Rapid Microscale Synthesis, a New Method for Lead Optimization Using Robotics and Solution Phase Chemistry: Application to the Synthesis and Optimization of Corticotropin-Releasing Factor$_1$ Receptor Antagonists

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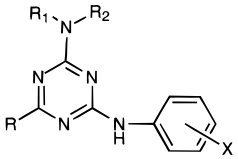
Corticotropin releasing factor (CRF), a 41-amino acid peptide primarily of hypothalamic origin, binds to the CRF receptor family of seven transmembrane domain (G-protein coupled) receptors. The CRF_1 receptor has been cloned and functionally characterized[1−4] and is distinct in its sequence, tissue distribution, and pharmacological profile from the CRF_2 receptor subfamily.[5−9] Hypothalamic CRF controls the release of adrenocorticotropin (ACTH) and other proopiomelanocortin-derived peptides from the anterior pituitary primarily through its action at the CRF_1 receptor subtype.[10] Recently, a substantial amount of clinical and laboratory data has been accumulated that suggests that CRF is a physiological mediator of stress and stress-related disorders and that antagonists to the CRF system may play a role in the treatment of depression and anxiety-related disorders.[11−16] As a result, the design of non-peptide, small molecule CRF receptor antagonists has been of substantial interest as a new approach for the treatment of these disorders.[10]

We report the optimization of **5u** (see Table 1), a compound synthesized as part of our program to obtain non-peptide CRF receptor antagonists, with a new solution phase robotics-driven synthesis method called rapid microscale synthesis (RMS) that utilizes a readily modified version of the commercially available Hewlett-Packard 7686 PrepStation.[17] Automated solution phase organic synthesis was pioneered by Fuchs and coworkers[18] for optimization of reaction conditions with a Zymark robot. Extensive programming was required for each reaction sequence, and close attention to the robot was required. Lindsey[19] has reviewed the status of automated solution phase synthesizers and notes that no general purpose synthesizers have been constructed. Very recently, a group from the Takeda laboratories[20] built an automated synthesis apparatus for preparing compounds in solution. However, no readily accessible or commercial instrument has been available for this approach. RMS provides a practical method for the preparation of between 25 and several hundred analogs of a lead molecule or "hit" obtained from screening, in only a few weeks, by an automated robotics method. This solution phase synthesis performs multistep syntheses with extractive workups between each step. Other automated methods for product purification are available including silica gel plug filtration (called SPE by the robot for solid phase extraction) and washing and isolation of crystals. It is important to note that RMS is amenable to the synthesis of known quantities (up to several milligrams) of each compound via a highly flexible approach and that the automated workup at each step in a reaction sequence provides a method to remove excess reagents. The RMS approach has been applied to the synthesis and optimization of non-peptide corticotropin-releasing factor (CRF) receptor antagonists (see accompanying paper[21]). Reports of small-molecule CRF receptor antagonists have appeared in recent European and World Patent applications[22−28] and have been reviewed;[10,29] however, no biological data are presented.

Table 1. Inhibition (K_i) Values for Triazines **5** in Stable Cell Lines Transfected with Human CRF_1 Receptors[a]



5u-5dd

compound	R	NR_1R_2	X	K_i (nM)
5u	Me	$PhCH_2CH_2NMe$	2,4,6-Me_3	2,100
5v	Me		2,4,6-Me_3	1,050
5x	Me	$(nBu)_2N$	2,4,6-Me_3	490
5z	Me	$PhCH_2NBu$	2,4,6-Me_3	1050
5aa	Me	$(nPr)_2N$	2,4,6-Me_3	130
5bb	Me	$nPrNCH_2cPr$	2,4-$(OMe)_2$	8,000
5cc	Et	$nPrNCH_2cPr$	2,4,6-Me_3	>10,000
5dd	Me	$nPrNCH_2cPr$	2,4-Me_2	>10,000

[a] Compounds were tested at 6−12 doses for their ability to inhibit [^{125}I]CRF binding as described in text. Data are representative of duplicate determinations with experiments repeated two or three times.

In order to prepare analogs of **5u** on a solid support, it was anticipated that a functional group would be required on the triazine core molecule to link to the solid support and could dramatically limit the diversity of the analogs prepared. It is important to note that techniques for the rapid synthesis of analogs on a solid support have received considerable recent attention as a method to enhance the discovery of new drugs.[30−45] (This approach is briefly reviewed in the Supporting Information.) The limitation of a linker led to the development of the solution phase RMS method for automated synthesis and was used to prepare milligram quantities of over 350 individual analogs of **5u** in about 4 weeks. A modified version of the commercially available HP 7686 PrepStation was required to carry out the automated synthesis and workup of the reactions (see Scheme 1).[17] Reaction conditions were programmed for the synthetic sequence on a PC computer terminal using a windows-based program called Bench Supervisor; all additions were performed by the robotic arm in series, but reactions were run in parallel in groups of 5−25 by heating the tray or heater block to the desired temper-

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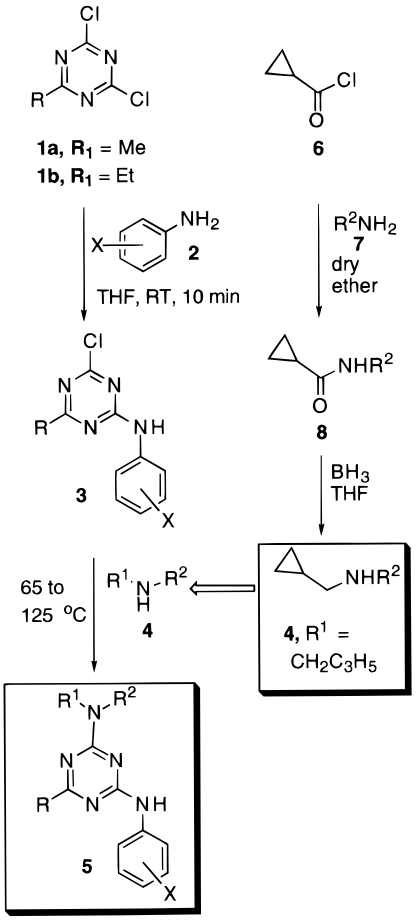
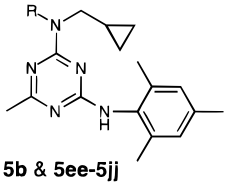
Scheme 1. Prepararion of **5** and Precursor Amines **4** (Where $R^1 = CH_2C_3H_5$) by RMS



-ature. 2-Methyl- or 2-ethyl-4,6-dichlorotriazine[46] in THF was added by syringe to each reaction vial (1.8 mL, sealed with a septum) and treated as outlined in Scheme 1 (see Supporting Information for a more detailed experimental details). Approximately two-thirds of the desired products were isolated in 70−95+% purity as determined by gas chromatography (total ion current mode of detection with HP 5972A mass selective detector) combined with mass spectroscopy analysis (GC MS), and only these compounds were used in SAR determinations. The most active compounds were resynthesized individually by hand to confirm the purity and accuracy of the method.



For the synthesis of the *N*-(cyclopropylmethyl)-*N*-alkyl- or -alkylarylamino analogs **5b** (R = *n*-Pr) and **5ee**−**jj**, (R = Et, *n*-Bu, *n*-pentyl, isopentyl, benzyl, and H, respectively) the amines **4** (R^1 = cyclopropylmethyl) which were used for coupling were prepared in an additional reaction sequence on the PrepStation as outlined in Scheme 1. The synthesis of these amines illustrates the utility of RMS for reactions that require dry solvents and an inert atmosphere.

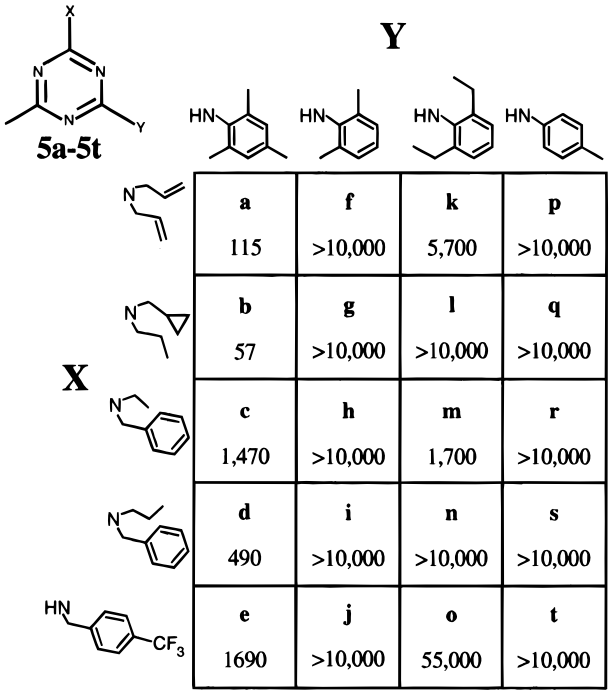


Figure 1. Example of a two-dimensional table created by varying both the amino and anilino groups on the triazine. K_i values (nM) for triazines **5a**−**t** prepared by RMS. (Inhibition of [^{125}I]CRF binding to human CRF_1 receptors. See text for details.)

CRF receptor binding data were obtained on the known quantities of over 350 triazine analogs of general structure **5** prepared by RMS on the modified HP PrepStation. In addition, K_i values were determined on resynthesized compounds **5a**−**e**, **5u**, **5x**, **5aa**, and **5bb**. The substantial effect of varying the X (amino) and Y (anilino) substituents on the 2-methyltriazines (**5**) on CRF_1 receptor binding activity is illustrated with the grid of compounds shown in Figure 1. Changing the methyl group at the 2-position to ethyl (**5cc**), while retaining the *N*-propyl-*N*-cyclopropylmethylamine and the 2,4,6-trimethylanilino group present in **5b** resulted in complete loss of receptor binding activity (Table 1). The effect of changing the amino and aryl group on activity is illustrated in Table 1 as well as the effect of substituting 2,4-dimethoxyanilino (**5bb**) and 2,4-dimethylanilino (**5dd**) for the 2,4,6-trimethylanilino group. The diversity in structure of the amino group was much greater with regard to retaining activity (Table 1), and over 250 different amines were substituted at this position by RMS (see Supporting Information); the *N*-propyl-*N*-cyclopropylmethylamine provided the best activity (i.e. **5b**). Replacement of the *N*-propyl group in **5b** (K_i = 57 nM) with *N*-ethyl (**5ee**) (K_i = 2260 nM) or *N*-butyl (**5ff**) (K_i = 1930 nM) resulted in a 40-fold loss in activity. Extending the chain from *N*-butyl to *N*-pentyl (**5gg**) resulted in complete loss in activity as did replacement of propyl with hydrogen (**5jj**). Substitution of an isopentyl group (**5hh**) (K_i = 4800 nM) for propyl provided a compound that was 2-fold less active than *N*-butyl, whereas the benzyl derivative (**5ii**) (K_i = 2250 nM) was equipotent with the butyl antagonist. It should be noted that RMS is best applied to one- to three-step reaction sequences that proceed in good yield and provide products and intermediates that are organic soluble and can be isolated by extractive workup.

In summary, potent CRF receptor antagonists in the range of 50 nM (**5b**) were identified by preparing over 350 analogs of an initial lead (**5u**) (K_i = 2100 nM) on a multimilligram scale using rapid microscale synthesis (RMS). RMS provides an alternative and convenient method to robotics driven solid phase synthesis for preparing up to several hundred analogs of a biologically active molecule in a relatively short period of time.

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Supporting Information Available: A description of modifications to the original PrepStation, Figure 2, illustrating capabilities/functions of the PrepStation, general experimental procedures (sequential steps in the computer programs) for the preparation of the triazines **5** and the cyclopropylmethylamines **4** on the HP PrepStation, and a table with compounds, CRF_1 receptor binding data, parent ion as determined by GC MS or M + 1 as determined by ion spray MS, amount of material submitted for testing, % yield, and purity as estimated by GC MS; in addition, an expansion of the introduction to solution and solid phase robotics chemistry is included (54 pages). Ordering information is given on any current masthead page.

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