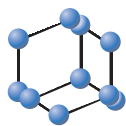
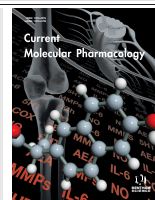


REVIEW ARTICLE

BENTHAM
SCIENCE

Structure and Function of Small Non-Peptide CRF Antagonists and their Potential Clinical Use

Hesham Fahmy^{a,*} Bhimanna Kuppast^b and Mohamed Teleb Ismail^a

^aDepartment of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Brookings, South Dakota 57007, USA; ^bDepartment of Pharmaceutical Sciences, Chicago College of pharmacy, Midwestern University, Downers Grove, IL-60151, USA

ARTICLE HISTORY

Received: July 23, 2015
Revised: December 24, 2015
Accepted: August 3, 2016

DOI:
10.2174/1874467209666161101144155

Abstract: Corticotropin-releasing factor (CRF) can be considered a very important hormone or a chemical mediator. It works closely with other systems to regulate the manner through which the body may respond to stress. Thus it affects many biological processes associated with stress. Dysfunction of this system has also been correlated with various diseases such as major depression, anxiety, drug addiction and eating disorders. Rationally, this means that interfering with binding of CRF to its intended receptors can be an attractive target for drug design aiming at developing new medications for many ailments that are associated with stress such as depression, anxiety and stress-induced relapse in drug addiction. Hundreds of accounts of small molecule antagonists have appeared in the literature and the preclinical and clinical pharmacology have been reported for many of these agents. Several classes of small molecule CRF receptor antagonists which belong to the non-peptide class have been developed with many being widely used for research purposes. Currently several major pharmaceutical companies are pursuing development of small non-peptide CRF1 receptor antagonists. In this review article we explain the importance of development of non-peptide CRF antagonists and their clinical relevance with emphasis on those members that showed great promise or those that were advanced to clinical trials.

Keywords: CRF antagonists, depression, anxiety, drug addiction, pyrimidines, hormone.

1. INTRODUCTION

Corticotropin-releasing factor (CRF) can be considered a very important hormone or a chemical mediator. It works closely with other systems to regulate the manner through which the body may respond to stress. Thus it affects many biological processes associated with stress. Many ailments which seem to involve anxiety or symptoms closely-related to anxiety are assumed to be associated with excessive activation of the CRF system. Dysfunction of this system has also been correlated with various diseases such as major depression, anxiety, eating disorders and drug addiction. Research points out that CRF may be involved in the stress-induced relapse and the anxiety-like behaviors observed during acute drug withdrawal and drug addiction [1]. CRF administration incites responses that are similar to stress such as sympathetic nervous system stimulation and parasympathetic nervous system inhibition. Consequently, it causes an increase in plasma concentration of adrenaline, noradrenaline

and glucose, increase in heart rate and hypertension, inhibition of gastrointestinal motility and secretion [2].

The general behavioral response to CRF administration is an increased excitement and emotional response to the environment [3]. In addition to the modulatory effect on the nervous system, CRF is also suggested to play a significant role in regulating the immune responses to physiological, psychological and immunological stressors [4].

The identification and characterization of a family of CRF peptides agonists and antagonists, coupled with the cloning and molecular characterization of the two main CRF receptor subtypes, CRF1 and CRF2, in addition to the discovery of antagonists selective to a particular CRF receptor subtypes, altogether provided new visions to further clarify the mechanism of how stress may affect different systems. It also signified the role of the CRF system and its implication in several stress-related ailments such as irritable bowel syndrome (IBS), anxiety, depression and others [5, 6].

Another important component of the CRF system is a 37 kDa glycoprotein known as the CRF binding protein or CRF-BP. This protein exists in the plasma and CNS of both rats and humans [7, 8]. The high concentration of CRF-BP in the synovial fluid of patients suffering from arthritis offers

*Address correspondence to this author at the Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University, Box 2202C, Brookings, SD 57007, USA; Tel: +1-605-688-4243; Fax: +1-605-688-5993; E-mail: Hesham.Fahmy@sdstate.edu

an important clue that CRF-BP may be involved in immune-therapy [9]. It was suggested that the main function of the peripheral CRF-BP is to offset the placental CRF hyper-secretion during the last trimester of pregnancy. The role of the membrane-bound central CRF-BP is currently under investigation for its potential therapeutic applications. The function of the CRF-BP is shifting from the assumptions that it simply acts a sponge to control the CRF concentration, to that it may act as a modulator of several central nervous system functions [10].

2. INVOLVEMENT OF CRF IN DISORDERS

Anxiety and its related disorders affect millions of adults aged eighteen and older. Anxiety-related disorders cost billions each year and it is considered as one of the largest total mental health bill [11]. Depressive disorders also affect approximately millions of adults [12]. Depression's annual cost amounts to billions in medical expenditures [13]. The comorbidity rate of depression with other disorders especially with anxiety, Parkinson's disease, eating disorders, substance abuse, heart attacks, strokes and cancer is also very high [14]. The currently used medications for treatment of mood disorders focus mainly on neural concentration of monoamine systems such as serotonin (5HT), norepinephrine (NE), and dopamine (DA). Thus, SSRIs (selective serotonin reuptake inhibitors) and NRIs (norepinephrine reuptake inhibitors) are among the first choice treatment options for depressive disorders. Similarly, benzodiazepines are among the first-choice treatment options for anxiety disorders [15]. Although the older traditional drugs such as the tricyclic antidepressants provided the prototype that eventually paved the way for the introduction of the new generation of antidepressants, their mechanism of action remains similar. Consequently, their efficacy and also their success rate remain within the same range [16, 17]. Also, they suffer from severe limitations such as delayed onset of therapeutic action and failure in sustaining disorder remission significant numbers of the patients are non-responsive [18].

Despite the presence of a large number of anti-anxiety, antidepressant and related drugs in the market, there is still an unmet medical need for new neuropsychiatric agents. Because a significant number of patients do not tolerate the current drugs well or because of their undesirable side effects, newer drugs that work through completely different mechanisms of actions are much needed [18].

Data from clinical research suggest an involvement of CRF in major depressive disorders. Earlier research demonstrated that patients who suffer from major depressive disorders usually have higher levels of CRF in the cerebrospinal fluid compared to non-depressed volunteers [19]. Moreover, clinical studies show that CRF levels are also higher in anorexic patients [20], or those with obsessive compulsive or post-traumatic disorders. Several other studies showed alterations in brain concentration of CRF in patients who are diagnosed with Alzheimer's and Parkinson's diseases [21, 22].

Clinical research also confirmed the involvement of CRF in the depression pathophysiology. This was concluded from observations such as the poor adaptation to chronic stress which is associated with long term elevated corticosteroids

levels. This hypercortisolemia can be a rational pathway to many forms of depression and appears to be a direct result of chronic hyper-secretion of hypothalamic CRF. The number of neurons expressing CRF in the hypothalamus as well as the concentration of CRF in the cerebrospinal fluid (CSF) in patients diagnosed with major depression were both found to be significantly higher compared to the normal population. [23-25].

Serious arguments confirming the role of CRF in anxiety disorders are also available. Intra-cerebroventricular injection of CRF in rats provokes many behavioral and physiological responses similar to those caused by stress. Furthermore, these responses can be blocked by the CRF peptide antagonists such as the α -helical CRF. Stress caused by chronic restraint in experimental animal models also causes chronic activation of the HPA axis. Transgenic mice, over-expressing CRF also were found to have higher plasma concentrations of corticotrophin and glucocorticoids and consequently display noticeable stress-related activities [26].

Convincing pre-clinical data demonstrated that CRF when administered centrally produced colonic responses similar to those caused by acute stress and that the visceral pain caused by colorectal distension in experimental animals was mediated by CRF1 receptors [27]. Likewise, peripheral CRF caused the reduction of the pain threshold to colonic distension and caused an increase in the colonic motility in rodents and also in humans. These observations resemble the IBS classic symptoms including the abdominal bloating and discomfort and the typical altered bowel habits [28]. Interestingly, the CRF1 pathways have been implicated in anxiety and depression and these same disorders, together with a stressful life style, are known to have high co-morbidity with IBS or considered a significant components of the IBS. Based on the above-mentioned findings, CRF1 receptors was suggested as a possible target for IBS. It was also suggested that peripherally-acting CRF1 antagonists may mend IBS symptoms associated with motility and secretion or the immune response. In the same context, the CNS effects of CRF1 antagonists might be beneficial for preventing psychopathologies associated with IBS by interfering with the CNS processing of signals related to stress and visceral pain [29].

Clinical studies show the involvement of CRF in behavioral responses and physiological symptoms during drug withdrawal, relapse and stress-induced drug-seeking behavior [30]. It's been noted that CRF is indeed responsible for elevated anxiety and negative emotional states during the development of dependence [31]. These data indicate the rationale behind the possible use of CRF antagonists for the management of drug addictive conditions.

Studies also show that overproduction of CRF at synovial joints may contribute to the development of rheumatoid arthritis. Higher expression of immunoreactive CRF, nearly 6 fold higher than normal individuals has been found in synovium of patients with rheumatoid arthritis [32-35]. Indirect effects of CRF on immune function [4] have been noted earlier by pituitary adrenocortical glucocorticoid secretion followed by their effective anti-inflammatory effects through suppression of immune and inflammatory responses and the related mediators [36]. These evidences indicate the role of

CRF and its importance as a novel target to develop medicines to treat rheumatoid arthritis and inflammatory diseases.

CRF is known to regulate both sympathetic and parasympathetic outflow [37, 38]. *In vivo* studies suggest that central CRF inhibits vagal output and stimulates sympathetic activity [39]. Therefore, CRF may function to inhibit undue vagal activation and thereby bradycardia. On the other hand, CRF1 antagonists increase cardiac vagal and decrease sympathetic activity, indicating CRF1 as a therapeutic target for autonomic disturbances leading to elevated sympathetic activity, such as hypertension and coronary heart disease [40].

3. CRF RECEPTORS AS DRUG TARGETS

The CRF role and its implication in the above-mentioned disorders and the above-mentioned behavioral, cardiovascular, gastrointestinal and immune systems suggest that new drugs that can interfere with the CRF function or CRF binding to its intended receptors may represent a different class of neuropsychiatric drugs for stress-related disorders such as anxiety and depressive and addictive disorders as well as other peripheral disorders as Irritable Bowel Syndrome.

Significant progress has been achieved in describing the structure-activity relationships of compounds acting as a selective agonists and antagonists for CRF1 and CRF2 receptors such as the peptides astressin and anti-sauvagine1-30 [41]. However, because of the peptide nature of these compounds, their physical properties posed a boundary for their use in clinical settings. This resulted in pursuing non-peptide antagonists as a better alternative [42]. Currently, only CRF1 selective non-peptide antagonists are available. Thus, the design of small molecule, non-peptide antagonists for the corticotropin-releasing factor receptor may afford new treatment options for many disorders and may represent a new class of antidepressants and anti-anxiety agents.

Hundreds of accounts of small molecule antagonists have appeared in the literature and the preclinical and clinical pharmacology have been reported for many of these agents. The main research into CRF antagonists to date has focused on non-peptide CRF1 receptor antagonists to target health problems arising as a result of chronic stress and thus as potential treatment options for anxiety-related and stress-related disorders [43, 44]. Several classes of small molecule non-peptide CRF receptor antagonists have been synthesized and many are commonly used in research. No data on CRF2 selective non-peptide agents have been published. Based on all these facts, many CRF antagonists, particularly non-peptide CRF1 receptor antagonists may be considered as potential treatment options for depression or anxiety. The synthesis of more non-peptide CRF1 receptor antagonists with a diverse structure scaffolds as well as further clinical investigations may shed more light on how CRF is implicated in mental disorders.

Currently several major pharmaceutical companies are pursuing development of small non-peptide CRF1 receptor antagonists including Ely-Lilly [45], DuPont pharmaceutical [46, 47], Pfizer [48, 49], Bristol-Myers-Squibb [50-53], Glaxo Smith Kline [54-56] and Neurocrine biosciences [57-61].

4. BASIC CRF ANTAGONISTS PHARMACOPHORE

The relationship between structures of non-peptide CRF1 antagonists and their affinity to CRF1 receptors indicates that CRF1 antagonists are typically built of three moieties: a hydrophobic moiety up, a proton accepting moiety in the middle, and an aromatic moiety down. Thus the basic pharmacophore is proposed to be made of a monocyclic or bicyclic heterocyclic ring carrying a hydrophobic dialkylamino group on one flank and an aryl ring, usually substituted at the 2-, 4- and 6-positions, on the opposite flank. Having the aromatic ring orthogonal to the heterocyclic core appears to be essential for the affinity. A methyl group on the heterocyclic core also appears to enhance the CRF1 antagonist properties [44, 62, 63] (Fig. 1).

X-ray structure of several non-peptide CRF1 antagonists confirms that extension of the aromatic or hetero-aromatic hydrophobic unit (down area) and the alkyl groups on the amino functionality (up area) being out of the plane of the core heterocyclic unit (central area) are required for optimum CRF receptor binding affinity [64].

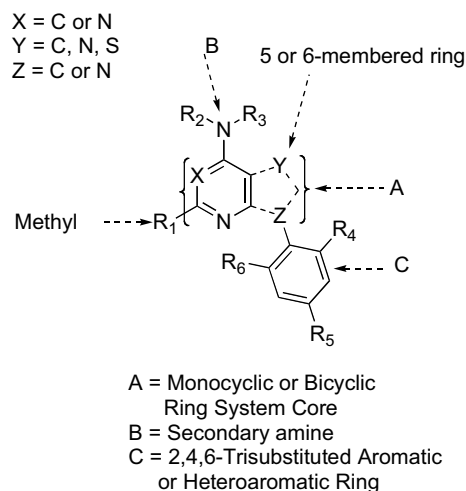


Fig. (1). Pharmacophore structure of CRF1 antagonists.

5. CHEMICAL CLASSIFICATION OF CRF1 RECEPTOR ANTAGONISTS

Based on the number of rings in the heterocyclic core ring system, CRF1 receptor antagonists can be classified to the following classes:

5.1. Monocyclic CRF1 Receptor Antagonists

Depending on the type of the heterocyclic ring system involved, this group can be further sub-classified into the following ring systems:

5.1.1. Pyrazines

Many pyrazines were developed as CRF1 receptor antagonists. The pyrazine proved to have greater potential compared to the previously-reported other heterocyclic-core counterparts; especially, in the areas of oral bioavailability, stability to metabolism and also brain accessibility.

A lead compound of the pyrazine-containing CRF1 receptor antagonist is NGD 98-2 (Fig. 2) which was extensively profiled [65]. During *in vitro* studies, it showed high affinity to CRF1 receptors in humans and rats. It showed an antagonist effect to the cAMP accumulation induced by CRF in human neuroblastoma cells. Also, the *in vivo* pharmacological effects of NGD 98-2 were evaluated in male rats following behavioral and biochemical models of stress.

This drug candidate was found to be orally bioavailable with good brain penetrability following oral administration to rats. It displayed highly selective CRF1 receptor antagonist activity. When quantitative assay of brain CRF-1 receptors occupancy was carried out, it was found that fifty percent occupancy was reached.

On the behavioral level, oral administration of NGD 98-2 at small doses resulted in a significant reduction of alteration in the locomotor activity induced by CRF. Moreover, experiments on rats suffering from physical restraint for ten minutes showed a significant increase in HPA axis activation, as demonstrated by augmented plasma ACTH levels. Oral administration of NGD 98-2 significantly reduced the restraint stress-induced elevated ACTH in a dose-dependent manner [65].

The Newly Developed CRF1 Receptor Antagonist, NGD 98-2 and its structurally-related analogue, NGD 9002 [66] (Fig. 2) were found to suppress both the exogenous (induced by administration of CRF) and endogenous (induced by exposure to water avoidance stress or repeated colorectal distension) CRF-induced stimulation of colonic function and visceral hypersensitivity (CRD) in conscious rats.

It is worth-mentioning that the long $t_{1/2}$ and thus the risk for accumulation in adipose tissues posed a safety concern for long term use in humans, and consequently, additional development of NGD 98-2 has come to an end.

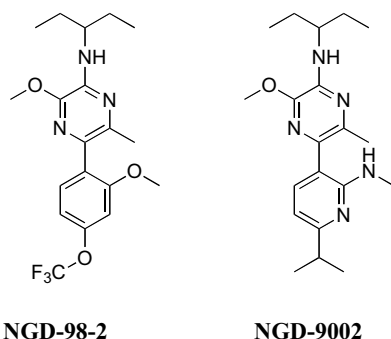


Fig. (2). Structures of pyrazine CRF antagonists.

5.1.2. Pyridines

Numerous pyridines were developed as novel, selective, and orally active CRF1 antagonists. Most exhibited potent CRF1 antagonist effect and showed good brain receptor occupancy following oral administration. This indicates oral bioavailability and accessibility to brain.

CP-316311 (Fig. 3) is a prominent potent member of this series of selective CRF1 receptor antagonists. It showed efficacy when given orally in the *in vivo* CNS experiments. CP-316311 was advanced to phase II trials for depression to ex-

amine the postulation that CRF1 antagonists may have a clinically use as antidepressant medications [49].

Many compounds from this series showed a significant positive effect for food following oral administration in both humans and dogs. In dogs, oral bioavailability was much lower during fasting than when the animals were fed. To address the food effect concerns, structural modifications were made in an effort to discover similar orally active compounds with an improved pharmacokinetic profile while retaining the desired pharmacological effect. This led to the development of compound CP-376395 [49].

CP-376395 (Fig. 3) is another prominent orally active clinical candidate of this group. It showed clear *in vivo* potency in animal models. Consequently, CP-376395 was chosen for additional development and was advanced to the clinic because of its better efficacy in CNS models and less interference of food with its effects [62]. Thus it may have a value in assessing the clinical usefulness of CRF1 receptor antagonists for stress or anxiety related illnesses.

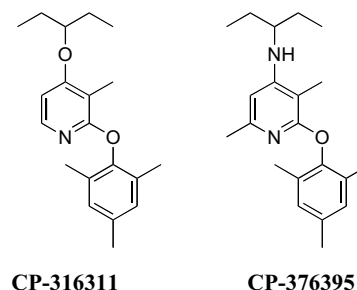


Fig. (3). Structures of pyridine CRF antagonists.

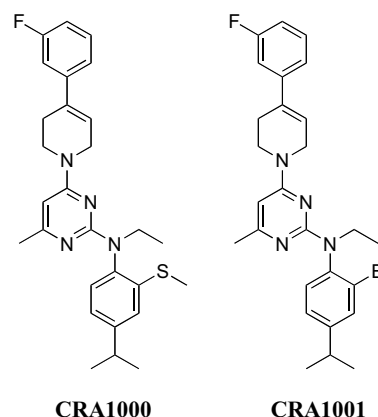


Fig. (4). Structures of pyrimidine CRF antagonists.

5.1.3. Pyrimidines

Several pyrimidines were developed as non-peptide CRF1 receptor antagonists with many showing great promise. Examples are CRA1000 and its bromo-analogue CRA1001 (Fig. 4). They displayed high affinity and CRF1 receptor subtype selectivity with strong anti-anxiety and antidepressant effects during animal investigations [67]. CRA1000 displayed a high affinity to rat frontal cortical CRF1 receptors. Unlike other CRF antagonists, it did not inhibit binding of radiolabelled ^{125} I-sauvagine CRF2 in rats.

It also inhibited, in a concentration-dependent manner, the accumulation of cAMP induced by CRF when tested in CRF1-expressing cells such as AtT-20 and COS-7 cell lines. During the *in vivo* testing, it reversed the shortening of sodium pentobarbital-induced sleeping time which was induced by stress caused by restraint in rats. This also served as evidence that the increase in arousal induced by stress is mediated by CRF1 receptors [68].

5.1.4. Thiazoles

A good example of thiazole-containing CRF antagonists is SSR125543A (Fig. 5). It is a potent CRF1 receptor antagonist [69]. It shows a nano-molar affinity for native or cloned human corticotrophin-releasing factor receptors and a thousand times more selectivity to CRF1 versus CRF2 receptors or CRF-BP. When tested using human retinoblastoma Y79 cells, SSR125543A inhibited the accumulation of cAMP induced by CRF. When tested in mouse pituitary tumor AtT-20 cells, it inhibited the secretion of adrenocorticotropin hormone. Brain accessibility by SSR125543A was established in rats when it displaced binding of the radiolabelled [^{125} I-Tyr0] to ovine brain CRF1 receptors. SSR125543A also reversed the increase in rat plasma ACTH levels which was induced by CRF injection.

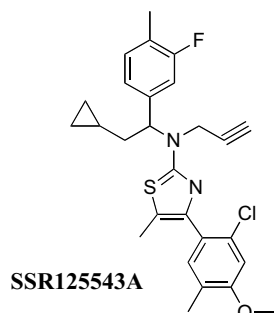


Fig. (5). Structures of thiazole CRF antagonist.

SSR125543A oral effectiveness was demonstrated by various tests. It reduced the stress-induced increase in plasma ACTH levels in rats caused by restraint. It also antagonized the increase in rat hippocampal acetylcholine release induced by an injection of CRF. Finally, it reduced the CRF-induced forepaw treading caused by injection of CRF in gerbils [69]. The collective results of these tests strongly confirmed the potential of SSR125543A as a selective potent CRF1 receptor antagonist which is orally active.

5.2. Bicyclic CRF1 Receptor Antagonists

Depending on the type of the heterocyclic ring system involved, this group can be further sub-classified into the following ring systems:

5.2.1. Pyrrolo[2,3-d]pyrimidines

Antalarmin (Fig. 6) is perhaps the most prominent member of non-peptide CRF antagonists in general and was studied extensively. Thus it is the best representative of the pyrrolo[2,3-d]pyrimidine series. Antalarmin showed anti-inflammatory properties and thus may have a potential value

in management of arthritis [70], as well as other inflammatory disorders which are stress-mediated such as irritable bowel syndrome [28, 71], and peptic ulcers [72]. Chronic CRF1 block with systemic antalarmin significantly improved rat adjuvant-induced arthritis, and reduced peripheral joints inflammation. Clinical and histopathological observations confirmed the results, and also the weight loss associated with disease onset. Despite the suppression in the levels of adjuvant-induced corticosterone which is the main anti-inflammatory glucocorticoid in rats, there was no induction nor exacerbation of arthritis expression by Antalarmin [70]. Systemic CRF1 block seems to mainly block the peripheral pro-inflammatory properties of immune CRF but does not block the systemic glucocorticoid-mediated anti-inflammatory properties of the hypothalamic CRF. Long term treatment with CRF1 antagonists was found to reduce the progression of degeneration of arthritic joints components including synovia, cartilage and bone which is induced by inflammation. This points out to a possible therapeutic value of antalarmin in treatment of autoimmune diseases and inflammatory ailments. Upon exposure to long-term stress, gastric ulcers and increase in colon motility accompanied with mucin depletion were developed in rats. Several indicators of physiological and behavioral arousal also appeared. Antidepressants such as fluoxetine and bupropion, and also anti-anxiety medications such as diazepam and buspirone in addition to antalarmin were all assessed for their potential to alter these responses. Antalarmin, fluoxetine, bupropion and diazepam suppressed the gastric ulceration induced by stress resulting from 4 hours restraint in male Sprague-Dawley rats. Antalarmin was found to produce the most noticeable anti-ulcer effect and also inhibited the other effects caused by stress including hyper-motility of the colon, depletion of mucin, and behaviors such as autonomic hyper-excitement and struggling [72]. Thus Antalarmin showed that non-peptide CRF1 antagonists may be of value as prophylactics for ulcers caused by stress in the critically-sick patients. It may also have a value as possible medication for similar gastrointestinal disorders such as gastric ulcers and irritable bowel syndrome.

Antalarmin was found to antagonize the pressor effect induced by central CRF [73]. It also showed promising results in treatment of CRF induced hypertension [74]. Similar promising results for antalarmin were also observed for disorders associated with drug addiction. When antalarmin was evaluated for its effects on cocaine dependence in monkeys, it showed a reduction of its use. Similarly, antalarmin tested on cocaine-addicted rats prevented dose escalation, suggesting that it might modulate the cocaine addictive effects over time [75]. Antalarmin showed a significant potential to reduce withdrawal symptoms associated with long-term use of opiates and to reduce self-administration of alcohol in rodents addicted to ethanol [76].

CP-154,526 (Fig. 6) is another potent and selective CRF1 receptor antagonist from this series developed by Pfizer [77, 78] and is in clinical trials. Systemic administration of CP-154,526 reduced the release of noradrenaline and plasma ACTH levels which were induced by stress [79, 80]. However, in anxiety related tests, CP-154,526 showed mixed results. This may be due to the differences in behavioral testing methods or compound itself may be weak anxiolytic

compared to benzodiazepines. But the preclinical data of CP-154,526 as an antidepressant showed promising results, indicating the potential application of CRF1 receptor antagonists in depression [81]. CP-154,526 also inhibited the self-administration of cocaine indicating potential use in cocaine abuse. It also reduced signs due to withdrawal from morphine and shows the potential application of CRF1 receptor antagonist in abstinence following opiate addiction. In addition, CP-154,526 also stopped ethanol, morphine, and cocaine administration caused by stress and shows strong potential in preventing the relapse during drug addiction caused by stress [82]. CP-154,526 is under investigation for the potential treatment of alcoholism [83].

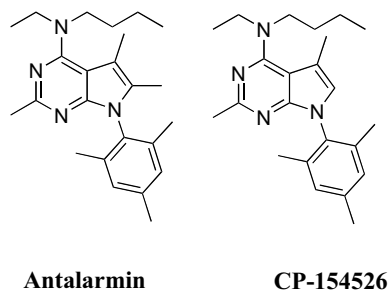


Fig. (6). Structures of Pyrrolo[2,3-d]pyrimidine CRF antagonists.

5.2.2. Pyrazolo[1,5-a]1,3,5-triazines

Structure-activity relationships studies of a new class of pyrazolo[1,5-a]1,3,5-triazine CRF1 receptor antagonists resulted in the discovery of BMS-562086 (Fig. 7), as potent, selective CRF1 antagonist. This compound, as well as other closely related analogues, showed great promise when tested in animal models. They showed a promising pharmacokinetic profile when tested in dogs and are orally active in anxiety models in rats including the defensive withdrawal model for the situational anxiety and the elevated plus maze anxiety model. Thus, they were advanced to clinical trials [84, 85].

Other pyrazolo[1,5-a]1,3,5-triazines have heterocyclic rings replacing one of the alkyl chains at the molecule C7 up-area. This structural modification produced derivatives which were less lipophilic. The most prominent derivative in this series was NBI-77860 which is also known as GSK561679 (Fig. 7) [86] which showed a high ligand affinity and a functional CRF1 antagonist activity. It has a promising pharmacokinetic profile and was advanced to the next level for additional preclinical evaluation.

Another potent, orally bioavailable CRF1 receptor antagonist is DMP696 (Fig. 7). It has a very promising oral activity and pharmacokinetic profile in rats as well as dogs. It displayed promising properties in situational anxiety models in rats whereas the literature-standard CP-154,526 was inactive. DMP-696 decreased the typical mouth movements in monkeys by almost fifty percent [87]. Thus, the profile of DMP-696 indicates that CRF1 receptor antagonists have the potential as anti-anxiety medications with less motor side effects.

5.2.3. Pyrazolo[1,5-a]pyrimidines

Some pyrazolo[1,5-a]pyrimidines was designed to have a pyridine ring at the 3-position of the bicyclic core which is

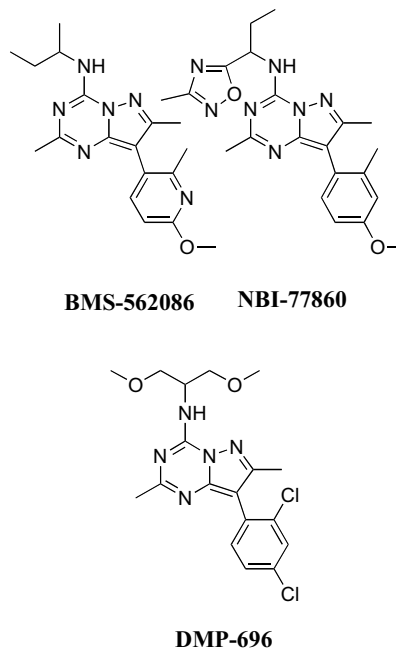


Fig. (7). Structures of Pyrazolo[1,5-a]1,3,5-triazine CRF antagonists.

weakly-basic in nature instead of the typical phenyl ring in order to decrease lipophilicity. These pyridine-substituted CRF1 antagonists showed great antagonism at CRF1 receptors in humans. A good example is NBI-30775, which has the code number R121919 (Fig. 8). The weakly-basic polar pyridine ring enhanced the solubility in water of the hydrochloride salt of NBI-30775. It showed a promising binding affinity for CRF1 receptors in humans as a functional antagonist. It reduced the CRF-induced cAMP accumulation in CRF1 receptor expressing cells, and CRF-induced ACTH release from rat pituitary cells. Both effects were in a concentration-dependent manner. Pharmacokinetic studies showed that it has good oral bioavailability in rats and also has promising brain penetrability. *In vivo* studies, demonstrated its antagonistic properties in behavioral models in animals designed to measure the anti-anxiety properties [88]. These results indicated that NBI-30775 (R121919) is a potent CRF1 receptor antagonist with good physicochemical and pharmacokinetic profiles. NBI-30775 moved to the next level and was developed into a clinical compound. It showed some efficacy in a minor open label clinical study for depression and anxiety [88].

Another prominent pyrazolo[1,5-a]pyrimidine is DMP-904 (Fig. 8). It has an excellent binding affinity. Its pharmacological profile strongly supports its potential as an anxiolytic agent with low motor side effect liability. DMP904 is a potent antagonist to the hCRF1-induced adenylate cyclase activity in HEK293 cells. It displayed good oral activity when tested in the situational anxiety model in rats and showed low motor side effects [89]. The antagonist activity profile of DMP904 to hCRF1 receptors has been processed through a Schild analysis where it has been shown to be a non-competitive antagonist. It was selective to hCRF1. It was also selective for CRF1 with no affinity for hCRF2 receptors

expressed in HEK293 cells and no affinity for the CRF-BP. When tested against other receptors, it showed no affinity for adenosine, adrenaline, dopamine, or serotonin, histamine, muscarine, GABA, opioid receptors or ion channels [90].

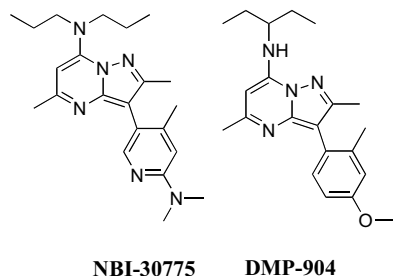


Fig. (8). Structures of Pyrazolo[1,5-a]pyrimidine CRF antagonists.

Pexacerfont (Fig. 9), also known as BMS-562,086 is a recently developed CRF1 antagonist developed by Bristol Myers Squibb which is currently in clinical trials to evaluate its potential in the treatment of anxiety disorders [91]. It has also been suggested as a possible medication that may be of value for depression and irritable bowel syndrome [92].

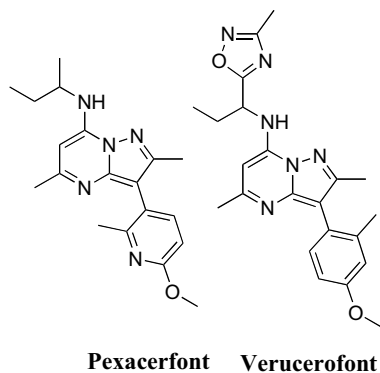


Fig. (9). Structures of Pyrazolo[1,5-a]pyrimidines Verucerofont and Pexacerfont.

Verucerofont (Fig. 9), also known as GSK-561,679 or NBI-77860, is a new drug developed by GlaxoSmith Kline [86]. It is a potent and specific CRF1 antagonist as confirmed by an array of *in vitro* and *in vivo* experiments and clinical trials in humans. NBI-77860 is under investigation as a possible treatment for the typical congenital adrenal hyperplasia [93]. It is also considered as a possible treatment for alcohol addiction, since long-term stress is usually a component during the development of alcohol addiction and also during relapse when alcoholic are attempting to quite [94]. It showed a potential in animal studies but still needs to be tested in humans.

5.2.4. Imidazo[1,2-b]pyridazines

Imidazo[1,2-b]pyridazines provided some novel CRF1 receptor antagonists with enhanced properties for clinical development. Among those MTIP (Fig. 10) which is a thiazolyl-imidazo[1,2-b]pyridazine [45]. It reduced binding of radiolabelled ^{125} I-sauvagine to pituitary membranes in rats

and cloned human CRF1 at sub-nanomolar concentrations without any detectable effects on CRF2 receptors. It also showed *in vivo* activity. Following administration orally, this compound reduced the binding of radiolabelled ^{125} I-sauvagine to the cerebellar membranes in rats with an excellent oral bioavailability reaching 91%. Compared with other prominent CRF1 antagonists in clinical trials such as R121919 and CP-154526, MTIP had a significant lower volume of distribution and clearance. MTIP, in a dose-dependent manner, reversed anxiogenic effects of alcohol withdrawal [45]. It also stopped ethanol self-administration behavior in rats which has a past of dependence. Also, this compound stopped reestablishment of ethanol seeking behavior which was induced by stress after dependence and also in the genetically-altered animals, at amounts which were ineffective in rats which were not alcohol-dependent rats. Thus, MTIP may have a particular value as a potential agent to treat of alcohol dependence.

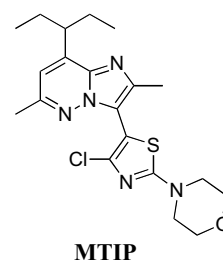


Fig. (10). Structures of Imidazo[1,2-b]pyridazines CRF antagonists.

5.3. Tricyclic CRF1 Receptor Antagonists

These compounds have the nitrogen of the five membered ring of the imidazo[4,5-b]pyridine and pyrazolo[4,3-b]pyridine heterocyclic core attached to the nitrogen at position 7, thus forming a tricyclic triaza- or tetraaza-acenaphthylene derivatives.

5.3.1. Triazaacenaphthylenes

In a series of tricyclic imidazo[4,5-b]pyridine-2-ones CRF1 receptor antagonists, the 5-(heptan-4-yl)-1-(4-methoxyphenyl) derivative (Fig. 11) was identified as a promising antagonist. This compound is very selective to CRF1 receptors and essentially without any affinity to the CRF2 receptors which are expressed in Chinese hamster ovary (CHO) cells. Moreover, it showed insignificant or no binding affinity for more than sixty various receptors, transporters or ion channels. Consequently, it was advanced to additional evaluation to assess its functional CRF1 antagonist properties both *in vitro* and *in vivo*. It inhibited, in a concentration-dependent manner, the accumulation of cyclic adenosine monophosphate (cAMP) induced by stress in the CHO cells expressing the CRF1 receptors. In a second functional assay, it also inhibited the release of CRF-induced adrenocorticotrophic hormone from primary anterior pituitary cell cultures obtained from rats [60]. These functional assays confirmed that this compound is a potent antagonist. Pharmacokinetics studies after either IV or oral administration in male Sprague-Dawley rats confirmed its oral bioavailability and good brain penetrability. Moreover, this analogue exhibited a favorably lower volume of distribution

and a moderate $t_{1/2}$. Consequently, it was subjected to an additional *in vivo* testing. The *in vivo* functional antagonist properties of this compound were determined by measuring the release of ACTH induced by stress in normal rats. Intravenous CRF administration in rats caused a vigorous increase in ACTH plasma levels. Administration of this compound weakened the increase in rat ACTH plasma level which was induced by CRF confirming that it is a functional CRF1 antagonist with enhanced pharmacokinetic profile [60].

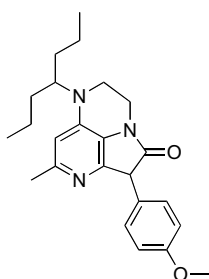
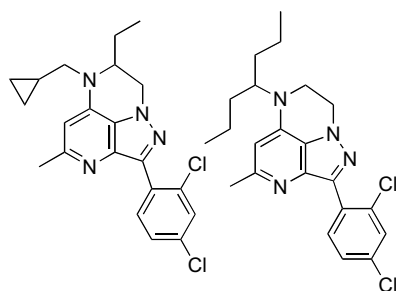


Fig. (11). Structure of Triazaacenaphthylene CRF antagonists.



NBI-35965 NBI-34041

Fig. (12). Structure of Tetraazaacenaphthylene CRF antagonists.

5.3.2. Tetraazaacenaphthylene

A prominent tetraazaacenaphthylene derivative is NBI 35965 (Fig 12). It was identified as an antagonist with a great affinity. This analogue was verified to have functional CRF1 antagonist properties when tested in the CRF-induced cAMP accumulation and ACTH production in the *in vitro* tests. Moreover, it also inhibited the release of ACTH *in vivo* regardless whether it was CRF-induced or caused by stress [59].

Another lead compound in the tetraazaacenaphthylene series is NBI-34041/SB723620 (Fig. 12) [95]. Preclinical studies carried out in various cells expressing human CRF receptors and also in Wistar and Sprague-Dawley rats showed that efficiency of NBI-34041 in inhibiting endocrine responses to pharmacological and behavioral stressors which are facilitated by CRF1 receptors. These particular effects in addition to its established safety profile allowed that compound to move forward to the first phase of clinical trials in 24 healthy males receiving NBI-34041 at doses ranging from 10 to 100 mgs, or placebo for two weeks. The effect on regulating the HPA axis was assessed by giving an IV injection of 100 mg of human CRF. Psychosocial stress response was

evaluated using the Trier Social Stress Test (TSST). Results indicated that NBI-34041 did not alter the secretion of the daytime adrenocorticotrophic hormone (ACTH) and cortisol or affect the CRF-induced ACTH and cortisol responses but weakened the neuro-endocrine reaction to psycho/social stress [95]. A possible explanation is that the neuroendocrine effects of this compound are mediated predominately by the anterior pituitary and the neuromodulatory effects are arbitrated by CRF receptors in the limbic and prefrontal areas. The same phenomenon is supported by another study using anti-CRF1 oligodeoxynucleotides in rats, in which the test compound caused anxiolytic effects [96, 97] without any change in plasma ACTH and corticosterone concentrations [96, 98]. These clinical data results indicate the safety of this compound and that it does not damage the HPA basal regulating system but enhances resistance to psycho/social stressors. Moreover, NBI-34041 endorses the postulation that inhibition of the CRF system can be a potential target in drug discovery efforts aiming to find new drugs against depression and anxiety disorders [95].

CONCLUSION

The vital function of CRF and its implication in several neurological illnesses and in behavioral, cardiovascular, gastrointestinal and immune systems suggest that novel agents which can interfere with the CRF role and binding to its intended receptors may present a new class of neuropsychiatric drugs for many disorders which are related to stress such as anxiety, depression and substance-abuse as well as other stress-related peripheral disorders as Irritable Bowel Syndrome. The physical properties of non-peptide CRF antagonists have a clinical advantage over the peptides counterparts in terms of the pharmacokinetic properties specially bioavailability and brain penetrability. Several small-molecule non-peptides CRF antagonists have been developed with many reaching clinical trial with great promise with the best known antagonists are Antalarmin, Verucerofont and Pexacerofont. Thus, the design of small molecule, non-peptide CRF antagonists may afford new treatment options and a new class of antidepressants and anti-anxiety agents from this class may emerge in the near future.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

- [1] Owens, M.J.; Nemeroff, C.B. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol. Rev.*, **1991**, 43(4), 425-473.
- [2] Fisher, L.A. Corticotropin-releasing factor: endocrine and autonomic integration of responses to stress. *Trends Pharmacol. Sci.*, **1989**, 10(5), 189-193.
- [3] Carpenter, R.E.; Korzan, W.J.; Bockholt, C.; Watt, M.J.; Forster, G.L.; Renner, K.J.; Summers, C.H. Corticotropin releasing factor influences aggression and monoamines: modulation of attacks and retreats. *Neuroscience*, **2009**, 158(2), 412-425.
- [4] Jain, R.; Zwickler, D.; Hollander, C.S.; Brand, H.; Saperstein, A.; Hutchinson, B.; Brown, C.; Audhya, T. Corticotropin-releasing

- factor modulates the immune response to stress in the rat. *Endocrinology*, **1991**, 128(3), 1329-1336.
- [5] Stahl, S.M.; Wise, D.D. The potential role of a corticotropin-releasing factor receptor-1 antagonist in psychiatric disorders. *CNS spectrums*, **2008**, 13(6), 467-483.
 - [6] Kehne, J.H.; Maynard, G.D. CRF1 receptor antagonists: treatment of stress-related disorders. *Drug Discovery Today: Therapeutic Strategies*, **2008**, 5(3), 161-168.
 - [7] Behan, D.P.; Maciejewski, D.; Chalmers, D.; De Souza, E.B. Corticotropin releasing factor binding protein (CRF-BP) is expressed in neuronal and astrocytic cells. *Brain Res.*, **1995**, 698(1-2), 259-264.
 - [8] Potter, E.; Behan, D.P.; Linton, E.A.; Lowry, P.J.; Sawchenko, P.E.; Vale, W.W. The central distribution of a corticotropin-releasing factor (CRF)-binding protein predicts multiple sites and modes of interaction with CRF. *Proc. Natl. Acad. Sci.*, **1992**, 89(9), 4192-4196.
 - [9] Woods, R.J.; David, J.; Baigent, S.; Gibbins, J.; Lowry, P.J. Elevated levels of corticotropin-releasing factor binding protein in the blood of patients suffering from arthritis and septicaemia and the presence of novel ligands in synovial fluid. *Br. J. Rheumatol.*, **1996**, 35(2), 120-124.
 - [10] Lowry, P.J.; Woods, R.J.; Baigent, S. Corticotropin releasing factor and its binding protein. *Pharmacol. Biochem. Behav.*, **1996**, 54(1), 305-308.
 - [11] Greenberg, P.E.; Sisitsky, T.; Kessler, R.C.; Finkelstein, S.N.; Berndt, E.R.; Davidson, J.R.; Ballenger, J.C.; Fyer, A.J. The economic burden of anxiety disorders in the 1990s. *J. Clin. Psychiatry*, **1999**, 60(7), 427-435.
 - [12] Kessler, R.C.; Chiu, W.T.; Demler, O.; Merikangas, K.R.; Walters, E.E. Prevalence, severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch. Gen. Psychiatry*, **2005**, 62(6), 617-627.
 - [13] Greenberg, P.E.; Kessler, R.C.; Birnbaum, H.G.; Leong, S.A.; Lowe, S.W.; Berglund, P.A.; Corey-Lisle, P.K. The economic burden of depression in the United States: how did it change between 1990 and 2000? *J. Clin. Psychiatry*, **2003**, 64(12), 1465-1475.
 - [14] Krishnan, K.R.; Delong, M.; Kraemer, H.; Carney, R.; Spiegel, D.; Gordon, C.; McDonald, W.; Dew, M.; Alexopoulos, G.; Buckwalter, K.; Cohen, P.D.; Evans, D.; Kaufmann, P.G.; Olin, J.; Otey, E.; Wainwright, C. Comorbidity of depression with other medical diseases in the elderly. *Biol. Psychiatry*, **2002**, 52(6), 559-588.
 - [15] Devane, C.L.; Chiao, E.; Franklin, M.; Kruep, E.J. Anxiety disorders in the 21st century: status, challenges, opportunities, and comorbidity with depression. *Am. J. Managed. Care*, **2005**, 11(12 Suppl), S344-S353.
 - [16] Andrews, J.; Pinder, R. In *Antidepressants*. Leonard, B. Ed.; Birkhäuser Basel, **2001**, pp 123-145.
 - [17] Broekkamp, C.L.; Laysen, D.; Peeters, B.W.; Pinder, R.M. Prospects for improved antidepressants. *J. Med. Chem.*, **1995**, 38(23), 4615-4633.
 - [18] Holmes, A.; Heilig, M.; Rupniak, N.M.; Steckler, T.; Griebel, G. Neuropeptide systems as novel therapeutic targets for depression and anxiety disorders. *Trends Pharmacol Sci*, **2003**, 24(11), 580-588.
 - [19] Arato, M.; Banki, C.M.; Bissette, G.; Nemeroff, C.B. Elevated CSF CRF in suicide victims. *Biol. Psychiatry*, **1989**, 25(3), 355-359.
 - [20] Gross, M.J.; Kahn, J.P.; Laxenaire, M.; Nicolas, J.P.; Burlet, C. [Corticotropin-releasing factor and anorexia nervosa: reactions of the hypothalamus-pituitary-adrenal axis to neurotropic stress]. *Annales d'endocrinologie*, **1994**, 55(6), 221-228.
 - [21] Raadsheer, F.C.; van Heerikhuize, J.J.; Lucassen, P.J.; Hoogendijk, W.J.; Tilders, F.J.; Swaab, D.F. Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. *Am. J. Psychiatry*, **1995**, 152(9), 1372-1376.
 - [22] Whitehouse, P.J.; Vale, W.W.; Zweig, R.M.; Singer, H.S.; Mayeux, R.; Kuhar, M.J.; Price, D.L.; De Souza, E.B. Reductions in corticotropin releasing factor-like immunoreactivity in cerebral cortex in Alzheimer's disease, Parkinson's disease, and progressive supranuclear palsy. *Neurology*, **1987**, 37(6), 905-909.
 - [23] Gold, P.W.; Loriaux, D.L.; Roy, A.; Kling, M.A.; Calabrese, J.R.; Kellner, C.H.; Nieman, L.K.; Post, R.M.; Pickar, D.; Gallucci, W.; W. Gallucci, Avgerinos, P.; Paul, S.; Oldfield, E.H.; Cutler, Jr. G.B.; Chrousos, G.P. Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. Pathophysiologic and diagnostic implications. *New England J. Med.*, **1986**, 314(21), 1329-1335.
 - [24] Carroll, B.J.; Curtis, G.C.; Davies, B.M.; Mendels, J.; Sugerman, A.A. Urinary free cortisol excretion in depression. *Psychological medicine*, **1976**, 6(1), 43-50.
 - [25] Nemeroff, C.B.; Bissette, G.; Akil, H.; Fink, M. Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy. Corticotrophin-releasing factor, beta-endorphin and somatostatin. *Br. J. Psychiatry*, **1991**, 158, 59-63.
 - [26] Kasahara, M.; Groenink, L.; Breuer, M.; Olivier, B.; Sarnyai, Z. Altered behavioural adaptation in mice with neural corticotrophin-releasing factor overexpression. *Genes Brain Behav.*, **2007**, 6(7), 598-607.
 - [27] Mailliot, C.; Million, M.; Wei, J.Y.; Gauthier, A.; Tache, Y. Peripheral corticotropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. *Gastroenterology*, **2000**, 119(6), 1569-1579.
 - [28] Martinez, V.; Tache, Y. CRF1 receptors as a therapeutic target for irritable bowel syndrome. *Curr. Pharma. Des.*, **2006**, 12(31), 4071-4088.
 - [29] Tache, Y.; Million, M.; Nelson, A.G.; Lamy, C.; Wang, L. Role of corticotropin-releasing factor pathways in stress-related alterations of colonic motor function and viscerosensitivity in female rodents. *Gender Med.*, **2005**, 2(3), 146-154.
 - [30] Sarnyai, Z.; Shaham, Y.; Heinrichs, S.C. The role of corticotropin-releasing factor in drug addiction. *Pharmacological reviews*, **2001**, 53(2), 209-243.
 - [31] Logrip, M.L.; Koob, G.F.; Zorrilla, E.P. Role of corticotropin-releasing factor in drug addiction: potential for pharmacological intervention. *CNS Drugs*, **2011**, 25(4), 271-287.
 - [32] Crofford, L.J.; Sano, H.; Karalis, K.; Webster, E.L.; Goldmuntz, E.A.; Chrousos, G.P.; Wilder, R.L. Local secretion of corticotropin-releasing hormone in the joints of Lewis rats with inflammatory arthritis. *The Journal of clinical investigation*, **1992**, 90(6), 2555-2564.
 - [33] Crofford, L.J.; Sano, H.; Karalis, K.; Friedman, T.C.; Epps, H.R.; Remmers, E.F.; Mathern, P.; Chrousos, G.P.; Wilder, R.L. Corticotropin-releasing hormone in synovial fluids and tissues of patients with rheumatoid arthritis and osteoarthritis. *J. Immunol.*, **1993**, 151(3), 1587-1596.
 - [34] Chikanza, I.C.; Petrou, P.; Kingsley, G.; Chrousos, G.; Panayi, G.S. Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. *Arthritis Rheum.*, **1992**, 35(11), 1281-1288.
 - [35] Sternberg, E.M.; Young, W.S.; Bernardini, R.; Calogero, A.E.; Chrousos, G.P.; Gold, P.W.; Wilder, R.L. A Central Nervous-System Defect in Biosynthesis of Corticotropin-Releasing Hormone Is Associated with Susceptibility to Streptococcal Cell Wall-Induced Arthritis in Lewis Rats. *Proc. Natl. Acad. Sci. USA.*, **1989**, 86(12), 4771-4775.
 - [36] Calogero, A.E.; Sternberg, E.M.; Bagdy, G.; Smith, C.; Bernardini, R.; Aksentijevich, S.; Wilder, R.L.; Gold, P.W.; Chrousos, G.P. Neurotransmitter-Induced Hypothalamic-Pituitary-Adrenal Axis Responsiveness Is Defective in Inflammatory Disease-Susceptible Lewis Rats: *In vivo* and *in vitro* Studies Suggesting Globally Defective Hypothalamic Secretion of Corticotropin-Releasing Hormone. *Neuroendocrinology*, **1992**, 55(5), 600-608.
 - [37] Curtis, A.L.; Lechner, S.M.; Pavcovich, L.A.; Valentino, R.J. Activation of the locus coeruleus noradrenergic system by intracerebral microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. *J. Pharmacol. Exp. Therapeutics*, **1997**, 281(1), 163-172.
 - [38] Valentino, R.J.; Pavcovich, L.A.; Hirata, H. Evidence for corticotropin-releasing hormone projections from Barrington's nucleus to the periaqueductal gray and dorsal motor nucleus of the vagus in the rat. *J. Comparative Neurol.*, **1995**, 363(3), 402-422.

- [39] Brown, M.R.; Fisher, L.A.; Spiess, J.; Rivier, C.; Rivier, J.; Vale, W. Corticotropin-releasing factor: actions on the sympathetic nervous system and metabolism. *Endocrinology*, **1982**, *111*(3), 928-931.
- [40] Wood, S.K.; Woods, J.H. Corticotropin-releasing factor receptor-1: a therapeutic target for cardiac autonomic disturbances. *Expert Opin. Ther. Targets*, **2007**, *11*(11), 1401-1413.
- [41] Gulyas, J.; Rivier, C.; Perrin, M.; Koerber, S.C.; Sutton, S.; Corrigan, A.; Lahrichi, S.L.; Craig, A.G.; Vale, W.; Rivier, J. Potent, structurally constrained agonists and competitive antagonists of corticotropin-releasing factor. *Proc. Natl. Acad. Sci. U.S.A.*, **1995**, *92*(23), 10575-10579.
- [42] Grigoriadis, D.E. Corticotropin-releasing factor receptor antagonists: potential novel therapies for human diseases. *Sigma-Aldrich Corporation: Celltransmissions*, **2003**, *19*(4), 3-10.
- [43] Gilligan, P.J.; Robertson, D.W.; Zaczek, R. Corticotropin Releasing Factor (CRF) Receptor Modulators: Progress and Opportunities for New Therapeutic Agents. *J. Med. Chem.*, **2000**, *43*(9), 1641-1660.
- [44] Zorrilla, E.P.; Koob, G.F. Progress in corticotropin-releasing factor-1 antagonist development. *Drug Discov. Today*, **2010**, *15*(9-10), 371-383.
- [45] Gehlert, D.R.; Cipitelli, A.; Thorsell, A.; Le, A.D.; Hipskind, P.A.; Hamdouchi, C.; Lu, J.; Hembre, E.J.; Cramer, J.; Song, M.; McKinzie, D.; Morin, M.; Ciccocioppo, R.; Heilig, M. 3-(4-Chloro-2-morpholin-4-yl-thiazol-5-yl)-8-(1-ethylpropyl)-2,6-dimethylimidazo [1,2-b]pyridazine: a novel brain-penetrant, orally available corticotropin-releasing factor receptor 1 antagonist with efficacy in animal models of alcoholism. *J. Neurosci.*, **2007**, *27*(10), 2718-2726.
- [46] Yoon, T.; De Lombaert, S.; Brodbeck, R.; Gulianello, M.; Krause, J.E.; Hutchison, A.; Horvath, R.F.; Ge, P.; Kehne, J.; Hoffman, D.; Chandrasekhar, J.; Doller, D.; Hodgetts, K.J. 2-Arylpurimidines: novel CRF-1 receptor antagonists. *Bioorganic Med. Chem. Lett.*, **2008**, *18*(16), 4486-4490.
- [47] Beck, J.P.; Arvanitis, A.G.; Curry, M.A.; Rescinito, J.T.; Fitzgerald, L.W.; Gilligan, P.J.; Zaczek, R.; Trainor, G.L. Purin-8-ones as corticotropin-releasing hormone (CRH-R1) receptor antagonists. *Bioorganic Med. Chem. Lett.*, **1999**, *9*(7), 967-972.
- [48] Yoon, T.; De Lombaert, S.; Brodbeck, R.; Gulianello, M.; Chandrasekhar, J.; Horvath, R.F.; Ge, P.; Kershaw, M.T.; Krause, J.E.; Kehne, J.; Hoffman, D.; Doller, D.; Hodgetts, K.J. The design, synthesis and structure-activity relationships of 1-aryl-4-aminoalkylisoquinolines: a novel series of CRF-1 receptor antagonists. *Bioorganic Med. Chem. Lett.*, **2008**, *18*(3), 891-896.
- [49] Chen, Y.L.; Braselton, J.; Forman, J.; Gallaschun, R.J.; Mansbach, R.; Schmidt, A.W.; Seeger, T.F.; Sprouse, J.S.; Tingley, F.D. 3rd; Winston, E.; Schulz, D.W. Synthesis and SAR of 2-aryloxy-4-alkoxy-pyridines as potent orally active corticotropin-releasing factor 1 receptor antagonists. *J. Med. Chem.*, **2008**, *51*(5), 1377-1384.
- [50] Hartz, R.A.; Arvanitis, A.G.; Arnold, C.; Rescinito, J.P.; Hung, K.L.; Zhang, G.; Wong, H.; Langley, D.R.; Gilligan, P.J.; Trainor, G.L. Synthesis and evaluation of 2-anilino-3-phenylsulfonyl-6-methylpyridines as corticotropin-releasing factor1 receptor ligands. *Bioorganic Med. Chem. Lett.*, **2006**, *16*(4), 934-937.
- [51] Han, X.; Civiello, R.; Pin, S.S.; Burris, K.; Balanda, L.A.; Knipe, J.; Ren, S.; Fiedler, T.; Browman, K.E.; Macci, R.; Taber, M.T.; Zhang, J.; Dubowchik, G.M. An orally active corticotropin releasing factor 1 receptor antagonist from 8-aryl-1,3a,7,8-tetraazacyclopenta[a]indenes. *Bioorganic Med. Chem. Lett.*, **2007**, *17*(7), 2026-2030.
- [52] Han, X.; Michne, J.A.; Pin, S.S.; Burris, K.D.; Balanda, L.A.; Fung, L.K.; Fiedler, T.; Browman, K.E.; Taber, M.T.; Zhang, J.; Dubowchik, G.M. Synthesis, structure-activity relationships, and anxiolytic activity of 7-aryl-6,7-dihydroimidazoimidazole corticotropin-releasing factor 1 receptor antagonists. *Bioorganic Med. Chem. Lett.*, **2005**, *15*(17), 3870-3873.
- [53] Han, X.; Pin, S.S.; Burris, K.; Fung, L.K.; Huang, S.; Taber, M.T.; Zhang, J.; Dubowchik, G.M. Synthesis and structure-activity relationship of imidazo[1,2-a]benzimidazoles as corticotropin-releasing factor 1 receptor antagonists. *Bioorganic Med. Chem. Lett.*, **2005**, *15*(18), 4029-4032.
- [54] Gentile, G.; Di Fabio, R.; Pavone, F.; Sabbatini, F.M.; St-Denis, Y.; Zampori, M.G.; Vitulli, G.; Worby, A. Novel substituted tetrahydrotriazacenaphthylene derivatives as potent CRF1 receptor antagonists. *Bioorganic Med. Chem. Lett.*, **2007**, *17*(18), 5218-5221.
- [55] St-Denis, Y.; Di Fabio, R.; Bernasconi, G.; Castiglioni, E.; Contini, S.; Donati, D.; Fazzolari, E.; Gentile, G.; Ghirlanda, D.; Marchionni, C.; Messina, F.; Micheli, F.; Pavone, F.; Pasquarello, A.; Sabbatini, F.M.; Zampori, M.G.; Arban, R.; Vitulli, G. Substituted tetraazaacenaphthylenes as potent CRF1 receptor antagonists for the treatment of depression and anxiety. *Bioorganic Med. Chem. Lett.*, **2005**, *15*(16), 3713-3716.
- [56] Sabbatini, F.M.; Di Fabio, R.; St-Denis, Y.; Capelli, A.M.; Castiglioni, E.; Contini, S.; Donati, D.; Fazzolari, E.; Gentile, G.; Micheli, F.; Pavone, F.; Rinaldi, M.; Pasquarello, A.; Zampori, M.G.; Di Felice, P.; Zarrantonello, P.; Arban, R.; Perini, B.; Vitulli, G.; Benedetti, R.; Olios, B.; Worby, A. Heteroaryl-substituted 4-(1H-pyrazol-1-yl)-5,6-dihydro-1H-pyrrolo[2,3-d]pyrimidine derivatives as potent and selective corticotropin-releasing factor receptor-1 antagonists. *Chem. Med. Chem.*, **2008**, *3*(2), 226-229.
- [57] Whitten, J.P.; Xie, Y.F.; Erickson, P.E.; Webb, T.R.; De Souza, E.B.; Grigoriadis, D.E.; McCarthy, J.R. Rapid microscale synthesis, a new method for lead optimization using robotics and solution phase chemistry: application to the synthesis and optimization of corticotropin-releasing factor1 receptor antagonists. *J. Med. Chem.*, **1996**, *39*(22), 4354-4357.
- [58] Chen, C.; Dagnino, R. Jr.; De Souza, E.B.; Grigoriadis, D.E.; Huang, C.Q.; Kim, K.I.; Liu, Z.; Moran, T.; Webb, T.R.; Whitten, J.P.; Xie, Y.F.; McCarthy, J.R. Design and synthesis of a series of non-peptide high-affinity human corticotropin-releasing factor1 receptor antagonists. *J. Med. Chem.*, **1996**, *39*(22), 4358-4360.
- [59] Gross, R.S.; Guo, Z.; Dyck, B.; Coon, T.; Huang, C.Q.; Lowe, R.F.; Marinkovic, D.; Moorjani, M.; Nelson, J.; Zamani-Kord, S.; Grigoriadis, D.E.; Hoare, S.R.; Crowe, P.D.; Bu, J.H.; Haddach, M.; McCarthy, J.; Saunders, J.; Sullivan, R.; Chen, T.; Williams, J.P. Design and synthesis of tricyclic corticotropin-releasing factor-1 antagonists. *J. Med. Chem.*, **2005**, *48*(18), 5780-5793.
- [60] Guo, Z.; Tellew, J.E.; Gross, R.S.; Dyck, B.; Grey, J.; Haddach, M.; Kiankarimi, M.; Lanier, M.; Li, B.F.; Luo, Z.; McCarthy, J.R.; Moorjani, M.; Saunders, J.; Sullivan, R.; Zhang, X.; Zamani-Kord, S.; Grigoriadis, D.E.; Crowe, P.D.; Chen, T.K.; Williams, J.P. Design and synthesis of tricyclic imidazo[4,5-b]pyridin-2-ones as corticotropin-releasing factor-1 antagonists. *J. Med. Chem.*, **2005**, *48*(16), 5104-5107.
- [61] Lowe, R.F.; Nelson, J.; Dang, T.N.; Crowe, P.D.; Pahuja, A.; McCarthy, J.R.; Grigoriadis, D.E.; Conlon, P.; Saunders, J.; Chen, C.; Szabo, T.; Chen, T.K.; Bozigian, H. Rational design, synthesis, and structure-activity relationships of aryltriazoles as novel corticotropin-releasing factor-1 receptor antagonists. *J. Med. Chem.*, **2005**, *48*(5), 1540-1549.
- [62] Chen, Y.L.; Obach, R.S.; Braselton, J.; Corman, M.L.; Forman, J.; Freeman, J.; Gallaschun, R.J.; Mansbach, R.; Schmidt, A.W.; Sprouse, J.S.; Tingley, J., F.D.; Winston, E.; Schulz, D.W. 2-aryloxy-4-alkylaminopyridines: discovery of novel corticotropin-releasing factor 1 antagonists. *J. Med. Chem.*, **2008**, *51*(5), 1385-1392.
- [63] Hodge, C.N.; Aldrich, P.E.; Wasserman, Z.R.; Fernandez, C.H.; Nemeth, G.A.; Arvanitis, A.; Cheeseman, R.S.; Chorvat, R.J.; Ciganek, E.; Christos, T.E.; Gilligan, P.J.; Krenitsky, P.; Scholfield, E.; Strucely, P. Corticotropin-releasing hormone receptor antagonists: framework design and synthesis guided by ligand conformational studies. *J. Med. Chem.*, **1999**, *42*(5), 819-832.
- [64] Wustrow, D.J.; Capiris, T.; Rubin, R.; Knobelsdorf, J.A.; Akunne, H.; Davis, M.D.; MacKenzie, R.; Pugsley, T.A.; Zoski, K.T.; Heffner, T.G.; Wise, L.D. Pyrazolo[1,5-a]pyrimidine CRF-1 receptor antagonists. *Bioorg. Med. Chem. Lett.*, **1998**, *8*(16), 2067-2070.
- [65] Hodgetts, K.J.; Ge, P.; Yoon, T.; De Lombaert, S.; Brodbeck, R.; Gulianello, M.; Kieptyka, A.; Horvath, R.F.; Kehne, J.H.; Krause, J.E.; Maynard, G.D.; Hoffman, D.; Lee, Y.; Fung, L.; Doller, D. Discovery of N-(1-ethylpropyl)-5-(2-methoxy-5-(2-methoxy-4-trifluoromethoxyphenyl)-6-methyl-pyrazin-2-yl)amine 59 (NGD

- 98-2): an orally active corticotropin releasing factor-1 (CRF-1) receptor antagonist. *J. Med. Chem.*, **2011**, 54(12), 4187-4206.
- [66] Million, M.; Zhao, J.F.; Luckey, A.; Czimmer, J.; Maynard, G.D.; Kehne, J.; Hoffman, D.C.; Tache, Y. The newly developed CRF1-receptor antagonists, NGD 98-2 and NGD 9002, suppress acute stress-induced stimulation of colonic motor function and visceral hypersensitivity in rats. *PLoS one*, **2013**, 8(9), e73749.
- [67] Nakazato, A.; Kumagai, T.; Okubo, T.; Tanaka, H.; Chaki, S.; Okuyama, S.; Tomisawa, K. Design, synthesis and structure-affinity relationships of 4-methylidenepiperidine and 4-aryl-1,2,3,6-tetrahydropyridine derivatives as corticotropin-releasing factor1 receptor antagonists. *Bioorganic Med. Chem.*, **2000**, 8(5), 1183-1193.
- [68] Arai, K.; Ohata, H.; Shibasaki, T. Non-peptidic corticotropin-releasing hormone receptor type 1 antagonist reverses restraint stress-induced shortening of sodium pentobarbital-induced sleeping time of rats: evidence that an increase in arousal induced by stress is mediated through CRH receptor type 1. *Neurosci. Lett.*, **1998**, 255(2), 103-106.
- [69] Gully, D.; Geslin, M.; Serva, L.; Fontaine, E.; Roger, P.; Lair, C.; Darre, V.; Marcy, C.; Rouby, P.E.; Simiand, J.; Guitard, J.; Gout, G.; Steinberg, R.; Rodier, D.; Griebel, G.; Soubrie, P.; Pascal, M.; Pruss, R.; Scatton, B.; Maffrand, J.P.; Le Fur, G. 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-methylphenyl)ethyl]-5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A): A potent and selective corticotropin-releasing factor(1) receptor antagonist. I. Biochemical and pharmacological characterization. *J. Pharmacol. Exper. Ther.*, **2002**, 301(1), 322-332.
- [70] Webster, E.L.; Barrientos, R.M.; Contoreggi, C.; Isaac, M.G.; Ligier, S.; Gabry, K.E.; Chrousos, G.P.; McCarthy, E.F.; Rice, K.C.; Gold, P.W.; Sternberg, E.M. Corticotropin releasing hormone (CRH) antagonist attenuates adjuvant induced arthritis: role of CRH in peripheral inflammation. *J. Rheumatol.*, **2002**, 29(6), 1252-1261.
- [71] Greenwood-Van Meerveld, B.; Johnson, A.C.; Cochrane, S.; Schulkin, J.; Myers, D.A. Corticotropin-releasing factor 1 receptor-mediated mechanisms inhibit colonic hypersensitivity in rats. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society*, **2005**, 17(3), 415-422.
- [72] Gabry, K.E.; Chrousos, G.P.; Rice, K.C.; Mostafa, R.M.; Sternberg, E.; Negrao, A.B.; Webster, E.L.; McCann, S.M.; Gold, P.W. Marked suppression of gastric ulcerogenesis and intestinal responses to stress by a novel class of drugs. *Mol. Psychiatry*, **2002**, 7(5), 474-483, 433.
- [73] Webster, E.L.; Lewis, D.B.; Torpy, D.J.; Zachman, E.K.; Rice, K.C.; Chrousos, G.P. *In vivo* and *in vitro* characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRH) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology*, **1996**, 137(12), 5747-5750.
- [74] Briscoe, R.J.; Cabrera, C.L.; Baird, T.J.; Rice, K.C.; Woods, J.H. Antalarmin blockade of corticotropin releasing hormone-induced hypertension in rats. *Brain Res*, **2000**, 881(2), 204-207.
- [75] Mello, N.K.; Negus, S.S.; Rice, K.C.; Mendelson, J.H. Effects of the CRF1 antagonist antalarmin on cocaine self-administration and discrimination in rhesus monkeys. *Pharmacol. Biochem. Behav.*, **2006**, 85(4), 744-751.
- [76] Funk, C.K.; Zorrilla, E.P.; Lee, M.J.; Rice, K.C.; Koob, G.F. Corticotropin-releasing factor 1 antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. *Biol. psychiatry*, **2007**, 61(1), 78-86.
- [77] Dautzenberg, F.M.; Hauger, R.L. The CRF peptide family and their receptors: yet more partners discovered. *Trends Pharmacol. Sci.*, **2002**, 23(2), 71-77.
- [78] Hauger, R.L.; Grigoriadis, D.E.; Dallman, M.F.; Plotsky, P.M.; Vale, W.W.; Dautzenberg, F.M. International Union of Pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. *Pharmacol. Rev.*, **2003**, 55(1), 21-26.
- [79] Kawahara, H.; Kawahara, Y.; Westerink, B.H. The role of afferents to the locus coeruleus in the handling stress-induced increase in the release of noradrenaline in the medial prefrontal cortex: a dual-probe microdialysis study in the rat brain. *Eur. J. Pharmacol.*, **2000**, 387(3), 279-286.
- [80] McCarthy, J.R.; Heinrichs, S.C.; Grigoriadis, D.E. Recent advances with the CRF1 receptor: design of small molecule inhibitors, receptor subtypes and clinical indications. *Curr. Pharma. Des.*, **1999**, 5(5), 289-315.
- [81] Seymour, P.A.; Schmidt, A.W.; Schulz, D.W. The pharmacology of CP-154,526, a non-peptide antagonist of the CRH1 receptor: a review. *CNS Drug Rev.*, **2003**, 9(1), 57-96.
- [82] Goeders, N.E.; Guerin, G.F. Effects of the CRH receptor antagonist CP-154,526 on intravenous cocaine self-administration in rats. *Neuropsychopharmacology*, **2000**, 23(5), 577-586.
- [83] Pastor, R.; McKinnon, C.S.; Scibelli, A.C.; Burkhart-Kasch, S.; Reed, C.; Ryabinin, A.E.; Coste, S.C.; Stenzel-Poore, M.P.; Phillips, T.J. Corticotropin-releasing factor-1 receptor involvement in behavioral neuroadaptation to ethanol: a urocortin1-independent mechanism. *Proc. Natl. Acad. Sci. USA.*, **2008**, 105(26), 9070-9075.
- [84] Gilligan, P.J.; Clarke, T.; He, L.; Lelas, S.; Li, Y.W.; Heman, K.; Fitzgerald, L.; Miller, K.; Zhang, G.; Marshall, A.; Krause, C.; McElroy, J.F.; Ward, K.; Zeller, K.; Wong, H.; Bai, S.; Saye, J.; Grossman, S.; Zaczek, R.; Arneric, S.P.; Hartig, P.; Robertson, D.; Trainor, G. Synthesis and structure-activity relationships of 8-(pyrid-3-yl)pyrazolo[1,5-a]-1,3,5-triazines: potent, orally bioavailable corticotropin releasing factor receptor-1 (CRF1) antagonists. *J. Med. Chem.*, **2009**, 52(9), 3084-3092.
- [85] Gilligan, P.J.; He, L.; Clarke, T.; Tivimahaissoon, P.; Lelas, S.; Li, Y.W.; Heman, K.; Fitzgerald, L.; Miller, K.; Zhang, G.; Marshall, A.; Krause, C.; McElroy, J.; Ward, K.; Shen, H.; Wong, H.; Grossman, S.; Nemeth, G.; Zaczek, R.; Arneric, S.P.; Hartig, P.; Robertson, D.W.; Trainor, G. 8-(4-Methoxyphenyl)pyrazolo[1,5-a]-1,3,5-triazines: selective and centrally active corticotropin-releasing factor receptor-1 (CRF1) antagonists. *J. Med. Chem.*, **2009**, 52(9), 3073-3083.
- [86] Tellew, J.E.; Lanier, M.; Moorjani, M.; Lin, E.; Luo, Z.; Slee, D.H.; Zhang, X.; Hoare, S.R.; Grigoriadis, D.E.; St Denis, Y.; Di Fabio, R.; Di Modugno, E.; Saunders, J.; Williams, J.P. Discovery of NBI-77860/GSK561679, a potent corticotropin-releasing factor (CRF1) receptor antagonist with improved pharmacokinetic properties. *Bioorganic Med. Chem. Lett.*, **2010**, 20(24), 7259-7264.
- [87] He, L.; Gilligan, P.J.; Zaczek, R.; Fitzgerald, L.W.; McElroy, J.; Shen, H.S.L.; Saye, J.A.; Kalin, N.H.; Shelton, S.; Christ, D.; Trainor, G.; Hartig, P. 4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)pyrazolo[1,5-a]-1,3,5-triazine: A Potent, Orally Bioavailable CRF1 Receptor Antagonist. *J. Med. Chem.*, **2000**, 43(3), 449-456.
- [88] Chen, C.; Wilcoxon, K.M.; Huang, C.Q.; Xie, Y.F.; McCarthy, J.R.; Webb, T.R.; Zhu, Y.F.; Saunders, J.; Liu, X.J.; Chen, T.K.; Bozigan, H.; Grigoriadis, D.E. Design of 2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylaminopyrazolo[1,5-a]pyrimidine (NBI 30775/R121919) and structure-activity relationships of a series of potent and orally active corticotropin-releasing factor receptor antagonists. *J. Med. Chem.*, **2004**, 47(19), 4787-4798.
- [89] Gilligan, P.J.; Baldauf, C.; Cocuzza, A.; Chidester, D.; Zaczek, R.; Fitzgerald, L.W.; McElroy, J.; Smith, M.A.; Shen, H.S.; Saye, J.A.; Christ, D.; Trainor, G.; Robertson, D.W.; Hartig, P. The discovery of 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)pyrazolo-[1,5-a]-pyrimidine: a corticotropin-releasing factor (hCRF1) antagonist. *Bioorganic Med. Chem.*, **2000**, 8(1), 181-189.
- [90] Zobel, A.W.; Nickel, T.; Kunzel, H.E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. Effects of the high-affinity corticotropin-releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatric Res.*, **2000**, 34(3), 171-181.
- [91] Coric, V.; Feldman, H.H.; Oren, D.A.; Shekhar, A.; Pultz, J.; Dockens, R.C.; Wu, X.; Gentile, K.A.; Huang, S.P.; Emison, E.; Delmonte, T.; D'Souza, B.B.; Zimbardo, D.L.; Grebb, J.A.; Goddard, A.W.; Stock, E.G. Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder. *Depression Anxiety*, **2010**, 27(5), 417-425.

- [92] Sweetser, S.; Camilleri, M.; Linker Nord, S.J.; Burton, D.D.; Castenada, L.; Croop, R.; Tong, G.; Dockens, R.; Zinsmeister, A.R. Do corticotropin releasing factor-1 receptors influence colonic transit and bowel function in women with irritable bowel syndrome? *American journal of physiology. Gastrointest. Liver Physiol.*, **2009**, *296*(6), G1299-1306.
- [93] NBI-77860 Development: Classical Congenital Adrenal Hyperplasia. <http://www.neurocrine.com/pipeline/nbi-77860-crf1-antagonist/> (8 Dec, 2015),
- [94] Zorrilla, E.P.; Heilig, M.; de Wit, H.; Shaham, Y. Behavioral, biological, and chemical perspectives on targeting CRF1 receptor antagonists to treat alcoholism. *Drug Alcohol Dependence*, **2013**, *128*(3), 175-186.
- [95] Ising, M.; Zimmermann, U.S.; Kunzel, H.E.; Uhr, M.; Foster, A.C.; Learned-Coughlin, S.M.; Holsboer, F.; Grigoriadis, D.E. High-affinity CRF1 receptor antagonist NBI-34041: preclinical and clinical data suggest safety and efficacy in attenuating elevated stress response. *Neuropsychopharmacology*, **2007**, *32*(9), 1941-1949.
- [96] Heinrichs, S.C.; Lapsansky, J.; Lovenberg, T.W.; De Souza, E.B.; Chalmers, D.T. Corticotropin-releasing factor CRF1, but not CRF2, receptors mediate anxiogenic-like behavior. *Regulatory Peptides*, **1997**, *71*(1), 15-21.
- [97] Liebsch, G.; Landgraf, R.; Gerstberger, R.; Probst, J.C.; Wotjak, C.T.; Engelmann, M.; Holsboer, F.; Montkowski, A. Chronic infusion of a CRH1 receptor antisense oligodeoxynucleotide into the central nucleus of the amygdala reduced anxiety-related behavior in socially defeated rats. *Regulatory Peptides*, **1995**, *59*(2), 229-239.
- [98] Liebsch, G.; Landgraf, R.; Engelmann, M.; Lörscher, P.; Holsboer, F. Differential behavioural effects of chronic infusion of CRH 1 and CRH 2 receptor antisense oligonucleotides into the rat brain. *J. Psychiatric Res.*, **1999**, *33*(2), 153-163.