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SYNTHESIS AND HIV-1 INHIBITION OF NOVEL BENZIMIDAZOLE DERIVATIVES.

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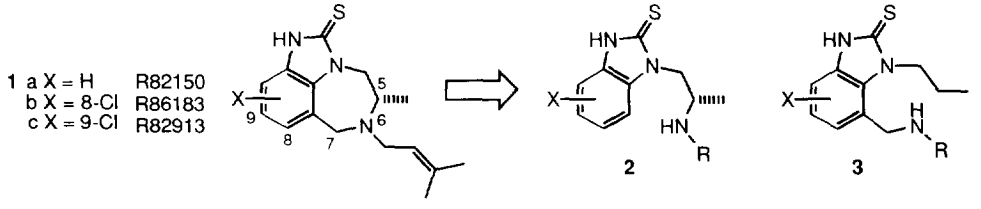
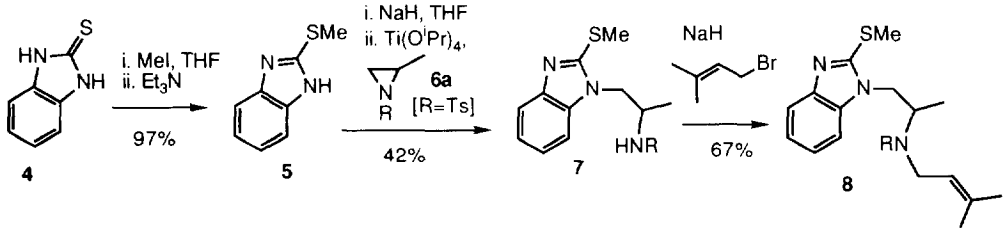
Abstract: A range of novel benzimidazole derivatives, some bearing analogy to TIBO, have been synthesized, and evaluated for inhibition of HIV-1 infectivity. The most active and selective compounds are a series of N-alkoxy-2-alkyl-benzimidazoles, several having $EC_{50} < 10\mu M$ (one sub-micromolar at 600nM), and selectivity ratios of 10-167. The most selective benzimidazoles, **18a**, **18c**, show modest RT inhibition, and binding assays indicate gp120-binding is not a target.

Clinical therapeutics for treatment of human immunodeficiency virus currently consist of the nucleoside analogues AZT, ddC, ddI and recently d4T, all of which, after intracellular phosphorylation, are inhibitors of HIV Reverse Transcriptase (RT). A large number of other nucleoside analogues, including sugar ring modified, carbocyclic and acyclic analogues, have been evaluated as potential HIV-RT inhibitors, and though a few are promising, none have yet been approved for clinical use. The search for other anti-HIV therapeutic avenues continues apace, targeting most stages of the viral life cycle. A current research focus is the search for lead agents with novel structures and novel mechanisms of action. A number of natural product[1] leads possessing wide structural diversity have emerged from the extensive screening programs established for HIV inhibition. Though some of these are RT inhibitors, alternative sites of action are proposed or have been confirmed for other compounds. Screening of *non-natural* products has also yielded a number of new anti-HIV agents, all specific for HIV-1, including several different types of non-nucleoside RT inhibitors, notably certain 2-pyridinones[2], bis-heteroarylpiperazines[3], HEPT[4] and derivatives[5], and benzodiazepine derivatives, nevirapine[6] , and those based on the TIBO ring system, **1**[7]. Nevirapine and TIBOs inhibit HIV-RT at a non-substrate, allosteric site[8a], and structural studies, including an X-ray structure of nevirapine bound to HIV-1 RT[8b], have been extensive. The TIBO-based compounds, first reported in 1990[7], attracted attention as several members showed potent viral inhibition but much lower toxicity than nucleoside antiviral analogues, though subsequent clinical evaluations have indicated that, like anti-HIV nucleoside analogues, development of resistant HIV-1 strains is problematic[9]. Numerous tricyclic TIBO analogues have been evaluated[10,11], however, until recently, no antiviral testing had been reported for mimics not possessing all three rings.
Amongst our interests in antiviral agents has been the synthesis of novel, acyclic analogues of the TIBO ring system, lacking the benzodiazepine ring, i.e. analogues of general structure, **2**, possessing a 1-(2-aminopropyl) substituent on a 2-mercaptobenzimidazole, or analogues of type **3**. We intended that such intermediates would also facilitate elaboration to the complete TIBO ring system, providing a common, divergent route to both TIBO derivatives, and novel acyclic analogues.

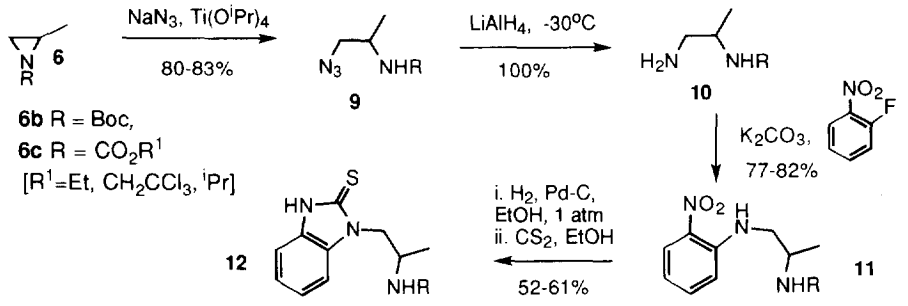


Recently, Townsend *et al*[12] have reported synthesis and anti-HIV activity of some 1-(2-aminopropyl)-mercapto-benzimidazole (analogous to **2**, with X=H), and herein we wish to report our route to similar compounds, as well as

serendipitous synthesis of more accessible benzimidazoles which proved to be more active. We envisaged that the 2-aminopropyl substituent of **2**, and analogues thereof, could be introduced, in a single step, *via* nucleophilic ring opening of simple 2-substituted aziridine precursors. This strategy was attractive for several reasons. The regioselective ring opening of N-protected-2-substituted (2-alkyl or carboxylate group) aziridines with S, O and N heteroatom nucleophiles has extensive precedent. 2-Methylcarboxylate aziridine and derivatives are readily prepared homochiral from L-serine[13a], while convenient resolution procedures have been reported for racemic 2-alkyl aziridines[13b], and a number of practical asymmetric synthetic approaches are also now available[13c]. Thus, aziridine ring opening chemistry promised a potentially short general route to a range of 1-(2-aminopropyl) substituted mercaptobenzimidazoles structurally related to the TIBO ring system. Two approaches were developed. The first involved Lewis acid promoted regioselective opening of N-activated 2-methyl aziridines by the sodium anion derived from **5**. This introduced the 1-(2-aminopropyl) function in two steps and 40% overall yield from 2-mercaptobenzimidazole, **4**. Best yields were obtained using the N-tosyl aziridine, **6a**. The resulting compound **7** [R=Ts][14] was alkylated in good yield with 3,3-dimethylallyl bromide to afford **8** [R=Ts], bearing key structural features of target benzimidazoles, **2**. Disappointingly, deprotection of **8** proved low yielding.



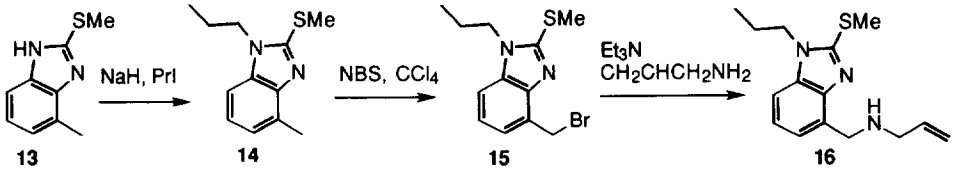
An alternative second route - which also directly provided compounds with the 2-mercaptobenzimidazole ring system - also utilized ring opening of 2-methylaziridine, but in a convergent strategy. N-Carbamate-2-methylaziridines, **6b,c**, were ring opened in good yields with NaN_3 using $Ti(O^iPr)_4$ as Lewis acid promoter [ethyl and trichloroethyl carbamates were concomitantly trans-acylated to the isopropylcarbamates]. The azides, **9**, were selectively reduced in quantitative yields by $LiAlH_4$ at -30°C to afford the 2-isopropylcarbamate protected 1,2-diaminopropanes, **10**. Nucleophilic aromatic substitution of 2-fluoronitrobenzene by **10** proceeded to give very good yields of the bright orange coloured nitroaniline derivatives, **11**. Hydrogenation then ring closure with CS_2 provided the acyclic TIBO analogues, **12**.



Evaluation of these compounds for inhibition of HIV-1 infectivity, showed **8** [R=Ts] and **7** [R=Ts] to be modest inhibitors, with EC_{50}s of 20 and 4μM respectively (comparable to closer analogues of **2**[12]). TC_{50} values were 100 and 8μM respectively, indicating **8** [R=Ts] is less toxic, but that both compounds have only very modest selectivity indices.

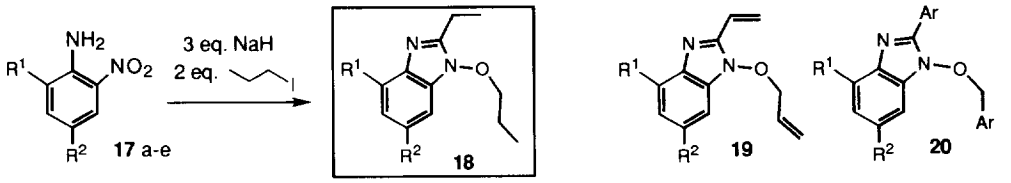
Since the 2-thiomethyl analogues seemed to have similar activity to acyclic 2-mercapto analogues of type **2**, we decided to also prepare other 2-thiomethyl, further substituted analogues, specifically, 4-bromomethyl- and 4-(N-allylaminomethyl)-1-propyl-2-thiobenzimidazoles **15** and **16** by the route outlined below from **13**. The 4-(N-allylaminomethyl)-1-propyl-2-thiobenzimidazoles **16** showed no selectivity for HIV-1, and cytotoxicity of 40μM. However, the 4-methyl- and 4-

bromomethyl- analogues, **14** and **15** were more active, with EC_{50} values of 4 and 8μM respectively, and selectivity indices of 25 and 10, showing modest antiviral selectivity, a little better than the 1-(N-2-aminopropyl) derivatives, **7** and **8**, evaluated.



13 **14** **15** **16**

Attempts to prepare 4-methyl-3-propyl-2-thiomethylbenzimidazole, the regioisomer of **14**, by N-alkylation of 2-methyl-6-nitroaniline led instead to a novel N-alkylation-cyclization-O-alkylation cascade reaction affording 2-ethyl-4-methyl-1-propyloxybenzimidazole as the major product in 73% yield[15]. This methodology proved applicable to synthesis of a range of benzimidazoles with various aromatic ring substitution patterns. Propyl iodide gave the benzimidazoles, **18**, and allyl and benzylic bromides provided the N-allyloxy-2-vinyl- and N-benzyloxy-2-aryl-benzimidazoles, **19** and **20**, respectively.



17 a-e **18** **19** **20**

a R^1=Me, R^2=H; b R^1=H, R^2=OMe; c R^1=H, R^2=Cl
d R^1=H, R^2=Me; e R^1=R^2=H

The benzimidazoles with *unsaturated* functionality showed varying toxicity, but *in all cases there was essentially no antiviral selectivity over host toxicity.* However, surprisingly, all the 2-ethyl-1-propyloxy-benzimidazoles, **18**, were found to inhibit HIV-1 infectivity with EC_{50} values of 0.6-6μM, with antiviral selectivity ratios of 12.5-167. Four of these compounds have selectivity ratios >30 [Table 1]. These 2-ethyl-1-propyloxy-benzimidazoles compare very favourably with the acyclic TIBO analogues reported recently[12]. Hydrogenolysis led to loss of the propyloxy group affording the 2-ethylbenzimidazole systems, which showed a complete loss of any antiviral selectivity.

Table 1

Compound	HIV-1 Infectivity[a] EC_{50} (μM)	Cytotoxicity TC_{50} (μM)	Selectivity Ratio TC_{50} / EC_{50}	RT Inhibition μM	RT Inhibition % inhibition
14	4	100	25		
15	8	80	10		
18 a	6	1000	167	100	66
18 b	3.2	100	31		
18 c	0.6	40	67	10	50
18 d	1.6	20 - 30	12.5 - 19		
18 e	2 - 3	100	33 - 50		
AZT	0.016	>1000			
R82913	0.002	62	31000	0.16	50

[a]Compounds were tested against HIV-1_{111B} in C8166 cells. Inhibition of viral replication was measured by determining reduction of syncytia formation and antigen gp120, estimated by ELISA. RT inhibition assay employed poly(rA).oligo(dT)$_{12-18}$ template, and RT from HIV-1_{IIIB}.

Evaluation of the two compounds with highest selectivity ratios, **18a** and **18c**, for inhibition of HIV-1 RT, showed that these compounds do inhibit RT in the 10-100μM range, but do not approach total inhibition at concentrations well above their EC_{50} values (table 1). These compounds showed no ability to prevent binding of monoclonal antibodies 358 or 388, indicating that gp120 is not a target for these compounds[16]. However, pre-incubation of **18a** with virus for 60 minutes at

37°C prior to infectivity assays, lead to slightly enhanced anti-HIV-1 activity (by a factor of ca. 1.5). Whether RT inhibition is the major mechanism of action of **18** is yet to be determined, since in the current assays R82913 shows a similar difference between HIV-1 infectivity and RT inhibition. [Other recently reported 1-(2-aminopropyl) acyclic TIBO analogues[12] provided data suggesting RT may not be their major target of action.]

In summary, the novel benzimidazoles reported here, are a new group of HIV-1 inhibitors, all aspects of whose mechanism of action is as yet uncertain. It should be noted that these compounds are readily prepared in a single pot reaction from commercially available 2-nitroanilines and propyl iodide. The reaction appears relatively general for other alkyl halides, the N-alkoxy group can be removed by hydrogenolysis and replaced, and thus, synthesis of a wide diversity of analogous compounds should be readily available. Further investigation of other possible viral targets of action will be undertaken.

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