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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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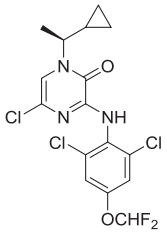
ABSTRACT

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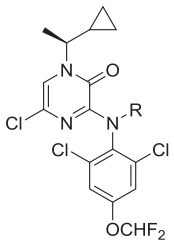
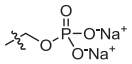
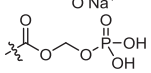
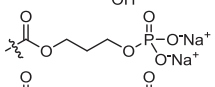
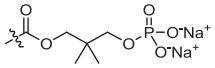
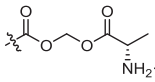
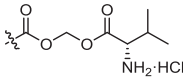
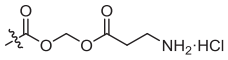
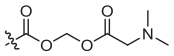
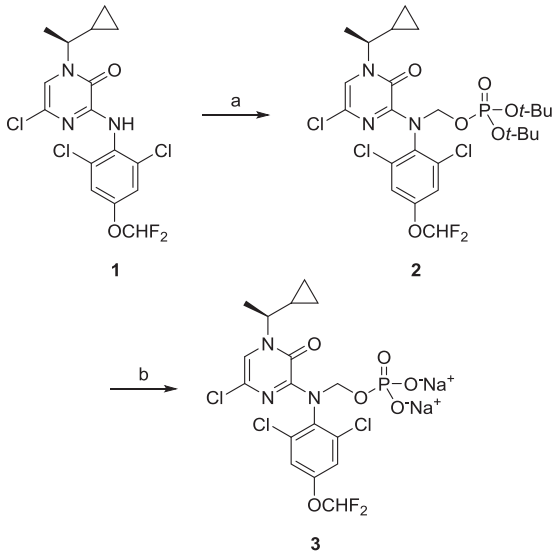
Table 1
Oral bioavailability of prodrugs **3**, **6**, **7**, **10**, **12**–**15**.



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.

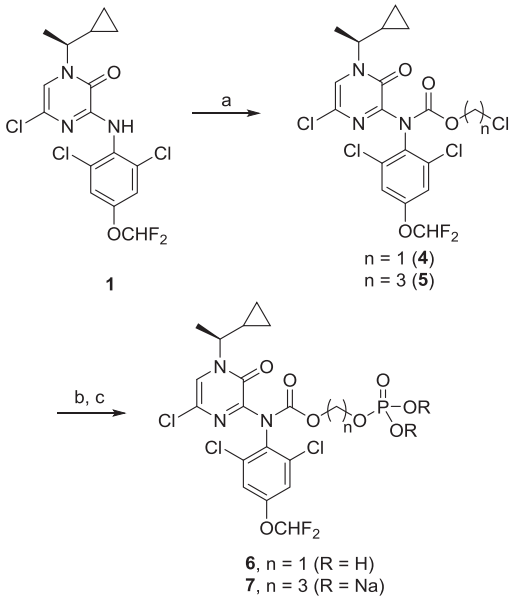


Scheme 1. Reagents and conditions: (a) NaH, I_2, di-*tert*-butyl (chloromethyl) phosphate,[14] THF (5–10%); (b) TFA (10 eq), CH_2Cl_2 then $NaHCO_3$ (61%).

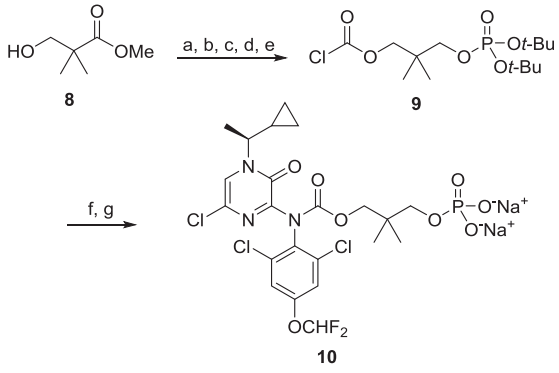
in vivo evaluation of prodrugs of **1** with the goal of improving its oral bioavailability in rats.

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**.



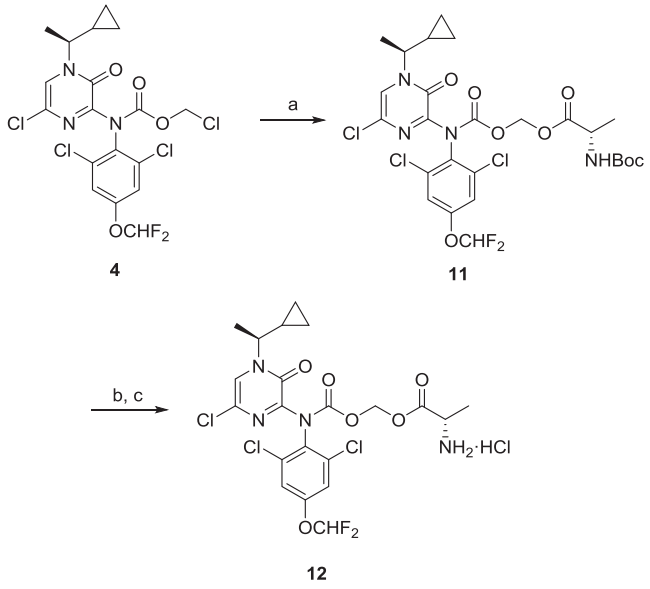
Scheme 2. Reagents and conditions: (a) NaH, DMAP, chloromethylchloroformate (for **4**) or chloropropylchloroformate (for **5**), THF (83–99%); (b) $Bu_4NO_2P(Ot\text{-}Bu)_2$, KI, DMF (59–82%); (c) 0.5 M TFA in CH_2Cl_2, 0 °C to room temperature then, for **7** only, 0.25 M $NaHCO_3$ (84% for **6**, 67% for **7**).



Scheme 3. Reagents and conditions: (a) NaH, benzyl bromide, THF (45%); (b) $LiAlH_4$, THF (94%); (c) $Et_2NP(Ot\text{-}Bu)_2$, 1*H*-tetrazole then MCPBA, THF; (d) H_2, Pd $(OH)_2$, EtOH, (42%, 2 steps); (e) 20% phosgene in toluene, *i*-Pr_2NEt, CH_2Cl_2 (58%); (f) NaH, DMAP, compound **1**, THF (97%); (g) 0.5 M TFA in CH_2Cl_2, 0 °C to room temperature then 0.25 M $NaHCO_3$ (68%).

The synthesis of **10** was completed in seven steps as shown in Scheme 3. Alcohol **8** was protected as the benzyl ether. The ester was then reduced with $LiAlH_4$ to give the corresponding alcohol. The alcohol was treated with di-*tert*-butyl *N*,*N*-diethylphosphoramidate and the resulting phosphite was oxidized *in situ* with *m*-CPBA to form the phosphate.[22] Removal of the benzyl group, followed by treatment with phosgene, furnished chloroformate **9**.[23] Chloroformate **9** was then coupled with **1** to form the carbamate product in 97% yield. Finally, deprotection of the phosphate by removal of the *tert*-butyl groups upon treatment with TFA provided **10** in high yield.

The synthesis of the amino acid prodrug **12** was carried out via the three step route illustrated in Scheme 4. Treatment of chloromethyl ether **4** with *N*-Boc-protected alanine in the presence of sodium bicarbonate and potassium iodide afforded **11** in 77% yield. Subsequent removal of the *N*-Boc group by treatment with TFA furnished **12** as the TFA salt, which was converted into the hydrochloride salt by ion exchange.[24] Compounds **13** and **14** were prepared



Scheme 4. Reagents and conditions: (a) $NaHCO_3$, KI, *N*-Boc-L-Ala, DMF (77%); (b) TFA (100%); (c) AG 1-X2 ion exchange resin (78%).

in a similar fashion, while the preparation of compound **15** was accomplished by treatment of **4** with *N*,*N*-dimethylglycine under conditions described to prepare **11**.

Prodrug **3** was evaluated in a rat pharmacokinetic study with oral dosing. The results presented in Table 1 show that this prodrug provided a five-fold increase in oral bioavailability versus parent compound **1**.[25] For prodrugs with the methyleneoxy linker, it was envisioned that enzymatic cleavage of either the phosphate or ester groups would then be followed by a cascade fragmentation of the linker to furnish the parent (**1**) along with carbon dioxide and formaldehyde.[19b] The risks associated with chronic low level oral exposure to formaldehyde in the expected dose ranges have been previously discussed and are minimal.[26] Prodrug **6**, which could be more readily prepared in sufficient quantities than **3**, was subsequently profiled. Prodrug **6** showed pH dependent stability, being stable at pH 1, but rapidly degrading (ca. 10 min) to parent at pH 6.5, presumably due to nucleophilic attack of the phosphate anion onto the carbonyl of the neighboring carbamate group. Nevertheless, **6** was dosed in rats to provide a preliminary assessment as to whether an enhancement in oral bioavailability would be observed. Upon dosing in a vehicle adjusted to low pH (10 mM HCl), prodrug **6** increased the oral bioavailability of **1** from 5% to 17%. However, this result was confounded by the instability of **6** as examination of the dosing solution revealed that 23% of the dose had converted to parent compound (**1**).

In an effort to improve upon the stability of phosphate-based prodrug **6**, compound **7** was prepared. This compound contains an extended linker that should reduce the propensity of the phosphate anion to undergo the undesired cyclization/degradation pathway. Stability studies with **7** showed that this prodrug was stable at pH 1 and 7.[27] It was anticipated that upon alkaline phosphatase catalyzed hydrolysis of the phosphate group in the gut,[28–31] subsequent cyclization of the newly formed alcohol (**16**) onto the carbamate functionality would result in liberation of the parent (**1**, Fig. 2).[32] We were gratified to find that a rat pharmacokinetic study with **7** resulted in an improved 18% oral bioavailability of the parent compound (**1**).

Prodrug **10** was subsequently prepared as it was envisioned that incorporation of a *gem*-dimethyl group would result in more rapid cyclization and subsequent liberation of parent compound due to the Thorpe-Ingold effect.[33] However, upon oral dosing no

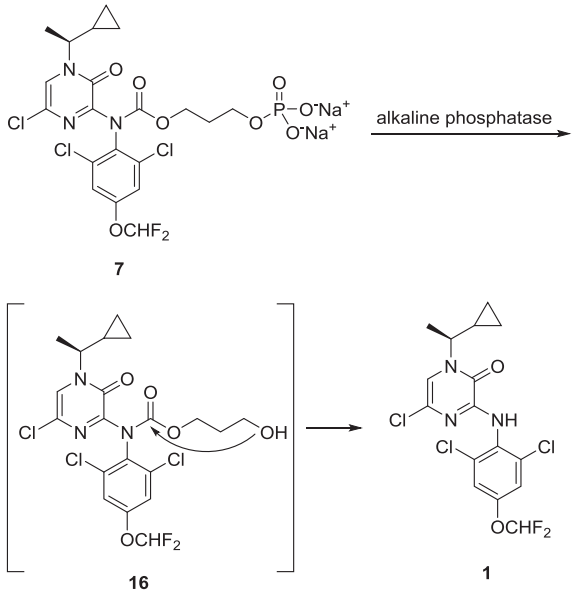


Fig. 2. A representative cascade fragmentation pathway for carbamate-linked prodrugs with an extended linker.

parent (**1**) was detected. Subsequent *in vitro* experiments demonstrated that the rate of alkaline phosphatase catalyzed hydrolysis was much slower for compound **10** versus compound **7**, presumably due to the increased steric hindrance presented by the *gem*-dimethyl group. Upon incubation of **7** and **10** separately with alkaline phosphatase at 23 °C it was observed that 50% conversion of **7** to the corresponding alcohol intermediate **16** occurred after 120 min, whereas only 22% of compound **10** underwent conversion during the same time interval.[34]

A series of ester based prodrugs was also tested *in vivo*. Compounds **12**–**15** are esters of alanine, valine, β-alanine, and *N*,*N*-dimethylglycine, respectively. Each of these prodrugs improved the solubility of the parent compound significantly at pH = 1; a smaller improvement in solubility was observed at pH = 6.5 (Table 1). Prodrugs **12**–**14** showed an improved oral bioavailability compared to **1** (36%, 32% and 27%, respectively). Prodrug **15**, however, did not improve the oral bioavailability of **1**, possibly because **15** was not cleaved by esterases.

In summary, our results demonstrate that an (acyloxy)alkoxy group can serve as a suitable linker for the preparation of phosphate and ester-based prodrugs of anilinopyrazinone **1** in lieu of the more commonly used methylene linker, which was synthetically challenging to prepare due to the poor reactivity of the anilinopyrazinone nitrogen. The resulting prodrugs exhibited improved aqueous solubility compared to **1** and amino acid prodrugs **12**, **13** and **14** provided a substantial increase in the oral bioavailability of **1** in rat pharmacokinetic studies. Alanine prodrug **12**, in particular, showed the greatest improvement in oral bioavailability compared to dosing the parent compound directly, with an increase from 5% for parent (**1**) to 36% for **12**.

Acknowledgments

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A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.02.015.

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24. The TFA salt of **12** (113 mg) was dissolved in MeOH/H_2O (20/80) and passed through a column of ca. 2 g of AG 1-X2 ion exchange resin, chloride form (Bio-Rad 100-200 mesh, catalog no. 140-1241). The fractions containing product were combined and concentrated under vacuum to remove the methanol. The remaining aqueous mixture was lyophilized to furnish the final product **12** as the HCl salt. Conversion of the TFA salt to the HCl salt was confirmed by ^{19}F NMR.
25. Rat Pharmacokinetic Studies. Pharmacokinetic parameters were estimated in Sprague-Dawley rats following intravenous (2 mg/kg; $n = 3$) and oral (10 mg/kg; $n = 3$) dosing. Intravenous doses were prepared in a vehicle consisting of PEG:ethanol, 90:10 (v/v) at a volume of 1 mL/kg. The oral doses were prepared in a vehicle described in Table 1 at a volume of 3 mL/kg. Blood samples were collected *via* a jugular vein catheter at 0, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hr post-dose for the intravenous experiment, and at 0, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 hr post-dose for the oral experiment. Plasma was separated by centrifugation and stored frozen at −20 °C until analysis. Concentrations were determined by LC/MS/MS.
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27. Compound **7** was stirred separately in solutions at pH 1 and pH 7 buffer for a period of 8 h at 23 °C and was monitored by HPLC. No degradation was observed over this time period.
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33. Keese R, Meyer M. *Tetrahedron Lett*. 1993;49:2055.
34. An 800 μL solution of **7** or **10** (2 mg/mL of either **7** or **10** in Tris buffer (50 mM)) at pH = 8, 100 μL of bovine serum albumin solution (10 mg/mL of 50 mM Tris buffer, pH = 8), and 100 μL of alkaline phosphatase solution (1 mg/mL of 900 μL 50 mM Tris buffer and 100 μL 10 mg/mL bovine serum albumin in 50 mM Tris buffer) were combined and allowed to stand at 23 °C. The reaction was monitored by LC/MS by sampling at the 0.5, 1, 2, 4 and 7 h time points.