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STRUCTURE-ACTIVITY RELATIONSHIPS OF ANTI-HIV-1 *N*-ALKOXY- AND *N*-ALLYLOXY-BENZIMIDAZOLES.

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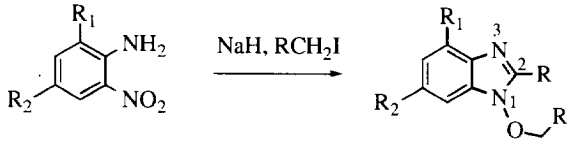
Abstract: One-pot benzimidazole syntheses have been used to prepare an extended series of novel analogues which were evaluated against HIV-1 infectivity, the most active having an EC_{50} of 0.5μM. There is a correlation between the length of saturated alkyl groups at O1 and C2 with antiviral selectivity. Replacing vinyl by the 2,2-dimethyl vinyl group increases antiviral selectivity.

A number of types of non-nucleoside reverse transcriptase inhibitors (NNRTIs),[1] generally HIV-1 specific, have been discovered since the TIBO (4,5,6,7-tetrahydro-5-methylimidazo[4,5,1-*jk*][1,4]benzodiazepino-2(1*H*)-thione) compounds were reported in 1990[2] to be potent RT inhibitors with much lower toxicity than the antiviral nucleoside analogues. Amongst these are several related families of non-nucleoside heteroaromatic compounds, notably 2-pyridinones,[3] bis-heteroarylpiperazines,[4] HEPT (1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine)[5] and derivatives,[6] and a group of benzodiazepine derivatives, nevirapine and analogues.[7]

Details regarding the mechanism of RT inhibition by these agents have emerged. NNRTIs appear to share a non-substrate binding pocket, allosterically linked to active site function, and X-ray structures of a range of NNRTIs bound to RT have been reported.[8] These agents, however, have proven problematic due to the appearance of resistant HIV-1 strains for which in several cases the mutations leading to resistance have been identified.[9] Resistance could potentially be overcome through combination/alternating therapy, or may even be turned to advantage through analogues effecting 'suicidal mutation' of RT.[10]



1 R=Me
2 R=Et
3 R=Ph

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

Our interests in synthesis of acyclic analogues of TIBO-related compounds led us recently to the serendipitous discovery of a new class of 2-alkyl-*N*-alkoxybenzimidazoles **1** and **2** (prepared in a novel one-pot synthesis from 2-nitroaniline derivatives) which inhibited HIV-1 at μM levels.[11] The first series of compounds indicated that those containing *alkyl* groups, **1**, **2**, **5** and **6**, generally showed anti-HIV

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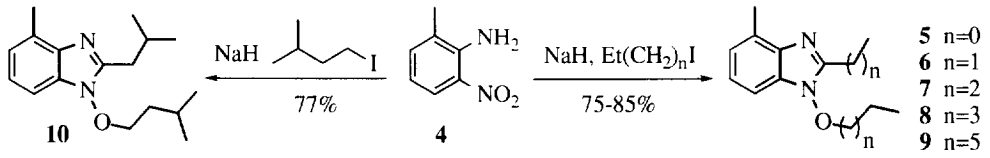
activity (EC_{50}=0.6-6μM) and antiviral selectivity (TC_{50}/EC_{50}=10-167), while those bearing *aryl* or *vinyl* functionalities, **3**, **14**, **16**, **18** and analogues, showed no selectivity. We thus undertook a structure-activity study relating to, firstly, derivatives of the most active benzimidazole **6**, and also related to the non-selective vinylic benzimidazoles. Herein we report the preparation and evaluation of a systematic group of new derivatives prepared to assess: (1) the effect of changing the size (length) of the (linear) C2- and *O*-alkyl groups and (2) to establish whether introducing methyl substituted vinyl groups would provide any selectivity for HIV-1. To probe specifically the role of these C2 and N1 substituents, 6-methyl-2-nitroaniline, **4**, was chosen (the precursor of the most active alkyl compound **6**) for preparation of these analogues. The new benzimidazoles **7-9** were prepared by modifying our previously reported methodology.[12] The conversion of **4** to **5-9** was substantially improved (to routinely ≥75%) by employing excess (~6 eq.) of alkyl iodide and NaH.



The homologous series of alkyl benzimidazoles **5-9** indeed shows that antiviral selectivity is substantially altered by the length of the alkyl group, with selectivities and activities differing by up to two orders of magnitude [Figures **1** and **2**] through this series. These results have also provided the most active, and one of the most selective, *N*-alkoxybenzimidazoles we have found to date, with **7** having an EC_{50} of 0.5μM, and a selectivity index of 80. The lower homologue, **6**, although approximately 12 times less active than **7**, displayed a better overall selectivity index of 167, twice that of **7**.[11] However, the selectivity indices for shorter chain homologue **5**, and longer chain homologues **8** and **9**, are significantly lower (5-10). *Thus, there is a very clear differentiation, of 1-2 orders of magnitude, of the more selective (medium length alkyl) homologues **6** and **7**, from lower and higher homologues* [Figure **2**.]

Figure 1: EC_{50} (μM) of **5-9**

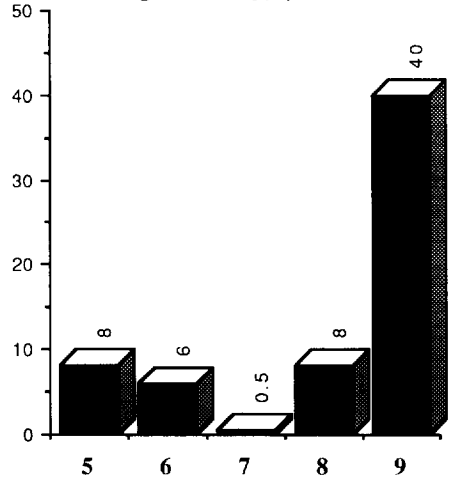
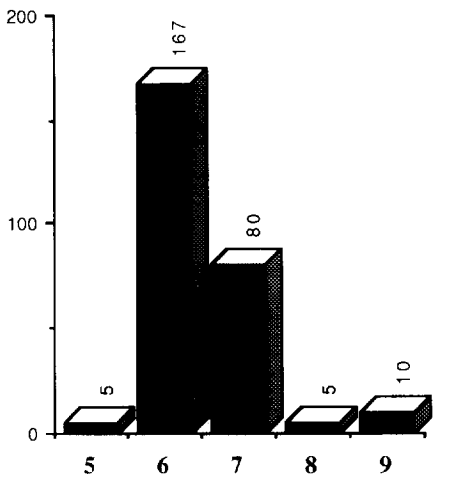
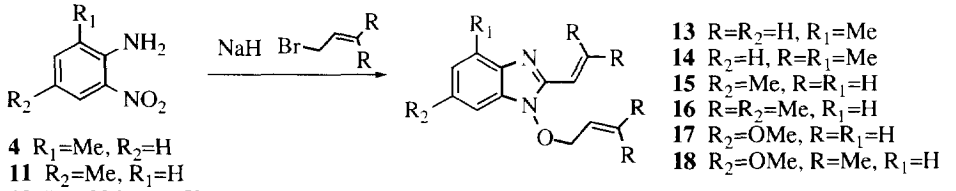


Figure 2: TC_{50}/EC_{50} of **5-9**



Attempts to prepare the branched isomeric analogue of **7** using 1-halo-2-methylpropanes (bromide or iodide) with **4** were unsuccessful, returning only starting material. This suggests perhaps that this reaction is sensitive to the steric effects of branching in the electrophile, a proposal supported by successful synthesis (77% yield) of **10**, a compound with branching more remote from the heterocycle.

Three pairs of vinylic benzimidazoles **13**-**18** were also prepared and the new 2,2-dimethyl vinyl analogues **14**, **16** and **18**, were evaluated against HIV-1 to compare with the vinyl analogues **13**, **15** and **17** [data previously reported[11]]. These are vinylic derivatives of the three nitroanilines **4, 11** and **12**, for each of which unsubstituted vinyl and 2,2-dimethylvinyl groups were introduced. This group of benzimidazoles was designed to systematically assess the effect of the extra methyl groups on the activity of these compounds. This also led to the simplification of the protocol for synthesis of vinylic (and benzylic) benzimidazoles where a single addition of 2 equivalents of alkyl iodide and 3.5 equivalents of NaH gave the products in very high yields (≥95%).



4 R_1=Me, R_2=H
11 R_2=Me, R_1=H
12 R_2=OMe, R_1=H

13 R=R_2=H, R_1=Me
14 R_2=H, R=R_1=Me
15 R_2=Me, R=R_1=H
16 R=R_2=Me, R_1=H
17 R_2=OMe, R=R_1=H
18 R_2=OMe, R=Me, R_1=H

Table 1[13]

Entry	Compound	HIV-1 Infectivity[a] EC_{50} (μM)	Cytotoxicity TC_{50} (μM)	HIV-1 Selectivity TC_{50} / EC_{50}
1	**13**	16	16	1
2	**14**	10	50	5
3	**15**	16	20	1.25
4	**16**	10	50	5
5	**17**	80	80	1
6	**18**	16	80	5
7	AZT	0.016	>1000	
8	R82913	0.002	62	31000

[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.

In all three cases the 2,2-dimethylvinyl substituted systems do show increased selectivity for the virus. While the 4-methyl and 6-methoxy vinyl-bearing benzimidazoles, **13** and **17**, showed no selectivity, the corresponding 2,2-dimethyl systems **14** and **18** gave selectivities of 5 [**Table 1**]. In the latter case this is due to a direct 5-fold improvement in antiviral efficacy when compared to the unsubstituted vinyl analogue. For the 6-methyl benzimidazoles, selectivity increases from 1.25 for **15** to 5 for **16**. These results appear consistent with our hope that adding vinylic methyl groups would make the alkenes '*pseudo*-saturated', increasing selectivity.

In summary, an improved protocol has been used to prepare an homologous series of 2-alkyl-*N*-alkoxybenzimidazoles to compare to the previously reported most active compound of this type. This methodology appears truly general to alkyl groups (except β-branched) and to substituted allylic systems. The homologue **7** has the highest anti-HIV-1 efficacy so far, providing the second sub-micromolar inhibitor from this class of compounds. A correlation has been established between activity and in particular selectivity to the length of the alkyl group. Furthermore, there appears to be a general trend that changing vinyl to 2,2-dimethyl vinyl groups improves selectivity. These results encourage pursuit of further categories of N1 and C2 substituents and ring modifications.

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