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Original article

Synthesis of substituted pyrimidines as corticotropin releasing factor (CRF) receptor ligands

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ABSTRACT

Corticotropin releasing factor (CRF) is a neuropeptide hormone produced from the hypothalamus that controls the secretion of corticotropin (ACTH) from the anterior pituitary gland that, in turn, prompts the adrenal glands to secrete glucocorticoids. This involvement in the hypothalamic–pituitary–adrenal axis (HPA) in response to stress and also playing a key role in behavioral, cardiovascular, immune and gastrointestinal systems made CRF binding to its receptors an important target in drug discovery aiming to develop lead compounds with the potential to treat various stress-related disorders including depression, anxiety and addictive disorders. Several non-peptide CRF_1 receptor antagonists were developed by pharmaceutical companies and are currently in clinical trials with the aim of improving the health consequences of chronic stress and for use in the clinical management of anxiety and stress. Many showed promising results not only in treatment of anxiety and depression but also in treatment of CRF-induced hypertension, as well as in treatment of arthritis, irritable bowel syndrome and peptic ulcers. In this manuscript, we describe the synthesis of substituted pyrimidines with close structural similarities to reported lead compounds with promising CRF_1 receptor affinities and carrying groups known to be associated with optimum affinity to CRF_1 receptors. The affinity of the newly prepared compounds in comparison to antalarmin, a potent CRF_1 receptor antagonist in clinical trials as a standard, is also described. Four compounds from the new series showed promising CRF_1 receptor affinity.

1. Introduction

Corticotropin releasing factor (CRF), also called corticotropin releasing hormone (CRH), is a hypothalamic hormone that plays a key role in the maintenance of homeostasis. CRF is secreted by the paraventricular nucleus of the hypothalamus in response to stress and is transported via the portal vein to the anterior lobe of the pituitary gland where it causes the release of corticotropin (also called adrenocorticotrophic hormone ACTH). The released ACTH is then transported by the blood to the adrenal glands where it stimulates the release of endogenous glucocorticoids [1,2]. In addition to the regulation of the hypothalamus–pituitary–adrenal axis (HPA), CRF plays an important role in stress as well as in many physiological and pathological processes such as the cardiovascular, gastrointestinal, behavioral, immune and reproductive systems [3–17].

CRF is a 41 amino acid neuropeptide and belongs to a family of structurally related highly homologous peptides from several species such as rat h/rCRF, human h/rCRF, goat, cow, pig and xenopus CRF [1,18–22]. In addition to the previously mentioned CRF peptides, the CRF family also includes peptides from various species such as sauvagine, urotensin and urocortins. These peptides are closely related to CRF and are considered CRF-like peptides. Sauvagine (SVG) and urotensin (URO) have been isolated from the frog *Phyllomedusa sauvagei* and the sucker fish *Catostomus commersoni*, respectively, whereas urocortin (Ucn), urocortin II (UcnII) and urocortin III (UcnIII) have been isolated from mammals. These are natural ligands that bind to and activate CRF receptors [23–27].

CRF and its analogs exert their actions by interacting with two types of plasma membrane receptors, CRF_1 and CRF_2. Both CRF receptor types belong to the superfamily of G protein-coupled receptors (GPCR) [28–30]. Several receptor sub-types exist for CRF_1 and CRF_2 [31]. The main functional receptor subtype of CRF_1 is $CRF_{1\alpha}$ that mediates most of the action of CRF family peptides. $CRF_{1\alpha}$ will be mentioned in this manuscript as CRF_1 for simplicity.

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Because of important role and involvement of CRF and its receptors in CNS, behavioral, cardiovascular, gastrointestinal, immune and reproductive systems, several non-peptide CRF antagonists were developed as new leads in drug discovery to treat various stress-related disorders including depression, anxiety [32,33] and addictive disorders [34–36]. Most non-peptide CRF antagonists discovered to date are substituted five or six membered heterocyclic or bicyclic rings [37,38]. These non-peptide antagonists bind selectively to CRF_1 and offer advantages over the peptide congeners in terms of stability, ease of preparation, ease of structural modification to enhance the pharmacokinetic profile and better blood–brain barrier penetration.

Several non-peptide CRF receptor antagonists were developed by pharmaceutical companies such as Antalarmin, CP-154526, DMP-904, DMP-696 and NBI-27914 (Fig. 1) and are currently in clinical trials with the aim of improving the health consequences of chronic stress and for use in the clinical management of anxiety and stress [38]. Antalarmin showed promising results in treatment of CRF-induced hypertension [39–42]. Promising results for antalarmin and other CRF_1 antagonists were also observed in the area of drug addiction disorders. Antalarmin also showed anti-inflammatory effects and has been suggested as having potential uses in treatment of arthritis [43], irritable bowel syndrome [44,45] and peptic ulcers [46].

In this manuscript we describe the synthesis and characterization of several substituted pyrimidines with close structural similarities to NBI-27914, a prominent CRF receptor antagonist with excellent binding affinity. The binding affinities of the synthesized compound were evaluated in comparison to antalarmin as a standard. Four of the newly synthesized compounds showed promising binding affinities and were selected as lead compounds for further structural modifications and for in vivo testing in animal models of anxiety.

2. Results and discussion

2.1. Chemistry

The general synthetic scheme for synthesis of the target compounds (**1**–**28**) is described in Fig. 2. The selected substituted phenyl isothiocyanate was treated with cynoacetamide and sulfur in presence of triethylamine following a Gewald type of reaction to give intermediate 4-amino-5-carboxamido-3-substituted phenylthiazol-2-thiones (**I a**,**b**) in excellent yields according to reported methods [47–51]. The thiazoles were then treated with acetic anhydride at reflux temperature to yield 6-methylthizolo[4,5-d]pyrimidin-6-ones (**II a**,**b**) [50] or with a 1:1 mixture of triethyl orthoformate and acetic anhydride to yield thiazolo[4,5-d]pyrimidin-6-one unsubstituted at position 6 (**II c**). It is worthmentioning that the selected isothiocyanates used were 2,4,6-trimethylphenylisothiocyanate and 2-bromo-4-isopropylphenyl isothiocyanate since previous studies have shown that this particular substitutions at that particular position is required for optimum CRF_1 binding antagonist activity [37,49].

The thiazolo[4,5-d]pyrimidin-6-ones (**II a**–**c**) were then subjected to thiazole ring opening using 4 N sodium hydroxide solution at reflux temperature in presence of positive pressure of nitrogen or argon gas. If the reaction is carried out in presence of air, oxidation reaction takes place where two molecules of the resulting pyrimidin-6-one-5-thiols are oxidized to form a dimer linked through an S–S bond [52].

Alkylation of the thiol group was spontaneously carried out in the same reaction mixture using methyl iodide or ethyl iodide in 1,4-dioxane, in presence of nitrogen or argon gas to give 5-alkylthio-pyrimidin-6-ones (**III a**–**f**) in excellent yields. Chlorination of **III a**–**f** with phosphorous oxychloride yielded the 6-chloro pyrimidine derivatives (**IV a**–**f**) in excellent yields. Because the chloro compounds (**IV a**–**f**) have electron donating groups (methyl, aminoaryl and thioalkyl at positions 2, 4 and 5), they are less reactive towards nucleophilic substitution reaction of the 6-chloro group with secondary amines. Thus synthesis of compounds (**1**–**28**) was carried out by a modification of the method reported by Beletskaya [53]. The chloro derivatives (**IV a**–**f**) were treated with secondary amines using tris(dibenzylideneacetone) dipalladium(0), Xphos and cesium carbonate, with the secondary amines as both nucleophile and solvent. Five secondary amines were selected for this reaction: *N*,*N*-diethylamine, *N*,*N*-di(*n*-propyl) amine, *N*-ethyl, *N*-(*n*-butyl)amine, *N*,*N*-*bis*(2-methoxyethyl)amine and *N*-[2-(cyclopropyl)ethyl], *N*-(*n*-propyl)amine. This selection was based on previous reports showing an optimum CRF_1 receptor antagonist activity for those particular amino groups in the target compounds [49].

The structures of the all intermediates were confirmed using ^1H NMR and Mass spectroscopy and the structures of the compounds (**1**–**28**) were elucidated using ^1H NMR and ^{13}C NMR and confirmed with Mass spectroscopy and elemental analysis. The detailed structural variations of the individual compounds (**1**–**28**) are shown in Table 1.

2.2. Receptor binding study

The series of the synthesized pyrimidines (**1**–**28**) were evaluated for their binding affinity to CRF_1 receptors. First, the ability of compounds (**1**–**28**) at a single concentration of 500 nM to inhibit the specific binding of [^{125}I]-Tyr^{0}sauvagine to membranes from HEK 293 cells stably expressing the CRF_1 receptor in binding experiments performed under equilibrium conditions was evaluated. In this initial screening experiment, compounds showing low affinity (1, 2, 4, 5, 7, 10–28) as measured by their inability to inhibit close to or more than 50% of [^{125}I]-Tyr^0-sauvagine specific binding (Fig. 3) were excluded from further pharmacological evaluation. The other compounds were further pharmacologically characterized by determining their binding affinities (IC_{50}) for CRF_1 receptors in competition experiments performed under equilibrium

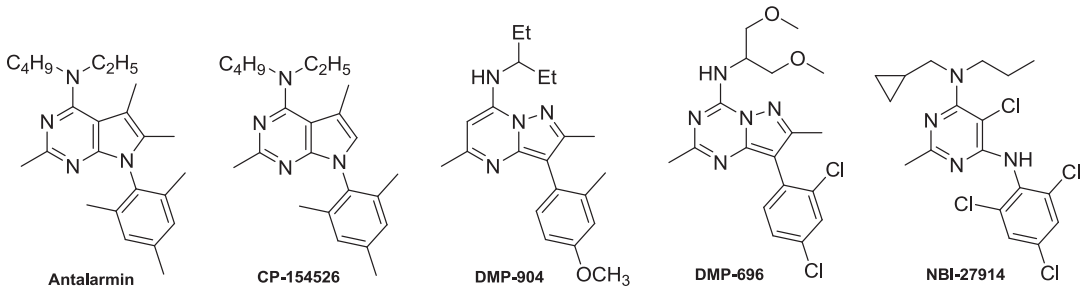


Fig. 1. Structures of CRF_1 receptor antagonists.

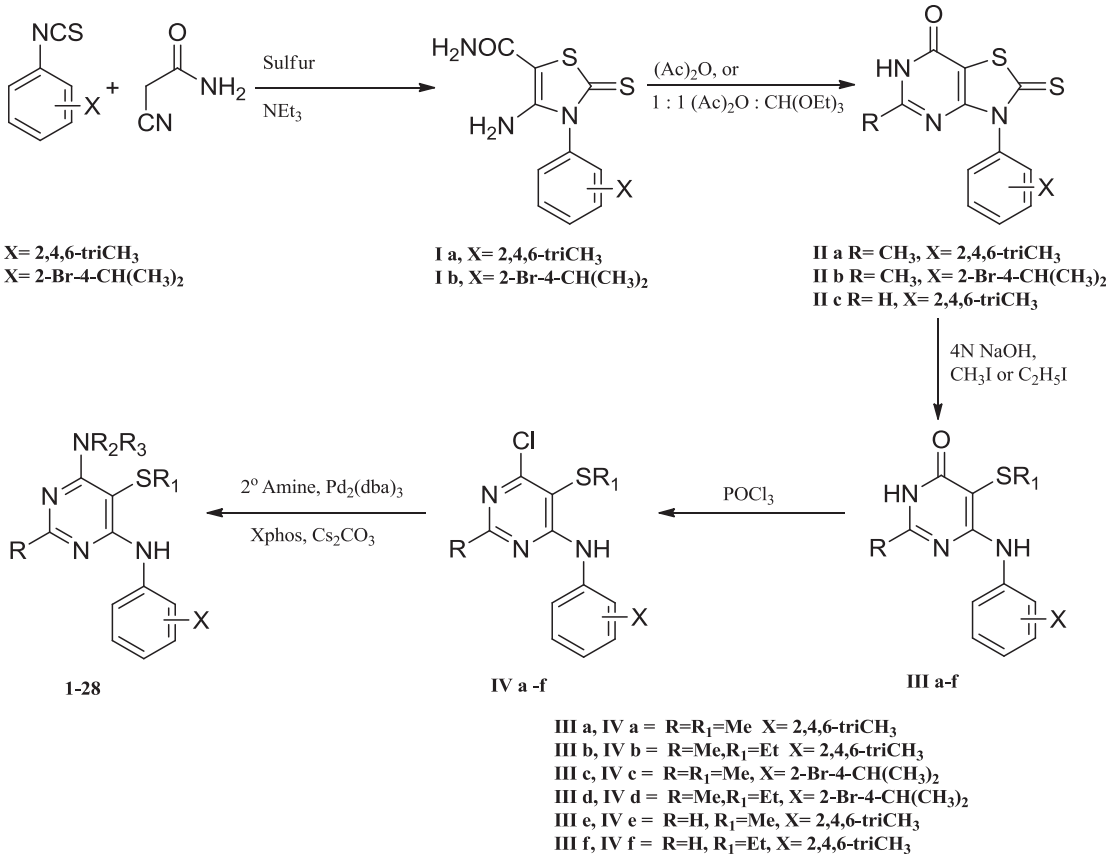


Fig. 2. Synthetic scheme of the compounds (**1**–**28**).

conditions in membranes from HEK 293 cells stably expressing the CRF_1 receptor. Four lead compounds (**3**, **6**, **8** and **9**) were identified (Fig. 3). These lead compounds were found to bind to CRF_1 receptors in a concentration dependent manner (Fig. 4) and with promising binding affinities (Table 2).

2.3. SAR study

Four compounds showed promising CRF receptor binding affinities and were identified as lead compounds (**3**, **6**, **8** and **9**). All the lead compounds have a methyl group at position 2. Compounds having a 2,4,6-trimethylphenyl group at −position 4 showed better binding affinities than compounds having a 2-bromo-4-isopropylphenyl group. Both the methyl and ethyl groups of the alkythiol group at position 5 showed promising affinity. At position 6, only the *N*,*N*-bis(methoxyethyl)amino, the *N*-cyclopropyl-*N*-methylpropylamino and the *N*,*N*-di-(*n*-propyl)amino displayed promising CRF receptor binding affinity.

3. Conclusion

A series of novel substituted pyrimidines were synthesized as CRF_1 receptor ligands. The newly synthesized compounds have the groups known for optimum binding affinity for CRF_1 receptors, particularly a methyl group at position 2, a substituted phenyl at position 4 and dialkylamino group at position 6 of the pyrimidine ring while having different thioalkyl groups at position 5. The synthesized compounds were evaluated for their binding affinity for CRF_1 receptor in comparison with antalarmin, a known non-peptide CRF_1 receptor antagonist, as a standard. Four lead compounds of the new series (**3**, **6**, **8** and **9**) were identified. These four compounds showed a promising CRF_1 receptor binding affinity. These compounds showed receptor binding affinity with (−log IC_{50}) values of 6.23–6.61 compared to 7.73 for antalarmin. This indicates that further structural modifications of these compounds may lead to compounds with superior CRF_1 receptor binding affinity and a potential for development as new anti-anxiety and antidepressant agents.

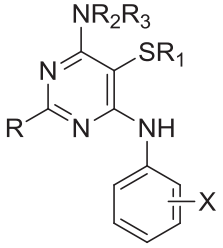
4. Experimental

4.1. Chemistry

4.1.1. General information

All chemicals were purchased from commercial sources. The substituted phenyl isothiocyantaes were purchased from Oakwood Products, Inc. SC, USA. Tris(dibenzylideneacetone)dipalladium(0), (±)-2, 2′-Bis(diphenylphosphino)-1,1′-binaphthyl, Copper(I) Iodide, and 2-(dicyclohexylphosphino) 2′,4′,6′-triisopropylbiphenyl were purchased from Acros organics, NJ,USA. Palladium (II) acetate was purchased from Tokyo chemical industry, Japan. cesium carbonate was purchased from Alfa Aesar, MA. All chemical were stored in desiccator. All laboratory grade, secondary amines and dry solvents were purchased from Sigma Aldrich USA, Acros organics, Fisher scientific USA and used without further purification. Flash column chromatography separation was performed using Acros organics silica gel 40–60 μm, 60 A using combination of ethyl acetate and hexanes. Whatman TLC plates were used for thin layer chromatography and visualization was done using UV fluorescence at 254 nm. Melting points were recorded on a Mel-Temp, Laboratory devices, Inc and are uncorrected. %CHN Analyzer by combustion/TCD and %S by O flask combustion/IC were used for elemental

Table 1
5-thioalkyl-6-*N*,*N*-diaminoalkyl-4-subsitituted phenylaminopyrimidines (**1**–**28**).



1-28

No	R	R_1	R_2	R_3	X
1	CH_3	CH_3	C_3H_7	C_3H_7	2,4,6-triCH_3
2	CH_3	CH_3	C_2H_5	C_2H_5	2,4,6-triCH_3
3	CH_3	CH_3	$C_2H_4OCH_3$	$C_2H_4OCH_3$	2,4,6-triCH_3
4	CH_3	CH_3	*c*-propylCH_2	C_3H_7	2,4,6-triCH_3
5	CH_3	CH_3	C_2H_5	C_4H_9	2,4,6-triCH_3
6	CH_3	C_2H_5	C_3H_7	C_3H_7	2,4,6-triCH_3
7	CH_3	C_2H_5	C_2H_5	C_2H_5	2,4,6-triCH_3
8	CH_3	C_2H_5	$C_2H_4OCH_3$	$C_2H_4OCH_3$	2,4,6-triCH_3
9	CH_3	C_2H_5	*c*-propylCH_2	C_3H_7	2,4,6-triCH_3
10	CH_3	C_2H_5	C_2H_5	C_4H_9	2,4,6-triCH_3
11	CH_3	CH_3	C_3H_7	C_3H_7	2-Br-4-$CH(CH_3)_2$
12	CH_3	CH_3	C_2H_5	C_2H_5	2-Br-4-$CH(CH_3)_2$
13	CH_3	CH_3	$C_2H_4OCH_3$	$C_2H_4OCH_3$	2-Br-4-$CH(CH_3)_2$
14	CH_3	CH_3	*c*-propylCH_2	C_3H_7	2-Br-4-$CH(CH_3)_2$
15	CH_3	CH_3	C_2H_5	C_4H_9	2-Br-4-$CH(CH_3)_2$
16	CH_3	C_2H_5	C_3H_7	C_3H_7	2-Br-4-$CH(CH_3)_2$
17	CH_3	C_2H_5	C_2H_5	C_2H_5	2-Br-4-$CH(CH_3)_2$
18	CH_3	C_2H_5	$C_2H_4OCH_3$	$C_2H_4OCH_3$	2-Br-4-$CH(CH_3)_2$
19	CH_3	C_2H_5	*c*-propylCH_2	C_3H_7	2-Br-4-$CH(CH_3)_2$
20	CH_3	C_2H_5	C_2H_5	C_4H_9	2-Br-4-$CH(CH_3)_2$
21	H	CH_3	C_3H_7	C_3H_7	2,4,6-triCH_3
22	H	CH_3	$C_2H_4OCH_3$	$C_2H_4OCH_3$	2,4,6-triCH_3
23	H	CH_3	*c*-propylCH_2	C_3H_7	2,4,6-triCH_3
24	H	CH_3	C_2H_5	C_4H_9	2,4,6-triCH_3
25	H	C_2H_5	C_3H_7	C_3H_7	2,4,6-triCH_3
26	H	C_2H_5	C_2H_5	C_2H_5	2,4,6-triCH_3
27	H	C_2H_5	$C_2H_4OCH_3$	$C_2H_4OCH_3$	2,4,6-triCH_3
28	H	C_2H_5	C_2H_5	C_4H_9	2,4,6-triCH_3

analysis of final compounds and performed by Micro Analysis Inc., Wilmington DE, USA and are within ±0.4%. ^1H and ^{13}C NMR spectra were obtained on a Bruker Avance 400 MHz instrument using $CDCl_3$ as solvent unless otherwise stated. ^1H NMR Spectra are

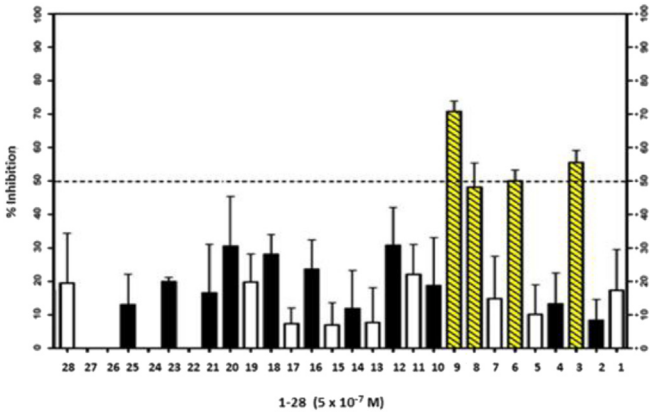


Fig. 3. Screening of compounds for binding to human CRF_1 receptor. Inhibition of [^{125}I]-Tyr^0-sauvagine specific binding by 500 nM of compounds **1**–**28** on membranes from HEK 293 cells stably expressing the human CRF_1 receptor. The bars represent the % inhibition of radioligand specific binding by the compounds, determined from 2 to 5 experiments (with their means and S.E.). The dashed line represents the 50% inhibition of radioligand specific binding.

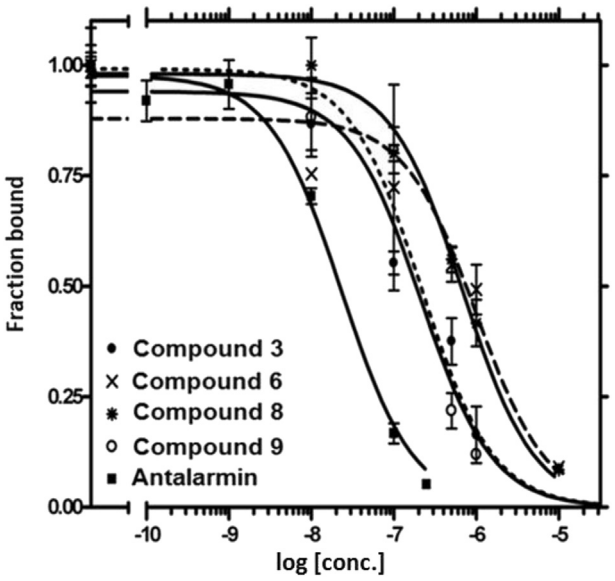


Fig. 4. Competition binding isotherms of compounds 3, 6, 8 and 9 to human CRF_1 receptor. Competition of [^{125}I]-Tyr^0-sauvagine specific binding by increasing concentrations of the 4 lead compounds on membranes from HEK 293 cells stably expressing the human CRF_1 receptor. The means and S.E. (duplicate determination) are shown and the data were fit to a one-site competition model by nonlinear regression.

reported in order; multiplicity, number of protons and signals were characterized as s (singlet), dd (doublet of doublet), t (triplet), m (multiplet), br s (broad signal), q (quartet), quin (quintet), tquin (triplet of quintet), sxt (sextet), spt (septet). Chemical shifts are relative to TMS as an internal standard. Mass spectra were recorded on Finnigan LCQTM DECA by Thermo Quest San Jose, CA, using electrospray ionization method. All the reactions were carried out in flame dried glassware under an atmosphere of nitrogen unless otherwise stated.

*4.1.2. 4-Amino-5-carboxamido-3-(substituted phenyl)-2,3-dihydrothiazole-2-thiones (**I a,b**) [50]*

To a stirred solution of triethylamine (1.0 mmol) and cynoacetamide (1.0 mmol) in dimethyl sulfoxide (2.5 mL), sulfur was added (1.0 mmol) and the reaction mixture was stirred for 10 min at ambient temperature, followed by addition of the selected substituted phenyl isothiocyanate (1.0 mmol). The reaction mixture was stirred at ambient temperature for 4 h and completion of the reaction was monitored by TLC using 9:1 dichloromethane and methanol. The reaction mixture was then diluted with water and extracted with ethyl acetate. The combined organic layer was washed with water and brine solution and dried over anhydrous sodium sulfate and concentrated under vacuum to give the product as a solid residue Ia and Ib.

*4.1.2.1. 4-amino-3-mesityl-2-thioxo-2,3-dihydrothiazole-5-carboxamide (**I a**). Yield:* yellow solid (73%); MP: 203 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.08 (s, 2H), 5.85 (br. s., 2H), 5.10 (br. s., 2H), 2.37 (s, 3H), 2.11 (s, 6H); ESI–MS *m*/*z*: 294.95 (MH^+).

Table 2
The IC_{50} values of the lead compounds compared to antalarmin.

Compound	-LogIC50 ± S.E
Antalarmin	7.73 ± 0.09
Compound **3**	6.56 ± 0.12
Compound **6**	6.27 ± 0.26
Compound **8**	6.23 ± 0.06
Compound **9**	6.61 ± 0.06

*4.1.2.2. 4-Amino-3-(2-bromo-4-isopropylphenyl)-2-thioxo-2,3-dihydrothiazole-5-carboxamide (**I b**). Yield:* yellow solid (71%); MP: 206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 1.8 Hz, 1H), 7.42 (dd, J = 1.8, 8.2 Hz, 1H), 7.30 (d, J = 8.2 Hz, 1H), 5.86 (br. s., 2H), 5.09 (br. s., 2H), 3.01 (spt, J = 7.1 Hz, 1H), 1.32 (d, J = 7.1 Hz, 6H); ESI–MS m/z: 371.94 (MH^+), 373.93 ($MH + 2^+$).

*4.1.3. 5-Substituted-2-thioxo-3-(substitutedphenyl)-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (**II a**–**c**) [50]*

A mixture of **I a,b** (1.0 mmol) in acetic anhydride (for **II a,b**) (3 mL) or 1:1 mixture of acetic anhydride and triethyl orthoformate (for **II c**) (3 mL) was stirred at reflux temperature for 3–4 h and completion of the reaction was monitored by TLC using 9:1 dichloromethane and methanol. The reaction mixture was cooled to room temperature. The separated product was filtered and washed with ethanol and dried to give **II a**–**c**.

*4.1.3.1. 3-Mesityl-5-methyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (**II a**).* Yield: brown crystals (69.7%); MP: 340 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.13 (br. s., 1H), 7.06 (s, 2H), 2.49 (s, 3H), 2.39 (s, 3H), 2.02 (s, 6H); ESI–MS m/z: 318.17 (MH^+).

*4.1.3.2. 3-(2-Bromo-4-isopropylphenyl)-5-methyl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (**II b**).* Yield: light yellow solid (70.4%); MP: 237 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.94 (br. s., 1H), 7.65 (d, J = 1.8 Hz, 1H), 7.39 (dd, J = 1.8, 8.2 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 3.02 (spt, J = 7.1 Hz, 1H), 2.50 (s, 3H), 1.34 (d, J = 7.1 Hz, 6H); ESI–MS m/z: 396.08 (MH^+), 398.06 ($MH + 2^+$).

*4.1.3.3. 3-Mesityl-2-thioxo-2,3-dihydrothiazolo[4,5-d]pyrimidin-7(6H)-one (**II c**).* Yield: brown color solid (84%); MP: 315 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.07 (br. s., 1H), 8.08 (s, 1H), 7.09 (s, 2H), 2.38 (s, 3H), 2.04 (s, 6H); ESI–MS m/z: 304.14 (MH^+).

*4.1.4. 6-(Substituted phenyl)-2-methyl-5-(alkylthio)pyrimidin-4(3H)-ones (**III a**–**f**)*

A mixture of **II a**–**c** (1.0 mmol) in 4 N NaOH (10 mL) was stirred at 100 °C for 3.5–4 h under the positive pressure of nitrogen. The reaction mixture was cooled to ambient temperature, and a solution of the selected alkyl iodide (1.5 mmol) in 1,4-dioxane (2 mL) was added slowly at ambient temperature and stirred for 2h under positive atmosphere of nitrogen. The reaction mixture was cooled to 0 °C, and pH was adjusted to 4 pH using conc. HCl. The precipitated solid was filtered and washed with ice cold water and dried to give a crude residue. The pure compound was isolated by flash chromatography using gradient ethyl acetate in hexanes to give **III a**–**f**.

*4.1.4.1. 6-(Mesitylamino)-2-methyl-5-(methylthio)pyrimidin-4(3H)-one (**III a**).* Yield: whitish solid (87%); MP: 335 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.54 (br. s., 1H), 7.48 (s, 1H), 6.93 (s, 2H), 2.31 (s, 6H), 2.29 (s, 3H), 2.17 (s, 3H), 2.14 (s, 3H); ESI–MS m/z: 290.12 (MH^+).

4.1.4.2. 5-(Ethylthio)-6-(mesitylamino)-2-methylpyrimidin-4(3H)-one (III b). Yield: whitish solid (87%); MP: 244 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.03 (br. s., 1H), 7.49 (s, 1H), 6.93 (s, 2H), 2.82 (q, J = 7.4 Hz, 2H), 2.34 (s, 3H), 2.29 (s, 3H), 2.19 (s, 6H), 1.30 (t, J = 7.4 Hz, 3H); ESI–MS m/z: 304.07 (MH^+).

*4.1.4.3. 6-((2-Bromo-4-isopropylphenyl)amino)-2-methyl-5-(methylthio)pyrimidin-4(3H)-one (**III c**). Yield*: light yellow solid (87%); MP: 238 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.05 (br. s., 1H), 8.64 (s, 1H), 8.21 (d, J = 8.3 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.21 (dd, J = 2.0, 8.3 Hz, 1H), 2.92 (spt, J = 6.9, Hz, 1H), 2.55 (s, 3H), 2.40 (s, 3H), 1.24 (d, J = 6.9 Hz, 6H); ESI–MS m/z: 368.1 (MH^+), 370.07 ($MH + 2^+$).

*4.1.4.4. 6-((2-Bromo-4-isopropylphenyl)amino)-5-(ethylthio)-2-methylpyrimidin-4(3H)-one (**III d**). Yield*: light yellow solid (87%); MP: 207 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.07 (br. s., 1H), 8.68 (s, 1H), 8.19 (d, J = 8.3 Hz, 1H), 7.44 (d, J = 1.8 Hz, 1H), 7.19 (dd, J = 1.8, 8.3 Hz, 1H), 2.95 (spt, J = 6.8 Hz, 1H), 2.76 (q, J = 7.4 Hz, 2H), 2.49 (s, 3H), 1.30 (t, J = 7.4 Hz, 3H), 1.25 (d, J = 6.8 Hz, 6H); ESI–MS m/z: 382.07 (MH^+), 384.07 ($MH + 2^+$).

4.1.4.5. 6-(Mesitylamino)-5-(methylthio)pyrimidin-4(3H)-one (III e). Yield: white solid (90%); MP: 247 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.52 (br. s., 1H), 7.95 (s, 1H), 7.52 (s, 1H), 6.95 (s, 2H), 2.34 (s, 3H), 2.30 (s, 3H), 2.17 (s, 6H); ESI–MS m/z: 276.25 (MH^+).

*4.1.4.6. 5-(Ethylthio)-6-(mesitylamino)pyrimidin-4(3H)-one (**III f**). Yield*: white solid (90%), MP: 218 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.51 (br. s., 1H), 7.92 (s, 1H), 7.50 (s, 1H), 6.92 (s, 2H), 2.73 (q, J = 7.3 Hz, 2H), 2.29 (s, 3H), 2.19 (s, 6H) 1.24 (t, J = 7.3 Hz, 3H); ESI–MS m/z: 290.14 (MH^+).

*4.1.5. 6-Chloro-2-substituted-4-(substitutedphenylamino)-5-alkylthiopyrimidines (**IV a**–**f**)*

A mixture of **III a**–**f** (1.0 mmol) and phosphorous oxychloride (2.5 mL) were stirred at reflux temperature for 3–4 h and completion of the reaction was monitored by TLC using ethyl acetate and hexanes mixture. The reaction mixture was cooled to room temperature and carefully diluted with ice cold water, extracted with ethyl acetate. The combined organic layer was washed with water, brine and dried over anhydrous sodium sulfate and concentrated under vacuum to get residue. The pure compound was isolated by flash chromatography using gradient ethyl acetate in hexanes to give **IV a**–**f**.

*4.1.5.1. 6-Chloro-N-mesityl-2-methyl-5-(methylthio)pyrimidin-4-amine (**IV a**).* Yield: off white solid (78%); MP: 114 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (br. s., 1H), 6.95 (s, 2H), 2.39 (s, 3H), 2.38 (s, 3H), 2.32 (s, 3H), 2.16 (s, 6H); ESI–MS m/z: 308.17 (MH^+), 310.15 ($MH + 2^+$).

*4.1.5.2. 6-Chloro-5-(ethylthio)-N-mesityl-2-methylpyrimidin-4-amine (**IV b**).* Yield: off white solid (81%); MP: 77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (br. s., 1H), 6.95 (s, 2H), 2.90 (q, J = 7.4 Hz, 2H), 2.38 (s, 3H), 2.32 (s, 3H), 2.16 (s, 6H), 1.34 (t, J = 7.4 Hz, 3H); ESI–MS m/z: 322.13 (MH^+), 324.14 ($MH + 2^+$).

*4.1.5.3. N-(2-Bromo-4-isopropylphenyl)-6-chloro-2-methyl-5-(methylthio)pyrimidin-4-amine (**IV c**).* Yield: off white solid (81%); MP: 94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.44 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.24 (dd, J = 2.0, 8.6 Hz, 1H), 2.90 (spt, J = 7.1, 1H), 2.57 (s, 3H), 2.40 (s, 3H), 1.26 (d, J = 7.1 Hz, 6H); ESI–MS m/z: 386.04 (MH^+), 388.03 ($MH + 2^+$), 390.03 ($MH + 4^+$).

*4.1.5.4. N-(2-bromo-4-isopropylphenyl)-6-chloro-5-(ethylthio)-2-methylpyrimidin-4-amine (**IV d**).* Yield: off white solid (65%); MP: 65 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.48 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 2.0 Hz, 1H), 7.24 (dd, J = 2.0, 8.6 Hz, 1H), 2.95 (spt, J = 6.8 Hz, 1H), 2.84 (q, J = 7.4 Hz, 2H), 2.53 (s, 3H), 1.32 (t, J = 7.4 Hz, 3H), 1.26 (d, J = 6.8 Hz, 6H); ESI–MS m/z: 400.15 (MH^+), 402.11 ($MH + 2^+$), 404.08 ($MH + 4^+$).

*4.1.5.5. 6-Chloro-N-mesityl-5-(methylthio)pyrimidin-4-amine (**IV e**).* Yield: off white solid (70%); MP: 194 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (s, 1H), 7.69 (br. s., 1H), 6.97 (s, 2H), 2.43 (s, 3H), 2.31 (s, 3H), 2.16 (s, 6H); ESI–MS m/z: 294.48 (MH^+), 296.44 ($MH + 2^+$).

*4.1.5.6. 6-Chloro-5-(ethylthio)-N-mesitylpyrimidin-4-amine (**IV f**).* Yield: off white solid (94%); MP: 115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.55 (s, 1H), 6.94 (s, 2H), 2.75 (q, J = 7.3 Hz, 2H), 2.29 (s, 3H), 2.18 (s, 6H), 1.23 (t, J = 7.3 Hz, 3H); ESI–MS *m/z*: 308.47 (MH^+), 310.56 ($MH + 2^+$).

*4.1.6. 2-Substituted-6-dialkylamino-4-(substitutedphenylamino)-5-alkylthiopyrimidines (**1**–**28**)*

An oven-dried 3 necked flask equipped with a magnetic stir bar and fitted with a condenser and a rubber septum was evacuated and backfilled with nitrogen. This step was repeated two times with a total of three times. The chloro compound (**IV a**–**f**) (1.0 mmol) and cesium carbonate (1.5 mmol) were added and flask was evacuated and backfilled with nitrogen. *tris*(dibenzylideneacetone)dipalladium(0) (0.1 mmol, 10 mol%) and 2-dicyclohexyl phosphine-2′,4′,6′-triisopropyl biphenyl (0.1 mmol, 10 mol%) were added and flask was evacuated and backfilled with nitrogen. 10 mL of the selected secondary amine was added to the reaction mixture via a syringe and the flask was evacuated and backfilled with nitrogen for 3 times. The flask was placed in an oil bath and the temperature was maintained at 100 °C under positive pressure of nitrogen and the reaction was monitored by TLC. After the reaction completion, the reaction mixture was cooled to room temperature, filtered through celite and washed with dichloromethane (3 × 10 mL). The combined dichloromethane layer was washed with 0.1 N HCl solution until the pH was neutral, followed by water (3 × 10 mL) and brine solution (1 × 10 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to semisolid residue. The residue was purified by flash chromatography with a gradient eluant of 0–10% ethyl acetate in hexanes to give the desired product (**1**–**28**).

*4.1.6.1. N^4-mesityl-2-methyl-5-(methylthio)-N^6,N^6-dipropylpyrimidine-4,6-diamine (**1**).* Yield: semisolid (61%); MP: 206 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 6.91 (s, 2H), 3.56 (t, J = 7.6 Hz, 4H), 2.29 (s, 3H), 2.20 (s, 3H), 2.19 (s, 3H), 2.18 (s, 6H), 1.67 (sxt, J = 7.4, Hz, 4H), 0.90 (t, J = 7.4 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 163.9, 163.4, 135.5, 134.1, 128.7, 88.9, 52.6, 26.3, 21.5, 21.0, 18.9, 18.4, 11.4; ESI–MS *m/z*: 373.38 (MH^+).

*4.1.6.2. N^4,N^4-diethyl-N^6-mesityl-2-methyl-5-(methylthio)pyrimidine-4,6-diamine (**2**).* Yield: light yellow solid (55%); MP: 68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 6.91 (s, 2H), 3.66 (q, J = 6.9 Hz, 4H), 2.29 (s, 3H), 2.21 (s, 3H), 2.20 (s, 3H), 2.18 (s, 6H), 1.23 (t, J = 6.9 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.5, 163.8, 163.4, 135.5, 134.1, 128.7, 88.9, 44.5, 26.3, 21.0, 18.8, 18.3, 13.6; ESI–MS *m/z*: 345.25 (MH^+).

*4.1.6.3. N^4-mesityl-N^6,N^6-bis(2-methoxyethyl)-2-methyl-5-(methylthio)pyrimidine-4,6-diamine (**3**).* Yield: off white semisolid (61%); MP: 60 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (s, 1H), 6.92 (s, 2H), 3.93 (t, J = 6.0 Hz, 4H), 3.67 (t, J = 6.0 Hz, 4H), 3.38 (s, 6H), 2.31 (s, 3H), 2.22 (s, 3H), 2.22 (s, 3H), 2.18 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.5, 163.7, 163.6, 135.7, 135.5, 133.9, 128.7, 89.2, 71.5, 58.8, 50.8, 26.2, 21.0, 18.8, 18.2; ESI–MS *m/z*: 405.26 (MH^+).

*4.1.6.4. N^4-(cyclopropylmethyl)-N^6-mesityl-2-methyl-5-(methylthio)-N^4-propylpyrimidine-4,6-diamine (**4**).* Yield: Yellow semisolid (69%); ^{1}H NMR (400 MHz, $CDCl_3$) 7.57 (s, 1H), 6.93 (s, 2H), 3.69 (t, J = 7.3 Hz, 2H), 3.52 (d, J = 6.5 Hz, 2H), 2.32 (s, 3H), 2.24 (s, 3H), 2.23 (s, 3H), 2.20 (s, 6H), 1.71 (sxt, J = 7.3 Hz, 2H), 1.19 (tquin, J = 6.8 Hz, 1H), 0.93 (t, J = 7.4 Hz, 3H), 0.52 (dt, J = 6.5 Hz, 2H), 0.26 (dt, J = 6.5, 12.6 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 164.5, 163.4, 135.5, 134.1, 128.7, 89.6, 55.1, 52.2, 26.2, 21.4, 21.0, 18.8, 18.1, 11.4, 10.3, 3.7; ESI–MS *m/z*: 385.28 (MH^+).

*4.1.6.5. N^4-butyl-N^4-ethyl-N^6-mesityl-2-methyl-5-(methylthio)pyrimidine-4,6-diamine (**5**).* Yield: light yellow semisolid (66%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58 (s, 1H), 6.93 (s, 2H), 3.70 (q, J = 7.1 Hz, 2H), 3.60 (t, J = 7.5 Hz, 2H), 2.31 (s, 3H), 2.23 (s, 3H), 2.22 (s, 3H), 2.20 (s, 6H), 1.67 (quin, J = 7.5 Hz, 2H), 1.36 (sxt, J = 7.5 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 163.9, 163.4, 135.5, 134.1, 128.7, 88.9, 49.8, 45.3, 30.5, 26.2, 21.0, 20.3, 18.8, 18.3, 14.0, 13.6; ESI–MS *m/z*: 373.35 (MH^+).

*4.1.6.6. 5-(Ethylthio)-N^4-mesityl-2-methyl-N^6,N^6-dipropylpyrimidine-4,6-diamine (**6**).* Yield: Light yellow solid (59.0%); MP: 85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 6.92 (s, 2H), 3.57 (t, J = 7.4 Hz, 4H), 2.67 (q, J = 7.5 Hz, 2H), 2.30 (s, 3H), 2.23 (s, 3H), 2.18 (s, 6H), 1.66 (sxt, J = 7.6 Hz, 4H), 1.21 (t, J = 7.4 Hz, 3H), 0.91 (t, J = 7.4 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.3, 164.5, 163.7, 135.6, 134.1, 128.7, 87.3, 52.7, 28.9, 26.2, 21.5, 20.9, 18.9, 14.3, 11.4; ESI–MS *m/z*: 387.27 (MH^+).

*4.1.6.7. N^4,N^4-diethyl-5-(ethylthio)-N^6-mesityl-2-methylpyrimidine-4,6-diamine (**7**).* Yield: off white semisolid (44.5%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H), 6.92 (s, 2H), 3.67 (q, J = 6.9 Hz, 4H), 2.68 (q, J = 7.4 Hz, 2H), 2.31 (s, 3H), 2.24 (s, 3H), 2.18 (s, 6H), 1.28 (t, J = 6.9 Hz, 6H), 1.24 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 164.7, 163.7, 135.6, 134.1, 128.7, 87.5, 44.6, 28.8, 26.2, 20.9, 18.9, 14.3, 13.6; ESI–MS *m/z*: 359.22 (MH^+).

*4.1.6.8. 5-(Ethylthio)-N^4-mesityl-N^6,N^6-bis(2-methoxyethyl)-2-methylpyrimidine-4,6-diamine (**8**).* Yield: semisolid (60%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 6.92 (s, 2H), 3.92 (t, J = 6.2 Hz, 4H), 3.64 (t, J = 6.1 Hz, 4H), 3.37 (s, 6H), 2.69 (q, J = 7.5 Hz, 2H), 2.30 (s, 3H), 2.23 (s, 3H), 2.18 (s, 6H), 1.22 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 164.4, 163.9, 135.5, 133.9, 128.7, 87.8, 71.4, 58.8, 50.8, 28.7, 26.2, 20.9, 18.8, 14.3; ESI–MS *m/z*: 419.27 (MH^+).

*4.1.6.9. N^4-(cyclopropylmethyl)-5-(ethylthio)-N^6-mesityl-2-methyl-N^4-propylpyrimidine-4,6-diamine (**9**).* Yield: off white solid (72.7%); MP: 70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 6.92 (s, 2H), 3.67 (t, J = 7.3 Hz, 2H), 3.50 (d, J = 6.5 Hz, 2H), 2.69 (q, J = 7.4 Hz, 2H), 2.30 (s, 3H), 2.24 (s, 3H), 2.18 (s, 6H), 1.69 (sxt, J = 7.3 Hz, 2H), 1.21 (t, J = 7.4 Hz, 3H), 1.16 (tquin, J = 5.0, 6.5 Hz 1H), 0.91 (t, J = 7.3 Hz, 3H), 0.50 (dt, J = 5.0 2H), 0.24 (dt, J = 5.0 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 166.1, 165.2, 163.8, 135.6, 134.1, 128.7, 88.0, 55.1, 52.3, 28.7, 26.2, 21.3, 21.0, 18.9, 14.4, 11.4, 10.3, 3.7; ESI–MS *m/z*: 399.27 (MH^+).

*4.1.6.10. N^4-butyl-N^4-ethyl-5-(ethylthio)-N^6-mesityl-2-methylpyrimidine-4,6-diamine (**10**).* Yield: semisolid (66.7%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (s, 1H), 6.93 (s, 2H), 3.69 (q, J = 7.1 Hz, 2H), 3.62 (t, J = 7.1 Hz, 2H), 2.68 (q, J = 7.6 Hz, 2H), 2.31 (s, 3H), 2.24 (s, 3H), 2.19 (s, 6H), 1.65 (quin, J = 7.6, Hz, 2H), 1.37 (sxt, J = 7.5, 2H), 1.22 (t, J = 7.3 Hz, 6H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.3, 164.6, 163.7, 135.6, 134.1, 128.7, 87.4, 49.9, 45.3, 30.4, 28.8, 26.2, 20.9, 20.3, 18.9, 14.3, 14.0, 13.5; ESI–MS *m/z*: 387.27 (MH^+).

*4.1.6.11. N^4-(2-bromo-4-isopropylphenyl)-2-methyl-5-(methylthio)-N^6,N^6-dipropylpyrimidine-4,6-diamine (**11**).* Yield: semisolid (72.9%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.56 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.59 (t, J = 7.4 Hz, 4H), 2.86 (spt, J = 7.1 Hz, 1H), 2.41 (s, 3H), 2.19 (s, 3H), 1.67 (sxt, J = 7.4 Hz, 4H), 1.25 (d, J = 7.1 Hz, 6H), 0.91 (t, J = 7.4 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.9, 161.9, 143.8, 135.7, 130.0, 126.0, 121.5, 114.0, 91.3, 52.7, 33.3, 26.1, 24.0, 21.5, 18.7, 11.3; ESI–MS *m/z*: 451.24 (MH^+), 453.20 ($MH + 2^+$).

*4.1.6.12. N^4-(2-bromo-4-isopropylphenyl)-N^6,N^6-diethyl-2-methyl-5-(methylthio)pyrimidine-4,6-diamine (**12**).* Yield: semisolid (76.7%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.55 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.69 (q, J = 7.1 Hz, 4H), 2.86 (spt, J = 6.9 Hz, 1H), 2.42 (s, 3H), 2.20 (s, 3H), 1.26 (t, J = 7.0 Hz, 6H) 1.20 (t, J = 7.1 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.9, 164.0, 161.8, 143.9, 135.7, 130.0, 126.0, 121.5, 114.1, 91.3, 44.7, 33.3, 26.1, 24.0, 18.6, 13.6; ESI−MS *m/z*: 423.19 (MH^+), 425.17 (MH + 2^+).

*4.1.6.13. N^4-(2-bromo-4-isopropylphenyl)-N^6,N^6-bis(2-methoxyethyl)-2-methyl-5-(methylthio)pyrimidine-4,6-diamine (**13**).* Yield: semisolid (56%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.90 (s, 1H), 8.53 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.96 (t, J = 6.0 Hz, 4H), 3.65 (t, J = 6.0 Hz, 4H), 3.37 (s, 6H), 2.87 (spt, J = 6.9 Hz, 1H), 2.41 (s, 3H), 2.21 (s, 3H), 1.25 (d, J = 6.9 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.0, 163.9, 162.0, 144.1, 135.5, 130.0, 126.0, 121.6, 114.1, 91.7, 71.4, 58.8, 51.0, 33.3, 26.1, 23.9, 18.3; ESI−MS *m/z*: 483.2 (MH^+), 485.17 (MH + 2^+).

*4.1.6.14. N^4-(2-bromo-4-isopropylphenyl)-N^6-(cyclopropylmethyl)-2-methyl-5-(methylthio)-N^6-propylpyrimidine-4,6-diamine (**14**).* Yield: semisolid (68.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.56 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.70 (t, J = 7.5 Hz, 2H), 3.53 (d, J = 6.8 Hz, 2H), 2.87 (spt, J = 6.9 Hz, 1H), 2.42 (s, 3H), 2.21 (s, 3H), 1.68 (sxt, J = 7.5 Hz, 2H), 1.25 (d, J = 6.9 Hz, 6H), 1.17 (tquint, J = 5.0, 6.8 Hz, 1H), 0.91 (t, J = 7.4 Hz, 3H), 0.51 (dt, J = 2.0, 5.0 Hz, 2H), 0.25 (dt, J = 2.0, 5.0 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.9, 164.7, 161.9, 143.9, 135.7, 130.0, 126.0, 121.5, 114.0, 92.0, 55.2, 52.3, 33.3, 26.1, 23.9, 21.4, 18.4, 11.3, 10.3, 3.7; ESI−MS *m/z*: 463.23 (MH^+), 465.19 (MH + 2^+).

*4.1.6.15. N^4-(2-bromo-4-isopropylphenyl)-N^6-butyl-N^6-ethyl-2-methyl-5-(methylthio)pyrimidine-4,6-diamine (**15**).* Yield: semisolid (72%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.54 (d, J = 8.6 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.18 (dd, J = 2.0, 8.6 Hz, 1H), 3.69 (q, J = 7.0 Hz, 2H), 3.60 (t, J = 7.2 Hz, 2H), 2.85 (spt, J = 6.9 Hz, 1H), 2.40 (s, 3H), 2.18 (s, 3H), 1.63 (quin, J = 7.4 Hz, 2H), 1.34 (sxt, J = 7.3 Hz, 2H), 1.25 (d, J = 6.9 Hz, 6H), 1.18 (t, J = 7.0 Hz, 3H), 0.95 (t, J = 7.3 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.9, 164.1, 161.9, 143.8, 135.7, 130.0, 126.0, 121.5, 114.0, 91.3, 49.9, 45.4, 33.3, 30.5, 26.1, 24.0, 20.2, 18.6, 14.0, 13.6; ESI−MS *m/z*: 451.23 (MH^+), 453.20 (MH + 2^+).

*4.1.6.16. N^4-(2-bromo-4-isopropylphenyl)-5-(ethylthio)-2-methyl-N^6,N^6-dipropylpyrimidine-4,6-diamine (**16**).* Yield: semisolid (66.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (s, 1H), 8.58 (d, J = 8.6 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.18 (dd, J = 2.0, 8.6 Hz, 1H), 3.59 (t, J = 7.3 Hz, 4H), 2.86 (spt, J = 6.9 Hz, 1H), 2.64 (q, J = 7.5 Hz, 2H), 2.42 (s, 3H), 1.66 (sxt, J = 7.3 Hz, 4H), 1.24 (d, J = 6.9 Hz, 6H), 1.18 (t, J = 7.4 Hz, 3H), 0.90 (t, J = 7.3 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.8, 164.8, 162.3, 143.7, 135.8, 130.0, 126.0, 121.2, 113.8, 89.6, 52.8, 33.3, 29.5, 26.2, 24.0, 21.4, 14.1, 11.3; ESI−MS *m/z*: 465.24 (MH^+), 467.20 (MH + 2^+).

*4.1.6.17. N^4-(2-bromo-4-isopropylphenyl)-N^6,N^6-diethyl-5-(ethylthio)-2-methylpyrimidine-4,6-diamine (**17**).* Yield: semisolid (54%); ^{1}H NMR (400 MHz, $CDCl_3$) δ δ 8.91 (s, 1H), 8.57 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.68 (q, J = 7.1 Hz, 4H), 2.86 (spt, J = 6.9, 1H), 2.65 (q, J = 7.4 Hz, 2H), 2.43 (s, 3H), 1.28 (d, J = 6.9 Hz, 6H), 1.26 (t, J = 7.1 Hz, 6H), 1.18 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.9, 162.3, 143.8, 135.7, 130.0, 126.0, 121.3, 113.9, 89.7, 44.8, 33.3, 29.4, 26.2, 24.0, 14.2, 13.5; ESI−MS *m/z*: 437.4 (MH^+), 439.20 (MH + 2^+).

*4.1.6.18. N^4-(2-bromo-4-isopropylphenyl)-5-(ethylthio)-N^6,N^6-bis(2-methoxyethyl)-2-methylpyrimidine-4,6-diamine (**18**).* Yield: semisolid (69.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.56 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.95 (t, J = 6.0 Hz, 4H), 3.63 (t, J = 6.0 Hz, 4H), 3.36 (s, 6H), 2.86 (spt, J = 6.8 Hz, 1H), 2.67 (q, J = 7.4 Hz, 2H), 2.42 (s, 3H), 1.24 (d, J = 6.8 Hz, 6H), 1.19 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.9, 164.6, 162.4, 144.0, 135.5, 130.0, 126.0, 121.4, 113.9, 90.1, 71.2, 58.8, 51.0, 33.3, 29.2, 26.1, 23.9, 14.1; ESI−MS *m/z*: 497.23 (MH^+), 499.19 (MH + 2^+).

*4.1.6.19. N^4-(2-bromo-4-isopropylphenyl)-N^6-(cyclopropylmethyl)-5-(ethylthio)-2-methyl-N^6-propylpyrimidine-4,6-diamine (**19**).* Yield: semisolid (59.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 8.59 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 1.8, 8.6 Hz, 1H), 3.74 (t, J = 7.5 Hz, 2H), 3.53 (d, J = 6.5 Hz, 2H), 2.86 (spt, J = 6.9 Hz, 1H), 2.66 (q, J = 7.3 Hz, 2H), 2.43 (s, 3H), 1.67 (sxt, J = 7.5 Hz, 2H), 1.25 (d, J = 7.1 Hz, 6H), 1.17 (tquin, J = 5.0, 6.8 Hz, 1H), 1.19 (t, J = 7.4 Hz, 3H), 0.90 (t, J = 7.3 Hz, 3H), 0.51 (dt, J = 2.0, 5.0 Hz, 2H), 0.25 (dt, J = 2.0, 5.0 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 165.4, 164.8, 162.3, 143.8, 135.7, 130.0, 126.0, 121.3, 113.8, 90.2, 55.2, 52.4, 33.3, 29.2, 26.2, 24.0, 21.3, 14.2, 11.3, 10.2, 3.7; ESI−MS *m/z*: 477.27 (MH^+), 479.21 (MH + 2^+).

*4.1.6.20. N^4-(2-bromo-4-isopropylphenyl)-N^6-butyl-N^6-ethyl-5-(ethylthio)-2-methylpyrimidine-4,6-diamine (**20**).* Yield: semisolid (59.6%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 8.58 (d, J = 8.6 Hz, 1H), 7.41 (d, J = 2.0 Hz, 1H), 7.19 (dd, J = 2.0, 8.6 Hz, 1H), 3.70 (q, J = 7.1 Hz, 2H), 3.60 (t, J = 7.2 Hz, 2H), 2.86 (spt, J = 6.9 Hz, 1H), 2.64 (q, J = 7.3 Hz, 2H), 2.42 (s, 3H), 1.63 (quin, J = 7.2 Hz, 2H), 1.34 (sxt, J = 7.3 Hz, 2H), 1.25 (d, J = 6.9 Hz, 6H), 1.20 (t, J = 7.1 Hz, 3H), 0.96 (m, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.8, 164.8, 162.3, 143.7, 135.7, 130.0, 126.0, 121.3, 113.8, 89.7, 50.0, 45.5, 33.3, 30.4, 29.4, 26.2, 24.0, 20.2, 14.2, 14.0, 13.5; ESI−MS *m/z*: 465.25 (MH^+), 467.21 (MH + 2^+).

*4.1.6.21. N^4-mesityl-5-(methylthio)-N^6,N^6-dipropylpyrimidine-4,6-diamine (**21**).* Yield: off white solid (67.2%); MP: 83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 7.58 (s, 1H), 6.94 (s, 2H), 3.63 (t, J = 7.4 Hz, 4H), 2.29 (s, 3H), 2.26 (s, 3H), 2.19 (s, 6H), 1.70 (sxt, J = 7.5 Hz, 4H), 0.93 (t, J = 7.4 Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.8, 156.6, 136.4, 135.9, 133.6, 128.9, 91.6, 52.7, 21.4, 21.0, 18.6, 18.2, 11.3; ESI−MS *m/z*: 459.25 (MH^+).

*4.1.6.22. N^4-mesityl-N^6,N^6-bis(2-methoxyethyl)-5-(methylthio)pyrimidine-4,6-diamine (**22**).* Yield: light yellow solid (60.9%); MP: 89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.59 (s, 1H), 6.94 (s, 2H), 3.96 (t, J = 6.0 Hz, 4H), 3.66 (t, J = 6.0 Hz, 4H), 3.37 (s, 6H), 2.29 (s, 3H), 2.27 (s, 3H), 2.18 (s, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 163.9, 163.6, 156.6, 136.6, 135.9, 129.0, 92.0, 71.4, 58.9, 51.0, 21.0, 18.5; ESI−MS *m/z*: 391.54 (MH^+).

*4.1.6.23. N^4-(cyclopropylmethyl)-N^6-mesityl-5-(methylthio)-N^4-propylpyrimidine-4,6-diamine (**23**).* Yield: white solid (75.4%); MP: 85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.56 (s, 1H), 6.94 (s, 2H), 3.70 (t, J = 7.3 Hz, 2H), 3.53 (d, J = 6.5 Hz, 2H), 2.30 (s, 3H), 2.27 (s, 3H), 2.20 (s, 6H), 1.71 (sxt, J = 7.4, 2H), 1.20 (tquin, J = 12.5, 6.5 Hz, 1H), 0.94 (t, J = 7.3 Hz, 3H), 0.52 (dt, J = 6.5, 12.6 Hz, 2H), 0.26 (dt, J = 6.5, 12.6 Hz, 2H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.4, 163.8, 156.6, 136.5, 135.9, 133.5, 129.0, 92.3, 55.2, 52.2, 21.3, 21.0, 18.6, 17.9, 11.3, 10.2, 3.7; ESI−MS *m/z*: 371.29 (MH^+).

*4.1.6.24. N^4-butyl-N^4-ethyl-N^6-mesityl-5-(methylthio)pyrimidine-4,6-diamine (**24**).* Yield: off white solid (73.8%); MP: 57 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.57 (s, 1H), 6.94 (s, 2H), 3.70 (q, J = 7.1 Hz, 2H), 3.60 (t, J = 7.5 Hz, 2H), 2.31 (s, 3H), 2.23 (s, 3H), 2.20 (s, 6H), 1.67 (quin, J = 7.5 Hz, 2H), 1.36 (sxt, J = 7.5 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H), 0.97 (t, J = 7.4 Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$)

δ 163.8, 163.7, 156.7, 136.4, 135.9, 133.6, 129.0, 91.5, 50.0, 45.4, 30.5, 21.0, 20.3, 18.6, 18.1, 14.0, 13.5; ESI−MS *m/z*: 359.27 (MH^+).

*4.1.6.25. 5-(Ethylthio)-N^4-mesityl-N^6,N^6-dipropylpyrimidine-4,6-diamine (**25**).* Yield: light yellow semisolid (76.4%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.55 (s, 1H), 6.94 (s, 2H), 3.60 (t, $J = 7.4$ Hz, 4H), 2.72 (q, $J = 7.4$ Hz, 2H), 2.29 (s, 3H), 2.18 (s, 6H), 1.68 (sxt, $J = 7.6$ Hz, 4H), 1.23 (t, $J = 7.4$ Hz, 3H), 0.92 (t, $J = 7.3$ Hz, 6H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.4, 164.1, 156.5, 136.4, 135.9, 129.0, 90.0, 52.8, 28.7, 21.4, 21.0, 18.6, 14.3, 11.3; ESI−MS *m/z*: 373.29 (MH^+).

*4.1.6.26. N^4,N^4-diethyl-5-(ethylthio)-N^6-mesitylpyrimidine-4,6-diamine (**26**).* Yield: (42.03%); MP: 45 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.55 (s, 1H), 6.94 (s, 2H), 3.69 (q, $J = 6.8$ Hz, 4H), 2.73 (q, $J = 7.3$ Hz, 2H), 2.29 (s, 3H), 2.19 (s, 6H), 1.28 (t, $J = 6.9$ Hz, 6H), 1.24 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.4, 164.1, 156.6, 135.9, 133.5, 128.9, 90.0, 44.8, 28.7, 21.0, 18.6, 14.3, 13.5; ESI−MS *m/z*: 345.27 (MH^+).

*4.1.6.27. 5-(Ethylthio)-N^4-mesityl-N^6,N^6-bis(2-methoxyethyl)pyrimidine-4,6-diamine (**27**).* Yield: light yellow semisolid (61.5%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.55 (s, 1H), 6.94 (s, 2H), 3.95 (t, $J = 6.0$ Hz, 4H), 3.64 (t, $J = 6.0$ Hz, 4H), 3.36 (s, 6H), 2.75 (q, $J = 7.3$ Hz, 2H), 2.29 (s, 3H), 2.18 (s, 6H), 1.23 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.2, 156.5, 136.5, 135.8, 133.4, 129.0, 90.5, 71.2, 58.8, 50.9, 28.5, 21.0, 18.6, 14.3; ESI−MS *m/z*: 405.28 (MH^+).

*4.1.6.28. N^4-butyl-N^4-ethyl-5-(ethylthio)-N^6-mesitylpyrimidine-4,6-diamine (**28**).* Yield: off white semisolid (76%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.54 (s, 1H), 6.94 (s, 2H), 3.69 (q, $J = 7.0$ Hz, 2H), 3.60 (t, $J = 7.1$ Hz, 2H), 2.72 (q, $J = 7.4$ Hz, 2H), 2.29 (s, 3H), 2.18 (s, 6H), 1.61 (quin, $J = 7.6$, Hz, 2H), 1.34 (sxt, $J = 7.5$, 2H), 1.23 (t, $J = 7.3$ Hz, 6H), 0.96 (t, $J = 7.4$ Hz, 3H); ^{13}C NMR (400 MHz, $CDCl_3$) δ 164.4, 164.1, 156.6, 136.4, 135.9, 133.6, 129.0, 90.1, 50.1, 45.5, 30.4, 28.7, 21.0, 20.2, 18.6, 14.3, 14.0, 13.4; ESI−MS *m/z*: 373.32 (MH^+).

4.2. CRF_1 receptor binding study

Binding studies were performed in membrane homogenates from human embryonic kidney cells (HEK 293) stably expressing CRF_1 receptors and using [^{125}I]-Tyr^0-sauvagine as radioligand. Membrane homogenates were prepared according to the method of Gkountelias [54] CRF_1-expressing HEK 293 cells, grown in DMEM/F12 (1:1) containing 3.15 g/L glucose, 10% bovine calf serum and 300 μg/ml of the antibiotic, Geneticin at 37 °C and 5% CO_2, were washed with phosphate-buffered saline (PBS) (4.3 mM Na_2H-$PO_4.7H_20$, 1.4 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.2−7.3 at R.T). Then the cells were briefly treated with PBS containing 2 mM EDTA (PBS/EDTA), and then dissociated in PBS/EDTA. Cells suspensions were centrifuged at 1000× g for 5 min at room temperature, and the pellets were homogenized in 1.5 mL of buffer H (20 mM HEPES, containing 10 mM $MgCl_2$, 2 mM EGTA, 0.2 mg/ml bacitracin and 0.93 μg/ml aprotinin pH 7.2 at 4 °C) using a Janke & Kunkel IKA Ultra Turrax T25 homogenizer, at setting ~20, for 10−15 s, at 4 °C. The homogenates were centrifuged at 16,000× g, for 10 min, at 4 °C. The membrane pellets were re-suspended by homogenization, as described above, in 1 mL buffer B (buffer H containing 0.1% BSA, pH 7.2 at 20 °C). The membrane suspensions were then diluted in buffer B and aliquots of suspensions (50 μl) were added into tubes containing buffer B and 20−25 pM [^{125}I]-Tyr^0-sauvagine without or with compounds **1**−**28** at a single concentration of 500 nM or with increasing concentrations of these compounds (competition binding experiments) in a final volume of 0.2 mL. The mixtures were incubated at 20−21 °C for 120 min and then filtered through Whatman 934AH filters, presoaked for 1 h in 0.3% polyethylenimine at 4 °C. The filters were washed 3 times with 0.5 mL of ice-cold PBS, pH 7.1 containing 0.01% Triton X-100 and assessed for radioactivity in a gamma counter. The amount of membranes used was adjusted to insure that the specific binding was always equal to or less than 10% of the total concentration of the added radioligand. Specific [^{125}I]-Tyr^0-sauvagine binding was defined as total binding less nonspecific binding in the presence of 1000 nM antalarmin. Data for competition binding were analyzed by nonlinear regression analysis, using Prism 4.0 (GraphPad Software, San Diego, CA). IC_{50} values were obtained by fitting the data from competition studies to a one-site competition model.

4.3. Statistical analysis

Prism 4.0 was used for all statistical analysis (GraphPad Software, San Diego, CA). Competition binding was analyzed by nonlinear regression analysis, and IC_{50} values were obtained by fitting the data from competition studies to a one-site competition model by nonlinear regression.

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