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Original article

Synthesis of some new 1,4-benzothiazine and 1,5-benzothiazepine tricyclic derivatives with structural analogy with TIBO and their screening for anti-HIV activity[#]

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Abstract – Several new tricyclic derivatives with structural analogy to TIBO were prepared starting from properly substituted 1,4-benzothiazines and 1,5-benzothiazepine. All synthesized compounds were submitted to screenings for in vitro anti-HIV-1 activity. Only two compounds showed moderate activity. © 1999 Éditions scientifiques et médicales Elsevier SAS

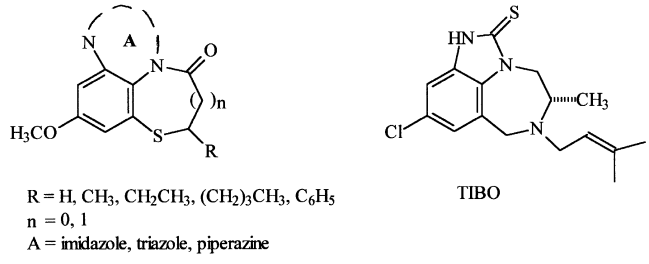
1,4-benzothiazine and 1,5-benzothiazepine derivatives / anti-HIV-1 activity / non-nucleoside reverse transcriptase inhibitor (NNRTI)

1. Introduction

The dramatic increase of spread of the acquired immunodeficiency syndrome (AIDS) has stimulated considerable efforts in the research in this field. As a result of the difficulties encountered in the development of an effective vaccine, research is aimed at the discovery of new chemotherapeutic agents.

Good results were obtained with the class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) for both their antiviral activity and their low toxicity.

These considerations prompted us to prepare some 1,4-benzothiazine and 1,5-benzothiazepine derivatives with structural analogy with TIBO [1–5], a non-nucleoside inhibitor of HIV-1 reverse transcriptase *(figure 1)*. In this paper we describe the synthesis and the preliminary anti-HIV screening of some tricyclic derivatives *(figure 1)*.



R = H, CH_3, CH_2CH_3, $(CH_2)_3CH_3$, C_6H_5
n = 0, 1
A = imidazole, triazole, piperazine

Figure 1. General structure of new tricyclic 1,4-benzothiazine and 1,5-benzothiazepine derivatives with structural analogy with TIBO.

2. Chemistry

Starting materials for our work program were compounds **2** (**a–f**) in which the amino group is properly located to react with bifunctional reagents to give the desired tricyclic compounds. The 5- or 6-amino derivatives **2** *(table I)* were obtained by reduction of 7-methoxy-5-nitro-3,4-dihydro-2H-1,4-benzothiazin-3-ones or 8-methoxy-6-nitro-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one **1** already synthesized by us [6] *(figure 2)*.

[#] A preliminary account of this work was presented at the 4th International Conference on Heteroatom Chemistry, Seoul, Korea, July 30–August 4, 1995

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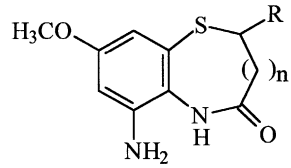
Table I. Physical and chemical data of compounds **2a–f.**



Compound	R	n	Yield (%)	M.p. (°C)	Formula (MW)	^{1}H-NMR, δ
2a	H	0	78	258–260	$C_9H_{10}N_2O_2S$ (210.25)	3.35 (s, 2H, SCH_2), 3.62 (s, 3H, OCH_3), 5.25 (s, 2H, NH_2), 6.10–6.20 (m, 2H, Ar), 9.50 (s, 1H, NH) (DMSO-d_6)
2b	CH_3	0	89	178–179	$C_{10}H_{12}N_2O_2S$ (224.28)	1.30 (d, *J* = 9.5 Hz, 3H, $SCH\mathit{CH}_3$), 3.50 (q, 1H, $SC\mathit{H}CH_3$), 3.65 (s, 3H, OCH_3), 5.25 (s, 2H, NH_2), 6.10–6.20 (m, 2H, Ar), 9.48 (s, 1H, NH) (DMSO-d_6)
2c	CH_2CH_3	0	70	153–154	$C_{11}H_{14}N_2O_2S$ (238.30)	1.05 (t, 3H, $CH_2\mathit{CH}_3$), 1.50–2.10 (m, 2H, CH_2CH_3), 3.25 (q, 1H, SCH), 3.75 (s, 3H, OCH_3), 4.05 (brs, 2H, NH_2), 6.15 (d, *J* = 2.4 Hz, 1H, Ar), 6.35 (d, *J* = 2.4 Hz, 1H, Ar), 9.40 (brs, 1H, NH) ($CDCl_3$)
2d	$(CH_2)_3CH_3$	0	51	104–106	$C_{13}H_{18}N_2O_2S$ (266.36)	0.90 (t, 3H, CH_3), 1.22–1.70 (m, 4H, $CH_2\mathit{CH}_2\mathit{CH}_2CH_3$), 1.85–2.03 (m, 2H, $\mathit{CH}_2CH_2CH_2CH_3$), 3.35 (q, 1H, SCH), 3.73 (s, 3H, OCH_3), 3.88 (br s, 2H, NH_2), 6.20 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.35 (d, J_{meta} = 2.4 Hz, Ar), 8.75 (br s, 1H, NH) ($CDCl_3$)
2e	C_6H_5	0	62	220–221	$C_{15}H_{14}N_2O_2S$ (286.35)	3.61 (s, 3H, OCH_3), 4.82 (s, 1H, $S\mathit{CH}C_6H_5$), 5.38 (s, 2H, NH_2), 6.10 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.20 (d, J_{meta} = 2.40 Hz, 1H, Ar), 7.27 (s, 5H, Ar), 9.83 (s, 1H, NH) (DMSO-d_6)
2f	H	1	51	198–199	$C_{10}H_{12}N_2O_2S$ (224.28)	2.33, 3.33 (A_2B_2 system, 4H, CH_2CH_2), 3.65 (s, 3H, OCH_3), 5.12 (s, 2H, NH_2), 6.30–6.40 (m, 2H, Ar), 8.67 (s, 1H, NH) (DMSO-d_6)

Unfortunately, because of the problems encountered during the synthesis of **1** [3], to date only compounds with a methoxy substituent at the 7 or 8 position could be prepared.

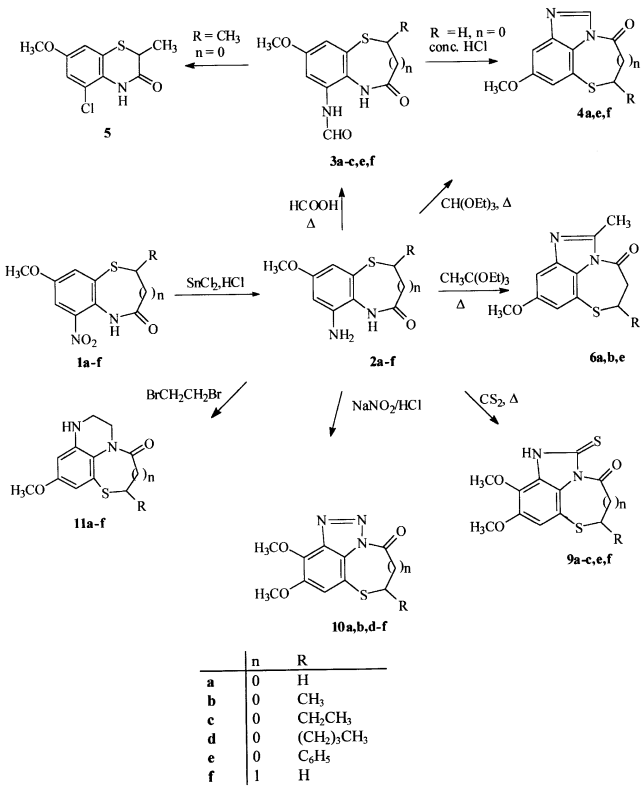
Our first attempt to synthesize the imidazo derivatives **4** (**a–f**) was performed by heating **2** (**a–f**) with an excess of formic acid under reflux [7]. In this case the *N*-formylderivatives **3 (a–c, e** and **f)** were obtained *(table II)*. Tentative cyclizations were performed by treating **3** with concentrated HCl but only for **3a** (R = H, n = 0) the desired tricyclic **4a** was obtained.

Treatment of **3b** (R = CH_3, n = 0) with concentrated HCl gave rise to the replacement of the N-formyl group with a chlorine atom with the formation of the 5-chloro-7-methoxy-2-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one **5**.

Another tentative synthesis of **4** (**a–f**) was achieved by refluxing compound **2** (**a–f**) with triethyl orthoformate in xylene (mixture of isomers) [7], in this way only compounds **4e** and **4f** were obtained *(table III)*.

Condensation of compounds **2** with triethyl orthoacetate in xylene was successful only for compounds **6a, b** and **e** *(table III)*. 5-Amino-2-ethyl-7-methoxy-3,4-dihydro-2H-1,4-benzothiazin-3-one **2c** and 5-amino-2-butyl-7-methoxy-3,4-dihydro-2H-1,4-benzothiazin-3-one **2d** afforded only a variety of decomposition products which have not yet been identified. When the same reaction was performed with the benzothiazepine **2f**, the 2-ethoxy-9-methoxy-2-methyl-1H-5,6-dihydro-imidazo[3,4,5-*e*,*f*]-1,5-benzothiazepin-4-one **7** *(figure 3)* was obtained. Operating in the presence of pyridinium hydrochloride, compound **8** was formed *(figure 3)*.

Compounds **9a–c** and **e** *(table IV, figure 2)* were obtained by refluxing the corresponding amines **2a–c** and **e** with carbon disulfide in anhydrous pyridine. Reaction of carbon disulfide with **2d** and **2f** either in anhydrous



	n	R
a	0	H
b	0	CH_3
c	0	CH_2CH_3
d	0	$(CH_2)_3CH_3$
e	0	C_6H_5
f	1	H

Figure 2. Synthetic pathway for new tricyclic derivatives.

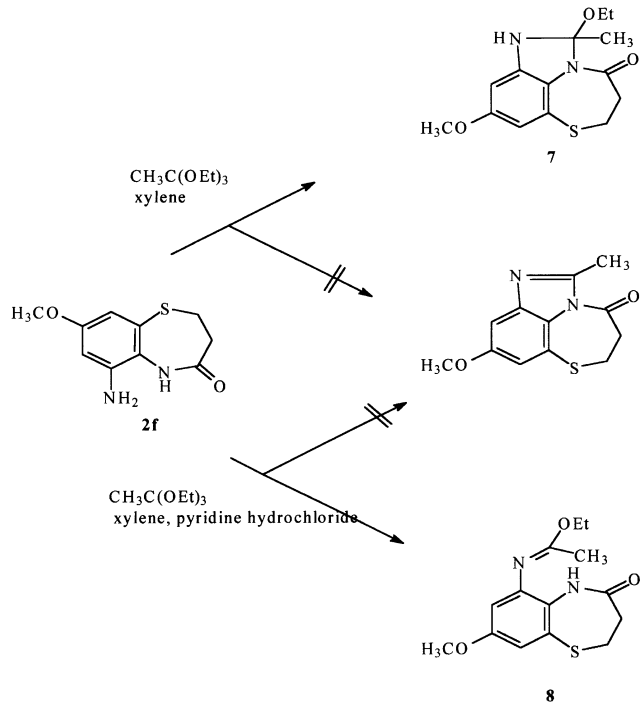


Figure 3. Tentative synthesis of 2-methyl-9-methoxy-4H-5,6-dihydroimidazo[3,4,5-e,f]-1,5-benzothiazepin-4-one.

pyridine or in anhydrous xylene afforded only decomposition of the starting products. A further attempt was realized by lowering the reaction temperature, thus **2d** and **2f** were reacted with carbon disulfide in tetrahydrofuran in the presence of triethylamine. Only compound **9f** *(table IV)* was obtained, although in low yields. Unfortunately this attempt to synthesize the 2-butylderivative was unsuccessful.

Diazotization reaction of the primary amino group gave rise to the triazoloderivatives **10a, b** and **d–f** *(table V)*. It is noteworthy that this reaction occurred immediately for 2-unsubstituted-1,4-benzothiazine and needed 2–40 h of stirring for 2-substituted benzothiazines.

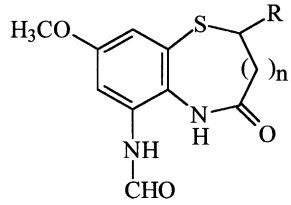
Annulation of a six-membered ring on the 1,4-benzothiazine or 1,5-benzothiazepine system was very difficult. Many attempts were made with 1,2-dibromoethane, phenacylbromide, ethylchlorooxalate and ethylpyruvate, but only reaction of **2** with 1,2-dibromoethane was successful and gave rise to compounds **11a–f** *(table VI)*, operating in the presence of tetrabutylammonium bromide and powdered potassium hydroxide in tetrahydrofuran according to phase-transfer catalysis conditions (PTC) for **11a–d** and refluxing in xylene for **11e** and **f**.

It is noteworthy that the building of a third nucleus was easier on the 1,5-benzothiazepine system than on the 1,4-benzothiazine one and among the 1,4-benzothiazines it was easier on the 2-unsubstituted than on the 2-substituted derivatives, perhaps because of the hindrance exerted by the substituent at the 2 position. The annulation of a six-membered ring was more difficult than that of a five membered one.

3. Biological investigation and results

All synthesized compounds were submitted to the National Cancer Institute (NCI) of Bethesda (MD) and were evaluated for in vitro anti-HIV-1 activity. All compounds were inactive with the exception of **9c** which showed moderate activity (CC_{50} = 0.155 mM, EC_{50} = 0.0323 mM), the ratio between the two concentrations (therapeutic index = CC_{50}/EC_{50}) being 4.80.

Table II. Physical and chemical data of compounds **3a–c, e** and **f.**



Compound	R	n	Yield (%)	M.p. (°C)	Formula (MW)	^{1}H-NMR, δ
3a	H	0	50	208–210	$C_{10}H_{10}N_2O_3S$ (238.26)	3.45 (s, 2H, SCH_2), 3.75 (s, 3H, OCH_3), 6.83 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.15 (d, J_{meta} = 2.4 Hz, 1H, Ar), 8.25 (s, 1H, NH*CH*O), 9.60, 9.77 (2s, 2H, 2NH) (DMSO-d_6)
3b	CH_3	0	77	203–204	$C_{11}H_{12}N_2O_3S$ (252.29)	1.29 (d, *J* = 7.8 Hz, 3H, CH*CH*$_3$), 3.60 (q, 1H, *CH*CH_3), 3.70 (s, 3H, OCH_3), 6.80 (d, *J* = 2.4 Hz, 1H, Ar), 7.15 (d, *J* = 2.4 Hz, 1H, Ar), 8.25 (s, 1H, NH*CH*O), 9.61, 9.80 (2s, 2H, 2NH) (DMSO-d_6)
3c	CH_2CH_3	0	23	58–60	$C_{12}H_{14}N_2O_3S$ (266.31)	0.75 (t, 3H, CH_2*CH_3*), 1.10–1.70 (m, 2H, *CH_2*CH_3), 3.10–3.20 (m, 1H, SCH), 3.50 (s, 3H, OCH_3), 6.70 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.90 (d, J_{meta} = 2.4 Hz, 1H, Ar), 8.00 (s, 1H, NH*CH*O), 8.10, 8.15 (2s, 2H, 2NH) (DMSO-d_6)
3e	C_6H_5	0	27	117–119	$C_{16}H_{14}N_2O_3S$ (314.36)	3.72 (s, 3H, OCH_3), 6.00 (s, 1H, S*CH*C_6H_5), 6.70 (br d, 1H, Ar), 6.88 (br d, 1H, Ar), 7.25–7.50 (m, 7H, C_6H_5 + NHCHO), 8.15 (s, 1H, NH) (DMSO-d_6)
3f	H	1	33	186–188	$C_{11}H_{12}N_2O_3S$ (252.29)	2.55, 3.40 (A_2B_2 system, 4H, CH_2CH_2), 3.85 (s, 3H, OCH_3), 7.00 (d, *J* = 2.4 Hz, 1H, Ar), 7.77 (d, *J* = 2.4 Hz, 1H, Ar), 8.20 (s, 1H, NH*CH*O), 8.48, 8.56 (2s, 2H, 2NH) ($CDCl_3$)

4. Experimental protocols

4.1. Chemistry

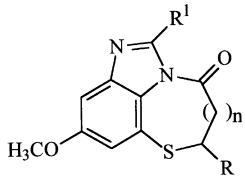
Melting points were taken on a Kofler hot-stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded in the solvent indicated, using a Bruker AC-200 (200 MHz) instrument. The chemical shift values are reported in δ (ppm) relative to tetramethylsilane as an internal standard. Mass spectra were recorded on a Varian MAT 311A spectrometer. Elemental microanalyses were performed for C, H and N on a Carlo Erba Elemental Analyser model 1106 and results were within ± 0.4% of the theoretical values. The purity of the compounds was checked by TLC (pre-coated silica-gel plates, Merck Kieselgel 60 F_{254}). Flash chromatographies were performed on columns packed with Merck silica gel, 230–400 mesh.

4.1.1. General procedure for 5-amino-7-methoxy-3,4-dihydro-2H-1,4-benzothiazin-3-ones **2a–e** *and 6-amino-8-methoxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-ones* **2f** *(table I)*

Nitrobenzothiazinederivative **1a–e** (0.03 mol) was added portionwise under stirring to a solution of stannous chloride dihydrate (6.77 g, 0.03 mol) in concentrated HCl (10 mL). For thiazepine compound **1f** (0.03 mol) a suspension of stannous chloride dihydrate (9.02 g, 0.04 mol) in tetrahydrofuran (30 mL) and concentrated HCl (2 mL) was used. The mixture was heated in a steam bath for 10 min for compound **1d**, 30 min for **1a**, 1 h for **1b**, **e** and **f,** and 1.5 h for **1c**.

After cooling, in the case of **1a**, an abundant precipitate was formed. It was collected by filtration, alkalinized with 5 N NaOH solution, filtered again, washed with water and finally recrystallized from EtOH to give **2a**.

Table III. Physical and chemical data of compounds **4a, e** and **f** and **6a, b** and **e.**



Compound	R	R^1	n	Yield (%)	M.p. (°C)	Formula (MW)	^{1}H-NMR, δ
4a	H	H	0	71	210–212	$C_{10}H_8N_2O_2S$ (220.25)	3.78 (s, 3H, OCH_3), 3.97 (s, 2H, SCH_2), 6.75 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.90 (d, J_{meta} = 2.4 Hz, 1H, Ar), 8.15 (s, 1H, N=CH)(DMSO-d_6)
4e	C_6H_5	H	0	44	158–159	$C_{16}H_{12}N_2O_2S$ (296.34)	3.81 (s, 3H, OCH_3), 5.90 (s, 1H, S*CH*C_6H_5), 7.04 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.21 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.39 (s, 5H, Ar), 8.87 (s, 1H, N=CH) (DMSO-d_6)
4f	H	H	1	10	176–178	$C_{11}H_{10}N_2O_2S$ (234.27)	3.17, 3.51 (A_2B_2 system, 4H, CH_2CH_2), 3.85 (s, 3H, OCH_3), 6.87 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.10 (d, J_{meta} = 2.4 Hz, 1H, Ar), 8.71 (s, 1H, N=CH) ($CDCl_3$)
6a	H	CH_3	0	54	151	$C_{11}H_{10}N_2O_2S$ (234.27)	2.85 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 4.10 (s, 2H, SCH_2), 6.75 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.85 (d, J_{meta} = 2.4 Hz, 1H, Ar) (DMSO-d_6)
6b	CH_3	CH_3	0	63	166–167	$C_{12}H_{12}N_2O_2S$ (248.30)	1.65 (d, J = 6.3 Hz, 3H, SCH*C*H_3), 2.85 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.06 (q, 1H, S*CH*CH_3), 6.78 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.00 (d, J_{meta} = 2.4 Hz, 1H, Ar) ($CDCl_3$)
6e	C_6H_5	CH_3	0	50	140–141	$C_{17}H_{14}N_2O_2S$ (310.37)	2.73 (s, 3H, CH_3), 3.78 (s, 3H, OCH_3), 5.76 (s, 1H, S*CH*C_6H_5), 6.94 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.05 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.36 (s, 5H, Ar) (DMSO-d_6)

In the case of **1f**, the mixture was evaporated in vacuo, the residue was dissolved in $CHCl_3$ and extracted with 2 N HCl. The aqueous solution was alkalinized with 50% NaOH solution, extracted with $CHCl_3$, dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo. The pure solid residue was recrystallized from EtAc to give **2f**.

For other compounds, the reaction mixture was alkalinized with 30% ammonia solution. Compounds **2c** and **2d** crystallized and were collected by filtration, washed with water and recrystallized from EtOH. In the other cases the alkalinized mixture was extracted with $CHCl_3$, washed with water, dried over Na_2SO_4 and dried in vacuo. The solid residue was recrystallized from EtOH.

4.1.2. General procedure for 5-formylamino-7-methoxy-1,4-benzothiazine derivatives **3a–c** *and* **e** *and 6-formylamino-8-methoxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one* **3f** (table II)

A solution of **2** (0.01 mol) in 30 mL formic acid (excess) was refluxed under stirring for 40 min for **2f**, 2 h for **2a–c** and 9 h for **2e**. After cooling, the solution was poured into ice-water and alkalinized with 30% ammonia solution and the resulting precipitate was collected and recrystallized from EtOH with the exception of **3b** where MeOH was used.

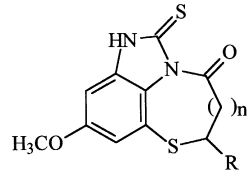
4.1.3. 8-Methoxy-2H-4,5-dihydro-imidazo[3,4,5-d,e]-1,4-benzothiazin-4-one **4a** (table III)

N-(formyl)derivative **3a** (2.38 g, 0.01 mol) was refluxed in concentrated HCl (10 mL) for 1 h. After cooling, the solution was poured into ice-water and alkalinized with 30% ammonia solution. The resulting precipitate was collected by filtration and recrystallized from EtOH.

4.1.4. Tentative synthesis of 5-methyl-8-methoxy-4,5-dihydro-2H-imidazo[3,4,5-d,e]-1,4-benzothiazin-4-one. Formation of 5-chloro-7-methoxy-2-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one **5**.

When the reaction described above (4.1.3) was performed using **3b**, the 5-chloro-7-methoxy-2-methyl-3,4-dihydro-2H-1,4-benzothiazin-3-one **5** was obtained (38% yield), m.p. 143–144 °C, recrystallized from EtOH [($C_{10}H_{10}ClNO_2S$)]; ^{1}H-NMR, δ (DMSO-d_6): 1.5 (d, J =

Table IV. Physical and chemical data of compounds **9a–c, e** and **f.**



Compound	R	n	Yield (%)	M.p. (°C)	Formula (MW)	^{1}H-NMR, δ
9a	H	0	48	242–243	$C_{10}H_8N_2O_2S_2$ (252.31)	3.78 (s, 3H, OCH_3), 4.05 (s, 2H, SCH_2), 6.50 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.80 (d, J_{meta} = 2.4 Hz, 1H, Ar), 13.18 (s, 1H, NH) (DMSO-d_6)
9b	CH_3	0	89	246–248	$C_{11}H_{10}N_2O_2S_2$ (266.33)	1.48 (d, *J* = 6.6 Hz, 3H, $CHCH_3$), 3.78 (s, 3H, OCH_3), 4.37 (q, 1H, $CHCH_3$), 6.50 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.78 (d, J_{meta} = 2.4 Hz, 1H, Ar), 13.20 (s, 1H, NH) (DMSO-d_6)
9c	CH_2CH_3	0	10	172–175	$C_{12}H_{12}N_2O_2S_2$ (280.36)	1.00 (t, 3H, CH_2CH_3), 1.75–2.05 (m, 2H, CH_2CH_3), 3.52 (q, 1H, SCH), 3.80 (s, 3H, OCH_3), 6.75 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.90 (d, J_{meta} = 2.4 Hz, 1H, Ar), 11.05 (br s, 1H, NH) ($CDCl_3$)
9e	C_6H_5	0	25	263–265	$C_{16}H_{12}N_2O_2S_2$ (328.41)	3.79 (s, 3H, OCH_3), 5.65 (s, 1H, $SCHC_6H_5$), 6.52 (d, *J* = 2.4 Hz, 1H, Ar), 6.83 (d, *J* = 2.4 Hz, 1H, Ar), 7.35 (m, 5H, Ar), 13.28 (s, 1H, NH) (DMSO-d_6)
9f	H	1	13	117–120	$C_{11}H_{10}N_2O_2S_2$ (266.34)	2.62, 3.15, 4.20 (AB_2X system, 4H, CH_2CH_2), 3.73 (s, 3H, OCH_3), 6.75 (d, *J* = 2.4 Hz, 1H, Ar), 6.83 (d, *J* = 2.4 Hz, 1H, Ar), 11.28 (s, 1H, NH) ($CDCl_3$)

8.3 Hz, 3H, CH_3), 3.55 (q, *J* = 8.3 Hz, 1H, $CHCH_3$), 3.78 (s, 3H, OCH_3), 6.75 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.83 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.80 (br s, 1H, NH) ppm.

4.1.5. 8-Methoxy-2-phenyl-4,5-dihydro-2H-imidazo[3,4,5-d,e]-1,4-benzothiazin-4-one **4e** *and 9-methoxy-2,4,5,6-tetrahydro-imidazo[3,4,5-e,f]-1,5-benzothiazepin-5-one* **4f** (table III)

A solution of triethyl orthoformate (1.50 g, 0.01 mol) in anhydrous xylene (5 mL) was added slowly under stirring to a suspension of **2e** and **f** (0.005 mol) in anhydrous xylene (20 mL). The reaction mixture was refluxed with stirring for 4 h for **2e** and 7 h for **2f**. After cooling, the solvent was evaporated in vacuo and the solid residue was recrystallized from EtOH.

4.1.6. General procedure for 2-methyl-8-methoxy-4,5-dihydro-imidazo[3,4,5-d,e]-1,4-benzothiazin-4-ones **6a, b** *and* **e** (table III)

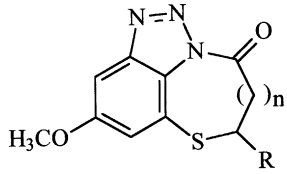
A solution of triethyl orthoacetate (3.24 g, 0.02 mol) in anhydrous xylene (5 mL) was added slowly and with stirring to a suspension of **2a, b** and **e** (0.01 mol) in anhydrous xylene (100 mL). The mixture was refluxed for 6–12 h, then filtered while hot. The filtrate was concentrated under reduced pressure until ca. 40 mL. The resulting precipitate was isolated by filtration and recrystallized from EtOH.

4.1.7. Tentative synthesis of 2-methyl-9-methoxy-4H-5,6-dihydroimidazo[3,4,5-e,f]-1,5-benzothiazepin-4-one. Formation of 2-ethoxy-9-methoxy-2-methyl-1H-5,6-dihydro-imidazo[3,4,5-e,f]-1,5-benzothiazepin-4-one **7** *and 6-{[(E)-1-ethoxyethylidene]amino}-8-methoxy-2,3,4,5-tetrahydro-1,5-benzothiazepin-4-one* **8**

4.1.7.1. Cyclocondensation reaction in anhydrous xylene

The reaction mixture, prepared as described above using **2f** as starting material, was refluxed for 16 h and was evaporated under reduced pressure. The residue was purified by flash chromatography using $CHCl_3$ as eluent and recrystallized from EtOH to give **7** (19% yield), m.p. 157–159 °C [($C_{14}H_{18}N_2O_3S$)]; ^{1}H-NMR, δ ($CDCl_3$): 1.33 (t, *J* = 6.9 Hz, 3H, CH_2CH_3), 1.82 (s, 3H, CH_3), 2.63, 3.44 (A_2B_2 system, 4H, CH_2CH_2), 3.78 (s, 3H,

Table V. Physical and chemical data of compounds **10a, b** and **d–f.**



Compound	R	n	Yield (%)	M.p. (°C)	Formula (MW)	^{1}H-NMR, δ
10a	H	0	69	250–251	$C_9H_7N_3O_2S$ (221.23)	3.82 (s, 3H, OCH_3), 4.08 (s, 2H, SCH_2), 6.80 (d, J = 1.5 Hz, 1H, Ar), 6.95 (d, J = 1.5 Hz, 1H, Ar) (DMSO-d_6)
10b	CH_3	0	24	191–194	$C_{10}H_9N_3O_2S$ (235.26)	1.50 (d, J = 7.5 Hz, 3H, $CHCH_3$), 3.80 (s, 3H, OCH_3), 4.50 (q, 1H, $CHCH_3$), 6.85–7.15 (m, 2H, Ar) (DMSO-d_6)
10d	$(CH_2)_3CH_3$	0	35	192–195	$C_{13}H_{15}N_3O_2S$	0.85 (t, 3H, CH_3), 1.20–1.50 (m, 4H, $CH_2CH_2CH_2CH_3$), 1.70–1.95 (m, 2H, $CH_2CH_2CH_2CH_3$), 3.83 (s, 3H, OCH_3), 4.25–4.42 (m, 1H, SCH), 6.95 (br s, 2H, Ar) (DMSO-d_6)
10e	C_6H_5	0	23	215–217	$C_{15}H_{11}N_3O_2S$ (297.33)	3.80 (s, 3H, OCH_3), 5.85 (s, 1H, CHC_6H_5), 6.80–7.55 (m, 7H, Ar) (DMSO-d_6)
10f	H	1	10	85–86	$C_{10}H_9N_3O_2S$ (235.26)	2.70, 3.40 (A_2B_2 system, 4H, CH_2CH_2), 3.88 (s, 3H, OCH_3), 7.00 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.05 (d, J_{meta} = 2.4 Hz, 1H, Ar) ($CDCl_3$)

OCH_3), 4.20 (q, J = 6.9 Hz, 2H, CH_2CH_3), 6.32 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.85 (d, J_{meta} = 2.4 Hz, 1H, Ar) ppm.

4.1.7.2. Cyclocondensation reaction in anhydrous xylene in the presence of pyridinium hydrochloride

To the reaction mixture, prepared as above, was added 1.16 g (0.01 mol) of pyridinium hydrochloride. The reaction mixture was refluxed for 14 h and finally was evaporated under reduced pressure. The residue was purified by flash chromatography using $CHCl_3$ as eluent and was recrystallized from EtOH to give **8** (72% yield), m.p. 234–235 °C [($C_{14}H_{18}N_2O_3S$)]; ^{1}H-NMR, δ ($CDCl_3$): 1.25 (t, J = 6.9 Hz, 3H, CH_2CH_3), 2.60 (s, 3H, CCH_3), 2.60, 3.20 (A_2B_2 system, 4H, CH_2CH_2), 3.80 (s, 3H, OCH_3), 4.16 (q, J = 6.9 Hz, 2H, CH_2CH_3), 6.90 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.98 (d, J_{meta} = 2.4 Hz, 1H, Ar), 8.90 (s, 1H, NH) ppm.

4.1.8. General procedure for 8-methoxy-4-oxo-1H,4,5-dihydro-imidazo[3,4,5-d,e]-1,4-benzothiazin-2-thiones **9a–c** *and* **e** (table IV)

Carbon disulfide (20 mL) was added slowly to a solution of **2a–c** and **e** (0.01 mol) in anhydrous pyridine (50 mL). The reaction mixture was refluxed for 8 h for **2a**, 14 h for **2b** and **e** and for 30 h for **2c**. After cooling the solvent was evaporated in vacuo. The solid residue was recrystallized from EtOH to give **9a, b** and **e**. In the case of **9c** the oily residue was induced to crystallize by adding a few drops of EtOH. The compound was purified by flash chromatography using $CHCl_3$ as eluent and finally was recrystallized from EtOH.

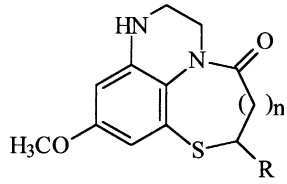
4.1.9. 9-Methoxy-4-oxo-1,4,5,6-tetrahydroimidazo[3,4,5-e, f]-1,5-benzothiazepin-2-thione **9f** (table IV)

A mixture of **2f** (2.24 g, 0.01 mol), carbon sulfide (20 mL) and triethylamine (1.52 g, 0.015 mol) in anhydrous tetrahydrofuran (50 mL) was refluxed under stirring for 16 h. After cooling the reaction mixture was evaporated in vacuo and the residue was purified by flash chromatography using $CHCl_3$ as eluent. The purified compound was crystallized from EtOH.

4.1.10. General procedure for 8-methoxy-4,5-dihydro-1,2,3-triazolo[3,4,5-d, e]-1,4-benzothiazin-4-ones **10a, b, d** *and* **e** *and 9-methoxy-4H-5,6-dihydro-1,2,3-triazolo[3,4,5-e, f]-1,5-benzothiazepin-4-one* **10f** (table V)

A solution of sodium nitrite (1.04 g, 15 mmol) in 10 mL of water was added slowly to an ice-cooled suspension of the aminoderivative **2a, b, d–f** (0.01 mol) in 2 N HCl (10 mL).

Table VI. Physical and chemical data of compounds **11a–f.**



Compound	R	n	Yield (%)	M.p. (°C)	Formula (MW)	^{1}H-NMR, δ ($CDCl_3$)
11a	H	0	18	136–138	$C_{11}H_{12}N_2O_2S$ (236.29)	3.20–3.43 (superimposed d and t, 4H, *CH*$_2$CH$_2$ + SCH$_2$), 3.67 (s, 3H, OCH$_3$), 3.92 (t, *J* = 10.0 Hz, 2H, CH$_2$*CH*$_2$), 4.25 (s, 1H, NH), 6.00 (d, J_{meta} = 2.5 Hz, 1H, Ar), 6.22 (d, J_{meta} = 2.5 Hz, 1H, Ar)
11b	CH_3	0	27	118–120	$C_{12}H_{14}N_2O_2S$ (250.32)	1.50 (d, *J* = 5.1 Hz, 3H, CH*CH*$_3$), 3.40 (t, 2H, *CH*$_2$CH$_2$), 3.55 (q, 1H, *CH*CH$_3$), 3.73 (s, 3H, OCH$_3$), 3.75–4.23 (m, 3H, CH$_2$*CH*$_2$ + NH), 6.05 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.25 (d, J_{meta} = 2.4 Hz, 1H, Ar)
11c	CH_2CH_3	0	5	oil	$C_{13}H_{16}N_2O_2S$ (264.34)	1.08 (t, 3H, CH$_2$*CH*$_3$), 1.50–2.10 (m, 2H, *CH*$_2$CH$_3$), 3.25–3.45 (m, 2H, CH$_2$), 3.60–3.80 (m, 2H, CH$_2$), 3.72 (s, 3H, OCH$_3$), 4.08 (br s, 1H, NH), 4.20–4.35 (m, 1H, SCH), 6.05 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.25 (d, J_{meta} = 2.4 Hz, 1H, Ar)
11d	$(CH_2)_3CH_3$	0	4	oil	$C_{15}H_{20}N_2O_2S$ (292.40)	0.86 (t, 3H, CH$_3$), 1.20–1.70 (m, 4H, CH$_2$*CH*$_2$*CH*$_2$CH$_3$), 1.80–2.00 (m, 2H, *CH*$_2$CH$_2$CH$_2$CH$_3$), 3.30–3.46 (m, 3H, NH + *CH*$_2$CH$_2$), 3.72 (s, 3H, OCH$_3$), 4.15–4.28 (m, 2H, CH$_2$*CH*$_2$), 6.05 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.25 (d, J_{meta} = 2.4 Hz, 1H, Ar)
11e	C_6H_5	0	24	160–162	$C_{17}H_{16}N_2O_2S$ (312.39)	3.70 (s, 3H, OCH$_3$), 3.82, 4.25 (A$_2$B$_2$ system, 4H, CH$_2$CH$_2$), 4.07 (s, 1H, NH), 4.68 (s, 1H, S*CH*C$_6$H$_5$), 6.03 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.25 (d, J_{meta} = 2.4 Hz, 1H, Ar), 7.20–7.40 (m, 5H, Ar)
11f	H	1	45	oil	$C_{12}H_{14}N_2O_2S$ (250.32)	2.65–3.50 (m, 8H, SCH$_2$CH$_2$ + NCH$_2$CH$_2$), 3.75 (s, 3H, OCH$_3$), 4.20 (s, 1H, NH), 6.15 (d, J_{meta} = 2.4 Hz, 1H, Ar), 6.50 (d, J_{meta} = 2.4 Hz, 1H, Ar)

After the addition was complete, compound **10a** was immediately obtained as an abundant precipitate which was collected by filtration and recrystallized from MeOH. In all the other cases the reaction mixture was stirred at room temperature for 2 h for compound **2d**, for 5h for compounds **2b** and **f** and for 40 h for **2e**. The resulting precipitate was filtered to give compounds **10b, d** and **e** which were recrystallized from EtOH (**10d** and **e**) or EtAc (**10c** and **d**).

In the case of the benzothiazepine derivative, as no precipitate was formed, the solution was alkalinized with dilute $NaHCO_3$, extracted with $CHCl_3$, washed with water, dried over anhydrous Na_2SO_4 and dried in vacuo. The oily residue was purified by flash chromatography using $CHCl_3$ as eluent to give **10f**.

4.1.11. General procedure for 9-methoxy-2,3,6,7-tetrahydro-5H-[1,4]thiazino[4,3,2-d,e]quinoxalin-3-ones **11a–d** (table VI)

To a stirred solution of the aminoderivatives **2a–d** (0.01 mol), tetrabutylammonium bromide (0.32 g, 0.001 mol) and 1,2-dibromoethane (1.88 g, 0.01 mol) in tetrahydrofuran, finely powdered potassium hydroxide (0.56 g, 0.01 mol) was added. The reaction mixture was kept at room temperature for 20 h for **2d**, 48 h for **2a**, 3 d for **2b** and 6 d for **2c,** and then filtered. The filtrate was evaporated in vacuo and the residue was taken up with chloroform, the chloroform extract washed with water, dried over anhydrous Na_2SO_4 and dried in vacuo. The oily residue was purified by flash chromatography using $CHCl_3$ as eluent.

4.1.12. Preparation of 9-methoxy-2-phenyl-2,3,6,7-tetrahydro-5H-1,4-thiazino[4,3,2-d,e]quinoxalin-3-one **11e** *and 10-methoxy-2,3,6,7-tetrahydro-1H,5H-1,4-thiazepino [4,3,2-d,e]quinoxalin-5-one* **11f** (table VI)

A suspension of aminoderivative **2e** and **f** (0.01 mol), 1,2-dibromoethane (1.88 g, 0.01 mol), $NaHCO_3$ (2.52 g, 0.03 mol) in anhydrous xylene was refluxed under stirring for 24 h. After cooling the reaction mixture was filtered, the filtrate was dried in vacuo and the resulting residue was purified by flash chromatography using $CHCl_3$ as eluent.

5. Biological evaluation

The procedure [8] used in the NCI's test for anti-HIV-1 screening is designed to detect agents acting at any stage of the virus reproductive cycle.

Tested compounds were dissolved in dimethylsulfoxide and then diluted 1:100 in cell culture medium and then serial half-log_{10} dilutions were prepared. T4 lymphocytes (CEM cell line) were added and after a brief interval HIV-1 was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound were used as a toxicity control and uninfected cells without the compound as basic controls. Cultures were incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 d.

The tetrazolium salt XTT was added to all wells and cultures were incubated to allow formazan colour development by viable cells. Individual wells were analysed spectrophotometrically to quantitate formazan production and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity.

Drug-treated virus-infected cells were compared with drug-treated noninfected cells and with other controls (untreated-infected and noninfected cells, drug-containing wells without cells) on the same plate. Data were reviewed in comparison with other tests done at the same time and a determination about activity was made.

Anti-HIV-1 activity was expressed as 50% effective concentration (EC_{50}) against HIV-1 cytopathic effects and drug cytotoxicity as 50% cytotoxic concentration (CC_{50}).

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