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Optimization of CRF_1R binding affinity of 2-(2,4,6-trichlorophenyl)-4-trifluoromethyl-5-aminomethylthiazoles through rapid and selective parallel synthesis

Dmitry Zuev,* Jodi A. Michne,† Sokhom S. Pin, Jie Zhang,‡ Matthew T. Taber and Gene M. Dubowchik*

Bristol–Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, USA

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Abstract—An efficient approach was developed to synthesize 2-(2,4,6-trichlorophenylamino)-4-trifluoromethyl-5-aminomethylthiazoles, corticotropin-releasing factor type 1 receptor (CRF_1R) antagonists, by monoalkylation of amines with chloromethyl intermediate **5**. The effect of variations in aminomethyl side chain of **6** on binding affinity is discussed.

Corticotropin-releasing factor (CRF), an endogenous neuropeptide involved in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis, is a principal modulator of mammalian physiological and behavioral responses to stress.[1,2] Extensive pre-clinical and clinical studies have provided significant evidence of the involvement of CRF in pathology of major depression and anxiety disorders.[3–5] The potential of corticotropin-releasing factor type 1 receptor (CRF_1R) antagonists to provide a novel mechanism for the treatment of anxiety and depression[6] has prompted many groups to search for suitable nonpeptide small molecules.[7,8] We have previously shown that 2-arylamino-4-trifluoromethyl-5-aminomethylthiazoles **1** (Fig. 1) are potent CRF_1R antagonists that are readily accessible from commercially available starting materials.[9] Systematic modulation of the steric and electronic properties of the aniline portion of the molecule led to the identification of lead compound **2** with $K_i = 8.6$ nM ($hCRF_1R$), possessing an optimal 2,4,6-trichlorosubstitution on the phenyl ring. To address the issue of further optimization of 2-(2,4,6-trichlorophenylamino)-4-trifluoromethyl-5-aminomethylthiazoles, here we discuss the effect of variations in the aminomethyl side chain on binding affinity in the series, and report a quick and efficient parallel synthesis of derivatives **6** by a highly selective amination of a chloromethyl intermediate **5**.

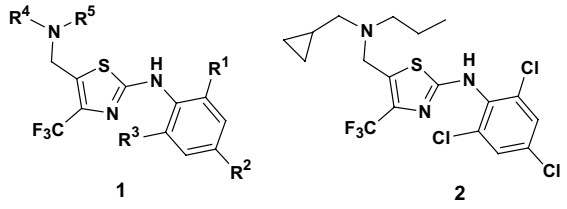

Figure 1.

We previously reported[9] that target aminomethylthiazole derivatives **6** could be prepared from ester **3** by sequential Weinreb amidation and borane–tetrahydrofuran complex reduction in good overall yields. However, we found that this pathway was inconvenient for rapid parallel synthesis of amines **6**, due to the moisture-sensitivity of both reactions and tedious aqueous work-ups. As an alternative, ester **3** was quantitatively reduced to alcohol **4** with DIBAL-H, followed by the conversion of the latter compound to chloromethyl

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* Corresponding authors. Tel.: +1 203 677 6714; fax: +1 203 677 7702; e-mail addresses: dmitry.zuev@bms.com; gene.dubowchik@bms.com
† Present address: AstraZeneca R&D Boston, Infection Discovery, 35 Gatehouse Park, Waltham, MA 02451, USA.
‡ Present address: Aventis Pharmaceuticals, PO Box 6800, Bridgewater, NJ 08807-0800, USA.

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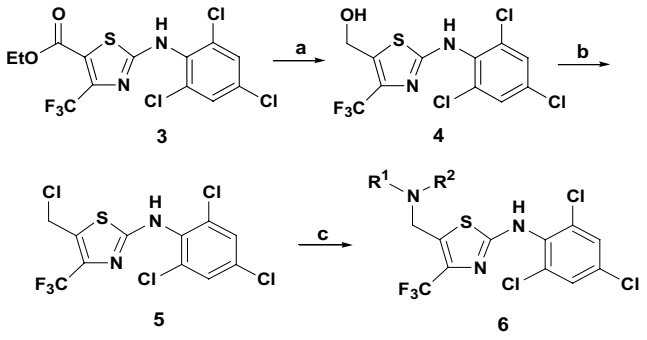


Scheme 1. Reagents and conditions: (a) DIBAL-H, THF, 0 °C to rt, 2 h, 100%; (b) $SOCl_2$, 85 °C, 5 min, 91%; (c) R^1R^2NH, Et_3N, diethyl ether, rt, 15 min, 50–95%.

intermediate **5** by heating in neat thionyl chloride (Scheme 1). Happily, alkylation of both primary and secondary amines with **5** in ether cleanly provided corresponding monoalkylated products without any detectable overalkylation.[10] The reaction occurred at room temperature and was usually complete within 15 min. For convenience, no aqueous work up was necessary, as triethylammonium chloride could be removed from the reaction mixture by filtration.[11]

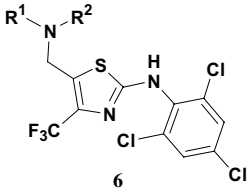
This selective monoalkylation of amines with chloromethyl intermediate **6** is in sharp contrast with the majority of analogous alkylation reactions, where a mixture of alkylated amines along with a significant amount of quaternary ammonium salt is the usual outcome. We believe that the nucleophilicity of the nitrogen atom in product **6** is significantly reduced by the strong electron-withdrawing properties of the trifluoromethylthiazole core. As a result, the nitrogen atom losses its ability to undergo further alkylation.

The general approach presented in Scheme 1 was applied successfully to the parallel synthesis of a number of analogs **6** in 50–95% yields.[12] Their CRF_1R binding affinities were determined by displacement of [^{125}I] Tyr-*o*-CRF from *h*CRF_1R endogenously expressed on IMR-32 human neuroblastoma cells[13] and are summarized in Table 1.

Initially, we decided to explore structural changes in analogs **6a**–**f** with various groups R_2, while retaining *N*-cyclopropylmethyl group as R_1. Derivatives **6a** (R_2 = Et) and **6b** (R_2 = *c*$PrCH_2$) were less active than **2**. Compounds **6a** and **2** were somewhat more potent than their trifluorinated analogs **6c** and **6d**. A single example of a thiazole with a secondary amino side chain was only weakly active (K_i = 29 μM). Replacement of the cyclopropylmethyl group in **2** with *n*-propyl resulted in an almost 20-fold loss in activity for **6g**. However, allyl analogs **6j** and **6k** regained some of that potency.

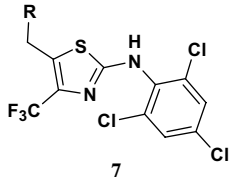
The tolerance of the CRF_1 receptor to the steric size of substituents R_1 and R_2 was next investigated. We found that the expansion of the cyclopropyl ring in **2** to

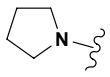
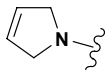
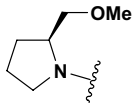
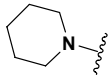
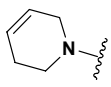
Table 1. *h*CRF_1R binding affinities of *N*,*N*-dialkylaminomethylthiazoles **6**



Compound	R^1	R^2	K_i (nM)
2	*c*$PrCH_2$	*n*Pr	8.6
6a	*c*$PrCH_2$	Et	23
6b	*c*$PrCH_2$	*c*$PrCH_2$	26
6c	*c*$PrCH_2$	CF_3CH_2	43
6d	*c*$PrCH_2$	$CF_3CH_2CH_2$	46
6e	*c*$PrCH_2$	$Me_2NCH_2CH_2$	13,000
6f	*c*$PrCH_2$	H	29,000
6g	*n*Pr	*n*Pr	160
6h	*n*Pr	Et	150
6i	*n*Bu	Et	290
6j	Allyl	Allyl	57
6k	Allyl	*n*Pr	100
6l	*c*$BuCH_2$	*n*Pr	73
6m	*c*$HexCH_2$	*n*Pr	6300
6n	*i*Bu	*n*Pr	240
6o	*i*Pr	*i*Pr	9800
6p	*c*$PrCH_2CH_2$	*n*Pr	420
6q	*c*$PrCH_2CH_2$	Et	1100
6r	$Me_2NCH_2CH_2$	Et	19,000

Table 2. *h*CRF_1R binding affinities of cyclic aminomethylthiazoles **7**



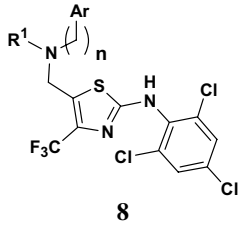
Compound	R	K_i (nM)
7a		10,000
7b		6700
7c		5100
7d		18,000
7e		1800
7f		55,000
7g		12,000

cyclobutyl led to an 8-fold decrease in binding affinity for **6l**. Analog **6m**, with the bulkier cyclohexyl ring, was nearly inactive. The introduction of β- and, especially, α-methyl branching to the nitrogen atom also led to a decrease in potency. This is exemplified by a 3-fold loss of activity for **6n**, compared to **6g**, followed by a further 40-fold decrease in activity for **6o**. Homologation of the cyclopropylmethyl group resulted in a 50-fold decrease in potency of **6p** and **6q**, compared to **2** and **6a**. Analogs **6e** (K_i = 13 μM) and **6r** (K_i = 19 μM), containing dimethylamino groups, were weakly active, suggesting very little tolerance of the CRF_1 receptor for polar atoms in the side chain.

Thiazoles **7a**–**g**, possessing cyclic amino side chains, displayed significantly lower binding affinities than acyclic amines **6** (Table 2). Olefins **7b** and **7g** were slightly more potent than their saturated analogs **7a** and **7f**. Imidazole derivative **7d** was less active than pyrrolidine **7a** and pyrroline **7b**. The introduction of an ethyl group at the 2-position of the imidazole ring led to a 10-fold enhancement in activity for **7e**, compared to its unsubstituted analog **7d**. This 'branching effect' could also be responsible for the marginally higher binding affinity of **7c** compared to that of **7a**.

In the course of our optimization study we prepared and tested a number of arylalkylthiazoles **8** (Table 3). The highest binding affinity was displayed by benzyl 3,3,3-trifluoropropyl amines **8m**–**p**, which were more potent, than our initial lead **2**. These analogs were also three to six times more potent than the corresponding *n*-propyl amines **8c**–**g**. Pyridyl-containing derivatives **8i**–**k** were less active than benzyl amine **8a**, again suggesting that polar atoms in the side chain are not well-tolerated by the receptor. Indeed, the more lipophilic 2-thienyl compound **8g** was 6-fold more potent than 2-furanylmethyl analog **8h**. Some of the phenethyl alkyl amines **8q**–**v** showed good binding to the CRF_1 receptor. The highest potency in the series was displayed by phenethyl *n*-propyl amine **8q** (K_i = 28 nM), and it rapidly diminished as the size of the alkyl substituent R_2 was reduced. 5-(2-Methylthiothiazolyl) amine **8w** was nine times more potent than benzyl analog **8t**.

Table 3. *h*CRF_1R binding affinities of arylalkylaminomethylthiazoles **8**



8

Compound	*n*	Ar	R^1	K_i (nM)
8a	1	Ph	*n*Pr	38
8b	1	Ph	Et	120
8c	1	*p*-F–Ph	*n*Pr	25
8d	1	*p*-Cl–Ph	*n*Pr	11
8e	1	*p*-MeO–Ph	*n*Pr	37
8f	1	*m*-F–Ph	*n*Pr	42
8g	1	2-Thiophenyl	*n*Pr	130
8h	1	2-Furanyl	*n*Pr	770
8i	1	4-Pyridyl	*n*Pr	100
8j	1	3-Pyridyl	*n*Pr	290
8k	1	2-Pyridyl	*n*Pr	1300
8l	1	Ph	CF_3CH_2	390
8m	1	Ph	$CF_3CH_2CH_2$	6.1
8n	1	*p*-F–Ph	$CF_3CH_2CH_2$	4.8
8o	1	*m*-F–Ph	$CF_3CH_2CH_2$	3.7
8p	1	*p*-Cl–Ph	$CF_3CH_2CH_2$	3.2
8q	2	Ph	*n*Pr	28
8r	2	Ph	Et	79
8s	2	Ph	Me	560
8t	2	Ph	H	>10,000
8u	2	Ph	CF_3CH_2	50
8v	2	Ph	$CF_3CH_2CH_2$	130
8w	0	5-(2-MeS-thiadiazolyl)	H	1100

In summary, we have developed an efficient parallel synthesis of CRF_1R antagonists, 2-(2,4,6-trichlorophenyl)-4-trifluoromethyl-5-aminomethylthiazoles, by selective monoamination of the common chloromethyl intermediate. Analogs containing benzyl 3,3,3-trifluoropropyl amino side chains were found to have the highest binding affinities.

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10. All new compounds gave satisfactory analytical data. For **6i**: ^{1}H NMR ($CDCl_3$) 2.27 (m, 2H), 2.75 (ABq, 2H), 3.62 (s, 2H), 3.74 (s, 2H), 7.28 (m, 5H), 7.48 (s, 2H), 8.75 (br s, 1H). Mass spec.: 563.98 (MH^+).
11. *General procedure for monoalkylation of amines*: To a stirred solution of the appropriate primary or secondary amine (1 mmol) and triethylamine (1 mmol) in ether (3 mL) at 0 °C was added (5-chloromethyl-4-trifluoromethylthiazol-2-yl)-(2,4,6-trichlorophenyl)amine **5** (1 mmol). The reaction mixture was warmed to room temperature and stirred for 15 min. Triethylamine hydrochloride was removed by filtration and the filtrate was concentrated in vacuo. The final purification of the products **6** was achieved by column chromatography.
12. The purity of analogs **6** was found to be >90% by reversed phase analytical LC–MS analysis under following conditions: column–PHENOMENEX-LUNA 4.6 × 50 mm S10;

solvent A—CH_3OH (10%)–H_2O (90%)–TFA (0.1%); solvent B—CH_3OH (10%)–H_2O (90%)–TFA (0.1%), starting %B—0, final %B—100; flow rate—4 mL/min, gradient time—2 min, end time—3 min.

13. Membranes were prepared from transformed IMR-32 cells as previously described,[14] and incubated with [^{125}I]Tyr-*o*-CRF (100 pM) and increasing concentrations of test compound for 100 min at 25 °C (assay buffer: 50 mM Tris (pH 7.2), 10 mM $MgCl_2$, 0.5% BSA, 0.005% Triton X-100, 10 μg/mL aprotinin, and 10 μg/mL leupeptin). Assays were stopped by addition of ice-cold wash buffer (50 mM Tris, pH 7.4, and 0.2% BSA). Nonspecific binding was defined with 10 μM *o*-CRF. The present compounds are full antagonists of the CRF_1R as determined by their ability to inhibit CRF stimulated *c*AMP production in IMR-32 cells.
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