Bis(heteroaryl)piperazine (BHAP) Reverse Transcriptase Inhibitors: Structure-Activity Relationships of Novel Substituted Indole Analogues and the Identification of 1-[(5-Methanesulfonamido-1*H*-indol-2-yl)-carbonyl]-4-[3-[(1-methylethyl)amino]-pyridinyl]piperazine Monomethanesulfonate (U-90152S), a Second-Generation Clinical Candidate

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Retroviruses such as human immunodeficiency virus type 1 (HIV-1) contain an enzyme, reverse transcriptase (RT), which catalyzes the conversion of the genomic viral RNA into the proviral DNA. This process of reverse transcription is an essential step in the life cycle of HIV-1 and is absolutely required for viral replication.[1] For this reason, the enzyme reverse transcriptase is an ideal target for the development of therapeutics for the treatment of HIV infection.

Reverse transcriptase inhibitors (RTIs) such as 3′-azido-3′-deoxythymidine (AZT)[2] and 2′,3′-dideoxyinosine (ddI)[3] are nucleoside drugs which mimic the normal deoxynucleoside triphosphate substrates for RT and act as chain terminators. Since these drugs have been shown to benefit HIV-1-infected patients, they have been approved by the FDA for the treatment of AIDS. Unfortunately, administration of these compounds to patients often causes serious toxic side effects,[4] thought to be due to the inhibition of cellular DNA polymerases (e.g. lack of selectivity).[2b,3b,4,5] Moreover, treatment with AZT or ddI results in the emergence of resistant viral strains.[6] The successful treatment of HIV-1 infection will most likely require the discovery of drugs which inhibit RT via disparate mechanisms, possibly to be administered in combination with one another. In order to maximize the chances of discovering an inhibitor with a mechanism of action different from that of AZT and ddI, we pursued a strategy which targeted the identification of *non-nucleoside* inhibitors of RT and their subsequent optimization via molecular modification.

A recent disclosure from our laboratories described the discovery and preliminary elucidation of the structure-activity relationships (SAR) of the bis(heteroaryl)piperazine (BHAP) class of RT inhibitors.[7] The BHAPs were discovered via a computationally[8] directed broad screening of the Upjohn chemical repository for RT inhibitors. Chemical modification of the original lead compound, through incorporation of an indole moiety, resulted in dramatically improved anti-HIV-1 activity and led to the identification of atevirdine mesylate (Table I, 2), which is currently undergoing phase II clinical evaluation.[7]

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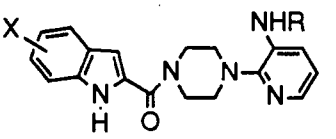
Table I. Biological Activities of 5- and 6-Substituted Indole Analogues



compound	X	R	RT inhibition[a] *in vitro* % 100 μM	RT inhibition[a] *in vitro* IC_{50} (μM)	MT-2/IIIb[b] ED_{50} (μM)	PBMC/D34[b,c] ED_{50} (μM)
1	H	Et	96	4.0	0.3	0.01
2[d]	5-OCH_3	Et	92	5.2	<0.2	0.001
3	5-OH	Et	89	2	0.27	0.01-0.1
4[d]	H	*i*-Pr	96	1.2	0.3	0.001
5	5-OH	*i*-Pr	98	1.3	<0.26	0.001
6	5-OCH_2Ph	*i*-Pr	74	4.3	<0.21	0.01
7	5-CH_3	*i*-Pr	68	34	0.26-2.6	0.01
8	5-NH_2	*i*-Pr	94	1	ND	0.01-0.1
9	6-OCH_3	*i*-Pr	97	1.3	<0.25	0.01
10	6-F	*i*-Pr	96	4.4	0.26-2.6	0.001-0.01
11	6-OCH_2Ph	*i*-Pr	65	37	ND	0.1
12	6-OH	*i*-Pr	97	1.2	ND	ND
13	6-CN	*i*-Pr	74	3.9	ND	<0.0001
14	6-CHO	*i*-Pr	94	1.4	<0.26	0.0001
nevirapine	NA	NA	93	5.5	ND	0.01-0.1
L-697,661	NA	NA	98	2.7	<0.3	<0.0001

[a] The HIV-1 RT *in vitro* assay was carried out with recombinant enzyme[23] using the template:primer poly (rA):$(dT)_{10}$ and dTTP as the mononucleotide substrate as described.[24] IC_{50}s were determined by assaying at four drug concentrations. [b] See footnote 26 for description of assay. A less than symbol (<) indicates that the ED_{50} was not reached at the lowest concentration tested. [c] See footnote 25 for description of assay. CC_{50}s for all compounds were >10 μM. ED_{50} = 50% effective antiviral dose. ND = not determined. [d] Compounds tested as mesylate salts in PBMC assay.

Other classes of potent and selective non-nucleoside reverse transcriptase inhibitors (NNRTIs) including the thiobenzimidazolones (TIBO),[9] dipyridodiazepinones,[10] pyridinones,[11] TSAO compounds,[12] and the HEPT[13] compounds have also been recently reported.[14] The rapid emergence of drug-resistant HIV-1 to several of these NNRTIs, such as the pyridinone L-697,661[11] and nevirapine,[10] requires the discovery of more potent NNRTIs that might be used in combination to inhibit such resistance development.[15-18] Therefore, we embarked on a program to discover and identify a second-generation BHAP candidate which retained the desirable attributes of atevirdine mesylate (i.e. animal safety, desirable pharmacokinetics), but which improved upon its antiviral potency. Toward this end, we concentrated on three types of structural modifications: substitution of the indole moiety, alteration of the central linker (piperazine surrogates), and substitution of the aminopyridine portion. Herein, we focus primarily on the SAR of the indole nucleus and describe the synthesis, inhibitory activity vs recombinant HIV-1 RT, and antiviral activities of 5- and 6-monosubstituted indole analogues.

We initially became interested in preparing 5-substituted indoles in order to deter potential metabolic 5-hydroxylation of the indole nucleus.[19] As a result, the 5-methoxyindole (atevirdine mesylate, 2) and 5-fluoroindole analogues described previously were synthesized and shown to possess good anti-HIV-1 activities.[7] In order to further explore optimum substitution patterns, a variety of other 5-substituted and 6-substituted analogues were prepared and evaluated. These compounds were readily prepared by the route outlined in Scheme I. Coupling of

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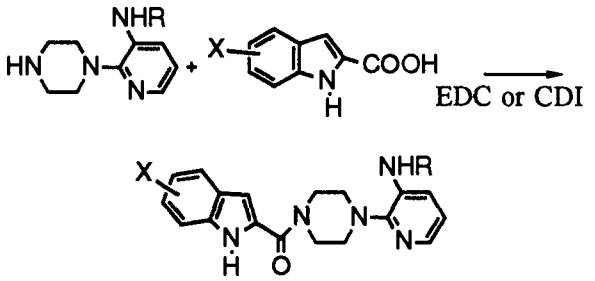
Scheme I



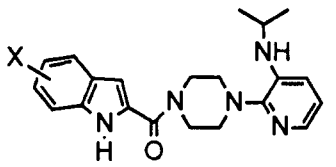
a pyridylpiperazine[20] with a 5- or 6-substituted indole-2-carboxylic acid was accomplished with EDC (1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide) or CDI (1,1'-carbonyldiimidazole). Subsequent modification of the indole substituent, e.g. reduction of the nitro group and acylation or sulfonylation of the resulting amine, afforded the desired derivatives. The requisite indoles were prepared by condensing methyl azidoacetate with substituted benzaldehydes to form the vinyl azides which were thermolyzed according to the procedure of Hemetsberger.[21,22]

Initially each compound was evaluated for inhibitory activity in an *in vitro* recombinant HIV-1 RT screening assay.[23,24] Active compounds identified via this screen were further examined to determine their inhibition of HIV-1 replication in human lymphocytes [peripheral blood mononuclear cells (PBMC)[25] and/or MT-2 cells[26]] as previously described.[27] A comparison of the data obtained from these two assays aided in future molecular design by allowing us to assess each compound's inherent potency against RT as well as the effect of structural modifications on cellular pharmacokinetics such as penetration and metabolism. Presented in Table I are results obtained in an investigation of the effect of indole substitution on activity in the above assays (nevirapine and L-697,661 are included for comparison).

We were encouraged to find that certain indole substitution patterns exert a beneficial effect on RT inhibitory activity and reduced replication of HIV-1 as compared to the unsubstituted indoles. For the purposes of discussing the SAR, indole substitutions were compared within the same structural series, either the 3-(ethylamino)pyridyl series (1–3) or the 3-(isopropylamino)pyridyl series (5–24). For example, 6-cyano- (14) and 6-formyl- (13) substituted indole congeners are more potent at blocking HIV-1 replication than the unsubstituted parent 4. In both the ethylamino or isopropylamino series, 5-methoxy- (2) and 5-hydroxy- (3,5) substituted analogues possess potencies comparable to their respective parents 1 or 4. A 5-methyl substituent (7) adversely effected potency compared to its (isopropylamino)pyridyl parent 4. Sterically demanding groups exemplified by 5-benzyloxy (6) and 6-benzyloxy (11) clearly lessen RT inhibition and potency in cell culture as compared to the unsubstituted congener 4. Even though the factors governing the effects of nuclear indole substitution were not well understood, we hoped to increase anti-HIV-1 activity and potentiate other pharmaceutical properties by manipulating such substituents.

At the time a chemical strategy was being formulated, preliminary biological results indicated an improvement in potency for the hydroxylated indoles. These results compelled us to attempt substitution of the indole ring with known phenolic bioisosteres[28,29] (Table II) in order

Table II. Biological Activities of BHAP Analogues Incorporating Hydroxy Bioisosteres



compound	X	RT inhibition[a] % 100 μM	RT inhibition[a] IC_{50} (μM)	MT-2/IIIb[b] ED_{50} (μM)	PBMC/D34[b,c] ED_{50} (μM)
15	5-OSO_2CH_3	97	5.5	ND	<0.0001
16[d]	5-$NHSO_2CH_3$	98	1.1	0.01	0.0001
17	5-$NHCOCH_3$	94	1.8	>10	0.01
18	5-$NHCO(OCH_3)$	96	2.5	ND	0.0001
19	5-$NHCOCF_3$	85	27	0.21	0.001
20	5-$NHSO_2CF_3$	94	8.3	0.2–2	0.1
21	6-$NHSO_2CH_3$	98	0.50	<0.02	0.0001
22	6-OSO_2CH_3	97	2.2	ND	<0.0001
23	6-CH_2OH	98	0.76	<0.25	0.001
24	5-$NCH_3SO_2CH_3$	95	5.6	ND	0.001
AZT	NA	NA	NA	0.07	0.001

[a–c] See legend, Table I. [d] CC_{50} (MT-2) >30 μM; CC_{90} (PBMC) ≈ 10 μM. NA = not applicable. ND = not determined.

to capitalize on the increased potency while avoiding the anticipated phase 2 conjugative metabolism at the hydroxyl group.[30] Fortunately, several surrogates effectively mimicked a 5- or 6-hydroxy group in terms of biological activity. Analogues bearing 5-methanesulfonamido (16), 5-(methoxycarbonyl)amino (18), or 6-methanesulfonamido (21) effectively inhibited replication of HIV-1 in cell culture at concentrations less than the parent 5-hydroxy compound 5. Interestingly, the 5-acetamido (17) and 5-trifluoromethanesulfonamido (20) groups exert a deleterious effect on potency in cell culture compared to the unsubstituted parent, 4. The excellent potency of the methanesulfonamido surrogate prompted the preparation of several additional analogues. For example, a methanesulfonamido analogue of a secondary 5-aminoindole (24) was prepared and determined to decrease inhibition of RT compared to 16 which paralleled the results obtained in cell culture. Furthermore, preparation of the methanesulfonyloxy-containing analogues 15 and 22 afforded two of the most potent compounds tested in the antiviral assay conducted in PBMC. At 0.1 nM, the lowest concentration tested, HIV-1 replication was inhibited >50% making these compounds much more potent than AZT.

In order to select an appropriate second-generation candidate, a representative of the substituted indole series was evaluated more thoroughly in parallel with several BHAPs with alternative molecular modifications (e.g. changes in central linker, etc.). A 5-substituted indole analogue was selected for this in-depth evaluation due to its greater chemical accessibility and concerns that a 6-substituted indole (especially an indole with an electron rich substituent) might have a greater propensity to undergo oxidative metabolism at the 5-position.[31] To quantitate the improvement in biological activity introduced by the newer indole substituents, 5-methanesulfonamidoindole 16 was selected for a direct comparison with AZT and atevirdine mesylate (U-87201E, 2) in a more rigorous RT assay. Determination of the IC_{50} from the slopes of median effect plots (12 concentrations) with at

least two independent determinations using highly purified RT showed that compound 16 had an IC_{50} comparable to that of AZT-triphosphate (0.26 and 0.15 μM, respectively) and approximately 5-fold lower than that of 2.[32] The ED_{50}s of 16, AZT, and 2 against HIV-1_{IIIB} in PBMC were 0.1–1, 1, and 1–10 nM, respectively.[32] Assays which measured the spread of HIV-1_{IIIB} in human lymphocytes over extended times (85 days, 500 MT-4 cells mixed 1:1000 with uninfected cells) *in vitro* indicated that 16 was much more effective than AZT.[32] AZT at a concentration of 3 μM delayed rapid viral growth for 7 days, whereas 16 at a concentration of 3 μM totally prevented the spread of HIV-1. Analogous to other BHAPs,[7] AZT and 16 worked synergistically to block HIV-1 replication in this type of assay.[32] On the basis of these and other more in-depth evaluations of the potency, and safety and pharmacokinetic profile in animals, phase I clinical evaluations of 16 (U-90152S)[33] were initiated.

The acquisition of HIV-1 resistance to several potent NNRTIs such as nevirapine, TIBO, and pyridinone derivatives has been described[15–18] and is an important issue relating to the potential clinical utility of 16. The observation that these structurally diverse inhibitors interact with RT at a common binding site,[11,34] and the observation of cross resistance among some of these inhibitors has led to the view that the emergence of HIV-1 variants resistant to one of these NNRTIs could compromise the effectiveness of the others.[34] Therefore, we quantitated the *in vitro* sensitivities of 16, L-697,661, and nevirapine to recombinant HIV-1_{IIIB} RTs which carried the previously described amino acid substitutions[15–18] conferring resistance to nevirapine or L-697,661 [tyrosine-181 to cysteine (Y181C) and lysine-103 to asparagine (K103N)].[35] Both of these mutations conferred resistance to all NNRTIs tested, but we were surprised to find that compound 16 retained significant inhibitory activity against the recombinant RT with the Y181C substitution (IC_{50} = 8.3 μM) while nevirapine and L-697,661 failed to achieve 50% inhibition at 60 μM, the highest concentration tested. This suggests that amino acid 181, while contributing to the affinity of all three drugs for RT, is a less important factor in the binding of 16. Moreover the significant inhibitory activity of 16 against the known non-nucleoside-resistant RTs, combined with the attainment of serum drug levels in laboratory animals well in excess of those required for this *in vitro* activity, bodes well for the potential clinical utility of 16.

In order to derive BHAP resistant HIV-1 variants, HIV-1 was serially passaged *in vitro* in the presence of increasing concentrations of 16. Variants 100-fold resistant were obtained and utilized to characterize the mode of BHAP resistance. Remarkably a novel substitution in RT at conserved proline 236 to leucine (P236L) was found to be responsible for conferring resistance.[35] Determination of the sensitivity of the mutant RT with the P236L substitution to other drugs indicated that it was not cross resistant to nevirapine,[10] or L-697,661,[11] but remarkably was 7–10-fold more sensitive to inhibition than the wild-type enzyme.[35] To confirm this sensitization cell culture experiments were performed, and in fact, lower concentrations of nevirapine or L-697,661 (5–20-fold) were required to completely inhibit viral replication of BHAP-resistant HIV-1 variants as compared to wild-type HIV-1. If the mutations that arise upon treatment of HIV-1 infected patients with U-87201E or 16 parallel those derived in cell culture, initial treatment with a BHAP may result in a population of virus that is sensitized to inhibition by other NNRTIs.

In summary, inclusion of certain 5- or 6-substituents on the indole nucleus illustrated that slight structural modifications in the BHAP template can cause dramatic increases in potency. Several BHAPs with potencies greater than AZT were designed employing this approach. Moreover experiments with one of these compounds, clinical candidate U-90152S (16), demonstrated that significant activity against known non-nucleoside-resistant forms of RT can be obtained by a non-nucleoside drug. Furthermore, the development of HIV-1 resistance to U-90152S can increase the vulnerability of HIV-1 to other inhibitors. This intriguing property of U-90152S clearly indicates that HIV-1 resistant to one NNRTI will not necessarily be cross resistant to another NNRTI and emphasizes the importance of investigating the combination of different NNRTIs in HIV-1 infected patients.

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Supplementary Material Available: Experimental procedures, including analytical and spectral data, for 8 and 16 (2 pages). Ordering information is given on any current masthead page.

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(26) MT-2 cells were infected with HIV-1 (IIIb isolate) at a multiplicity of infection of 0.001. Syncytium formation was determined 4 days after infection at the peak of the viral cytopathic effect. Drugs were tested at three concentrations via a series of 10-fold dilutions. When the apparent ED_{50} fell between two such dilutions the range is reported. Cell viability was determined by trypan blue exclusion.

(27) Comparisons of compounds should be made within a particular assay. Discrepancies between the enzyme assay and the whole-cell assays could be introduced by variables such as cell penetration and uptake, metabolism, and use of unnatural template:primer. The outcome of these cell based assays is dependent on factors such as cell type, amount of infectious innocula, method of endpoint determination, strain of virus and time line of the assay.

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