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Design, synthesis and in vitro/in vivo evaluation of orally bioavailable prodrugs of a catechol-*O*-methyltransferase inhibitor

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ABSTRACT

Compound **1** is an investigational, nanomolar inhibitor of catechol-*O*-methyltransferase (COMT) that suffers from poor oral bioavailability, most probably due to its low lipophilicity throughout most of the gastrointestinal tract and, to a lesser extent, its rapid systemic clearance. Several lipophilic esters were designed as prodrugs and synthesized in an attempt to optimize presystemic drug absorption. A modest twofold increase in 6-h exposure of **1** was observed with two prodrugs, compared to that of **1**, after oral treatment in rats.

Parkinson's disease (PD) is a slowly progressive, neurodegenerative disorder which is characterized by the destruction of dopaminergic cells in the pars compacta region of the substantia nigra in the mid brain region, leading to a deficiency of dopamine.[1] Current PD treatment involves oral administration of levodopa, a precursor of dopamine, combined with an inhibitor of DOPA decarboxylase (DDC), such as carbidopa, to inhibit decarboxylation of levodopa in the periphery.[2] During such treatment, catechol-*O*-methyltransferase (COMT) becomes the main enzyme to metabolize levodopa.[3] Compound **1** is an investigational, very potent, nanomolar inhibitor of COMT (Fig. 1). However, **1** displays less than ideal pharmacokinetic properties, such as oral absorption, similar to another structurally related COMT inhibitor, entacapone.[4] The main barrier to achieving high bioavailability for entacapone has been suggested to be due to its high systemic clearance.[4] Extensive ionization associated with the poor lipophilicity (log *D* values) of entacapone at intestinal pH and, to a lesser extent, its high systemic clearance, are the main factors contributing to the poor bioavailability of **1** based in preliminary studies.

Prodrugs have been utilized to overcome several drug delivery problems by introducing lipophilicity and masking hydrogen bonding groups.[5,6] In the present study, several prodrug esters were studied in an attempt to improve the oral absorption of **1**. The rational for embarking on a prodrug approach to address the poor bioavailability of **1** is based on increasing its lipophilicity, and also saturation of systemic clearance by optimizing presystemic drug absorption. The prodrugs designed, synthesized and evaluated in this letter are alkyl esters (**2–6**), acyloxyalkyl esters (**7–10**), aminoacyloxy alkyl esters (**11**) and cyclic carbonate esters (**12**).

Synthetic pathways to prodrugs are shown in the Scheme 1. The alkyl esters **2–5** were prepared by heating **1** in the corresponding alcohol in the presence of thionyl chloride. All other prodrugs were synthesized by starting from the acetate-protected compound **1b** to avoid alkylation to the catechol. The acetate proved to be the most convenient protecting group for the catechol and was easily hydrolyzed in most cases in a ACN–water solution by adding a few drops of 25% aqueous ammonia, with only low to moderate losses of the desired promoiety. Prodrugs **6** and **12** were synthesized by reacting **1b** with benzyl bromide and 4-(bromomethyl)-5-methyl-1,3-dioxol-2-one,[7] respectively, using potassium carbonate as a base. Acyloxyalkyl chlorides used in the preparation of **7–11** were synthesized as previously described[8,9], and reacted with compound **1b** in presence of sodium iodide and cesium carbonate. The overall reaction yields were low to moderate, partly due to tailing of the target compounds during the flash chromatography, which allowed the collection of only a narrow window of pure fractions.

Chemical and enzymatic stabilities of selected prodrugs were assessed in aqueous buffers, fasted state simulated intestinal fluid (FaSSIF), human serum and rat liver homogenate. As seen in Table 1, the prodrugs showed varying levels of stability to hydrolysis relative to the structure of promoiety. Esterification of an acidic functional group with a simple alkyl or aryl alcohol is the most commonly used approach in successful prodrugs when increasing

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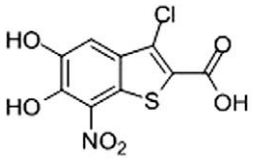


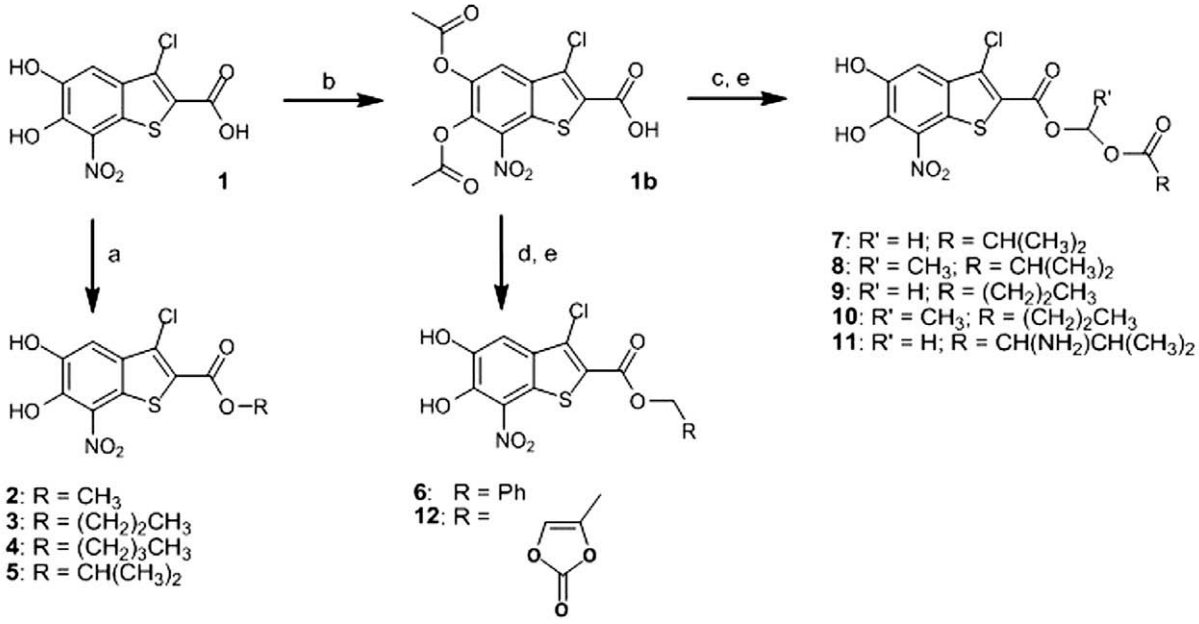
pK_a = 2.57, 4.14, 10.09
log D = 3.40 (pH 1.0), 0.04 (pH 5.0), -4.71 (pH 7.4)

Figure 1. Structure and selected properties of **1**.

lipophilicity is the desired objective; for example, angiotensin-converting enzyme (ACE) inhibitors.[10]

In the present set of prodrugs, however, the simple alkyl (**2–5**) and aryl (**6**) esters were not susceptible to esterase activity in human serum. Although bioconversion of **2–6** took place in rat liver homogenate, double esters **7–10** were also prepared to increase the recognition by esterases through the second ester. Previous examples of such double prodrugs include orally active acyloxyalkyl esters of β-lactam antibiotics and nucleoside monophosphate anti-viral agents.[11–14] The isopropyloxycarbonylmethyl (**7**) and propyloxycarbonylmethyl (**9**) analogues were synthesized along with their homologated derivatives with a 1-ethyl group (**8** and **10**). Upon hydrolysis by esterases, prodrugs **8** and **10** released acetaldehyde instead of formaldehyde, which was released by the prodrugs **7** and **9**. Although the amount of formaldehyde generated from prodrugs would be small, formaldehyde is a probable human carcinogen.[15]

As expected, the acyloxyalkyl esters **7–10** were susceptible to enzymatic hydrolysis in human serum and hydrolyzed quantitatively to **1** with half-lives ranging from 5.1 to 32.3 h. The



Scheme 1. Synthesis routes of compounds **1b–12**. Reagents: (a) thionyl chloride, R–OH, reflux; (b) acetic anhydride, pyridine; (c) Cs_2CO_3, acyloxyalkyl chloride, NaI, ACN or DMF; (d) K_2CO_3, alkyl bromide, acetone; (e) ACN–water, 25% ammonia.

Table 1
Physicochemical, pharmacokinetic and pharmacologic properties for prodrugs of **1**

Compound	COMT inhibition (K_i) (nM)	Uncoupling (μM)	$T_{1/2}$					AUC, fold increase[e]
			pH 4.0 (days)	pH 7.4 (h)	FaSSIF (h)	Human serum (h)	Rat liver homogenate (min)	
1	<1	>50						
2	2.2	>10	Stable[a]	Stable[a]	—[d]	—[b]	19.8	
3	9.2	>5	31	Stable[a]	—[d]	—[b]	20.4	—[d]
4	1.3	>2.5	—[c]	Stable[a]	—[d]	—[b]	41.9	—[d]
5	1.1	>1	23	Stable[a]	—[d]	—[b]	130.9	—[d]
6	10.2	>10	—[c]	21.6	—[d]	—[b]	51.9	—[d]
7	17.0	>50	8.4	7.6	—[b]	5.1	⩽1	2.0
8	17.0	>50	—[c]	48	—[b]	32.3	⩽1	0.5
9	10.6	>50	—[c]	2.5	—[d]	5.3	⩽1	—[d]
10	16.6	>50	—[c]	4.5	—[d]	25.0	⩽1	—[d]
11	6.0	25	1.7	2.0	31.7	0.06	⩽1	0.4
12	1.6	—[d]	3.9	11.7	167	0.03	⩽1	2.1

[a] No degradation was detected during one week.
[b] No degradation was detected within 8 h.
[c] Not determined due to poor aqueous solubility.
[d] Not determined.
[e] Adult male Wistar rats were orally administered the test compounds at 10 μmol/kg, and area under the curve (AUC) was determined over a 6-h interval relative to the parent compound, **1**.

acyloxyethyl prodrugs **8** and **10** had 5–6-fold longer half-lives compared to their acyloxymethyl analogues **7** and **9**. These results are in good agreement with a previously published study of oxymethyl- and oxyethylphosphates, in which the latter prodrug showed a twofold slower bioconversion rate.[16] In rat liver homogenate **7–10** underwent rapid bioconversion to **1** with half-lives less than 1 min.

Amino acid prodrugs have previously been reported to be substrates for the dipeptide intestinal transporter.[17,18] The aminoacyloxyalkyl prodrug **11**, which contains the amino acid valine, was also prepared and evaluated. The cyclic carbonate prodrug **12** was designed to be labile only in plasma, while avoiding undesired metabolism by esterases in the enterocytes during absorption. The clinically novel prodrugs olmesartan medoximil[19] and lenampicillin[20] are examples of such cyclic carbonate prodrugs. Both **11** and **12** hydrolyzed rapidly to **1** in both human serum and rat liver homogenate, with the latter most probably metabolized by the plasma enzyme called paraoxonase.[21,22]

Both **1** and prodrugs **2–12** were subjected to COMT inhibition and uncoupling assays (Table 1).[23,24] Although **2–12** were designed to function as prodrugs; that is, to be inactive or less active derivatives of **1**, they all displayed nanomolar inhibition of COMT, indicating that these prodrugs are also COMT inhibitors by themselves. Moreover, based on its catechol structure, **1** is a potential mitochondrial uncoupler of oxidative phosphorylation, which may be linked to its mechanism of hepatotoxicity, due to impairment of energy production, which was a serious drawback seen with another COMT inhibitor, tolcapone.[25,26] While simple alkyl (**2–5**) and aryl (**6**) esters and the aminoacyloxyalkyl ester (**11**) were shown to be in vitro uncouplers at low micromolar concentrations, high uncoupling efficacy was not seen either with **1** or acyloxyalkyl ester prodrugs **7–10**.

Before testing compounds **1–12** in rats, their apparent partition coefficients (log *D*) were determined along with aqueous solubilities in various buffer solutions. The aqueous solubility of **1** was significantly higher at pH values of 1.2, 4 and 7.4 (3.9, 53.0 and 15,000 μg/ml, respectively) than those of any prodrugs **2–12**, except for the ionized valinate ester **11** at pH 1.2 (Supplementary data). As predicted, masking of the hydrophilic carboxylic acid group by the addition of various lipophilic promoieties resulted in prodrugs having log *D* values that were generally higher than those of **1** within the pH range of 1.2–7.4. The log *D* values of these derivatives typically varied between 1 and 3, which is in the optimal range for good oral absorption by passive diffusion.

Based on solubility and bioconversion studies, **1**, **7**, **10–12** were orally administered to male Wistar rats at 10 μmol/kg. Because all administered prodrugs showed reasonable good stability in FaSSIF their conversion to **1** at the absorption should be negligible. Plasma concentrations of prodrugs were undetectable during the 6-h exposure, which is consistent with prodrug bioconversion by ubiquitous esterases (**7**, **8** and **11**) or the plasma enzyme paraoxonase (**12**) after or during absorption. However, only prodrugs **7** and **12** presented a very little benefit for the delivery of **1**, and that being only a twofold increase in plasma levels of **1**. For example, in contrast to previous experience with nucleoside-amino ester prodrugs[17,18], the valinate prodrug (**11**) did not appear to be a substrate for active transport, and **11** did not enhance oral bioavailability. Therefore, our attempts to improve drug absorption and address the poor bioavailability of **1** resulted in only modest success.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.02.057.

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