Discovery of the Hemifumarate and (α-L-Alanyloxy)methyl Ether as Prodrugs of an Antirheumatic Oxindole: Prodrugs for the Enolic OH Group

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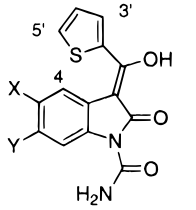
Ether, ester, and carbonate derivatives of the antirheumatic oxindole **1** were prepared and screened as potential prodrugs of **1**. This effort led to the discovery of the (α-L-alanyloxy)-methyl ether and hemifumarate derivatives of **1** which deliver the drug efficiently into the circulation of test animals, are stable in the solid state, and possess good stability in solution at low pH as required to ensure gastric stability. Success in achieving acceptable bioavailabilties of **1** across species (rats, dogs, and monkeys) followed the inclusion of ionizable functionality within the promoiety to compensate for masking the polar enolic OH group of the free drug. However, the introduction of ionizable functionality was often associated with decreased stability, as demonstrated by the hemisuccinate, hemiadipate, hemisuberate, and α-amino ester derivatives of **1** which could not be isolated. A clear exception was the hemifumarate derivative of **1** which was not only isolable but actually more stable at neutral pH than the nonionizable ester analogues. The solution and solid state stability of the hemifumarate, together with its activity as a prodrug of **1**, suggests that hemifumarate be considered as an alternative to hemisuccinate as a prodrug derivative for alcohols, particularly in situations where solution state stability is an issue.

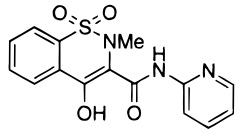
Introduction

Antirheumatic oxindoles constitute a new class of drugs for the treatment of arthritis.[1] The lead example, tenidap, has demonstrated excellent activity in rheumatoid arthritis and osteoarthritis clinical trials[2] and is now awaiting regulatory approval for the treatment of these diseases. Although being a potent inhibitor of cyclooxygenase (CO),[3] tenidap can be clearly distinguished from classical nonsteroidal anti-inflammatory agents (NSAIDs) by its cytokine-modulating properties.[4]

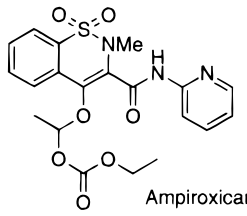
Like the NSAID piroxicam, tenidap and other antirheumatic oxindoles undergoing evaluation possess an acidic enolic OH group which is essential for CO inhibitory activity.[5] In the case of piroxicam, the enolic OH group has been exploited as a "handle" for the preparation of piroxicam derivatives which are inactive against CO but converted to piroxicam during or after absorption, thus minimizing the potential for gastrointestinal irritation due to topical CO inhibition and prostaglandin depletion by the free drug.[6−9] Several of these piroxicam prodrugs are now marketed including ampiroxicam, an ether carbonate derivative of piroxicam discovered in these laboratories.[6,7] Here we report on our parallel efforts to discover prodrugs of the antirheumatic oxindole **1**, a close analogue of tenidap with a similar pharmacologic profile. As in the case of piroxicam, the strategy was to prepare derivatives with a masked enolic OH group to prevent direct exposure of the stomach to the free drug. These derivatives would be stable under gastric conditions but sufficiently labile toward hydrolysis to allow release of **1**, preferably during or immediately after absorption from the gastrointestinal track.[10]



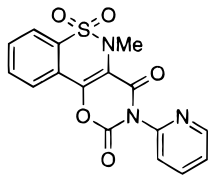
Tenidap: X = Cl, Y = H
1: X = F, Y = Cl



Piroxicam



Ampiroxicam



Droxicam

Particular structural and physicochemical properties of **1** were of special concern in considering the preparation of prodrugs of this agent. Compound **1** itself is poorly soluble in water (40 μg/mL at pH 6.2); masking of the enolic OH group was likely to produce compounds with even lower aqueous solubility unless the promoiety itself possessed solubilizing functionality. Compound **1** is also quite acidic ($pK_a = 3.5$), and consequently the enolate of **1** is a better leaving group than that of piroxicam ($pK_a = 6.5$). Thus, there was concern that carbonyl-linked derivatives (e.g., esters and carbonates) would be too readily hydrolyzed and that the enolate of **1** would lack sufficient reactivity for derivatization under some circumstances. Since **1** is known to exist predominantly as a mixture of *E* and *Z* enol tautomers, another chemical issue was the likelihood that mixtures of geometric isomers would be formed upon acylation or alkylation of the exocyclic enol group.

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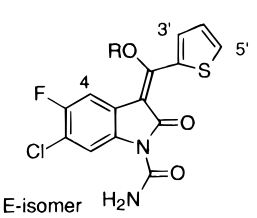
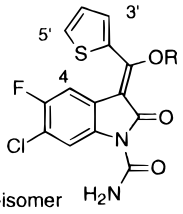
Table 1



E-isomer



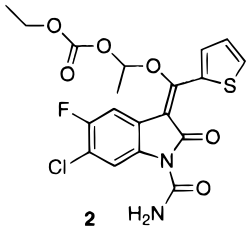
Z-isomer

no.	R	*E*,[a] %	formula[b]	mp, °C	recryst solv	aq solubility, μg/mL (pH)	Rb rat	Rb dog	Rb mon
1	H		$C_{14}H_8ClFN_2O_3S$	220−221		40 (6.2)	100	100	100
2	CH(Me)OCO_2Et[c]	>95	$C_{19}H_{16}ClFN_2O_6S$	176−179	EtOAc	0.009 (6.2)	26	1.4	
3	CH(Me)OCO_2*i*-Pr[c]	>95	$C_{20}H_{18}ClFN_2O_6S$	183−184	Et_2O		12		
4	CH(Me)OCOMe[c]	94	$C_{18}H_{14}ClFN_2O_5S\cdot H_2O$	209	MeCN	0.04 (4.8)	2.3		
5	CH_2OCOt-Bu	>95	$C_{20}H_{18}ClFN_2O_5S$[d]	199−200	EtOAc		0		
6	CH_2OCOPh	>95	$C_{22}H_{14}ClFN_2O_5S$	202−203	EtOAc		0		
7	CH(Me)OCOCH_2OMe[c]	>95	$C_{19}H_{16}ClFN_2O_6S$	175−176	MeCN	0.34 (6.7)	47	5.2	
8	CH_2OCOCH_2OMe	83[e]	$C_{18}H_{14}ClFN_2O_6S$	144−147	hexane/EtOAc		62		
9	CH_2OCOCH_2OCH_2Ph	>95	$C_{24}H_{18}ClFN_2O_6S$	130−131	hexane/EtOAc		42		
10	CH_2OCOCH_2OAc	>95	$C_{19}H_{14}ClFN_2O_7S$	176−177	MeCN	<0.02[g]	17		
11	CH_2OCOCH_2OCONEt_2	>95	$C_{22}H_{21}ClFN_3O_7S$	157−158	Et_2O		36		
12	CH_2OCOCH(Me)OH[c]	>95	$C_{18}H_{14}ClFN_2O_6S$	184−187	hexane/EtOAc	0.38 (6.5)	90		22
13	CH_2OCOCH(Me)OMe[c]	92	$C_{19}H_{16}ClFN_2O_6S$	149−151	hexane/EtOAc		45		
14	CH_2OCOCH(Et)OMe[c]	>95	$C_{20}H_{18}ClFN_2O_6S$	134−135	hexane/EtOAc		93		
15	CH_2OCOCH(Et)OCH_2Ph[c]	>95	$C_{26}H_{22}ClFN_2O_6S$	111−113	hexane/EtOAc		86		12
16	CH_2OCOCH(*n*-Pr)OCH_2Ph[c]	>95	$C_{27}H_{24}ClFN_2O_6S$	121−123	cyclohexane/EtOAc		58		7.5
17	$CH_2OCH_2NH_2\cdot$TsOH	95	$C_{24}H_{21}ClFN_3O_8S_2$	210−211	EtOH/EtOAc		46		78
18	CH_2OCH(Me)$NH_2\cdot$TsOH[f]	>95	$C_{25}H_{23}ClFN_3O_8S_2$	210−214	MeOH/EtOAc	150 (6.5)	80	78	78
19	CH_2OCH(*i*-Pr)$NH_2\cdot$TsOH[f]	>95	$C_{27}H_{27}ClFN_3O_8S_2$	168−173	EtOH/EtOAc		27		
20	CH_2OCH(*i*-Bu)$NH_2\cdot$TsOH[f]	>95	$C_{28}H_{29}ClFN_3O_8S_2$	210−211	EtOH/EtOAc		81		
21	CH_2OCH[$(CH_2)_4NH_2$]$NH_2\cdot$ 2HCl$\cdot H_2O$[f]	88	$C_{21}H_{26}Cl_3FN_4O_6S$	135	EtOH/EtOAc		49		
22	$CH_2OCH_2NHCO_2$*t*-Bu	83	$C_{22}H_{21}ClFN_3O_7S$	171−174	hexane/EtOAc	0.06[g]	0		
23	CH_2OCH(Me)$NHCO_2$*t*-Bu[f]	>95	$C_{23}H_{23}ClFN_3O_7S$	165−170	hexane/EtOAc		20		

[a] Determined by ^{1}H NMR. [b] Except where indicated, all compounds analyzed within ±0.4% for C, H, and N. [c] Racemic. [d] Anal. Calcd: C, 53.04; H, 4.01; N, 6.19. Found: C, 52.73; H, 3.53; N, 6.10. [e] The other 17% is C-alkylated material. [f] *S*-Configuration. [g] Unbuffered distilled water; final pH not determined.

Chemistry

The ether derivatives **2**−**16**, **22**, and **23** (Table 1) were prepared in low to moderate yields by alkylation of a salt of **1** with a 2−3-fold excess of an α-haloalkyl ester or carbonate: compound **2** was obtained by the reaction



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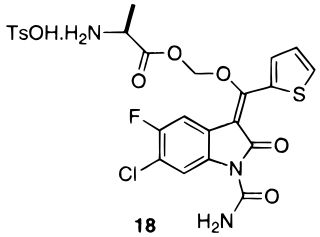
of the triethylammonium salt with 1-chloroethyl ethyl carbonate in chloroform in the presence of silver nitrate; compounds **3**, **4**, and **7** were obtained by reaction of the corresponding chloroethyl carbonate or ester with the sodium salt in acetone in the presence of sodium iodide;[7] the (acyloxy)methyl ethers **5**, **6**, **8**−**16**, **22**, and **23** were prepared by treatment of the sodium salt with the corresponding iodomethyl ester in refluxing acetone. In nearly all cases, chromatography and recrystallization were required for isolation of the desired product which often included separation of the unwanted product arising from C-alkylation of the enolate of **1**.

The [(aminoacyl)oxy]methyl ethers **17**−**21** were prepared by deprotection of the corresponding *N*-*tert*-butoxycarbonyl (*N*-*t*-BOC) amino acid derivatives (e.g., **22** and **23**). This was accomplished by treatment of the latter with neat TFA and 1 equiv of *p*-toluenesulfonic acid or with HCl in dioxane. The method chosen was based on pilot experiments to determine whether the tosylate or hydrochloride salt of the final product was crystalline. The *N*-*t*-BOC precursors to compounds **19**−**21** were prepared as described for compounds **22** and **23**.

In all cases, the ethers **2**−**23** were predominantly to exclusively the *E* isomers. This was established by single-crystal X-ray structure analysis of compounds **12** and **18** and correlation of the ^{1}H NMR spectra with those of the other ether derivatives. In each case, the chemical shifts and coupling constants of the aromatic protons of the major (or exclusive) isomer closely matched those of **12** and **18**. The possibility that the *E* and *Z* isomers could produce closely similar spectra was discounted by the ^{1}H NMR spectra of the ester and carbonate derivatives (**24**−**45**), where it was clear that the chemical shifts of the aromatic protons in the *E* and *Z* isomers were significantly and consistently different (vide infra).



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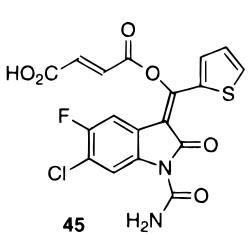

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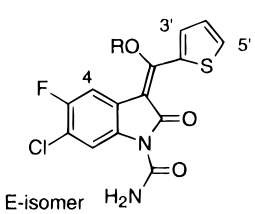
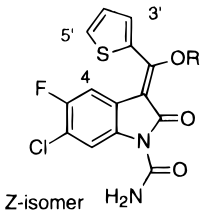
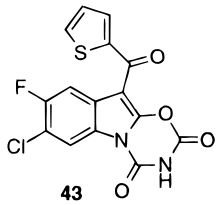
Table 2







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no.	R	*E*,[a] %	formula[b]	mp, °C	recryst solv	aq solubility, μg/mL (pH)	Rb rat	Rb dog	Rb mon
1	H		$C_{14}H_8ClFN_2O_3S$	220−221		40 (6.2)	100	100	100
24	COMe	40	$C_{16}H_{10}ClFN_2O_4S$	200−203	acetone/*i*-PrOH	0.011 (6.2)	14		
25	COEt	5	$C_{17}H_{12}ClFN_2O_4S$	182−188	hexane/EtOAc	0.003 (6.2)	6.6		
26	CO*n*-Bu	8	$C_{19}H_{16}ClFN_2O_4S$	181−183	hexane/EtOAc	0.013 (6.2)	17		
27	CO*t*-Bu	7	$C_{19}H_{16}ClFN_2O_4S$	196−197	hexane/EtOAc		3.7		
28	COPh	20	$C_{21}H_{12}ClFN_2O_4S$	216−222	*i*-PrOH		0		
29	COCH=CHPh (*E*)	90	$C_{24}H_{16}ClFN_2O_4S$	214−215	acetone/*i*-PrOH		4		
30	CO_2Me	40	$C_{16}H_{10}ClFN_2O_5S$	203−208	*i*-PrOH		13		
31	CO_2Et	15	$C_{17}H_{12}ClFN_2O_5S$	164−166	*i*-PrOH	0.023 (6.2)	3.5		
32	CO_2i-Pr	>95	$C_{18}H_{14}ClFN_2O_5S$	197−199	*i*-PrOH		0.6		
33	CO_2i-Pr	24	$C_{18}H_{14}ClFN_2O_5S$	175−178	*i*-PrOH		9.9		
34	CO_2n-C_8H_{17}	>95	$C_{23}H_{24}ClFN_2O_5S$	142−145	*i*-PrOH		5.7		
35	COCH=CHCO$_2$Et (*E*)	19	$C_{20}H_{14}ClFN_2O_6S$	165−168	*i*-PrOH	<0.02 (6.7)	21	6.4	
36	$CO(CH_2)_2CO_2Et$	>95	$C_{20}H_{16}ClFN_2O_6S$	182−183	MeCN		3.5		
37	CO-2-pyridyl	*c*	$C_{20}H_{11}ClFN_3O_4S$	184−185	*i*-PrOH	<0.06 (2), <0.06 (7.5)	1.6		
38	CO-3-pyridyl	*c*	$C_{20}H_{11}ClFN_3O_4S$	201−203	*i*-PrOH	<0.06 (2), <0.06 (7.5)	0		
39	CO-4-pyridyl	*c*	$C_{20}H_{11}ClFN_3O_4S$	219−220	*i*-PrOH	0.32 (2), <0.07 (7.5)	2.2		
40	$COCH_2OMe$	28	$C_{17}H_{12}ClFN_2O_5S$	199−200	MeCN		8.9		
41	$COCH_2OAc$	8	$C_{18}H_{12}ClFN_2O_6S$	188−189	MeCN		6.6		
42	$CO(CH_2)_2CO_2CH_2CONEt_2$	>95	$C_{24}H_{23}ClFN_3O_7S$	165−167	toluene		16		
43			$C_{15}H_6ClFN_2O_4S$	>260	MeCN	17[d]	25		
44	CO-3-$C_6H_4CO_2H$	>95	$C_{22}H_{12}ClFN_2O_6S$[e]	244−248	AcOH	0.58[d]	4.2		
45	COCH=CHCO$_2$H (*E*)	99	$C_{18}H_{10}ClFN_2O_6S$	229−232	EtOAc[f]	0.08 (2), 2.5 (6.5), 24 (7)	95	91	73

[a] Determined by ^{1}H NMR except compound **45** for which HPLC was used. [b] Except where indicated, all compounds analyzed within ±0.4% for C, H, and N. [c] Stereochemistry not assigned. **37**: ratio of isomers, 95:5. **38**: ratio of isomers, 90:10. **39**: ratio of isomers, 88:12. [d] Unbuffered distilled water; final pH not determined. [e] Anal. Calcd: C, 54.27; H, 2.48; N, 5.75. Found: C, 53.80; H, 2.18; N, 5.71. [f] Trituration.

The ester and carbonate derivatives of **1** (Table 2) were prepared by the reaction of **1** with acid chlorides or chloroformates in the presence of a base, Et_3N, or *i*-Pr_2NEt. These conditions invariably gave rise to mixtures of geometric isomers. The products were typically isolated and tested as mixtures, although, in certain instances, isolation of the *E* isomer was achieved by fractional crystallization. Assignments of olefin geometry in the ester and carbonate series are based on ^{1}H NMR studies with the octyl carbonate isomers, where a significant NOE (16%) between the oxindole C4 and thiophene C3′ hydrogens was detected with the *Z* isomer and not the *E* isomer (**34**). Stereochemical assignments for other carbonates and esters are based on comparisons of the ^{1}H NMR spectra to those of the octyl carbonate isomers which allowed characteristic patterns to be discerned. For the *E* isomer series in $CDCl_3$, the thiophene hydrogens are typically well separated by chemical shift; of these, the C3′ hydrogen appears furthest downfield. In the *Z* isomers series, the thiophene C3′ hydrogen is typically shifted 0.4 ppm upfield, often overlapping with the signal due to the C5′ hydrogen. This is typically accompanied by a 0.2 ppm upfield shift of the oxindole C4 proton. Both shifts are ascribed to mutually interacting anisotropies of the oxindole and thiophene aromatic rings.

The hemiisophthalate and hemifumarate derivatives **44** and **45** were prepared by selective cleavage of the corresponding mixed 2,2,2-trichloroethyl and 2-(trimethylsilyl)ethyl esters using Zn/AcOH and HF/pyridine, respectively.[11] In the latter case, a fortuitous olefin isomerization took place under the conditions for removal of the protecting group. Thus, while the *E*/*Z* ratio of the 2-(trimethylsilyl)ethyl ester was about 2:1, that of the product (**45**) was about 99:1. Attempts to prepare the corresponding hemisuccinate, hemiadipate, and hemisuberate derivatives failed during cleavage of the corresponding mixed 2-(trimethylsilyl)ethyl esters. In these cases, quantitative regeneration of **1** took place upon treament with HF/pyridine. For the hemisuccinate especially, this was most likely due to intramolecular displacement of **1** (pK_a = 3.5) by the free ω-carboxy group.[12]

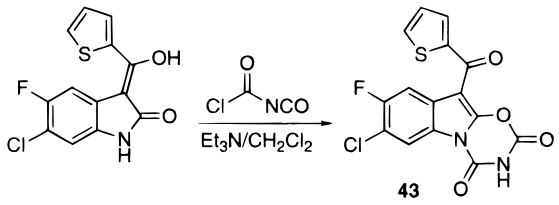
The relatively high stability of the enolate of **1** undoubtedly played a role in the failure to isolate other ester derivatives: α-amino acid esters after treatment of **1** with various activated α-amino acid derivatives (recovered **1**) and 4-(aminomethyl)benzoyl esters[13] by treatment of the 4-(chloromethyl)benzoate derivative with ammonia or alkylamines (ester aminolysis and regeneration of **1**).

The cyclic carbamate derivative **43** was prepared as shown in Scheme 1.[14]

Results and Discussion

The prodrug activity of all compounds was first assessed in the rat. Compounds were administered at a dose equivalent to 3 mg of **1**/kg by oral gavage in 0.1% methyl cellulose. Relative bioavailabilities were determined by measurement of plasma AUCs of **1** and comparison with plasma AUCs of **1** achieved following

Scheme 1



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oral dosing of the free drug. Select compounds were advanced to dogs and/or monkeys for determination of relative bioavailabilities in these species. The relative bioavailability (Rb) data are included in Table 1 for ether derivatives and in Table 2 for esters and carbonates.

Ether-linked derivatives of **1** were prepared to avoid the potential for hydrolytic instability anticipated for the carbonyl-linked compounds (esters and carbonates) due to the good leaving group ability of the enolate of **1**. Based on its structural relationship to ampiroxicam, the ether carbonate **2** was of particular interest as a potential prodrug of **1**. However, this compound and the other nonpolar ether-linked derivatives **3**−**6** were only weakly active as prodrugs of **1** in the rat (Table 1). Compound **2** did have the highest activity among these nonpolar ether derivatives (27%) and was therefore advanced into dogs; however, in this species the bioavailability of **1** was even lower (1.4%). These results were attributed to a lack of absorption since in no instance were circulating levels of these potential prodrugs detected in the plasma of test animals and since, as expected, masking the enolic OH group led to compounds having very low aqueous solubility (e.g., **2**: 0.009 μg/mL at pH 6.2). Stability measurements on the ester carbonate **2** were encouraging, however, being within acceptable limits: In solution (1:2:1 H_2O/MeCN/MeOH) times for 10% decomposition ($t_{10\%}$) of 3.6 and 28 h were determined at pH 2 and 7.4, respectively, and in the solid state, 93% of **2** remained unchanged after 4 weeks at 50°C under 75% relative humidity.

Also promising was the moderate to excellent bioavailability of **1** achieved in the rat for the more polar ethers (**7**−**16**) derived from **1** and the chloromethyl esters of various glycolic, lactic, 2-hydroxybutyric, and 2-hydroxyvaleric acid derivatives. Unfortunately, the four compounds among these selected for testing in dogs or monkeys failed to deliver **1** with even moderate bioavailability in these higher species. What appeared to be a lack of correlation between the improved rat bioavailability of **1** and aqueous solubility (Table 1) suggested a variable other than solubility to be determinant of activity in the rat. This was not necessarily true for the other species, and we thus hypothesized that although significant increases in solubility had been achieved in some cases (e.g., **12**: 0.38 μg/mL), the aqueous solubilities were still too low on an absolute scale to allow good absorption in dogs and monkeys.

The [(aminoacyl)oxy]methyl ether derivatives **17**−**21** were prepared in the hope that the ionizable amino functionality would further increase aqueous solubility.[15] This appears to have been the case based on the >300-fold increase in solubility of the (α-L-alanyloxy)-methyl ether tosylate **18** (150 μg/mL) over the lactate ether **12**. Like the earlier series (**7**−**16**) derived from α-hydroxy acids, the rat bioavailabilities of **1** for the amino acid ethers ranged from moderate (27% for the (α-L-valinyloxy)methyl ether tosylate **19**) to excellent (81% for the (α-L-leucinyloxy)methyl ether tosylate **20**). Fortunately, in this series, the goal of achieving good bioavailability in dogs and monkeys was attained; the bioavailability of **1** for the (glycinyloxy)methyl ether tosylate **17** was 78% in monkeys, and that for the (α-L-alanyloxy)methyl ether tosylate **18** was 78% in both dogs and monkeys. Not unexpectedly, the [[(*N*-*t*-BOC-amino)acyl]oxy]methyl ethers **22** and **23**, the immediate nonionizable precursors of compounds **17** and **18**, respectively, exhibited poor prodrug activity in the rat.

Relative to the ether carbonate **2**, the (α-L-alanyloxy)-methyl ether tosylate **18** was quite unstable in solution, another factor possibly contributing to the high bioavailabilities of **1** in the three test species. At pH 7.4, **18** was highly unstable, with a time for 10% decomposition ($t_{10\%}$) to **1** of only 0.9 min. At lower pH, stability improved; at pH 6.0, $t_{10\%}$ increased to 9.1 min, and at pH 2, $t_{10\%}$ was 90 min.[16] Thus a reasonable assumption was that **18** would remain mostly unchanged during passage through the stomach, as desired, but would hydrolyze to **1** to some extent prior to absorption upon encountering the higher pH within the small intestine. The stability of **18** in the solid state, in contrast to that in solution, was excellent with no decomposition occurring during 4 weeks at 50 °C under 75% relative humidity.

Owing to their ease of synthesis, ester derivatives of **1** were also explored as potential prodrugs despite the concerns over stability. The simple nonpolar esters **24**−**29** and carbonates **30**−**34** uniformly exhibited poor activities as prodrugs of **1** in the rat (Table 2). With the exception of the valerate **26**, bioavailabilities of **1** were <10%. Not surprisingly, these ester and carbonate derivatives exhibited very low aqueous solubility (<0.02 μg/mL), and as anticipated, the solution state stabilities were poor at pH 7.4 (e.g., **27**, $t_{10\%}$ = 0.6 min; **31**, $t_{10\%}$ = 5.1 min). However, since solution state stabilities increased markedly at lower pH (e.g., at pH 2: **27**, $t_{10\%}$ = 4.9 h; **31**, $t_{10\%}$ = 5.9 h) and since the solid state stabilities were good, the ester series was further pursued to improve aqueous solubility and bioavailability of **1**.

Introduction of nonionizable or poorly ionizable polar functionality, as in the various mixed diester, pyridine carboxylates, and glycolates (**35**−**41**), was not sufficient to improve bioavailability of **1**. The mixed succinate glycolamide **42**, a potential prodrug of the unstable hemisuccinate derivative (a pro-prodrug of **1**), likewise displayed poor activity.[17,18] These results were again attributed to poor absorption resulting from low aqueous solubility as confirmed for several of these compounds (Table 2). Despite having comparatively good aqueous solubility (17 μg/mL), the cyclic carbamate **43** (analogous to the piroxicam prodrug droxicam[8]) delivered **1** with only slightly improved bioavailability (25% in the rat).

In parallel with the ethers of **1**, ionizable ester derivatives were next examined. Unfortunately, all attempts to prepare amino-functionalized esters (α-amino esters and 4-(aminomethyl)benzoates[13]) were unsuccessful as were efforts to prepare the acidic hemisuccinate, hemiadipate, and hemisuberate derivatives (Chemistry). In the latter cases, the results were attributed to susceptibility of the hemiesters to intramolecular attack by the free acid group. Thus, acidic

compounds were sought in which intramolecular attack would be precluded by geometric restriction of the tether between the ester and the ω-acid functionalities. In the event, both the hemiisophthalate **44** and the hemifumarate **45** proved to be stable and isolable.[11] The rat bioavailability of **1** for **44** was very low (4.2%), probably a reflection of its low aqueous solubility (0.58 μg/mL). On the other hand, the prodrug activity of the hemifumarate **45** proved to be excellent, not only in the rat (95%) but in dogs (91%) and monkeys (73%) as well. Interestingly, the aqueous solubility of this compound was found to increase markedly from pH 2 (0.08 μg/mL) to pH 7.0 (24 μg/mL), a finding in keeping with the high bioavailability of **1** since, in the small intestine, **45** was likely to have a solubility toward the higher end of this range.

In sharp contrast to the nonionizable ester and carbonate derivatives as well as the (α-L-alanyloxy)-methyl ether **18**, **45** was quite stable in solution at pH 7.4 ($t_{10\%}$ = 3 h). The stability at pH 2, although relatively low ($t_{10\%}$ = 70 min), was considered to be sufficient enough to allow passage of **45** through the stomach without significant hydrolysis. This assumption was supported by studies *in vitro* which demonstrated **45** to be stable (no detectable decomposition over 30 min) in gastric fluids obtained from rats and monkeys. Like (α-L-alanyloxy)methyl ether **18**, **45** exhibited excellent stability in the solid state with 98% remaining unchanged after 4 weeks at 50 °C under 75% relative humidity.

Although both the (α-L-alanyloxy)methyl ether **18** and the hemifumarate **45** fulfilled the initial bioavailability and stability criteria as prodrugs of **1**, **45** was selected for further profiling based largely on its better overall solution state stability profile. In all species (rats, dogs, and monkeys), circulating levels of **45** were below the assay limit of quantitation (<0.02 μg/mL) following oral administration of the prodrug, indicating that, as desired, systemic exposure to **45** was extremely low. This was confirmed by the observation that, after oral administration of **45** to hepatic portal vein-cannulated dogs, concentrations of intact prodrug within the portal and systemic circulation were undetectable and systemic concentrations of **1** were similar to systemic concentrations achieved following oral administration of **1** to noncannulated dogs.

Further *in vitro* studies were undertaken in order to determine the sites and kinetics of the conversion of the hemifumarate **45** to **1**. The prodrug was rapidly hydrolyzed to the parent drug in monkey intestinal homogenates and in rat, dog, monkey, and human plasma with *in vitro* half-lives of <1 min. Together with the *in vitro* studies showing stability of **45** in rat and monkey gastric fluid, these data support the "ideal" scenario wherein **45** is stable within the upper gastrointestinal tract but rapidly hydrolyzed to **1** as it is absorbed and circulated.

Conclusion

We have described here the discovery of the (α-L-alanyloxy)methyl ether **18** and the hemifumarate **45** as prodrugs of the antirheumatic oxindole **1**. These compounds deliver **1** efficiently into the circulation of test animals, are stable in the solid state, and possess sufficient stability in solution at low pH to ensure gastric stability. Success in achieving acceptable bioavailabilties of **1** across species followed the inclusion of ionizable functionality within the promoiety to compensate for masking the polar enolic OH group of the free drug and the accompanying decrease in aqueous solubility observed with nonionizable derivatives. As demonstrated by the low stability of **18** at neutral pH and our inability to isolate the hemisuccinate, hemiadipate, hemisuberate, and α-amino ester derivatives of **1**, the introduction of ionizable functionality was often associated with decreased stability. A clear exception to this observation was the hemifumarate **45** which was not only isolable but actually more stable at neutral pH than its nonionizable analogues. We ascribe the stability of **45** relative to the hemisuccinate and other diacid hemiesters to the presence of the *trans* olefinic double bond which prevents intramolecular attack on the ester by the free carboxylate. On the other hand, the stability of **45** relative to the nonionizable ester derivatives at neutral pH may be due to the presence of the ionized, negatively charged carboxylate group (pH = 4.5) which may electronically or electrostatically hinder hydrolysis of the adjacent ester linkage.

Despite limited solution phase stability, hemisuccinates have been commonly used to increase the aqueous solubility of compounds containing free hydroxyl groups (particularly corticosteroids).[10] On the other hand, to our knowledge, hemifumarates have never been studied as prodrug derivatives of alcohols or enolic OH compounds. Together with the activity of **45** as a prodrug of **1**, the stability of **45** relative to the corresponding hemisuccinate (which could not be isolated) and its stability in solution relative to the nonionizable esters suggest that hemifumarate be considered as an alternative to hemisuccinate as a prodrug derivative for alcohols, particularly in situations where solution state stability is an issue.

Experimental Section

The ^{1}H NMR spectra were recorded at 300 MHz (Bruker AC 300 or Varian XL-300 spectrometer) or at 250 MHz (Bruker AC 250 spectrometer) as indicated. Flash chromatography was carried out with use of silica gel (J. T. Baker Inc.; 40 μm flash chromatography packing). All reactions requiring anhydrous conditions were run in flame-dried glassware under nitrogen. Melting points are uncorrected.

1-Chloroethyl ethyl carbonate (used for preparing **2**) was used as purchased from Fluka. 1-Chloroethyl 2-propyl carbonate (used for preparing **3**) was prepared from 1-chloroethyl chloroformate.[19] 1-Chloroethyl acetate (used for preparing **4**) and 1-chloroethyl methoxyacetate (used for preparing **7**) were prepared by the reaction of acetyl chloride and methoxyacetyl chloride, respectively, with acetaldehyde in the presence of catalytic zinc chloride.[20] The iodomethyl esters used in the preparation of **5**, **6**, and **8**−**23** were prepared by reacting the tetrabutylammonium or cesium salt of the appropriate carboxylic acid with a large excess of bromochloromethane followed by a Finkelstein reaction.[21] This method gave reasonable yields of the desired esters with only minimal *gem*-diester formation.[15]

Except for the cases of compounds **35**, **42**, **44**, and **45**, the acid chlorides and chloroformates used for preparing the ester and carbonate derivatives of **1**, respectively, were obtained from commercial sources. Fumaric acid monoethyl ester monochloride (used in the preparation of **35**) was prepared according to the published procedure for the corresponding methyl ester.[22]

Carbonic Acid 1-[[1-(Aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethoxy]-ethyl Ethyl Ester (2). To a solution of **1** (49.8 g, 147 mmol) and triethylamine (22.5 mL, 161 mmol) in chloroform (1 L) were added 1-chloroethyl ethyl carbonate (67.3 g, 441 mmol) and silver nitrate (25 g, 147 mmol). The resulting mixture was stirred at room temperature for 36 h and then cooled in an ice bath, and the reaction was quenched by addition of concentrated HCl (14 mL). After filtration to remove silver salts, the filtrate was washed with aqueous 1 N HCl solution, saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. The residue was chromatographed twice on silica gel eluting with 5% MeOH/$CHCl_3$, and the appropriate fractions were concentrated. The solid residue was recrystallized from EtOAc to provide **2** as a yellow solid, 7.4 g (11%). Mp: 176−179 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.25 (d, J = 7.1 Hz, 1 H), 8.05 (dd, J = 1.0, 4.9 Hz, 1 H), 7.95 (br s, 1 H), 7.81 (br s, 1H), 7.77 (d, J = 9.7 Hz, 1 H, overlapped), 7.67 (dd, J = 1.0, 3.5 Hz, 1 H), 7.28 (dd, J = 3.5, 4.9 Hz, 1 H), 6.14 (q, J = 5.4 Hz, 1 H), 4.01 (q, J = 7.1 Hz, 2 H), 1.70 (d, J = 5.4 Hz, 3 H), 1.03 (t, J = 7.1 Hz, 3 H). Anal. ($C_{19}H_{16}ClFN_2O_6S$) C, H, N.

2-Hydroxypropanoic Acid [[1-(Aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethoxy]methyl Ester (12). (a) To a solution of benzyl lactate (21.26 g, 0.118 mol) and diisopropylethylamine (51 mL, 0.293 mol) in CH_2Cl_2 (60 mL) was added [2-(trimethylsilyl)-ethoxy]methyl chloride (25 mL, 0.141 mol). The mixture was stirred at room temperature for 2 h and then at reflux for 1 h. After cooling, the mixture was washed with aqueous 1 N HCl (3 × 50 mL) and concentrated to afford an oil which was chromatographed on silica gel eluting with 1% and then 10% EtOAc/hexane as eluant. Concentration of the appropriate fractions gave 2-[[2-(trimethylsilyl)ethoxy]methoxy]propionic acid benzyl ester as an oil, 27.1 g (74%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.32 (s, 5 H), 5.20 (s, 2 H), 4.75 (s, 2 H), 4.31 (q, J = 6.8 Hz, 1 H), 3.69−3.60 (m, 2 H), 1.44 (d, J = 6.8 Hz, 3 H), 0.92−0.87 (m, 2 H), 0.00 (s, 9 H).

(b) A solution of [[2-(2-trimethylsilyl)ethoxy]methoxy]-propionic acid benzyl ester (26.7 g, 86 mmol) in methanol was hydrogenated over 10% Pd on charcoal (2.65 g) in a Parr shaker for 1 h at 2 atm of pressure. The catalyst was removed by filtration. Evaporation of the solvent provided 2-[[2-(trimethylsilyl)ethoxy]methoxy]propionic acid as an oil, 9.97 g (58%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 4.76 (s, 2 H), 4.28 (q, J = 7.1 Hz, 1 H), 3.71−3.63 (m, 2 H), 1.47 (d, J = 7.1 Hz, 3 H), 0.95−0.88 (m, 2 H), 0.00 (s, 9 H).

(c) A mixture of 2-[[2-(trimethylsilyl)ethoxy]methoxy]-propionic acid (8.95 g, 40.6 mmol), $CsHCO_3$ (9.47 g, 48.8 mmol), $BrCH_2Cl$ (27 mL, 0.415 mol), and dioxane was heated at 80 °C for 3 h. The reaction mixture was taken up in EtOAc, washed twice with water, dried ($MgSO_4$), and concentrated to afford an oil (a mixture of the chloromethyl and bromomethyl esters, 6.36 g) which was combined with sodium iodide (14.2 g, 94.7 mmol) in acetone (100 mL) and refluxed for 18 h. The mixture was filtered, and the filtrate was concentrated to leave an oil. This was dissolved in EtOAc and washed with 10% aqueous sodium thiosulfate, water, and brine. Drying ($MgSO_4$) and concentration yielded crude 2-hydroxypropionic acid iodomethyl ester as an oil, 2.49 g (46%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.98 (ABd, J = 6.5 Hz, 1 H), 5.93 (ABd, J = 6.5 Hz, 1 H), 4.32 (q, J = 7 Hz, 1 H), 1.40 (d, J = 7 Hz, 3 H).

(d) Crude 2-hydroxypropionic acid iodomethyl ester (2.40 g, 10.4 mmol) and the sodium salt of **1** (1.62 g, 4.5 mmol) were combined in acetone (50 mL) and refluxed for 4 h. After concentration of the reaction mixture, the residue was dissolved in EtOAc. The solution was washed with water, dried ($MgSO_4$), and concentrated. The residue was taken up in CH_2Cl_2, filtered to remove unreacted **1**, and concentrated. The residue was chromatographed on silica gel three times eluting with CH_2Cl_2 and then 1% MeOH/CH_2Cl_2. Combination and concentration of the appropriate fractions gave a yellow solid which was recrystallized from EtOAc/hexane to yield **12**, 382 mg (19%). Mp: 184−187 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.25 (d, J = 6.8 Hz, 1 H), 8.06 (dd, J = 1.4, 4.9 Hz, 1 H), 7.94 (br s, 1 H), 7.79 (d, J = 9.5 Hz, 1 H, overlapped), 7.79 (br s, 1 H), 7.65 (dd, J = 1.4, 3.5 Hz, 1 H), 7.29 (dd, J = 3.5, 4.9 Hz, 1 H), 5.80 (ABd, J = 6.2 Hz, 1 H), 5.72 (ABd, J = 6.2 Hz, 1 H), 5.56 (d, J = 6.0 Hz, 1 H), 4.18−4.09 (m, 1 H), 1.18 (d, J = 6.9 Hz, 3 H). Anal. ($C_{18}H_{14}ClFN_2O_6S$) C, H, N. The *E* stereochemistry was assigned by X-ray crystallographic analysis.

General Method for the Preparation of (Acyloxy)-methyl Ether Derivatives of 1. *N*-[(1,1-Dimethylethoxy)-carbonyl]-L-alanine [[1-(Aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienyl-methoxy]methyl Ester (23). (a) A solution of *N*-*t*-BOC-L-alanine (9.46 g, 50 mmol) in ethanol (100 mL) was neutralized with a solution of 40% aqueous tetrabutylammonium hydroxide using phenolphthalein as indicator. The solution was concentrated under vacuum leaving the tetrabutylammonium salt which was dissolved in $BrCH_2Cl$ (200 mL) and stirred for 48 h in the dark. After removal of the excess $BrCH_2Cl$ under vacuum, the mixture was taken up in $CHCl_3$ and washed with water and brine. Drying ($MgSO_4$) and concentration gave an oil. This was chromatographed on silica gel eluting with CH_2Cl_2 to afford *N*-*t*-BOC-L-alanine chloromethyl ester as an oil, 6.25 g (53%) (containing a trace of the corresponding bromomethyl ester). ^{1}H NMR (250 MHz, $CDCl_3$): δ 5.83 (ABd, J = 5.9 Hz, 1 H), 5.63 (ABd, J = 5.9 Hz, 1 H), 5.10−4.90 (br m, 1 H), 4.40−4.28 (m, 1 H), 1.43 (s, 9 H), 1.40 (d, J = 7.3 Hz, 3 H).

(b) *N*-*t*-BOC-L-alanine chloromethyl ester (6.20 g, 26.1 mmol) and sodium iodide (19.5 g, 130 mmol) were combined in acetone (150 mL) and stirred for 18 h. The mixture was then filtered, and the filtrate was concentrated to leave an oil. This was dissolved in EtOAc and washed with 10% aqueous sodium thiosulfate, water, and brine. Drying ($MgSO_4$) and concentration yielded crude *N*-*t*-BOC-L-alanine iodomethyl ester as an oil, 7.40 g (86%).

(c) Crude *N*-*t*-BOC-L-alanine iodomethyl ester (7.30 g, 22.2 mmol) and the sodium salt of **1** (2.67 g, 7.4 mmol) were combined in acetone (75 mL) and refluxed for 6 h. The reaction mixture was concentrated, and the residue, after preadsorption onto silica gel, was chromatographed eluting with CH_2Cl_2 and then 5% MeOH/CH_2Cl_2. Combination and concentration of the appropriate fractions gave a yellow solid which was recrystallized from EtOAc/hexane to yield **23**, 1.16 g (29%). Mp: 165−170 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.25 (d, J = 7.0 Hz, 1 H), 8.07 (d, J = 4.9 Hz, 1 H), 7.96 (br s, 1 H), 7.81 (d, J = 9.7 Hz, 1 H, overlapped), 7.79 (br s, 1 H), 7.63 (d, J = 3.5 Hz, 1 H), 7.39 (d, J = 7.0 Hz, 1 H), 7.31−7.28 (m, 1 H), 5.78 (ABd, J = 6.5 Hz, 1 H), 5.71 (ABd, J = 6.5 Hz, 1 H), 4.09−3.94 (m, 1 H), 1.27 (s, 9 H), 1.15 (d, J = 7.4 Hz, 3 H). Anal. ($C_{23}H_{23}ClFN_3O_7S$) C, H, N.

L-Alanine [[1-(Aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethoxy]-methyl Ester Tosylate Salt (18). To cold (0°C) TFA (12 mL) was added **23** (998 mg, 1.85 mmol). The resulting solution was allowed to stand for 1 h at 0 °C, and then TsOH·H_2O (352 mg, 1.85 mmol) was added. The TFA was removed under vacuum chasing with toluene, and the residual solid was crystallized from MeOH/EtOAc to afford **18** as a yellow solid, 352 mg (31%). Mp: 210−214 °C. ^{1}H NMR (250 MHz, DMSO-d_6): δ 8.31 (br s, 3 H, overlapped), 8.26 (d, J = 7.1 Hz, 1 H), 8.09 (dd, J = 1.1, 5.0 Hz, 1 H), 7.93 (br s, 1 H), 7.83 (d, J = 9.7 Hz, 1 H, overlapped), 7.82 (br s, 1 H), 7.67 (dd, J = 1.1, 3.7 Hz, 1 H), 7.48 (d, J = 8.1 Hz, 2 H), 7.34 (dd, J = 3.7, 5.0 Hz, 1 H), 7.12 (d, J = 8.1 Hz, 2 H), 5.90 (ABd, J = 6.5 Hz, 1 H), 5.82 (ABd, J = 6.5 Hz, 1 H), 4.27−4.13 (m, 1 H), 2.30 (s, 3 H), 1.34 (d, J = 7.2 Hz, 3 H). Anal. ($C_{25}H_{23}ClFN_3O_8S_2$) C, H, N. The *E* stereochemistry was assigned by X-ray crystallographic analysis.

General Method for the Preparation of Ester and Carbonate Derivatives of 1. Carbonic Acid [1-(Amino-carbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethyl 2-Propyl Ester (32 and 33). To a solution of **1** (4.06 g, 12.0 mmol) and triethylamine (1.8 mL, 12.9 mmol) in chloroform (120 mL) was added 1 M isopropyl chloroformate in toluene (24 mL, 24 mmol). The mixture was allowed to stir overnight, concentrated, and then taken up in chloroform. The solution was washed successively

with aqueous 1 N HCl, water, saturated aqueous $NaHCO_3$, and brine and then dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica gel eluting with 5% MeOH/$CHCl_3$. Clean fractions containing the desired product were combined, concentrated, and recrystallized from 2-propanol to afford **32** (*E* isomer) as a yellow solid, 1.9 g (37%). Mp: 197−199 °C dec. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (br s, 1 H, NH), 8.44 (d, J = 7.0 Hz, 1 H, C7 H), 8.19 (dd, J = 1.5, 3.9 Hz, 1 H, C3′ H), 7.74 (dd, J = 1.5, 5.0 Hz, 1 H, C5′ H), 7.47 (d, J = 9.7 Hz, 1 H, C4 H), 7.21 (dd, J = 3.9, 5.0 Hz, 1 H, C4′ H), 5.36 (br s, 1 H, NH), 4.98 (septet, J = 6.3 Hz, 1 H, isopropyl CH), 1.37 (d, J = 6.3 Hz, 6 H, 2 × CH_3). Anal. ($C_{18}H_{14}ClFN_2O_5S$) C, H, N.

The mother liquor from the recrystallization was concentrated and recrystallized again from 2-propanol to afford a 3:1 mixture of the *Z*/*E* isomers (**33**), 320 mg (6%). Mp: 175−178 °C. ^{1}H NMR (*Z* isomer) (300 MHz, $CDCl_3$): δ 8.48 (br s, 1 H, NH), 8.41 (d, J = 7.0 Hz, 1 H, C7 H), 7.75−7.73 (m, 2 H, C3′ H, C5′ H), 7.27 (d, J = 9.7 Hz, 1 H, C4 H), 7.22 (dd, J = 4.0, 5.0 Hz, 1 H, C4′ H), 5.40 (br s, 1 H, NH), 4.98 (septet, J = 6.3 Hz, 1 H, isopropyl CH), 1.38 (d, J = 6.3 Hz, 6 H, 2 × CH_3). Anal. ($C_{18}H_{14}ClFN_2O_5S$) C, H, N.

Butanedioic Acid [1-(Aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethyl 2-(Diethylamino)-2-oxoethyl Ester (42). (a) A mixture of 2-chloro-*N*,*N*-diethylacetamide (11.9 g, 79.5 mmol), succinic acid monobenzyl ester[23] (15.0 g, 72.0 mmol), sodium iodide (1.1 g, 7.4 mmol), and triethylamine (11.2 mL, 80.4 mmol) in EtOAc (280 mL) was heated at reflux for 3 h. After cooling, the mixture was filtered, and the filtrate was washed successively with aqueous 1 N HCl, water, saturated aqueous $NaHCO_3$, and brine. The solution was then dried ($MgSO_4$) and concentrated to afford an oil which was chromatographed on silica gel eluting with 50% EtOAc/hexane as eluant. Combination and concentration of the appropriate fractions provided succinic acid benzyl 2-(diethylamino)-2-oxoethyl ester as a clear oil, 14.6 g (63%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 7.34 (s, 5 H), 5.13 (s, 2 H), 4.70 (s, 2 H), 3.38 (q, J = 7.1 Hz, 2 H), 3.22 (q, J = 7.1 Hz, 2 H), 2.86−2.71 (m, 4 H), 1.21 (t, J = 7.1 Hz, 3 H), 1.12 (t, J = 7.1 Hz, 3 H).

(b) A solution of succinic acid benzyl 2-(diethylamino)-2-oxoethyl ester (14.6 g, 45.4 mmol) in EtOH (250 mL) was hydrogenated at 4 atm over 10% Pd on carbon (1.0 g) in a Parr shaker for 12 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to leave succinic acid mono[2-(diethylamino)-2-oxoethyl] ester as a white solid, 10.5 g (100%). ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.33 (br s, 1 H), 4.73 (s, 2 H), 3.37 (q, J = 7.1 Hz, 2 H), 3.24 (q, J = 7.1 Hz, 2 H), 2.80−2.64 (m, 4 H), 1.21 (t, J = 7.1 Hz, 3 H), 1.11 (t, J = 7.1 Hz, 3 H).

(c) A solution of succinic acid mono[2-(diethylamino)-2-oxoethyl] ester (5.0 g, 21.6 mmol) and oxalyl chloride (2.0 mL, 23.5 mmol) in dry benzene (100 mL) was heated at reflux for 1 h. After cooling, the solution was concentrated to afford the crude acid chloride as an oil. This was taken up in CH_2Cl_2 (20 mL) and added to a solution of **1** (3.40 g, 10.0 mmol) and diisopropylethylamine (3.8 mL, 21.8 mmol) in CH_2Cl_2 (100 mL). The resulting mixture was stirred at room temperature for 66 h and then diluted with CH_2Cl_2 and washed with aqueous 1 N HCl and brine. After drying ($MgSO_4$), the solution was concentrated, and the residue was chromatographed twice on silica gel eluting with CH_2Cl_2 and then 2.5% MeOH/$CHCl_3$ as eluants. Fractions containing the desired product were combined and concentrated to a dark solid which was triturated with 50% Et_2O/CH_2Cl_2 and then recrystallized from toluene to afford **42** as a yellow solid, 512 mg (5%). Mp: 165−167 °C. ^{1}H NMR (250 MHz, $CDCl_3$): δ 8.54 (br s, 1 H, NH), 8.45 (d, J = 7.0 Hz, 1 H, C7 H), 8.19 (dd, J = 1.0, 3.9 Hz, 1 H, C3′ H), 7.71 (dd, J = 1.0, 5.0 Hz, 1 H, C5′ H), 7.51 (d, J = 9.3 Hz, 1 H, C4 H), 7.21 (dd, J = 3.9, 5.0 Hz, 1 H, C4′ H), 5.32 (br s, 1 H, NH), 4.75 (s, 2 H, CH_2O), 3.40 (q, J = 7.1 Hz, 2 H, CH_2N), 3.24 (q, J = 7.1 Hz, 2 H, CH_2N), 3.19−3.14 (m, 2 H, CH_2CO), 2.97−2.92 (m, 2 H, CH_2CO), 1.23 (t, J = 7.1 Hz, 3 H, CH_3), 1.14 (t, J = 7.1 Hz, 3 H, CH_3). Anal. ($C_{24}H_{23}ClFN_3O_7S$) C, H, N.

1,3-Benzenedicarboxylic Acid Mono[[1-(aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethyl] Ester (44). (a) A solution of isophthaloyl dichloride (40.6 g, 200 mmol) and 2,2,2-trichloroethanol (30.9 g, 207 mmol) in dry toluene was heated at reflux for 24 h. The solvent was evaporated, and the remaining material was distilled under vacuum. The desired product distilled over along with some of the bis(2,2,2-trichloroethyl) ester between 125 and 170 °C at 0.2 mmHg. On standing, the unwanted side product separated out as a solid. The desired product, 3-[[(2,2,2-trichloroethyl)oxy]carbonyl]benzoyl chloride (19.0 g, 30%), was decanted off for use in the next step. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.82 (s, 1 H), 8.41−8.32 (m, 2 H), 7.69−7.63 (m, 1 H), 5.03 (s, 2 H).

(b) To a solution of **1** (5.0 g, 14.7 mmol) and diisopropylethylamine (5.1 mL, 29.3 mmol) in CH_2Cl_2 (100 mL) at room temperature was added 3-[[(2,2,2-trichloroethyl)oxy]carbonyl]-benzoyl chloride (9.3 g, 29.4 mmol). The mixture was stirred overnight, diluted with CH_2Cl_2, and then poured into water. The organic layer was washed with water and brine and then filtered to remove the suspended solid. The filtrate was concentrated to a yellow residue which, after trituration with $CHCl_3$, afforded the 2,2,2-trichloroethyl isophthalate mixed ester of **1** (*E* isomer), (1.4 g, 15%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.98 (t, J = 1.6 Hz, 1 H, isophthalate C2 H), 8.55−8.43 (m, 4 H, NH, C7 H, isophthalate C4 and C6 H), 8.21 (dd, J = 1.5, 3.9 Hz, 1 H, C3′ H), 7.78 (t, J = 7.9 Hz, 1 H, isophthalate C5 H), 7.69 (dd, J = 1.5, 4.9 Hz, 1 H, C5′ H), 7.24−7.17 (m, 2 H, C4′ H, C4 H), 5.35 (br s, 1 H, NH), 5.02 (s, 2 H, CH_2O).

(c) A mixture of the 2,2,2-trichloroethyl isophthalate mixed ester of **1** (1.0 g, 1.62 mmol) and zinc dust (1.0 g, 15.3 mmol) in glacial AcOH was stirred at 50 °C overnight. The warm solution was filtered and then poured into water (250 mL). The yellow precipitate was collected by filtration and recrystallized from AcOH to afford **44** as a yellow solid (130 mg, 16%). Mp: 244−248 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.71 (s, 1 H), 8.52 (d, J = 6.5 Hz, 1 H), 8.40 (d, J = 7.8 Hz, 1 H), 8.30−8.26 (m, 2 H), 8.11 (d, J = 4.9 Hz, 1 H), 8.05 (br s, 1 H), 8.01 (br s, 1 H), 7.88 (t, J = 7.8 Hz, 1 H), 7.31−7.24 (m, 2 H). Anal. Calcd for $C_{22}H_{12}ClFN_2O_6S$: C, 54.27; H, 2.48; N, 5.75. Found: C, 53.80; H, 2.18; N, 5.71.

Butenedioic Acid Mono[[1-(aminocarbonyl)-6-chloro-5-fluoro-1,2-dihydro-2-oxo-3*H*-indol-3-ylidene]-2-thienylmethyl] Ester (45). (a) A solution of 2-(trimethylsilyl)ethanol (50.0 g, 0.428 mol) in dry benzene (500 mL) was added slowly with stirring to fumaryl chloride (71.2 g, 0.466 mol) at room temperature. After stirring overnight, the mixture was filtered (to remove the precipitated fumaric acid bis[2-(trimethylsilyl)-ethyl] ester), and the solvent was evaporated to leave a yellow oil. This was distilled at 18 mmHg and then at 0.8 mmHg to remove volatiles including unreacted fumaryl chloride. Remaining in the pot was an oil (35.7 g) containing the desired product, fumaric acid mono[2-(trimethylsilyl)ethyl] ester monochloride, and a small amount of the unwanted diester. This was added slowly (rinsing with 50 mL of CH_2Cl_2) to a solution of **1** (46.8 g, 0.138 mol) and diisopropylethylamine (26.5 mL, 0.152 mol) in CH_2Cl_2 (1525 mL) at room temperature. The mixture was stirred overnight and then worked up by washing with aqueous 1 N HCl (1 L), saturated aqueous $NaHCO_3$ (1 L), and brine (1 L). After drying ($MgSO_4$), the solvent was evaporated to leave an oil which was chromatographed three times on silica gel (2 kg) eluting successively with CH_2Cl_2, 1% MeOH/CH_2Cl_2, and 1.2% MeOH/CH_2Cl_2. Fractions containing the desired product gave a yellow solid which was recrystallized from acetonitrile to afford the desired 2-(trimethylsilyl)ethyl fumarate mixed ester of **1** (24.6 g, 33% from **1**) as a mixture of *E* and *Z* isomers. Anal. ($C_{23}H_{22}ClFN_2O_6SSi$) C, H, N.

By careful chromatography on silica gel (using the above procedure), it was possible to separate the geometric isomers on a small scale for characterization by ^{1}H NMR. ^{1}H NMR (*E* isomer) (250 MHz, acetone-d_6): δ 8.45 (d, J = 6.9 Hz, 1 H, C7 H), 8.32−8.30 (m, 2 H, NH, C3′ H), 8.05 (dd, J = 1.1, 5.0 Hz, 1 H, C5′ H), 7.50 (d, J = 9.5 Hz, 1 H, C4 H), 7.31 (dd, J = 3.9, 4.9 Hz, 1 H, C4′ H), 7.28 (ABd, J = 15.7 Hz, 1 H, fumarate

CH), 7.20 (ABd, J = 15.7 Hz, 1 H, fumarate CH), 7.05 (br s, 1 H, NH), 4.42−4.36 (m, 2 H, CH_2O), 1.06−1.09 (m , 2 H, CH_2Si), 0.09 (s, 9 H, 3 × CH_3). ^{1}H NMR (*Z* isomer) (250 MHz, acetone-d_6): δ 8.43 (d, J = 7.0 Hz, 1 H, C7 H), 8.19 (br s, 1 H, NH), 8.10 (d, J = 4.9 Hz, 1 H, C5′ H), 8.01 (d, J = 3.7 Hz, 1 H, C3′ H), 7.41 (d, J = 9.7 Hz, 1 H, overlapped, C4 H), 7.40−7.37 (m, 1 H, C4′ H), 7.02 (s, 2 H, overlapped, 2 × fumarate CH), 7.01 (br s, 1 H, NH), 4.39−4.32 (m, 2 H, CH_2O), 1.14−1.07 (m, 2 H, CH_2Si), 0.08 (s, 9 H, 3 × CH_3).

(b) The 2-(trimethylsilyl)ethyl fumarate mixed ester of **1** (70.8 g, 0.131 mol) was added in three batches to HF/pyridine complex (900 mL) in a poly(ethylene) vessel at 0 °C. The mixture was occassionally manually agitated over 70 min and was then poured into 3 L of ice/water. The precipitated solid was collected by filtration, washed with water (1 L), and dried over P_2O_5 in a vacuum dessicator. The solid was mixed with ethyl acetate (6 L), and the mixture was warmed, with stirring, to 60 °C. After cooling to room temperature, the slurry was filtered to collect **45** as a fine yellow solid (43.8 g, 76%). Mp: 197−198 °C dec. ^{1}H NMR (250 MHz, acetone-d_6): δ 8.48 (d, J = 7.0 Hz, 1 H, C7 H), 8.34−8.33 (m, 2 H, NH, C3′ H), 8.07 (d, J = 4.5 Hz, 1 H, C5′ H), 7.55 (d, J = 9.6 Hz, 1 H, C4 H), 7.33 (t, J = 4.5 Hz, 1 H, C4′ H), 7.31 (ABd, J = 15.7 Hz, 1 H, fumarate CH), 7.22 (ABd, J = 15.7 Hz, 1 H, fumarate CH), 7.05 (br s, 1 H, NH). Anal. ($C_{18}H_{10}ClFN_2O_6S$) C, H, N.

It was possible to recrystallize **45** from anhydrous acetic acid giving analytically pure material with mp 229−232 °C. The *E* stereochemistry for the major isomer was assigned based on the close similarity of its ^{1}H NMR spectrum to that of the *E* isomer of the 2-(trimethylsilyl)ethyl ester precursor in acetone-d_6. The *E* stereochemistry for the latter was assigned based on correlation of the ^{1}H NMR spectrum in $CDCl_3$ with that of the (*E*)-octyl carbonate isomer **34**.

***In Vivo* Evaluation.** The relative bioavailabilities of **1** from test compounds were determined in all animals (Sprague−Dawley rats, beagle dogs, and cynomolgus monkeys) at a dose equivalent to 3 mg of **1**/kg administered by oral gavage in 0.1% methyl cellulose (10 mL/kg for rats, 1 mL/kg for dogs, and 1 mL/kg for monkeys). Each experiment was carried out in three animals fasted overnight prior to dosing. Plasma samples were obtained from rats at 1, 3, and 6 h postdose via sinus orbital bleeding; dogs were bled from the jugular vein at 0, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose, and plasma samples from monkeys were obtained at 0, 0.5, 1, 2, 4, 6, and 8 h postdose. For determination of plasma concentrations of **1**, plasma samples were precipitated with acetonitrile fortified with tenidap, the internal standard. The suspensions were centrifuged, and the supernatants were transferred to vials containing tris buffer. Analysis was carried out by reverse-phase HPLC with UV detection at 360 nm. The analytical column was a Waters Novapak C_{18} column (150 × 3.9 mm i.d.) fitted with a 2 cm LC-Si guard column by Supelco Pelliguard. The mobile phase consisted of 1:1 MeOH/tris buffer adjusted to pH 6.3 with H_3PO_4; a flow rate of 1 mL/min was used. Under these conditions, the retention times of **1** and tenidap were 4.7 and 3.3 min, respectively. Concentrations of **1** were measured from standard curves based on the peak height ratio of **1** to tenidap. The relative bioavailabilities were calculated by comparison of the averaged plasma AUCs of **1** with averaged plasma AUCs achieved of **1** following oral dosing of the free drug (3 mg/kg) to controls. A separate control group of three animals was used for determining rat bioavailabilities; for dogs and monkeys, each animal served as its own control. Experiments in hepatic portal vein-cannulated dogs (beagles) were carried out using **45** and **1** dosed orally as above in 0.1% methyl cellulose. Dogs were bled from the portal and jugular veins at 0, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4 h postdose. Additional plasma samples were obtained from the jugular vein at 6 and 8 h postdose.

Aqueous Solubilty, Solution State Stability, and Solid State Stability Determinations. Compounds selected for measurements of solution state stability, solid state stability, and/or aqueous solubilty were assayed by reverse-phase HPLC on a Waters Novapak C_{18} column (150 × 3.9 mm i.d.) using a UV detector at 214 nm and a flow rate of 1 mL/min. The mobile phase systems used were as follows:

compd	0.05 M AcOH (%, v/v)	MeOH (%, v/v)	MeCN (%, v/v)	*i*-PrOH (%, v/v)	H_2O (%, v/v)	0.01 M H_3PO_4 (%, v/v)	other
2, **24**−**34**	25	50	25				pH 2.5
4, **12**	40	35	25				pH 3.0
5, **6**, **35**	30	45	25				pH 3.0
7, **10**	35	35	30				pH 3.0
16	30		55	15			pH 3.0
18			35		65		0.1% (v/v) Et_3N, pH 3.0
22, **44**, **45**	35	30	35				pH 3.0
37−**39**		35	35			30	5 mmol/L $C_6H_{13}SO_3H$, pH 3.0
43	35	15	40	10			pH 3.0

The aqueous solubility of compounds was determined as follows. The test compound (1 mg) was placed in a screw cap vial to which unbuffered distilled water (6 mL) was added. The tightly capped vial was sonicated for 5 min,and the solution was filtered through a Millex HV_{13} 0.45 μm filter, discarding the first 0.5 mL. The solution was immediately measured for pH and assayed by HPLC. Solubility was determined by comparison of the peak area of the compound with that obtained from a reference solution of the compound dissolved in the mobile phase.

Solution state stabilities were routinely measured at pH 2.0 and 7.4. Limiting solubility usually precluded testing under 100% aqueous conditions. For solution state stability at pH 2.0, a test compound (0.5 mg) was typically dissolved in 1:1:2 (v/v/v) 0.02 M HCl/MeOH/MeCN (10 mL); for testing at pH 7.4, 1:1:2 (v/v/v) 0.01 M $NaHPO_4$/MeOH/MeCN (adjusted to pH 7.4 with 0.2 M NaOH) was typically used. After 10-fold dilution in the same system, samples (kept protected from light at room temperature) were assayed by HPLC immediately and thereafter at regular time intervals. The first-order rate constant and $t_{10\%}$ for decomposition were determined by linear regression of a plot of time versus ln(F) where F is the fraction of compound remaining. Dissolution of very unstable compounds was carried out in MeCN prior to dilution into the mixed aqueous/organic systems above. Compounds **18** and **45** were compared in 100% aqueous phosphate buffer systems except at low pH where the solubility of **45** was limiting. Consequently, for the pH 2.0 stability of **45**, various amounts of MeOH or MeCN were used with the aqueous buffer. From the observed rate constants in these systems and the respective dielectric constant of each mixture, the first-order rate constants and $t_{10\%}$ values for decomposition of **45** were extrapolated to pure water at pH 2.0.

To determine solid state stability, a test sample (0.5−0.8 mg) was placed in a clear screw cap vial and stored in a humidity chamber over a saturated solution of sodium acetate (75% relative humidity) at 50 °C for 4 weeks. The sample was dissolved in 10 mL of the mobile phase for HPLC analysis (ultrasonication was often required). After 10-fold dilution in mobile phase and filtration through a Millipore HV_{13} filter, the sample was analyzed by HPLC. The stability of the test compound was quantitated based on the percent of its peak area relative to the total detected area assuming the compound and its degradation products to have the same absorptivity. Peaks occurring in the solvent front were excluded from the total peak area count. The percentage of test compound remaining was compared to that obtained from a reference sample stored at 0 °C for 4 weeks.

***In Vitro* Stability of 45 in Biological Fluids.** Solution A was prepared by dissolving AcOH (100 μL) and H_3PO_4 (50 μL) in MeCN (800 mL); solution B was prepared by dissolving **45** (2.63 mg) in solution A (10 mL); solution C was prepared by dissolving the internal standard 5-chloro-*N*-ethyl-2,3-dihydro-3-(hydroxy-2-furanylmethylene)-2-oxo-1*H*-indole-1-carboxamide (2.0 mg) in MeCN (500 mL) containing H_3PO_4 (2.5 mL). Fresh plasma, gastric fluids, and intestinal homogenates (2 mL) were preincubated at 37 °C for 5−10 min and then spiked with solution B (100 μL). The samples were incubated at 37 °C over a period of 30 min; aliquots (50 μL) were removed

at 1, 2, 5, 10, 20, and 30 min after spiking and added to microvials containing solution C (100 μL). Zero-hour time point measurements were carried out by addition of solution C (2 mL) to the biological fluid (1 mL, preincubated at 37 °C) followed by spiking with solution B (50 μL). After centrifugation, aliquots of supernatant (75 μL) were removed, added to injection vials, and diluted with solution A (75 μL). Analysis of the samples was carried out by reverse-phase HPLC with UV detection at 360 nm. The analytical column was a Phenomenex Ultracarb 5 ODS (30) column (150 × 4.6 mm i.d.) fitted with a Phenomenex Ultracarb 5 ODS (30) guard column. The mobile phase consisted of 4:1 MeCN/H_2O containing 0.1% (v/v) AcOH and 0.05% (v/v) H_3PO_4; a flow rate of 1 mL/min. was used. Under these conditions, the retention times of **1**, **45**, and internal standard were 5.4, 2.3, and 11.4 min, respectively. Concentrations of **1** and **45** were measured from standard curves based on the peak height ratios of **1** and **45** to internal standard.

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Supporting Information Available: X-ray crystal structure experimental and data for compounds **12** and **18** (26 pages). Ordering information is given on any current masthead page.

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