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Synthesis and evaluation of prodrugs of corticotropin-releasing factor-1 (CRF_1) receptor antagonist BMS-665053 leading to improved oral bioavailability

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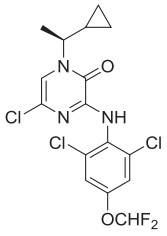
ABSTRACT

A series of phosphate and ester-based prodrugs of anilinopyrazinone **1** (BMS-665053) containing either a methylene or an (acyloxy)alkoxy linker was prepared and evaluated in rat pharmacokinetic studies with the goal of improving the oral bioavailability of the parent (**1**). The prodrugs, in general, had improved aqueous solubility and oral bioavailability compared to **1**. Prodrug **12**, which contains an (acyloxy)alkoxy linker, showed the greatest improvement in the oral bioavailability relative to the parent (**1**), with a seven-fold increase (from 5% to 36%) in rat pharmacokinetic studies.

Corticotropin-releasing factor (CRF), a 41 amino acid neuropeptide first isolated and characterized by Vale and coworkers,[1] is secreted from the paraventricular nucleus of the hypothalamus. CRF functions as the primary physiological regulator of the hypothalamic–pituitary–adrenal (HPA) axis, coordinating the body's endocrine response to stress by regulating the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary gland, which in turn initiates the synthesis and release of adrenal corticosteroid hormones (e.g. cortisol), enabling the body to respond to the stressor.[2,3] Two well-characterized receptor subtypes, CRF_1 and CRF_2, have been identified. These G-protein coupled receptors are widely distributed throughout the central and peripheral nervous systems.[4] Data suggests that the CRF_1 receptor subtype plays a significant role in the stress-related response.[4,5] Compelling evidence supports the hypothesis that excessive levels of CRF contribute to stress-related disorders such as depression and anxiety, and that antagonists of CRF_1 receptors may be able to successfully treat these conditions.[2,4,6–9]

A series of pyrazinones has demonstrated excellent antagonistic activity against CRF_1 receptors.[10–13] Among these, compound **1** (BMS-665053)[11] (Fig. 1) had high affinity for the CRF_1 receptor (IC_{50} = 1.0 nM) and was a potent inhibitor of CRF-stimulated cyclic adenosine monophosphate (cAMP) production in human Y-79 retinoblastoma cells (IC_{50} = 4.9 nM), indicating that it behaved as an antagonist. In addition, **1** was efficacious in the Defensive Withdrawal model of anxiety in rats and had low *in vivo* clearance (Cl = 17 mL/min/kg, $t_{½}$ = 7.8 h) in rats. However, when dosed as an oral suspension with methylcellulose, the oral bioavailability of **1** was low (5%). Compound **1** displayed very limited aqueous solubility (<0.001 mg/mL at pH = 6.5 and 0.007 mg/mL at pH = 1), which was likely the major cause of the low oral bioavailability of this compound. As a result, we turned our attention to the design and synthesis of solubility-enhancing prodrugs of **1**. Phosphate[14–17] and ester-based[18] prodrugs, either attached directly or via a linker to the parent compound, have been successfully used to increase the solubility and exposure of a variety of orally administered compounds. In this letter we describe the synthesis and



1
(BMS-665053)

Fig. 1. Structure of **1** (BMS-665053).

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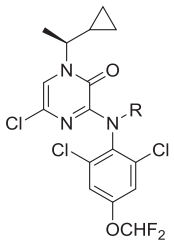
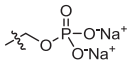
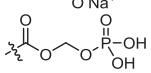
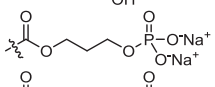
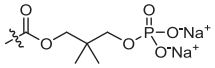
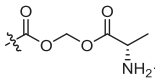
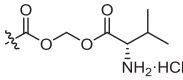
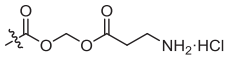
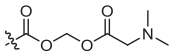
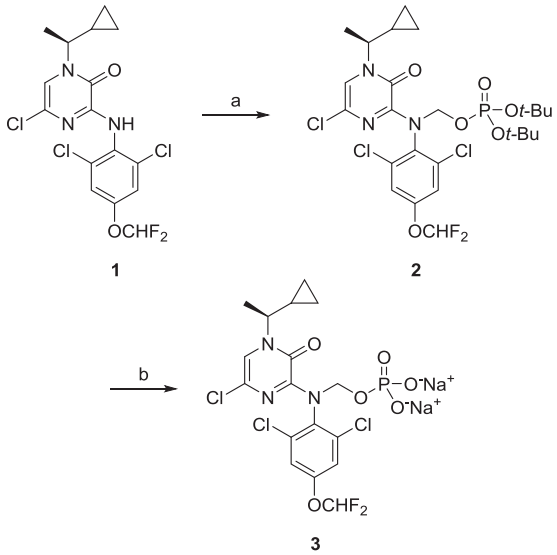
Table 1
Oral bioavailability of prodrugs **3**, **6**, **7**, **10**, **12**–**15**.



Compd	R	Solubility at pH 1 (mg/mL)	Solubility at pH 6.5 (mg/mL)	Oral AUC_{0-24h}[a] (nM h)	Oral C_{max} (nM)	%F
1	H	0.007	<0.001	1260	100	5%[b]
3		ND	ND	5990	2150	25%[c]
6		0.338	Unstable	3990	460	17%[d,e]
7		0.004	1.50	4230	295	18%[f]
10		0.05	>1.5	BQL	BQL	NA[f]
12		0.37	0.005	8250	730	36%[g]
13		1.82	0.01	7370	740	32%[h]
14		1.35	0.03	6320	490	27%[h]
15		1.72	0.53	BQL	BQL	NA[h,i]

[a] Prodrugs were administered at a 10 mg/kg equivalent dose to n = 3 rats for each compound (see endnote 25 for conditions).
[b] Vehicle: 0.5% methylcellulose with 0.1% Tween 80.
[c] Vehicle: 0.5% methylcellulose with 0.1% Tween 80, 5 mM $NaHCO_3$, pH 10.
[d] Vehicle: 0.75% methylcellulose with 0.1% Tween 80, 10 mM HCl.
[e] Examination of the dosing solution revealed that 23% of the dose converted back to **1**.
[f] Vehicle: 0.5% methylcellulose suspension with 0.1% Tween 80, 25 mM phosphate, pH = 7.
[g] Vehicle: 0.5% methylcellulose suspension with 0.1% Tween 80, 10 mM HCl.
[h] Vehicle: 0.5% methylcellulose suspension with 0.1% Tween 80, pH = 2.
[i] **1** was not detected. Based on an analysis of the dosing solution, it appeared that the entire dose was in the dosing solution as **15**; ND = not determined, BQL = below quantifiable limit, NA = not applicable.



Scheme 1. Reagents and conditions: (a) NaH, I_2, di-*tert*-butyl (chloromethyl) phosphate,[14] THF (5–10%); (b) TFA (10 eq), CH_2Cl_2 then $NaHCO_3$ (61%).

in vivo evaluation of prodrugs of **1** with the goal of improving its oral bioavailability in rats.

The phosphate and ester-based prodrugs shown in Table 1 were prepared for evaluation in rat pharmacokinetic studies in order to assess whether improved aqueous solubility translated to improved oral bioavailability of **1**. Several types of linkers were explored. Synthesis of a compound containing a methylene-linked di-*tert*-butyl phosphate group (Scheme 1) proved challenging due to difficulties in alkylating the electron deficient aniline nitrogen of **1**. Only low yields of alkylation product (**2**) were isolated (ca. 5–10%). Removal of the *tert*-butyl groups in **2** with TFA followed by treatment with sodium bicarbonate furnished **3** in 61% yield. We subsequently turned our attention to (acyloxy)alkoxy-linked prodrugs,[19] and were gratified to find that acylation of **1** proceeded in high yield as shown in Scheme 2.

Two phosphate-based prodrugs of **1** were prepared as described in Scheme 2. Treatment of **1** with either chloromethylchloroformate or chloropropylchloroformate using a modified procedure of Heckendorn[20] afforded **4** and **5**, respectively, in high yield. Displacement of the chloride with di-*tert*-butyl phosphate tetrabutylammonium salt[21] followed by removal of the *tert*-butyl groups upon treatment with TFA furnished the desired phosphate esters **6** and **7**.