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5-Alkyl-2-[(aryl and alkyloxylcarbonylmethyl)thio]-6-(1-naphthylmethyl) pyrimidin-4(3*H*)-ones as an unique HIV reverse transcriptase inhibitors of *S*-DABO series

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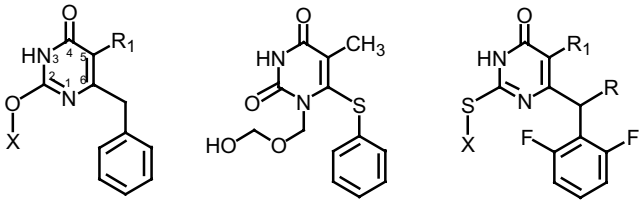
Abstract—The introduction of a β-carbonyl group to the C-2 side chain of *S*-DABO led to the finding of a series of novel potent anti-HIV agent. Some derivatives proved to be highly effective in inhibiting HIV-1 replication at nanomolar concentrations. Furthermore, the novel *S*-DABOs differ from the classical NNRTIs in that some compounds are active against both HIV-1 and HIV-2. They might interfere with another target or at least act on RT in a different way as compared to typical NNRTIs.

In the research of anti-AIDS agents, non-nucleoside inhibitors of reverse transcriptase (NNRTIs) have gained a definitive and important place due to their unique antiviral potency, high specificity and low toxicity.[1] To date, more than 30 different classes of NNRTIs have been reported. Among them, three compounds, viz. nevirapine,[2] delavirdine[3] and efavirenz,[4] have been approved by FDA for treatment of HIV-1-infected adults in combination with nucleoside analogues. Several other NNRTIs (MKC-442, calanolide A, tivirapine, HBY 097, etc.) have been or are currently being evaluated in human clinical trials.[5] Although these NNRTIs are structurally distinct, they share some common behaviours towards reverse transcriptase (RT): (i) All NNRTIs bind to the same allosteric site on HIV-1 RT, approximately 10 Å from the polymerase active site, and inhibit RT function by altering the shape of enzyme or by blocking the polymerase active site;[6] (ii) All NNRTIs are highly specific inhibitors of HIV-1 and, with the only exception of SJ3366,[7] have no efficacy against HIV-2 or other RTs.

Dihydro-alkoxy-benzyl-oxopyrimidines (DABOs, **1**), structurally related to HEPT[8] (**2**), were one of the most representative classes of NNRTIs developed in the past decade. Since the original synthesis of DABO,[9] many structural modifications have been performed with a view to increase its potency, which led to the discovery of a series of excellent DABO derivatives, including MTM-*S*-DABOs,[10] DATNOs,[11] F_2-*S*-DABOs[12] (**3**), etc. Their remarkable biological properties stimulated our interest in exploring these analogues. In particular, due to our recent success in the modification of C-6 position of HETPs,[13] we pay more attention to the research on the 6-(1-naphthylmethyl) substituted DABOs.



According to our molecular modeling investigations on the binding mode of DABOs to the NNBS of HIV-1 RT based on the crystallographic model of the RT/HEPT complex,[14] together with the previous structure–activity relationship (SAR) studies of DABOs,[15] we postulate that the introduction of an H-bond acceptor to the C-2

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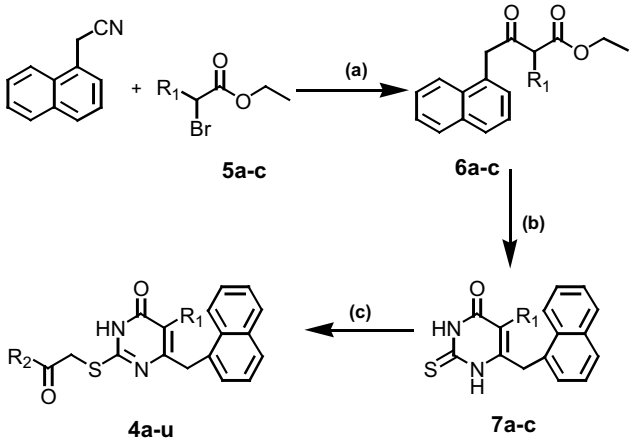
side chain of DABOs may be beneficial to enhance the interaction between the inhibitors and the RT. In order to examine our assumption and find more potent new NNRTIs, a series of 6-(1-naphthylmethyl) substituted *S*-DABO derivatives **4** bearing β-carbonyl on the C-2 side chain was designed.

Synthesis of the novel *S*-DABO derivatives **4a**–**u** is outlined in Scheme 1. Ethyl 2-alkyl-3-oxo-4-(1-napthyl) butyrates **6a**–**c** were prepared using the method of Hannick and Kishi[16] by reaction of 1-naphthylacetonitrile with activated zinc dust and ethyl 2-bromoalkanoates **5a**–**c**. Treatment of **6a**–**c** with thiourea in the presence of NaOEt in refluxing ethanol to give the 2-thiouracil **7a**–**c**, which were subjected to S-alkylation in anhydrous DMF with various halo-esters or halo-ketones in the presence of K_2CO_3 to afford the desirable *S*-DABO analogues **4a**–**u**, respectively. Both analytical and spectral data of all the synthesised compounds are in full agreement with the proposed structures.[17]

The novel *S*-DABO analogues (compounds **4a**–**u**) were evaluated for cytotoxicity and anti-HIV activity in MT-4 cells using the MTT method.[18] The used virus strains are HIV-1 strain III_B, HIV-2 strain ROD and SO561945 which is an HIV-1 (III_B) strain with typical NNRTI-selected mutations in the RT (K103N and Y181C), these mutations confer resistance towards the classical NNRTIs (nevirapine, delavirdine, sustiva, HEPT, etc.). The results, expressed as CC_{50}, IC_{50} and SI, are summarised in Table 1. The antiviral data on HEPT, nevirapine, DDI and efavirenz as reference compounds are also reported.

As shown in Table 1, many of these compounds inhibited HIV-1 replication in the lower micromolar concentration range, and the majority of them are noncytotoxic for MT-4 cells at doses as high as 170 μM, except for the five compounds, **4c**, **4h**, **4k**, **4p** and **4u**, which show CC_{50} values ranging from 6 to 85 μM.



Scheme 1. Reagents and conditions: (a) Zn, THF, then 10% HCl; (b) NH_2CSNH_2, NaOEt, reflux 6 h; (c) R_2COCH_2X (X = Br or Cl), K_2CO_3, DMF, rt, 6–14 h.

In this series, 5-isopropyl-2-[(4′-methoxy-phenyl-carbonylmethyl)thio]-6-(1-naphthylmethyl) pyrimidin-4(3*H*)-one **4o** was the most promising compound. It exhibited extremely potent inhibitory activity against HIV-1 replication with IC_{50} value of 0.030 μM, CC_{50} value of 203 μM and the viral selectivity amounted up to 6766, which were much better than those of HEPT and DDI. Besides compound **4o**, some compounds, **4m**, **4q**, **4i** and **4j**, were also endowed with the high anti-HIV-1 potency ($IC_{50} = 0.046$, 0.078, 0.045 and 0.078 μM, respectively) and good selectivity index (SI = 5173, 2384, 3955 and 3076, respectively). It is worth noting that the compound **4o** also showed activity against the double mutated strain SO561945 which was better than that observed with DDI.

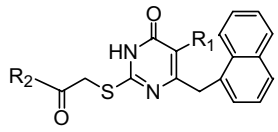
In terms of SARs, the uracils were generally more active by 2- to 10-fold than the corresponding thymines, and the 5-ethyl and 5-isopropyl substituted uracil derivatives exhibited similar anti-HIV activities. As with the SAR of previous *S*-DABOs, the presence of bulky substituents at the C-2 position of uracil and thymine rings correlated with the higher antiviral activity. Optimal anti-HIV-1 activity was obtained with compounds of a terminal phenyl moiety at the C-2 side chain. Moreover, the nature of the substituent on the 4-position of the phenyl ring also influenced on the antiviral activity of these novel *S*-DABOs. In fact, the 4′-OCH_3 and 4′-F derivatives (compounds **4i**, **4j**, **4o**, **4q**) were more potent than their 4′-CH_3 and 4′-Cl counterparts (compounds **4h**, **4k**, **4n**, **4r**).

Interestingly, when the present compounds were tested for their capability to inhibit the HIV-2 multiplication in acutely infected MT-4 cells, we were surprised to find that, different from what has been observed for DABOs or other classes of NNRTIs, some of our compounds exhibit the potency of anti-HIV-2 activity to some extent. The most interesting compounds from this point of view were **4b** and **4k**, which inhibited HIV-2 replication almost to the same extent as HIV-1 replication. Other compounds, such as **4o**, **4j** and **4e**, also showed an IC_{50} against HIV-2 ROD of 13.4, 16.3 and 19.8 μM, respectively.

It is clear that some of the members of the novel *S*-DABO analogues most probably act as genuine NNRTIs but, in addition, they might interfere with another target or act on RT in a different way as compared to the typical NNRTIs. This statement is based on the fact that some compounds show activity both against HIV-1 and HIV-2. Also loss of antiviral activity of those compounds when tested against SO561945 was much less pronounced as compared to the loss of activity of typical NNRTIs. Studies are in progress to pinpoint the exact mechanism that forms the basis for the anti-HIV activity of these compounds.

In summary, the bioassay results show that our approach has led to the development of unique potent anti-HIV agents. By comparing the structures and activities of these new compounds with those of previous *S*-DABOs,[11–15] we can assume that the carbonyl

Table 1. Anti-HIV activity in MT-4 cells of compounds **4a–u**



Compd	R_1	R_2	IC_{50} (μM)[a]			CC_{50} (μM)[b]	SI[c] (HIV-III_B)
			HIV-1 III_B	HIV-2 ROD	SO561945		
4a	Me	(4′-CH_3)Ph	0.67 ± 0.07	⩾ 13.61	⩾ 251	⩾ 251	⩾ 374
4b	Me	(4′-OCH_3)Ph	0.37 ± 0.07	⩾ 0.42	⩾ 290	>290	784
4c	Me	CH_3	1.12 ± 0.00	⩾ 84.9	⩾ 58.2	85 ± 10.41	76
4d	Me	(4′-F)Ph	0.180 ± 0.003	⩾ 275	⩾ 275	⩾ 275	⩾ 1527
4e	Me	(4′-Cl)Ph	0.55 ± 0.05	19.8 ± 0.27	⩾ 198	⩾ 198	⩾ 360
4f	Me	CH_3O	6.99 ± 0.10	⩾ 296	⩾ 296	⩾ 296	⩾ 42
4g	Me	CH_3CH_2O	7.00 ± 0.47	⩾ 293	⩾ 317	⩾ 287	⩾ 41
4h	Et	(4′-CH_3)Ph	0.32 ± 0.07	⩾ 1.25	⩾ 5.62	6 ± 0.17	19
4i	Et	(4′-OCH_3)Ph	0.045 ± 0.003	⩾ 18.9	>178	178 ± 21.05	3955
4j	Et	(4′-F)Ph	0.078 ± 0.009	16.3 ± 0.17	24.2 ± 0.88	⩾ 240	⩾ 3076
4k	Et	(4′-Cl)Ph	0.26 ± 0.01	⩾ 0.26	>56	56 ± 7.87	215
4l	Et	CH_3O	4.72 ± 0.16	⩾ 196	⩾ 196	⩾ 196	⩾ 42
4m	*i*-Pr	Ph	0.046 ± 0.005	22.0 ± 2.76	19.6 ± 0.42	238 ± 7.15	5173
4n	*i*-Pr	(4′-CH_3)Ph	0.24 ± 0.01	⩾ 225	⩾ 225	225 ± 14.12	937
4o	*i*-Pr	(4′-OCH_3)Ph	0.030 ± 0.002	13.4 ± 0.02	5.82 ± 0.09	⩾ 203	⩾ 6766
4p	*i*-Pr	CH_3	0.41 ± 0.008	>43.1	>43.1	43 ± 1.41	105
4q	*i*-Pr	(4′-F)Ph	0.078 ± 0.001	⩾ 32.2	188	186 ± 7.81	2384
4r	*i*-Pr	(4′-Cl)Ph	0.32 ± 0.02	>270	>270	>270	>843
4s	*i*-Pr	CH_3O	4.49 ± 0.39	>179	>179	179 ± 3.54	40
4t	*i*-Pr	CH_3CH_2O	1.41 ± 0.12	⩾ 41.1	>175	175 ± 9.09	>125
4u	*i*-Pr	(2′,4′-CH_3)Ph	0.24 ± 0.03	4.00 ± 0.33	2.67	27	112
DDI			5.37 ± 0.1	2.71 ± 0.25	7.15 ± 0.26	⩾ 529	⩾ 98
HEPT			5.06 ± 0.06	NA	>405	405 ± 3.21	80
Nevirapine			0.25	NA		>200	>800
Efavirenz			0.004	NA		80	20,000

NA: not active.

[a] Concentration required to protect the cell against viral cytopathogenicity by 50% in MT-4 cells.

[b] Concentration that reduces the MT-4 cell viability by 50%.

[c] Selectivity index: ratio CCtextsubscript50/IC_{50}, a higher SI means a more selective compound.

group of C-2 side chain plays an important role in the interaction between RT and our new compounds. Development of the novel *S*-DABO series is still ongoing with particular attention focused on the further synthesis of the analogues and the SAR studies to explore in detail the role of this structural feature.

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17. Selected data for compound **4o**: ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.15 (d, 6H, J = 6.85 Hz, $2CH_3$), 2.97 (m, 1H, J = 6.85 Hz, CH), 3.73 (s, 3H, OCH_3), 4.16 (s, 2H, SCH_2), 4.42 (S, 2H, CH_2naphthyl), 6.81–7.96 (m, 7H, naphthyl; 4H, Ph-$H_{2,3,5,6}$), 12.62 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 19.45 ($2CH_3$), 27.0 (CH), 36.8 (CH_2naphthyl), 37.2 (SCH_2), 55.3 (OCH_3), 113.5 (Ph-$C_{3,5}$), 123.7 (C-5), 125.2 (10C, naphthyl; 3C, Ph-$C_{1,2,6}$), 156.1 (C-6), 159.2 (C-2), 161.5 (Ph-C_4), 163.0 (C-4), 191.5 (C=O).
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