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Inhibitors of HIV-1 attachment. Part 9: An assessment of oral prodrug approaches to improve the plasma exposure of a tetrazole-containing derivative

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ABSTRACT

7-(2*H*-Tetrazol-5-yl)-1*H*-indole **3** was found to be a potent inhibitor of HIV-1 attachment but the compound lacked oral bioavailability in rats. The cause of the low exposure was believed to be poor absorption attributed to the acidic nature of the tetrazole moiety and, in an effort to address this liability, three more lipohilic tetrazole analogs, *N*-acetoxymethyl **4**, *N*-pivaloyloxymethyl **5**, and *N*-methyl **6**, were evaluated as potential oral prodrugs in rats. Prodrug **5** was ineffective in improving the plasma concentration of **3** in vivo but compound **4** provided a 15-fold enhancement of the plasma concentration of **3**. Most interestingly, oral dosing of analog **6** afforded a substantial increase in the plasma concentration of the parent in rats when compared to dosing of parent. This represents a novel example of a methyl tetrazole that acts as a prodrug for a free NH tetrazole-containing compound.

Highly active anti-retroviral therapy (HAART) has significantly reduced the incidence of mortality associated with human immunodeficiency virus-1 (HIV-1) infection/AIDS, and transformed the disease from an acute to a chronic one.[1] However, the emergence and persistence of drug-resistant HIV-1 mutant viruses, HAART-related toxicities, patient non-compliance and the appearance of co-morbidities associated with long-term therapy remain significant medical challenges.[2] Inhibitors of new viral targets thus continue to be evaluated for the treatment of HIV-1 infection in order to help overcome these major problems.[3]

A key first step during the HIV-1 entry into the host cells is the attachment of its envelope glycoprotein gp120 to the host cell receptor CD4. A subsequent conformational change in gp120 leads to the binding of this binary complex to a co-receptor, either the chemokine receptor CCR5 in the early stages of infection or CXCR4 in more advanced infection. A further conformational change in gp120 induced by co-receptor binding triggers activation of the transmembrane protein gp41, which inserts its hydrophobic N-terminal into the host cell membrane as a prelude to fusion between the viral and host cell membranes, resulting in the release of the viral genetic material into the host.[4] Indole- or azaindole-3-glyoxamide-derived small molecules that interfere with the attachment of gp120 to CD4 by binding to the CD4 binding site in gp120 were previously reported from these laboratories.[5–7] BMS-488043 (**1**, Fig. 1) was advanced into clinical studies and found to be safe and well tolerated in healthy human subjects. Proof-of-concept for this novel antiviral mechanism has been achieved with this compound in HIV-1 infected patients.[7e,8] After extensive optimization, this compound was supplanted in clinical development by a phosphonooxymethyl prodrug (BMS-663068) of an analog (BMS-626529) that showed an improved pre-clinical profile.[9,10]

During the optimization of the early lead compound **2**, an indole-containing HIV-1 attachment inhibitor, the C-7-tetrazole analog **3** (Fig. 2) was found to possess greatly enhanced potency toward a HIV-1 JRFL pseudotype virus in a single cycle infectivity assay. Further studies showed that **3** possessed nanomolar to sub-nanomolar potency against both M- and T-tropic virus strains in cell culture.[7h] Despite the fact that an NH tetrazole is a classical carboxylic acid isostere that usually results in compounds in which their tetrazolates are approximately 10 times more lipophilic than the corresponding carboxylates,[11] and certain tetrazole-containing drugs, for example the angiotensin II receptor antagonist, losartan, is well absorbed and orally bioavailable, **3** showed negligible oral exposure ($F = 2.3\%$) when administered orally to rats.[12] This was attributed to its low membrane permeability as a result of its ionized state at pH 6.5 (calculated pK_a of the tetrazole-NH of **3** = 4.2) and predicted by a 17 nm/sec Caco-2 permeability value measured at pH 6.5. As discussed in Part 8 of this series of reports,[7h] the oral bioavailability of the C7-heteroaryl substituted analogs of **2** appeared to correlate with membrane permeability, and a Caco-2 Pc of >100 ensured good bioavailability. Since **3** exhibited a

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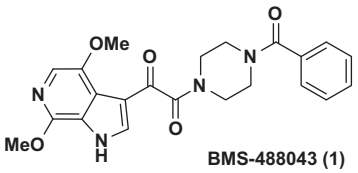


Figure 1. HIV-1 attachment inhibitor BMS-488043.

satisfactory terminal half-life (67 min) and a low total clearance (3.1 mL/min/kg) after IV dosing to rats, and given its superior in vitro antiviral profile compared to **2**, a prodrug approach designed to improve the oral exposure of **3** was considered as a means of establishing its potential to advance into further studies.

Although examples of prodrugs of carboxylic acids to improve absorption are abundant in the literature,[13] examples of prodrugs of tetrazoles are rare.[14] However, the precedent provided by the few literature examples of tetrazole prodrugs suggested that this could potentially be a viable approach. For example, a model tetrazole prodrug, 2-pivaloyloxymethyl-5-phenyltetrazole, underwent rapid hydrolysis to the parent compound in rat plasma.[15] Hydrolysis of a pivaloyloxymethyl-5-phenyltetrazole prodrug of an angiotensin II receptor antagonist to the parent was demonstrated in rat small intestinal homogenates, liver homogenates and plasma, with half-lives of 22.7, 9.80 and 3.94 min, respectively. These data were interpreted by the authors of the study to suggest that much of the intact prodrug had the potential to be absorbed through the gut wall and into the circulation without extensive hydrolysis, with facile hydrolysis of the prodrug by esterases in the liver and plasma. When the prodrug was actually dosed intravenously to rats, a time-dependent decrease in the AII-induced pressor response in the animals was observed.[16] In another published study, the 2-methyl-1-(2*H*-tetrazol-2-yl)propyl pivalate prodrug of the angiotensin II receptor antagonist BMS-183920 increased the oral bioavailability of the parent BMS-183920 in rats from 11% to 39% when dosed orally.[17–19]

Three potential prodrugs of the parent tetrazole **3**, *N*-acetoxymethyl derivative **4**, the *N*-pivaloyloxymethyl homolog **5** and the simple *N*-methyl tetrazole **6** were prepared, and their anti-viral potency and the extent of oral absorption and conversion of prodrug to parent in rats were evaluated. As shown in Table 1, compounds **4** and **6** exhibited a similar level of potency to the parent tetrazole **3** against the JRFL pseudotyped virus, while **5** was about sixfold less potent. The prodrugs retained the desirable low cytotoxicity exhibited by **3**. Both **4** and **5** were unstable in human liver microsomes (HLMs) in the absence of the CYP 450 enzyme co-factor, NADPH. Under these conditions, both compounds completely released the parent **3** over a 5 min interval in HLMs, thus indicating that the cleavage of the carboxymethyl group in **4** and **5** was not mediated by CYP 450 enzymes. The acetoxymethyl analog **4** was tested in the in vitro Caco-2 (pH 6.5) assay to assess its potential for absorption. Low recovery (<70%) of the compound was observed however, raising concern that **4** might be either chemically unstable in the assay medium or prematurely hydrolyzed in vivo by the esterases present in the enterocytes and gut lumen. When dosed orally to rats, the acetoxymethyl analog **4** nevertheless demonstrated a satisfying 15-fold increase in the concentration of parent tetrazole **3** in rat plasma at both 30 and 120 min post dosing when compared to levels of **3** obtained after dosing the parent compound (Table 1). However, the pivaloylxymethyl analog **5** was an ineffective prodrug and did not increase the exposure of parent tetrazole **3** in vivo. This compound likely possessed low intestinal solubility since a Caco-2 permeability value could not be determined for **5** because it was too insoluble in the medium used to conduct the Caco-2 assay. The rat plasma concentration of **3** increased threefold at the first 30 min after dosing with **5** but dropped to a concentration twofold less than that from dosing of **3** at the 120 min time point. The higher concentration at the earlier time point may indicate that the intact prodrug has improved absorption compared to the parent drug. The low solubility of the compound or the rapid conversion to parent prior to absorption could explain the observation of limited exposure at the later time point.

Interestingly, and somewhat unexpectedly, the plasma concentration of the parent tetrazole **3** obtained after dosing of the methyltetrazole **6** increased almost proportionally over time, and was 4- and 8-fold higher than after dosing with **3** at 30 and 120 min post-dosing, respectively. Methyltetrazole **6** was not metabolically stable in NADPH-supplemented HLMs, showing a half-life of 12 min, which compares to >200 min obtained for the parent tetrazole **3**. CYP 2B6-mediated *N*-demethylation of an *N*-methyl-5-phenyltetrazole-containing bradykinin B_1 receptor antagonist was previously found to be the major pathway of metabolism in human liver microsomes and hepatocytes.[20] CYP 450-mediated activation of prodrugs has been demonstrated as a viable prodrug release mechanism,[21] so it is plausible that the parent tetrazole **3** was released via CYP 450-mediated oxidative *N*-demethylation of **6** in the liver. Based on the plasma concentrations of **3** present at the two time points, it appears possible that the acetoxymethyl prodrug **4** underwent fast absorption while the methyltetrazole **6** was absorbed more slowly. However, the differences in the plasma exposure of **3** could have also resulted from differences in the rate of enzymatic conversion of **4** and **6** to the parent in vivo.

In conclusion, three tetrazole analogs, *N*-acetoxymethyl **4**, *N*-pivaloyloxymethyl **5** and *N*-methyl **6**, were evaluated as prodrugs of the potent HIV attachment inhibitor **3** in an effort to improve

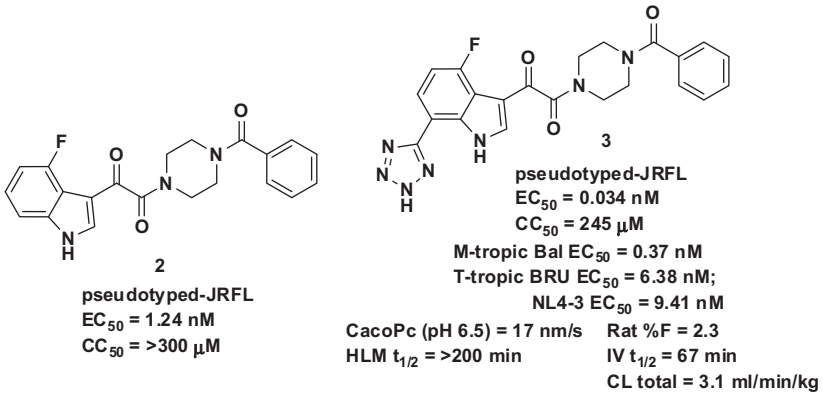
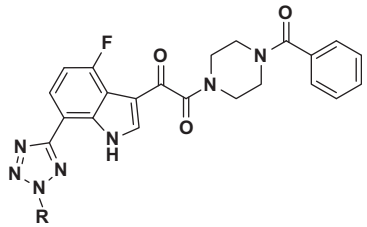


Figure 2. HIV-1 attachment inhibitor lead compound **2** and the C7-tetrazole analog **3**.

Table 1
Antiviral activity, cytotoxicity and rat oral plasma exposure of **3** and its prodrugs **4**, **5** and **6**

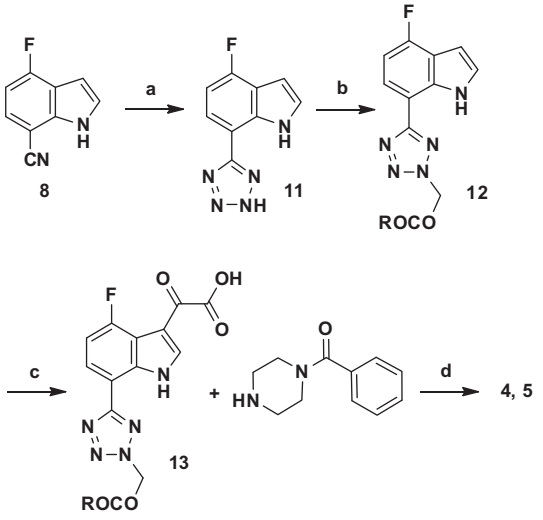


Inhibitor	**R**	EC_{50}[22] (nM)	CC_{50}[22] (μM)	Plasma [**3**] @30/120 min (ng/ml)[a]
3	H	34	245	19/25
4	CH_2OCOMe	60	36	261/378
5	CH_2OCOt-Bu	190	228	60/13
6	Me	20	>300	72/207

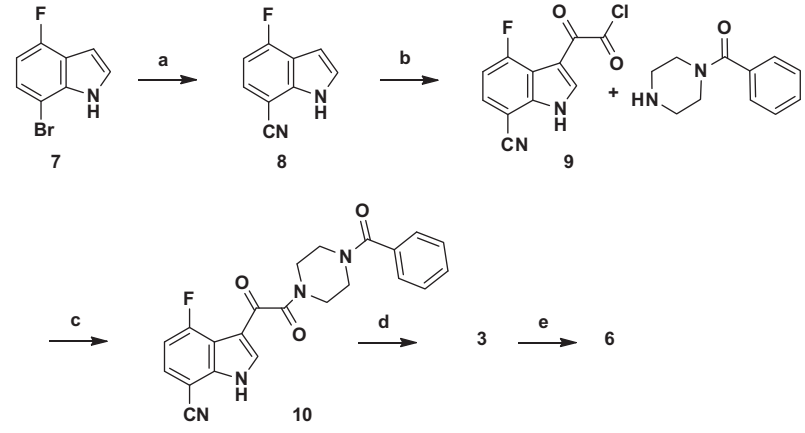
[a] 5 mg/kg P.O.; dosing vehicle 90% PEG 400/10% EtOH; each data was the average of those obtained from two rats.

upon the parent compound's poor oral bioavailability in rats. After oral dosing to rats, compound **5** had negligible effect on the plasma concentration of **3** compared to dosing the parent compound; however, analog **4** provided a 15-fold enhancement in exposure. Surprisingly, *N*-methyltetrazole **6** also conferred a substantial increase in the plasma concentration of the parent in rats over the time course of the study. Thus analog **6** represents a novel example of prodrug methodology for tetrazole-containing compounds. These studies exemplify the feasibility of prodrug approaches to improve the oral exposure of NH tetrazoles either through a hydrolytic or oxidative cleavage mechanism. These tactics, previously examined in only very limited examples and seldomly explored to date, are of potential application to discovery programs where such compounds exhibit insufficient oral exposure due to poor absorption.

The syntheses of **3** and analogs **4** to **6** are shown in Schemes 1 and 2. The 7-cyano-4-fluoroindole **8** was obtained from the cyanation of 7-bromo-4-fluoroindole **7** using copper cyanide. Friedel–Crafts acylation of **8** using oxalyl chloride provided acid chloride **9**, which was coupled with *N*-benzoylpiperazine to form the intermediate **10**. Reaction of the cyano group of **10** with sodium azide provided the parent tretrazole **3**, which was methylated using trimethylsilyldiazomethane to give the *N*-methyltetrazole **6**. The preparation of **4** and **5** relied on an alkylcarboxymethylation of the tetrazole **11** with the corresponding halomethyl reagents. After Friedel–Crafts acylation of **12**, it was more convenient to hydrolyze the intermediate acid chloride to the acid **13** since partial hydrolysis to the acid occurred during the coupling of the acid chloride with *N*-benzoylpiperazine. The acid **13** was then coupled to *N*-benzoylpiperazine using carbodiimide to furnish the final products **4** and **5**.



Scheme 2. Synthesis of compounds **4** and **5**. Reagents and conditions: (a) NaN_3, NH_4Cl, DMF, 100 °C; 17 h; (b) $BrCH_2OCOMe$ (for **4**), or $ClCH_2OCOt$-Bu (for **5**), K_2CO_3, MeCN, rt, 22 h; (c) (i) $(ClCO)_2$, CH_2Cl_2, rt, 21 h; (ii) *i*-Pr_2EtN, THF, rt, 19 h; (d) EDC, DMAP, DMF, rt, 20 r.[23]



Scheme 1. Synthesis of compounds **3** and **6**. Reagents and conditions: (a) CuCN, DMF, 145 °C, 17 h; (b) $(ClCO)_2$, CH_2Cl_2, reflux, 3 days; (c) *i*-Pr_2EtN, THF, rt, 16 h, (d) NaN_3, NH_4Cl, DMF, 85 °C; 12 h; (e) $TMSCHN_2$, MeOH/PhH, rt, 90 min.[23]

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