Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 14, pp. 1461-1466, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00257-X

SYNTHESIS AND ANTI HIV-1 ACTIVITY OF NEW THIADIAZEPINDIOXIDES

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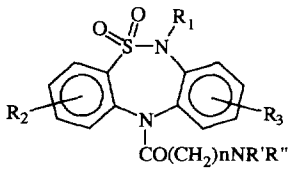
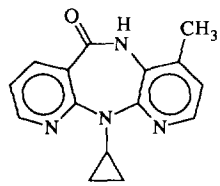
Abstract: New pyridobenzo-and dipyridothiadiazepindioxides **3-5** and two isomers **13-14** were synthetized and their anti HIV-1 activity and the cytotoxicity were evaluated *in vitro*. A good antiviral activity was shown by pyridobenzothiadiazepindioxides **4**, compound **4a** being the best.

The human immunodeficiency virus type 1 (HIV-1) reverse transcriptase is an important target for the development of potent and effective drugs against the acquired immunodeficiency syndrome (AIDS)[1], since the process of reverse transcription is an essential step in the HIV-1 life cycle.
Recently several non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as Nevirapine, TIBO, α-APA, Pyridinones and Bis(heteroaryl)piperazines have been introduced in clinical trials[2].
They are highly specific non-competitive inhibitors of HIV-1 and appear to bind to a site distinct from the substrate binding site where the nucleoside antagonists such as 3'-azido-3'-deoxythymidine (AZT)[3] interact. Although structurally distinct, NNRTIs have several features in common: all are extremely potent in cell culture assay on inhibition of HIV-1 replication at nanomolar concentrations, they have favorable oral bioavailability and lack of significant toxicity. These NNRTIs cause the developement of drug-resistant strains, neverthless clinical evidences are showing that this problem may be overcome by combination therapy[4].



1



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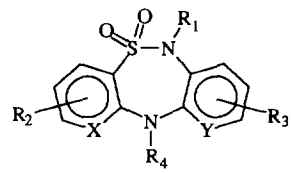


3 : X= CH, Y=N
4 : X= N, Y= CH
5 : X= N, Y= N

Among the NNRTIs in clinical trials, Nevirapine **2**[5] is one of the most promising expecially when used in combination with AZT[4]. Our previous experiences on dibenzothiadiazepindioxide derivatives **1**[6], as potential

antidepressants, prompted us to synthesize pyridobenzo-and dipyridothiadiazepindioxides **3-5**[7], strictly related to Nevirapine and its analogs[8], characterized by the presence of a sulfonamido instead of an amide group in the 7-membered central ring.

In this preliminary report we describe the synthesis and pharmacological evaluation as inhibitors of HIV-1 virus of some compounds having the tricyclic nucleus **3-5** and the two isomers **13-14** (schemes 1-2) as basic structures.

Synthesis

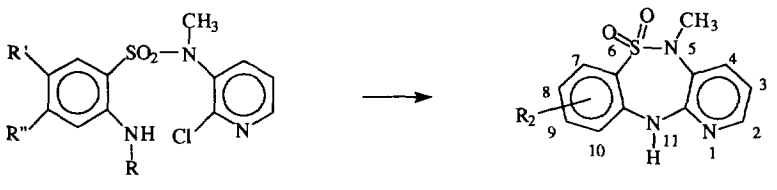
First, tricyclic compounds **7**, **10**, and **12** having an unsubstituited amine nitrogen in position 11 were prepared according to a modification of the previously described procedure for dibenzothiadiazepindioxides[6].

The 5,11-dihydropyrido[3,2-c][1,2,5]benzothiadiazepin-6,6-dioxides **7a-b** were prepared by cyclization of sulfonamides **6a-b**[9]: when R=H the reaction was done in the presence of K_2CO_3 in DMF at reflux temperature and when R=$COCH_3$, CuBr was added to the reaction mixture (Scheme 1).

The 6,11-dihydropyrido[2,3-f][2,1,5]benzothiadiazepin-5,5-dioxide **10** was prepared starting from sulfonamide **8**[9]. During reduction of the nitro group on the sulfonamide **8**, with boiling Fe/AcOH, the newly formed amino derivative **9** gave the product **10** directly (Scheme 2).

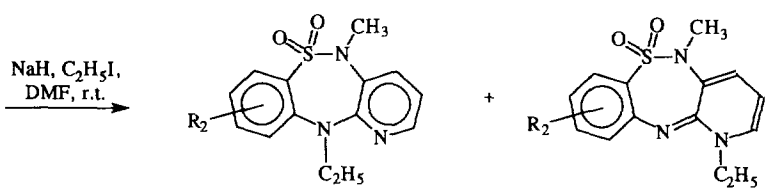
The 6,11-dihydrodipyrido[3,2-c:2',3'-f][1,2,5]thiadiazepin-5,5-dioxide **12** was synthetized by cyclization of sulfonamide **11**[9] as described for **6a-b** (Scheme 3).

Scheme 1



6 a R'= R" = H
b R'= CH_3, R" = Cl

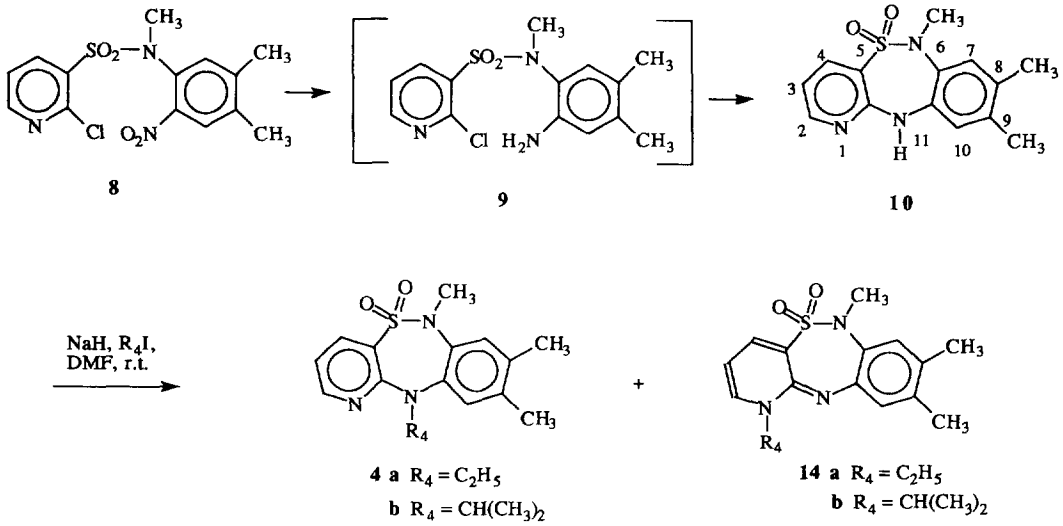
7 a R_2 = H
b R_2 = 8 CH_3, 9 Cl



3 a R_2 = H
b R_2 = 8 CH_3, 9 Cl

13 a R_2 = H
b R_2 = 8 CH_3, 9 Cl

Scheme 2

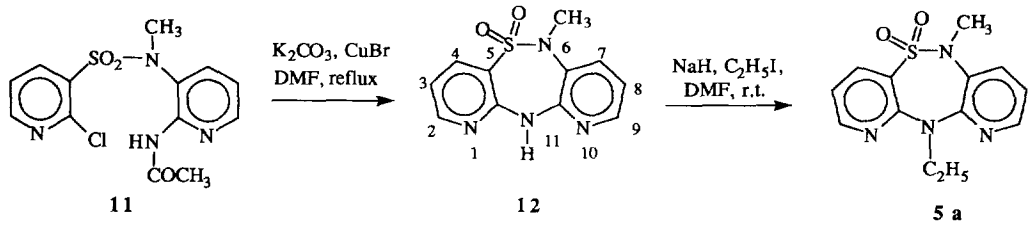


8 9 10

4 a $R_4 = C_2H_5$
b $R_4 = CH(CH_3)_2$

14 a $R_4 = C_2H_5$
b $R_4 = CH(CH_3)_2$

Scheme 3



11 12 5 a

Thiadiazepindioxides **7a-b**, **10** and **12** were obtained in good yield (70-90%) from the corresponding sulfonamides **6a-b**, **8** and **11**. Compounds **3-5** with R_1=H could not be obtained because the cyclization of the corresponding primary sulfonamides did not occur under the conditions used.

Next, the tricyclic intermediates **7a-b-10** and **12** were alkylated to the final compounds as shown in Schemes 1-3. Compounds **7a-b**, **10** and **12** were treated with NaH in DMF followed by alkyl iodide at room temperature[10].

Alkylation of compounds **7a-b** and **10** occurred either on the nitrogen in position 1 or on the nitrogen in position 11 obtaining predominantly compounds **13a-b** and **14a-b** with respectively their regioisomers **3a-b** and **4a-b** (overall yield 78-97 %). In the case of compound **12** only the product of the alkylation on nitrogen in position 11 (**5a** , yield 85%) was detected.

The structures of the compounds **3-5** and the isomers **13-14** were univocally determinated by NMR spectra, including steady state NOE measurements[11]. Moreover the analogous isomeric structures were also obtained in the synthesis of the corresponding Nevirapine derivatives[8].

Results and discussion

The anti HIV-1 activity and the cytotoxicity of thiadiazepindioxides of schemes 1-3 were evaluated by inhibition of HIV-1 replication in a CD4+ lymphoblastoid cell line, C8166 (containing the HTLV-I genome and expressing only the tax gene), infected with HIV/IIIB strain[12].

We can establish some relevant features on the structure-activity relationships of these molecules based on the results shown in Table 1.

First of all strong differences in the biological activity are evident among the different types of tricyclic nuclei. The best antiviral activity was found in compounds of structure **4** alkylated on nitrogen in position 11, while isomeric compounds **14**, alkylated on nitrogen in position 1, are less active. On the contrary, compounds of structure **3**, alkylated on nitrogen in position 11, are practically inactive while moderate antiviral activity was found in isomeric compounds **13**, alkylated on nitrogen in position 1. It is interesting to note that compound **5a**, which is more strictly related to nevirapine, is inactive and the compound **12**, which is not alkylated in position 11, is weakly active; showing that in this peculiar case alkylation on the nitrogen in position 11 does not produce an increase in activity, in contrast to that observed with derivatives of type **4**.

Among compounds of structure **4** the 11-ethyl-6,8,9-trimethyl-6,11-dihydro-pyrido[2,3-f][2,1,5] benzothiadiazepin-5,5-dioxide **4a** (MEN-10690)[13] inhibits HIV-1 replication at a 40 nanomolar concentration, 3750 times lower than its cytotoxic concentration. Compound **4a** exhibits good antiviral activity comparable to the data reported in the literature[8] for Nevirapine, but is less active than a TIBO (R82913) compound.

Table 1. Activity against HIV-1/III B virus in human C8166 cells

Compound	Antiviral activity IC_{50} (μM)[a]	Cytotoxicity CC_{50} (μM)[b]	Selectivity Index[c]
3a	>300	-	-
3b	48	>60	>1
4a	0.04	150	3750
4b	0.032	>60	>1875
5a	>300	300	<1
7a	>300	-	-
7b	>300	-	-
10	4	>125	>31
12	7	>125	>17
13a	3.7	80	22
13b	0.28	12	42
14a	0.33	>30	>90
14b	0.6	>30	>50
AZT	0.004	1400	350000
TIBO (R82913)	0.0015	34	22600
DDI	1	9000	9000
NEVIRAPINE[d]	0.04	321	8025

[a] 50% inhibitory concentration. [b] 50% cytotoxic concentration. [c] ratio between CC_{50} and IC_{50}.[d] reference 8.

Further studies were performed to evaluate the antiviral activity of our products on different viral strains and cellular systems and to examine closely their mechanism of action. A publication about these results is in preparation.

Acknowledgment: We thank Dr. Giuseppe Balacco for the discussion of NMR spectra and Dr. Antonio Triolo for ms data.

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3. The nucleoside drugs such as 3'-azido-3'-deoxythymidine (AZT) and 2',3'-dideoxyinosine (DDI) are a class of HIV reverse transcriptase inhibitors (RTIs) which mimic the normal deoxynucleosides in the substrate binding site of the reverse transcriptase (RT) enzyme and have demonstrated efficacy in the therapeutic treatment of HIV-1 infected patients. Unfortunately, treatment with these nucleoside drugs is limited by the emergence of serious clinical side effects, most of which are related to inhibition of cellular DNA polymerases: see references 1a-b.
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9. The sulfonamides were synthesized from amines and sulfonyl chlorides in analogy with our procedure:

see reference 6.

10. Typical procedure referred to derivate **4a**: NaH (0.170 g, 5.5 mmol) was added to **10** (1.4 g, 5 mmol), dissolved in anhydrous DMF (10 ml), the mixture was stirred at room temperature for 1h and ethyl iodide (1 ml) was added. The mixture was maintained at r.t. for 18 h under stirring and then was poured into water, extracted with ethyl acetate and washed with water. The organic layer was dried and evaporated to give a solid which was chromatographed on silica gel (ether/hexane 1/1), collecting 0.6g (39%) of **4a**, m.p. 149-150°C, MS (EI): m/z 317 (M^+) and 0.9 g (58%) of **14a**, m.p. 149-150°C, MS (TS): m/z 318 (MH^+).

11. ^1H NMR ($CDCl_3$, ppm) **4a**: 1.23 (3H, t, J = 7.0 Hz), 2.23 (6H, s), 3.30 (3H, s), 4.21 (2H, q, J = 7.0 Hz), 6.91 (1H, dd, J = 4.6, 7.7 Hz), 7.07 (1H, s), 7.21 (1H, s), 8.08 (1H, dd, J = 1.8, 7.7 Hz), 8.38 (1H, dd, J = 1.8, 4.6 Hz); ^1H NMR ($CDCl_3$, ppm) **14a**: 1.41 (3H, t, J = 7.0 Hz), 2.21 (3H, s), 2.23 (3H, s), 3.01 (3H, s), 4.16 (2H, q, J = 7.0 Hz), 5.92 (1H, dd, J = 6.6, 7.1 Hz), 6.98 (1H, s), 6.99 (1H, s), 7.42 (1H, dd, J = 2.0, 6.6 Hz), 7.88 (1H, dd, J = 2.0, 7.1). The structure of **14a** was determineted by the following steady-state ^1H-^1H NOEs measured in DMSO-d_6 (200 Hz): $f_{H2}\{CH_2\}$ = 23.8%, $f_{H3}\{CH_2\}$ = -2.3%, $f_{H10}\{CH_2\}$ = 3.7%, $f_{CH3}\{CH_2\}$ = 6.0%.
^{13}C NMR ($CDCl_3$, ppm) **4a**: 13.9, 19.2, 19.8, 37.7, 45.9, 115.7, 125.6, 125.9, 131.2, 133.1, 135.6, 138.6, 138.7, 142.6, 150.5, 151.5; ^{13}C NMR ($CDCl_3$, ppm) **14a**: 14.1, 18.8, 19.2, 38.1, 48.3, 101.2, 129.7, 129.9, 130.4, 131.1, 131.7, 136.9, 139.8, 140.4, 141.0, 143.5.

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(*Received in Belgium* 17 *February* 1995; *accepted* 19 *May* 1995)