocytes, and is highly expressed in the placenta[1]. Corticotropin-releasing factor receptors (CRFRs) belongs to the G protein-coupled receptor family, 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]. The CRF1 receptor is abundantly found in the pituitary and is involved in the regulation of ACTH, a key mediator of stress response.

The binding of Corticotropin Releasing-Hormone (CRH) with the Corticotropin-releasing factor receptors (CRFRs) activates the hypothalamic pituitary adrenal axis (HPA axis), one of the 2 parts of the fight or flight response to stress[4]. Increased CRH production has been observed to be associated with 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.

Central nervous system (CNS) CRF has been linked to a variety of disorders including depression, stress, anxiety, post-traumatic stress disorder, and addiction. CRF has been shown to be involved in the stress-induced phosphorylation of tau which implies a potential link between stress and Alzheimer's disease pathology[6]. CRF is also found in the periphery where it is involved in inflammation, and cancer. It has been suggested that CRF may be one of the links between stress and cancer. Also, a recent research suggested that CRF plays an important role in the development and maintenance of bone cancer pain via activation of neurons.

Several pharmaceutical research groups have focused on the discovery of CRF1 receptor antagonists for the treatment of depression or other stress-related disorders. Meanwhile, the benefits of blocking the CRF2 receptor remain uncertain. The available antagonists for CRF1 are Pexacerfont, Antalarmin, CP-316311 and CP-154,

Department of Bioinformatics, School of Bioengineering, SRM University, SRM Nagar, Kattankulathur, Chennai 603203, India

†Corresponding author : thiru.murthyunom@gmail.com, thirumurthy.m@ktr.srmuniv.ac.in
(Received: July 27, 2015, Revised: September 17, 2015, Accepted: September 25, 2015)

526. Pexacerfont is a recently developed CRF-1 antagonist which is currently in clinical trials for the treatment of anxiety disorders[7]. In case of Antalarmin, only animal studies for the treatment of anxiety, depression and other conditions, but no human trials have been carried out. Also, the results so far have had 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]. 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.

2. Computational Methods

2.1. Data Set

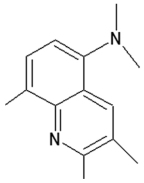
The structures of the 8-substituted-2-aryl-5-alkylaminoquinolines derivatives and their biological activities of 23 compounds were taken from the literature[11]. IC_{50} values of each inhibitor was converted into pIC_{50} ($-\log IC_{50}$) in order to use the data as dependent variable in CoMFA model. The test set molecules were selected which is the representative molecule for training set molecules. The test set molecules were selected manually so as to cover all the biological activity which is similar to the training set molecule. The total set of compounds was divided into a training set consist of 16 compounds and test set consist of 7 compounds. The structures and their activity values are displayed in Table 1.

2.2. Ligand-based Alignment Method

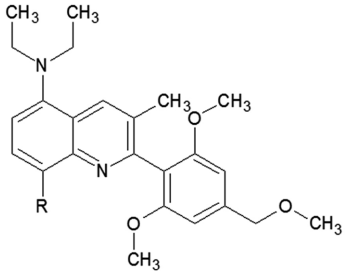
For each compound, the partial atomic charges were assigned by utilizing Gasteiger Hückel method available in SYBYLX 2.1 package (Tripos Inc., St. Louis, MO, USA). All rotatable bonds were searched with incremental dihedral angle from 120° by using systematic search conformation method. Conformational energies were computed with electrostatic term, and the lowest energy conformer was selected as template molecule. Then the template was modified for other ligands of the series. The common scaffold was constraint for each molecule and only the varying parts were energy minimized by Tripos force field with Gasteiger-Huckel

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

The indole/azaindole ROCK inhibitor scaffold

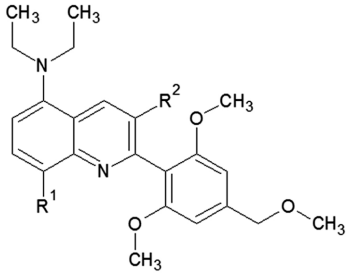


a) Compound 1-8



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

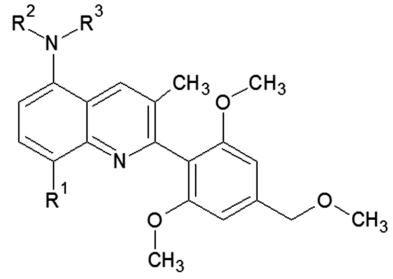
b) Compound 9-15



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

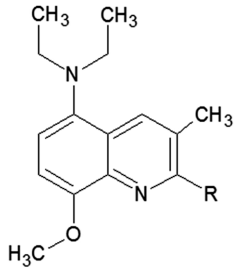
Table 1. Continued

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

d) 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

*Test set compounds

charge by using conjugate gradient method, and convergence criterion was 0.05 kcal/mol at 10,000 iteration. Using the atom fit method, the minimized structures were aligned over template and subsequently this alignment is used for Comparative molecular field analysis (CoMFA). The aligned molecules are represented in Fig. 1.

2.3. CoMFA Field Generation

SYBYLX 2.1 (Tripos Inc., St. Louis, MO, USA)

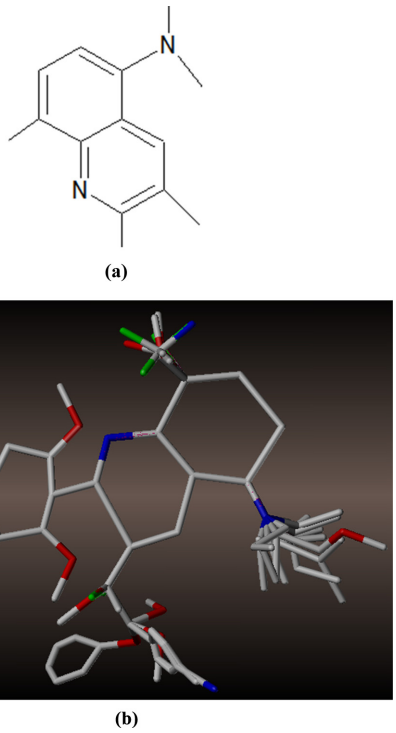


Fig. 1. (a) Maximum common substructure present in all molecules. (b) Alignment of molecules based on systematic search conformation of highly active compound 8.

package molecular modeling package was used for the 3D QSAR studies based on CoMFA. Generally used steric and electrostatic fields were used for this study. CoMFA studies helps in deriving a relation between the biological activities and three dimensional structures of the set of molecules of the dataset. The molecular alignment was placed in a 3D grid and the molecular field values of each conformation of a molecule are calculated. 2 Å lattice spacing was used. The CoMFA method was performed using steric and electrostatic fields with standard ±30 kcal/mol cutoffs. CoMFA calculated steric and electrostatic field values.

2.4. Partial Least Square (PLS) Analysis

PLS algorithm quantifies the relationship between the structural parameters and the biological activities[12,13]. CoMFA descriptors used as independent variables and pIC_{50} values used as dependent variables in PLS analysis for the generation of 3D-QSAR models. Leave-one-out (LOO) cross-validation procedures were used to obtain the cross-validated correlation coefficient (q2),

non-cross-validated correlation coefficient (r2), standard error estimate (SEE) and Fisher's values (F)[14,15]. A non-cross-validated analysis was carried out without column filtering was then followed. The cross-validated correlation coefficient (q^2) was calculated using the following equation:

$$q^2 = 1 - \frac{\sum_{\gamma}(\gamma_{pred} - \gamma_{actual})^2}{\sum_{\gamma}(\gamma_{actual} - \gamma_{mean})^2}$$

where γ_{pred}, γ_{actual}, and γ_{mean} are the predicted, actual, and mean values of the target property (pIC_{50}), respectively.

The predictive power of CoMFA models were determined from the set of seven test molecules which was excluded during model development. The predictive correlation coefficient (r^2_{pred}) based on the test set molecules, is defined as:

$$r^2_{pred} = \frac{(SD - PRESS)}{SD}$$

where *PRESS* is the sum of the squared deviation between the predicted and actual activity of the test set molecules, and SD is defined as the sum of the square deviation between the biological activity of the test set compounds and the mean activity of the training set molecules.

3. Results and Discussion

3.1. CoMFA Analysis

A realiable CoMFA model was derived with the combination of steric and electrostatic field contributions and Gasteiger-Hückel charge method with 2.0 Å grid space. Different combinations of training and test compounds were used for model generation. Many CoMFA models were obtained, of those only 5 models was selected based on the reliable q^2 and r^2_{pred} values. The statistical values of the 5 models are tabulated in Table 2. The Leave one out (LOO) analysis gave the cross-validated q^2 of 0.607 with 6 components and noncross-validated PLS analysis resulted in a correlation coefficient r^2 of 0.991, Fisher value as 491.002, and an estimated standard error of 0.135. The predictive ability of the developed CoMFA model was assessed by the test set (7 molecules) predictions, which were excluded during model generation. The predictive ability of the test set was 0.632. Predicted and experimental activities and their residual values of all inhibitors are shown in Table 3, and the corresponding scatter plot is depicted in Fig. 2.

Table 2. Statistical results of CoMFA models obtained from systematic search conformation based alignment

PLS statistics	Ligand-based CoMFA model (Systematic search conformation based alignment)				
	Model 1	Model 2	Model 3	Model 4	Model 5
q^2	**0.607**	0.565	0.565	0.604	0.461
N	**6**	5	6	6	5
r^2	**0.991**	0.987	0.990	0.990	0.983
SEE	**0.135**	0.148	0.139	0.131	0.145
F-value	**491.002**	254.852	385.529	414.892	223.568
r^2_{pred}	**0.632**	0.528	0.602	0.611	0.512
	Field contribution				
Steric	**0.504**	0.523	0.511	0.509	0.517
Electro static	**0.496**	0.477	0.489	0.491	0.483

q^2= cross-validated correlation coefficient; N= number of statistical components; r^2= non-cross validated correlation coefficient; SEE=standard estimated error; F=Fisher value; $r^2_{predictive}$= predictive correlation coefficient for test set.

The model chosen for analysis is highlighted in bold fonts.

Test set compounds

Model 1- compound no 4,5,11,13,19,21,23
Model 2- compound no 4,5,11,14,19,21,22
Model 3- compound no 4,5,11,14,19,21,23
Model 4- compound no 4,5,13,14,19,21,23
Model 5- compound no 4,5,13,14,19,21,22

Table 3. Predicted activities and experimental pIC_{50} values obtained from CoMFA models

Compound	Actual pIC_{50}	Predicted	Residual
1	7.102	6.908	0.194
2	6.928	6.850	0.078
3	6.842	6.865	-0.023
4*	6.967	6.815	0.152
5*	6.735	6.739	-0.004
6	6.842	6.856	-0.014
7	7.091	7.067	0.024
8	6.979	6.972	0.007
9	6.407	6.479	-0.072
10	6.807	6.795	0.012
11*	7.055	6.788	0.267
12	6.646	6.777	-0.131
13*	6.963	6.854	0.109
14	7.174	6.869	0.305
15	6.879	6.862	0.017
16	7.004	7.281	-0.277
17	7.208	7.249	-0.041
18	6.383	6.358	-0.025
19*	6.541	7.472	-0.931
20	6.991	6.784	0.207
21*	6.000	6.020	-0.020
22	6.959	6.892	0.067
23*	6.695	6.891	-0.196

*Test set compounds

3.2. CoMFA Contour Map

Color-coded contour maps were generated using CoMFA analyses which represent regions in 3D space where changes in the steric and electrostatic fields of a compound correlate strongly with changes in its biological activity. A scalar product of coefficients and standard deviation (SD*Coeff) associated with each column were generated as contour maps. Favored levels were fixed at 70% and disfavored levels were fixed at 30%.

The CoMFA contour map was generated based on the ligand-based (atom-by atom matching) alignment method. The CoMFA result is represented as a 3D 'coefficient contour' map. The steric contour map is displayed in Fig. 3. Green color in the steric contour maps depicts the more bulk molecules favored region whether yellow color region represent the less bulk molecules favored in the region. The green steric contour near the R_1 position of the phenyl ring indicates that substitution of bulky group is preferred at this position. This may be the reason that compounds 1, 6 and 8 with bulkier substituent at this position are more active. There was a yellow contour region which was very close to the green contour map in R position; the contour map clearly indicated that substitution of bulkier groups would decrease the activity. This may be the reason that compounds 4, 5 and 7 having bulkier substitution shows less activity.

The electrostatic contour map is displayed in Fig. 4. In case of the electrostatic field contours, red regions represent electronegative substituents favored regions and blue regions represent electropositive substituents favored regions. The electrostatic contour plot shows that there is a blue colored region situated close to the R positions. It indicates that the electropositive charges

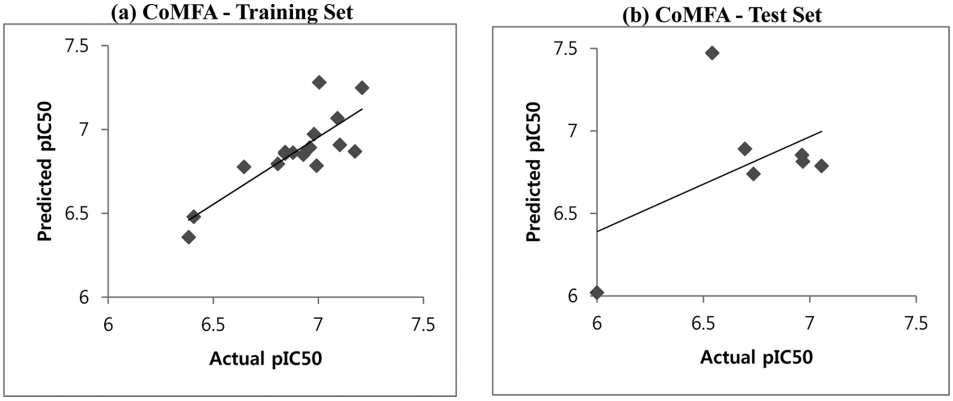


Fig. 2. (a and b) Plot of actual versus predicted pIC50 values for the training set and test set for the CoMFA values performed after atom-by atom matching alignment by systematic search.

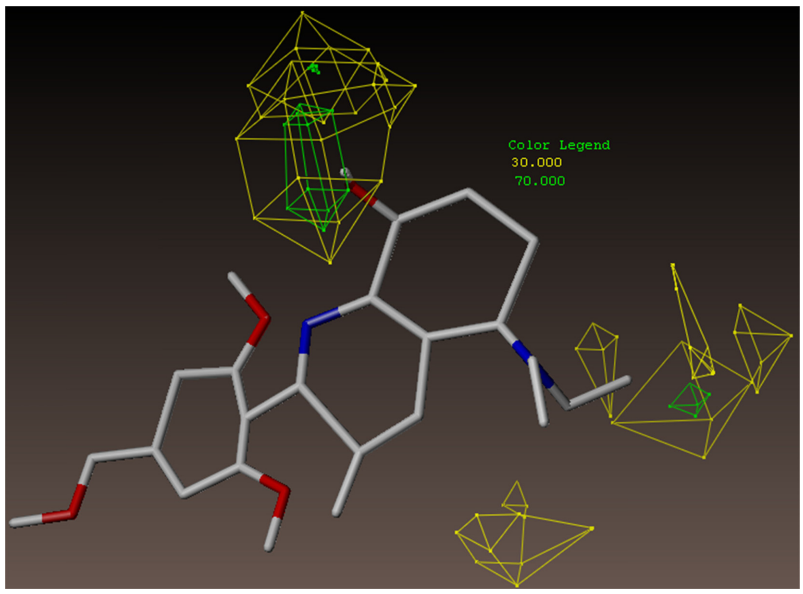


Fig. 3. CoMFA steric contour map with highly active compound 8 for systematic search based alignment. Here green contour indicates region where bulky group increases activity and yellow contours indicates bulky group decreases activity.

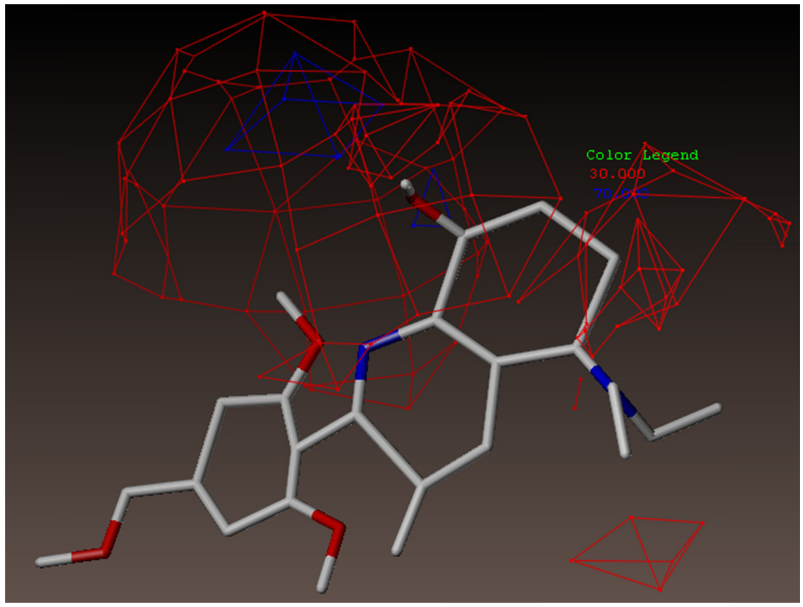


Fig. 4. CoMFA electrostatic contour map with highly active compound 8 for systematic search based alignment. Here blue contour indicates regions where electropositive groups increases activity and red contours indicates regions where electronegative groups increases activity.

in these regions are very important for ligand binding, and electropositive group linked to this position will enhance the biological activity.

4. Conclusion

In this study, a satisfactory 3D-QSAR model from 8-

substituted-2-aryl-5-alkylaminoquinolines derivatives as Corticotropin-releasing factor-1 receptor antagonists was developed using CoMFA method based on atom-by-atom matching alignment. The contour map indicated important sites, such as steric and electrostatic, can influence the bioactivities of the compounds. The steric contour map indicated that substitution of bulkier groups in the R position of the phenyl ring would enhance the biological activity. In addition to this, the electrostatic contour map shows that the substitution of electron donating group in R position could improve the biological activity. The results obtained from this study have thrown light on the important structural and chemical features in designing and developing new potent novel inhibitors for Corticotropin-releasing factor-1 receptor.

References

[1] B. P. Grone and K. P. Maruska, "A second corticotropin-releasing hormone gene (CRH2) is conserved across vertebrate classes and expressed in the hindbrain of a basal neopterygian fish, the spotted gar", J. Comp. Neurol., Vol. 523, pp. 1125-1143, 2015.

[2] R. L. Hauger, D. E. Grigoriadis, M. F. Dallman, P. M. Plotsky, W. W. Vale, and F. M. Dautzenberg, "International union of pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands", Pharmacol. Rev., Vol. 55, pp. 21-26, 2003.

[3] N. Sato, K. Takagi, T. Suzuki, Y. Miki, S. Tanaka, S. Nagase, H. Warita, S. Fukudo, F. Sato, H. Sasano, and K. Ito, "Immunolocalization of corticotropin-releasing Hormone (CRH) and its receptors (CRHR1 and CRHR2) in human endometrial carcinoma: CRHR1 as a potent prognostic factor", Int. J. Gynecol. Cancer, Vol. 24, pp. 1549-1557, 2014.

[4] F. Stamatelou, E. Deligeoroglou, N. Vrachnis, S. Iliodromiti, Z. Iliodromiti, S. Sifakis, G. Farmakides, and G. Creatsas, "Corticotropin-releasing hormone and progesterone plasma levels association with the onset and progression of labor", Clin. Exp. Obstet. Gyn., Vol. 40, pp. 568-571, 2013.

[5] F. C. Raadsheer, J. J. V. Heerikhuize, P. J. Lucassen, W. J. Hoogendijk, F. J. Tilders, and D. F. Swaab, "Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with alzheimer's disease and depression", Am. J. Psychiat., Vol. 152, pp. 1372-1376, 1995.

[6] H.-B. Fan, T. Zhang, K. Sun, S.-P. Song, S.-B. Cao, H.-L. Zhang, and W. Shen, "Corticotropin-releasing factor mediates bone cancer induced pain through neuronal activation in rat spinal cord", Tumor Biol., pp. 1-7, 2015.

[7] V. Coric, H. H. Feldman, D. A. Oren, A. Shekhar, J. Pultz, R. C. Dockens, X. Wu, K. A. Gentile, S.-P. Huang, E. Emison, T. Delmonte, B. B. D'Souza, D. L. Zimbroff, J. A. Grebb, A. W. Goddard, and E. G. Stock., "Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder." Depress. Anxiety, Vol. 27, pp. 417-425, 2010.

[8] E. M. Jutkiewicz, S. K. Wood, H. Houshyar, L.-W. Hsin, K. C. Rice, and J. H. Woods, "The effects of CRF antagonists, antalarmin, CP154, 526, LWH234, and R121919, in the forced swim test and on swim-induced increases in adrenocorticotropin in rats" Psychopharmacology, Vol. 180, pp. 215-223, 2005.

[9] B. Binneman, D. Feltner, S. Kolluri, Y. Shi, R. Qiu, and T. Stiger, "A 6-week randomized, placebo-controlled trial of CP-316,311 (a selective CRH1 antagonist) in the treatment of major depression", Am. J. Psychiat., Vol. 165, pp. 617-620, 2008.

[10] R. Pastor, C. S. McKinnon, A. C. Scibelli, S. Burkhart-Kasch, C. Reed, A. E. Ryabinin, S. C. Coste, M. P. Stenzel-Poore, and T. J. Phillips, "Corticotropin-releasing factor-1 receptor involvement in behavioral neuroadaptation to ethanol: A urocortin1-independent mechanism", P. Natl. Acad. Sci. U.S.A., Vol. 105, pp. 9070-9075, 2008.

[11] K. Takeda, T. Terauchi, M. Hashizume, K. Shikata, R. Taguchi, K. Murata-Tai, M. Fujisawa, Y. Takahashi, K. Shin, M. Ino, H. Shibata, and M. Yonaga, "Synthesis and structure–activity relationships of 8-substituted-2-aryl-5-alkylaminoquinolines: Potent, orally active corticotropin-releasing factor-1 receptor antagonists", Bioorgan. Med. Chem., Vol. 20, pp. 6559-6578, 2012.

[12] S. J. Cho and A. Tropsha, "Cross-validated R2-guided region selection for comparative molecular field analysis: A simple method to achieve consistent results", J. Med. Chem., Vol. 37, pp. 1060-1066, 1995.

[13] S. Wold, M. Sjöström, and L. Eriksson, "PLS-regression: a basic tool of chemometrics", Chemometr. Intell. Lab., Vol. 58, pp. 109-130, 2001.

[14] U. Debnath, S. Verma, S. Jain, S. B. Katti, and Y. S. Prabhakar, "Pyridones as NNRTIs against HIV-1 mutants: 3D-QSAR and protein informatics", J. Comput. Aid. Mol. Des., Vol. 27, pp. 637-654, 2013.

[15] D. Fernández, J. Ortega-Castro, and J. Frau, "Human farnesyl pyrophosphate synthase inhibition by nitrogen bisphosphonates: A 3D-QSAR study", J. Comput. Aid. Mol. Des., Vol. 27, pp. 739-754, 2013.