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Inhibitors of HIV-1 attachment. Part 2: An initial survey of indole substitution patterns ☆

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ABSTRACT

The effects of introducing simple halogen, alkyl, and alkoxy substituents to the 4, 5, 6 and 7 positions of 1-(4-benzoylpiperazin-1-yl)-2-(1*H*-indol-3-yl)ethane-1,2-dione, an inhibitor of the interaction between HIV gp120 and host cell CD4 receptors, on activity in an HIV entry assay was examined. Small substituents at C-4 generally resulted in increased potency whilst substitution at C-7 was readily tolerated and uniformly produced more potent HIV entry inhibitors. Substituents deployed at C-6 and, particularly, C-5 generally produced a modest to marked weakening of potency compared to the prototype. Small alkyl substituents at N-1 exerted minimal effect on activity whilst increasing the size of the alkyl moiety led to progressively reduced inhibitory properties. These studies establish a basic understanding of the indole element of the HIV attachment inhibitor pharmacophore.

The interaction of the human immunodeficiency virus-1 (HIV-1) surface glycoprotein gp120 with CD4, a glycoprotein receptor expressed on mammalian cells, is the critical first step of a series of several carefully choreographed events that allows virus access to host cells.[2,3] The binding of gp120 to CD4 induces a conformational change in the virus glycoprotein that exposes binding sites recognized by coreceptors expressed on the surface of host cells, either CCR5 or CXCR4, depending on virus tropism. The engagement of coreceptors by gp120 leads to its dissociation from gp41, the virus membrane-spanning protein that mediates the fusion of the virus with the host cell. We have recently described the discovery of indole-3-glyoxamide derivatives as the first small molecule inhibitors of the gp120-CD4 interaction (HIV-1 attachment inhibition) that demonstrate potent antiviral activity in cell culture.[1,4–7] These compounds, of which the 4-fluoro derivative **1b** is prototypical, appear to act by stabilizing a specific conformation of gp120 that is poorly recognized by CD4.[4–7] However, under certain circumstances, compounds of this class have been shown to form a ternary complex with gp-120 and CD4 and interfere with the CD4-induced exposure of the gp41 heptad repeats, providing a potential additional mode of action.[8,9] Resistance to these attachment inhibitors has been shown to map to a highly conserved, recessed binding pocket of gp120 that recognizes Phe_{43} of CD4.[4–7,10,11] Structural optimization to address deficiencies in the physical chemical profile of **1b** afforded BMS-378806 (**2**) and BMS-488043 (**3**), a compound that has demonstrated antiviral activity in HIV-1-infected patients following oral administration.[1,6,12] In this Letter, we elaborate the basic structure–activity relationships associated with the indole ring that identify the preferred patterns of substitution for this element of the pharmacophore that ultimately provided a foundation for the design of BMS-488043 (**3**).

The target compounds were synthesized by acylating a substituted indole with oxalyl chloride followed by coupling of the acid chloride with 1-benzoylpiperazine, a process depicted in Scheme 1.[13] For indole derivatives substituted with electron withdrawing elements an alternative process was employed that comprised reaction of the indole with methyl or ethyl chlorooxoacetate in the presence of a Lewis acid, typically $AlCl_3$,[14] followed by alkaline hydrolysis of the ester moiety and coupling with 1-benzoylpiperazine. Alkylation of the indole N atom was accomplished by

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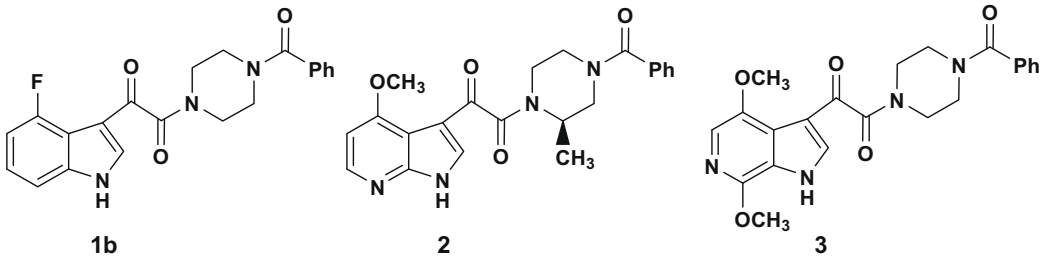


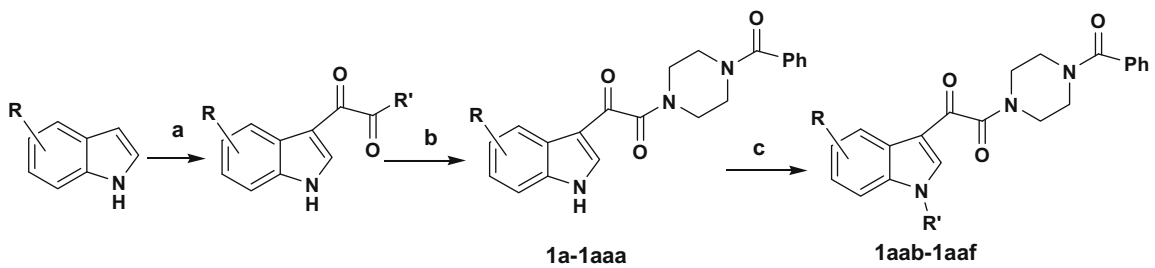
heating with an alkyl halide in DMF at 80 °C in the presence of a slight excess of the phosphazene base 2-*tert*-butylimino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosphorine (BEMP). The starting indoles were procured by methods described in the literature and the compounds surveyed are compiled in Table 1.

The antiviral activity of target compounds was determined by evaluation in a single-cycle viral infection system using luciferase activity as the endpoint.[4,15] To generate pseudotype virus, pJRFL-LucΔEnv, which contains full length HIV-1_{JRFL} in which the HIV-1 envelope sequences are replaced by the firefly luciferase gene sequence, and a plasmid expressing the HIV-1_{JRFL} envelope sequence were used to transfect HEK-293 cells and incubated for 48 hours. The titer of pseudovirus was determined by infecting HeLa67 cells expressing the primary HIV-1 receptor CD4 and the coreceptor CCR5 with a serial dilution of virus stock and quantifying luciferase activity (Luciferase Gene Reporter Assay Kit by Roche) on day 3 after infection. To assess the antiviral activity against the pseudovirus, fourfold serial dilutions of compounds were prepared in DMSO and applied to a luminescence plate containing pseudovirus and HeLa CD4/CCR5 cells. After three days, the resulting luciferase activity data were used to estimate the EC_{50} values of individual compounds in preventing pseudovirus infection. The cytotoxicity of compounds toward HeLa67 cells was determined in parallel and an XTT assay performed 3 days after compound addition. Where the data reported are the average of only 2 experiments, the individual results are provided as a measure of assay variability. In this assay, **1b** half-maximally inhibited infection by the pseudovirus at a concentration of 2.59 nM.

The prototype compound of the series, indole **1a** (Table 1), was discovered as an HIV-1 attachment inhibitor using a cell-based screening assay designed to query the Bristol-Myers Squibb proprietary collection.[1,4–7,15] This compound potently inhibits the entry of pseudotype virus and expresses antiviral activity towards a representative panel of HIV-1 strains in cell culture.[1] The initial effort to study this new chemotype focused on surveying the effect of introducing substituents to the indole ring with a view to increasing potency and establishing a basic understanding of this aspect of the pharmacophore, data that is presented in Table 1. Three substituents, fluorine, chlorine and a methoxy moiety, were introduced individually at each of the positions of the aromatic ring, providing a consistent series with which to systematically probe the effects of different electronic and hydrophobic elements. The results for this cluster of compounds, **1b,c,h** for C-4, **1k,l,o** for C-5, **1s,t,u** for C-6 and **1w,x,ab** for C-7, reveal a pattern of structure-activity correlates in which these substituents when installed at C-4 and C-7 lead to enhanced potency compared to the parent **1a**. However, these substituents produce markedly less active HIV-1 attachment inhibitors when deployed at C-5 and generally weaker inhibitors when installed at C-6. The tolerance for substitution at C-5 is particularly poor, as exemplified by the micromolar EC_{50}s recorded for **1k, 1l**, and **1o**, and confirmed by the data associated with the additional 5 examples **1m**, **1n**, and **1p–r**. At C-6, a fluorine atom improves the potency of the prototype by sevenfold (**1s**) but replacement by the larger chlorine (**1t**) and methoxy substituents (**1u**) leads to an erosion of antiviral activity that correlates with the size of the substituent.[16] However, the single additional C-6-substituted analog evaluated, the CF_3 derivative **1v**, exhibits markedly weaker activity than would be anticipated based simply on steric dimensions, suggestive of the contribution of an additional electronic component.

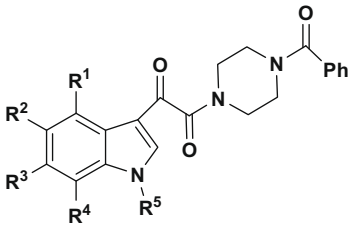
The introduction of a halogen at C-4 produced a homologous series of potent HIV-1 attachment inhibitors **1b–d** that offer a 30–60-fold advantage over **1a**, with virus entry inhibitory activity largely independent of the identity of the substituent. A methoxy (**1h**) or ethoxy (**1i**) substituent at C-4 provides a higher level of potency but the isopropoxy analog **1j** is over three orders of magnitude weaker. This observation indicates high sensitivity to steric effects in this region of the pharmacophore and can be attributed to branching since non-branched alkoxy substituents at C-4 have been shown to exert a less severe negative impact on potency.[17] The same principle may underlie the poor entry inhibition observed for the acetoxy derivative **1f**, which is over 160-fold weaker than **1a** and almost 50,000-fold weaker than **1h**. However, under the conditions of the assay, this compound may suffer cleavage to the simple C-4 hydroxy compound **1g** which is equally ineffective, perhaps pointing to a problem with the presence of polarity at this site. The final compound prepared in this series, the nitro derivative **1e**, exhibits potency comparable to the prototype **1a**.[17]

The introduction of substituents at C-7 in all cases examined enhanced the potency of the prototype molecule **1a**, with alkoxy



Scheme 1. Reagents and conditions: (a) oxalyl chloride/THF or Et_2O for R′ = Cl; or EtO·CO·CO·Cl or MeO·CO·CO·Cl/$AlCl_3$/CH_2Cl_2 for R′ = OEt or OMe when R is electron withdrawing; (b) *N*-benzoylpiperazine/*i*Pr_2NEt/THF for R′ = Cl or (i) NaOH or K_2CO_3/MeOH; (ii) *N*-benzoylpiperazine/DEPBT/*i*Pr_2NEt/DMF for R′ = OEt or OMe; (c) R′-X/BEMP/dry DMF/80 °C.

Table 1
Structure, HIV pseudotype virus inhibitory activity and cytotoxicity associated with indole glyoxamide derivatives



Compd #	R^1	R^2	R^3	R^4	R^5	EC_{50}[a] (nM)	CC_{50} (μM)
1a	H	H	H	H	H	152.97 ± 119.0 (*n* = 21)	338.8 ± 50.7 (*n* = 11)
1b	F	H	H	H	H	2.59 ± 2.46 (*n* = 17)	>300 (*n* = 7)
1c	Cl	H	H	H	H	4.3 ± 4.2 (*n* = 6)	212 ± 67 (*n* = 4)
1d	Br	H	H	H	H	4.5 (7.1, 1.8)	142 (*n* = 1)
1e	NO_2	H	H	H	H	149.8 (177.9, 121.6)	>300 (*n* = 2)
1f	$OCO{\cdot}CH_3$	H	H	H	H	24,857.5 (30,068.4, 19,646.6)	>300 (*n* = 2)
1g	OH	H	H	H	H	20,137.4 (20,477.4, 19,7973)	>300 (*n* = 2)
1h	OCH_3	H	H	H	H	0.52 (0.62, 0.41)	>300 (*n* = 2)
1i	OCH_2CH_3	H	H	H	H	0.45 (0.62, 0.27)	>94.8 (>94.8, 153.4)
1j	O^iPr	H	H	H	H	>500 (*n* = 2)	150.2 (135.2, 165.2)
1k	H	F	H	H	H	838.3 (1039.6, 646.9)	>300 (*n* = 2)
1l	H	Cl	H	H	H	395 (572, 218)	46 (44, 47)
1m	H	Br	H	H	H	1090.3 (1603.5, 577.1)	>149 (149.2, >300)
1n	H	CH_3	H	H	H	1548.6 (1648.2, 1449.0)	>300 (*n* = 2)
1o	H	OCH_3	H	H	H	21,100 (27,000, 15200)	>300 (*n* = 2)
1p	H	NO_2	H	H	H	3,800 (5900, 1700)	44.6 (17.4, 71.9)
1q	H	CN	H	H	H	120,523.1 (156,613.0, 84,433.1)	>300 (*n* = 2)
1r	H	$OCO{\cdot}CH_3$	H	H	H	952.2 (892.5, 1011.8)	95.2 (70.7, 119.6)
1s	H	H	F	H	H	21.1 (34.7, 7.4)	>245 (245.9, >300)
1t	H	H	Cl	H	H	208 (377, 38)	>110 (110.1, >300)
1u	H	H	OCH_3	H	H	328.8 (418.5, 239.0)	>300 (*n* = 2)
1v	H	H	CF_3	H	H	>5000 (*n* = 2)	54.5 (74.0, 35.0)
1w	H	H	H	F	H	7.3 (7.7, 6.9)	>152 (152.2, >300)
1x	H	H	H	Cl	H	4.4 (4.2, 4.5)	162.0 (*n* = 1)
1y	H	H	H	Br	H	17.4 (*n* = 1)	64.0 ± 16.3 (*n* = 3)
1z	H	H	H	CH_3	H	89.5 ± 48.7 (*n* = 3)	>218 (*n* = 1)
1aa	H	H	H	CH_3CH_2	H	24.2 ± 23.9 (*n* = 3)	>49 (49.2, >300)
1ab	H	H	H	OCH_3	H	6.6 ± 2.2 (*n* = 4)	153.6 ± 22.5 (*n* = 4)
1ac	H	H	H	OCH_2CH_3	H	0.5 (0.5, 0.6)	117.4 (163.6, 71.1)
1ad	H	H	H	OC_4H_9	H	0.14 (0.09, 0.2)	64.5 (48.8, 42.1)
1ae	H	H	H	CN	H	4.9 (3.7, 6.1)	234.3 (184.4, 284.3)
1af	F	Br	F	H	H	23.1 (17.3, 28.9)	20.9 (28.9, 12.9)
1ag	H	F	F	H	H	>290 (292.9, >500)	170.4 (112.1, 228.7)
1ah	H	Cl	Cl	H	H	500 (500, 500)[b]	52.3 (49.6, 55.0)
1ai	H	F	Cl	H	H	>500	98.5 (145.7, 51.2)
1aj	H	OCH_3	OCH_3	H	H	46,451.2 (43,771.5, 49,130.8)	>300 (*n* = 2)
1ak	H	F	H	Br	H	73.1 (110.4, 35.8)	22.5 (22.7, 22.3)
1al	F	H	H	F	H	0.35 ± 0.24 (*n* = 8)	>300 (*n* = 8)
1am	OCH_3	H	H	OCH_3	H	0.23 ± 0.33 (*n* = 7)	279.0 (277.3, 280.6)
1an	OCH_3	H	H	Cl	H	0.07 ± 0.05 (*n* = 4)	>300 (*n* = 1)
1ao	OCH_3	H	H	Br	H	0.13 (*n* = 1)	118 (*n* = 1)
1ap	OCH_3	H	H	CN	H	0.06 (0.06, 0.06)	>275 (275.4, >300)
1aq	OCF_3	H	H	Br	H	42.8 (28.1, 57.4)	75.7 (92.6, 58.8)
1ar	F	H	H	Br	H	0.13 ± 0.07 (*n* = 4)	39.8 ± 29.9 (*n* = 4)
1as	F	H	H	CH_3	H	0.56 ± 0.15 (*n* = 4)	87.5 ± 19.3 (*n* = 3)
1at	F	H	H	OCH_3	H	0.06 (0.008, 0.111)	>300 (*n* = 2)
1au	F	H	H	OCH_2CF_3	H	0.71 (*n* = 1)	196.1 ± 26.9 (*n* = 3)
1av	F	H	H	CN	H	1.9 (3.0, 0.8)	>300 (*n* = 2)
1aw	Br	H	H	F	H	8.2 (10.2, 6.1)	86.0 (98.1, 73.9)
1ax	CN	H	H	F	H	>500 (*n* = 2)	>300 (*n* = 2)
1ay	CH_3	H	H	CH_3	H	>500 (*n* = 2)	201.6 (109.7, 293.5)
1az	F	H	F	H	H	0.42 ± 0.04 (*n* = 3)	>188 (188.3, >300, >244.0)
1aaa	F	F	F	F	H	1.7 (1.5, 1.9)	55.7 (*n* = 1)
1aab	H	H	H	H	CH_3	265 (310, 220)	226 (300, 152)
1aac	H	H	H	H	CH_3CH_2	1450 (1900, 1000)	>300 (>300, >300)
1aad	H	H	H	H	$CH_3(CH_2)_3$	2650 (2400, 2900)	>300 (>300, >300)
1aae	H	H	H	H	CH_2=CH-CH_2	6760 (7720, 5800)	201.5 (195, 208)
1aaf	H	H	H	H	$PhCH_2$	13750 (22300, 5200)	182 (137, 227)

[a] Data are the means of two or more experiments with individual data provided for those experiments conducted twice.
[b] LAI envelope used rather than JRFL.

(**1ab–ad**) superior to alkyl (**1z and 1aa**) and both demonstrating a trend in which potency increases with the size of the substituent.

With a basic understanding of the SAR associated with substitution at each of the 4 sites established, it became of considerable interest to examine the effect of combinations, results that are captured by compounds **1af–1aaa** in Table 1. Dual substitution at C-5 and C-6 (**1ag–1aj**) produced compounds with unimpressive biological activity, reflecting an additive combination of the effects of the individual substituents. However, the poor potency associated with this pattern could be mitigated quite considerably by the introduction of a fluorine atom at C-4, as exemplified by **1af**, further underscoring the marked positive effect observed with this substituent in the context of the parent molecule. This property extends to the C-4, C-6 difluoro analog **1az** and the tetra-fluoro derivative **1aaa**, both of which are more potent than might be anticipated based on the data for the individually substituted compounds. The single C-5, C-7 combination studied, the fluoro, bromo derivative **1ak**, performed essentially as expected based on a composite of the individual components. It was anticipated that combinations of C-4 and C-7 substituents would be particularly productive, an aspect of SAR probed with compounds **1am–1aaa** and most effectively realized with analogs **1am–1ap** and **1ar–1au**. The most impressive representatives of this substitution pattern are **1an**, **1ap** and **1at**, all of which demonstrate EC_{50}s in the pseudotype virus assay of 60–70 picomolar. The results with **1ax** and **1ay** provide some insight into what may be expected of either a C-4 CN or CH_3 substituent, compounds not explicitly investigated but anticipated to show poor antiviral activity.

The effect of alkylating the indole N atom in the context of the parent molecule **1a**, surveyed with **1aab–1aaf**, indicates that only a CH_3 substituent (**1aab**) preserves significant HIV inhibitory activity with potency progressively eroded as the size of the alkyl group increases within this short series.[17]

The pharmacokinetic properties of compounds **1a**, **1b**, **1al** and **1am** were evaluated in the rat, data that is summarized in Table 2. The parent compound **1a**, selected to provide a basic understanding of the chemotype, demonstrated high clearance in the rat after intravenous administration and modest oral bioavailability of 29% after oral dosing. The 4-fluoro analog **1b** exhibited marginally reduced clearance and lower oral bioavailability, data that were predicted by rat liver microsomal studies where the rate of metabolic degradation predicted clearance in rats of 52 mL/min/kg, close to the observed figure reported in Table 2. However, compound **1b** is considerably more stable in human liver microsomes with clearance predicted to be 8 mL/min/kg, a figure that places this compound in the intermediate range of clearance. The 4,7-difluoro derivative **1al** performed similarly to **1b** in the rat, with high clearance also predicted well by rat liver microsomal stability data. For all three of these compounds, the terminal half life following oral dosing was longer than that measured after intravenous (IV) administration, indicative of an absorption rate that is slower than the rate of elimination. This appears to be a function of the dissolution properties of the drug in the gut since all three compounds readily permeate a confluent Caco-2 cell layer with rates of 228, 100 and 219 nm/s for **1a**, **1b** and **1al**, respectively, data consistent with high membrane permeability based on comparison to standard agents. This was supported by subsequent studies with **1b** in higher species, where bioavailability was complete after oral dosing of a solution of the drug in poly(ethylene glycol) 400 (PEG 400) and ethanol (90:10 v/v) to cynomolgus monkeys and dogs.[1] The 4,7-dimethoxy derivative **1am** offered superior pharmacokinetic properties in the rat, with low clearance (5.4 mL/min/kg), an observation predicted by good in vitro rat liver microsomal stability, and excellent absorption, anticipated based on the high Caco-2 cell permeability of 204 nm/s. Taken together, these properties predict the complete bioavailability of this compound that is observed in the rat. Whilst the potency of **1am** represents a 10-fold improvement compared to **1b**, further profiling of this compound was not pursued after in vitro studies with human liver microsomal preparations revealed that demethylation of each of the methoxy moieties were prevalent metabolic pathways, arousing concern for the potential of *para*-quinone formation in vivo should further oxidative metabolism of this electron-rich ring system occur. Quinones are chemically reactive electrophiles that have been shown to form covalent adducts with proteins and nucleotides and are associated with a number of toxicological effects.[18–20] Indoloquinones demonstrate a range of biological properties,[21] including cytotoxic acitvity in vitro, and metabolic

Table 2
Pharmacokinetic parameters of compounds **1a**, **1b**, **1al** and **1am** in the rat

	1a	1b	1al	1am
Rat IV: dose[a]	5 mpk (n = 3)	5 mpk (n = 3)	1 mpk (n = 2)	1 mpk (n = 2)
CL (mL/min/kg)	57 ± 11	48 ± 6	46.9 (44.5/49.2)	5.2 (5.4/5.1)
Vss (L/kg)	1.5 ± 0.3	0.99 ± 0.08	0.85 (0.9/0.79)	0.52 (.39/0.65)
Terminal $T_{1/2}$ (min)	30 ± 12	28 ± 7	17 (20/14)	138 (120/156)
MRT (min)	26 ± 4	21 ± 3	19 (23/16)	102 (72/126)
Rat PO: dose[a]	25 mpk, n = 3	25 mpk, n = 3	5 mpk, n = 2	5 mpk, n = 2
C_{max} (ng/mL)	1037 ± 871	649 ± 324	55 (80/30)	3200 (3100/3400)
T_{max} (min)	33 ± 12	53 ± 59	20 (10/30)	180 (240/120)
Terminal $T_{1/2}$ (min)	163 ± 131	72 ± 20	551 (684/416)	111 (108/114)
F (%)	29 ± 15	17 ± 9	11 (8.3/13)	107 (113/102)

[a] Dosed as solutions in PEG400/EtOH (90:10).

Table 3
Pharmacokinetic parameters for different formulations of compound **1b** in the rat and dog

Entry #	Species	Dose	Formulation	C_{max} (ng/mL)	T_{max} (min)	F (%)
1	Rat	5 mpk (n = 3)	PEG 400/EtOH (90:10)	57 ± 13	37 ± 46	9 ± 2
2	Rat	25 mpk (n = 3)	PEG 400/EtOH (90:10)	649 ± 324	53 ± 59	17 ± 9
3	Rat	5 mpk (n = 3)	Nanosuspension (184 nm)	18 ± 7	170 ± 165	4 ± 0.2
4	Dog	1 mpk (n = 3)	PEG 400/EtOH (90:10)	560 ± 84	72 ± 17	89 ± 14
5	Dog	1 mpk (n = 3	Micronized suspension (46 μm)	60 ± 26	240 ± 0	12 ± 6
6	Dog	1 mpk (n = 3)	Nanosuspension (184 nm)	160 ± 32	108 ± 17	36 ± 6

activation of **1am** in vivo may lead to toxicity, possibly idiosyncratic in nature.[22–24]

Consequently, from these four compounds attention was focused on the 4-fluoro analog **1b**, a compound that demonstrated a sufficiently promising antiviral profile towards a panel of HIV-1 viruses replicating in cell culture to be a useful vehicle for a more detailed evaluation. In probing **1b** in greater depth, the objective was to develop a greater appreciation for the potential of the chemotype and to identify any issues that might interfere with a drug development campaign. Whilst the pharmacokinetic profile of **1b** in higher species was quite encouraging following oral administration as a solution in PEG 400/ethanol and increases in exposure were observed with increased dosage in rats, problems were encountered when the compound was dosed to rats as a suspension in water containing 0.75% w/w of Methocel 4AM and 0.5% w/w of Pluronic F68.[1] The results of experiments conducted with different formulations in rats and dogs are summarized in Table 3. After oral administration of a solution of **1b** to rats at 5 mpk, a C_{max} of 57 ng/mL was observed at 37 min after dosing and bioavailability was 9% (Table 3, entry 1). Increasing the dose to 25 mpk as a solution resulted in an 11-fold increase in C_{max} with a T_{max} delayed to 53 min and an increase in bioavailability to 17% (Table 3, entry 2). However, dosing a nanosuspension in water gave a C_{max} that was only 31% of that of the solution formulation and the significant increase in T_{max} is indicative of slowed absorption (Table 3, entry 4). An analogous series of experiments in the dog revealed a similar pattern of exposure with the C_{max} after a micronized suspension ~10% of the solution formulation and the T_{max} markedly extended (Table 3, entries 5 and 6). A nanosuspension formulation of **1b** increased the C_{max} almost threefold relative to the micronized form whilst reducing T_{max}. The relative oral bioavailabilities of the micronized suspension and the nanosuspension compared to the solution formulation were 13% and 40%, respectively. These findings were reflected in the physical properties of **1b** which exhibited a melting point of 236 °C and solubility ranging from 5 ng/mL in water to 4.6 mg/mL in PEG 400. Taken together, these data indicated that the poor performance in vivo following oral administration of suspension formulations could be attributed to poor dissolution in the gut, a problem only partially addressed by reducing particle size.

In summary, structure–activity relationships associated with this series of indole-based HIV-1 attachment inhibitors provide fundamental insights into the optimal indole substitution patterns and compounds with increased potency were readily obtained by the introduction of relatively simple substituents at C-4 and C-7. Whilst representative compounds demonstrated good pharmacokinetic properties in the rat and dog following oral administration as solutions in PEG-400/ethanol, the physical properties associated with the chemotype resulted in poor performance in vivo following dosing of aqueous suspensions. As a consequence, attention was directed towards identifying analogs with improved intrinsic solubility that would demonstrate enhanced dissolution properties, the results of which will be communicated in due course.

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References and notes

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