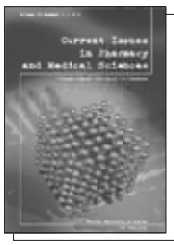

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The anti-HIV, anti-tumor activity of allyl derivatives of 1,2,4-triazole-5-thione, 1,3,4-thiadiazol-2-yl-amine and derivatives of bicyclic systems: 1,2,4-triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, thiazolo[2,3-c][1,2,4]triazole

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ABSTRACT

The isomerization of 5H-3,7-diphenyl-6,7-dihydro-1,2,4-triazole[3,4-b] 1,3-thiazine (**1**) in a sealed tube, in the presence of aqueous HBr, was carried out, and 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**2**) was obtained. The 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**2**), 5H-3-phenylmethyl-7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**3**), the 3-phenylmethyl-6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole (**4**), 3-(o-hydroxy-phenyl-)6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4] triazole (**5**) were evaluated against HIV-1. The anti-tumor activities of 3-(pyridin-2-yl)-4-allyl-1,2,4-triazole-5-thione (**6**), 2N-cinnamyl-(5-methyl-[1,3,4]thiadiazol-2-yl-) amine (**7**), 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**2**), 5H-3-phenylmethyl-7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**3**), the 3-phenylmethyl-6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole (**4**), 3-(o-hydroxy-phenyl-)6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole (**5**) were tested against the IP P388 Leukemia 3PS31.

Keywords: 1,2,4-Triazole[3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, thiazolo[2,3-c][1,2,4]triazole, 1,2,4-triazole-5-thione, 1,3,4-thiadiazole. The anti-HIV, *in vitro* and ati-tumor activity, *in vivo*

INTRODUCTION

The therapeutic properties of 1,2,4-triazole and 1,3,4-thiadiazole derivatives are well documented. The 1,2,4-triazole derivatives have been reported in the literature to show anti-microbial [26], anti-viral/anti-HIV, anti-tuberculosis [3, 10, 12] and anti-cancer activity [3, 5, 7]. Itraconazole, posaconazole, voriconazole and fluconazole that are used for the treatment of microbial infections [28] contain a 1,2,4-triazole ring in their structures. In addition, other important chemotherapeutics, such a Vorozole, Letrozole and Anastrozole that consist of the substituted 1,2,4-triazole ring, are used for the treatment of breast cancer [4].

The 1,3,4-thiadiazole derivatives are another important class of heterocycles due to their biological activities. They are known to exhibit anti-bacterial, anti-fungal, anti-viral, anti-inflammatory [27, 18, 6], anti-mycobacterial [13, 20] and anti-parasitic activities [8]. They possess anaestetic [14], anti-inflammatory, diuretic [19], anti-diabetic [18] and anti-cancer properties [15, 16].

The bicyclic systems comprising the 1,2,4-triazole or the 1,3,4-thiadiazole molecule possess a broad spectrum of biological activities. Among the more important effects are anti-inflammatory [25, 1], anti-microbial, anti-fungal [1, 2, 9, 17], anti-viral [11] properties. The present paper describes the anti-tumor and the anti-HIV activity screening of selected allyl derivatives of 1,2,4-triazole-5-thione, 1,3,4-thiadiazol-2-ylamine and some bicyclic systems: 1,2,4-triazole [3,4-b]1,3-thiazine, 1,2,4-triazole[3,2-b]1,3-thiazine, thiazolo[2,3-c][1,2,4]triazole .

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EXPERIMENTAL

Chemistry

The ^{1}H NMR spectrum of product (**2**) was measured with a Tesla BS-487C spectrometer (80 MHz), in $CDCl_3$ solution, at room temperature, with TMS as the internal standard. The chemical shifs are given in δ scale.

7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (2)

A mixture of 0.9 g (3 mmole) of (**1**) and 1.2 g (1.5 mmole) of 10% aqueous hydrogen bromide and 1.0 g of water was heated for 20 hrs in a sealed tube at 126°C. The content of the tube was washed with hot ethanol. The solvent was distilled off and the residue was neutralized with aqueous ammonia. The precipitate (0.9g) was crystallized from ethanol, 0.65 g (72% yield) m.p.185-186°C. ^{1}H NMR ($CDCl_3$), δ: 2.7 (2Hm) -N-CH_2-**CH_2**-CH-, 4.4 (2Hm) -N-**CH_2**-CH_2-CH-, 4.7 (1Hm) -N-CH_2-CH_2-**CH-**, 7.4 (8Hs), 8.0 (2Hs) for aro-

matic protons; anal. Calcd. for $C_{17}H_{15}N_3S$: N 14.3%, found: N 13.7%

Biological activity

The following compounds: 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**2**), 5H-3-phenylmethyl-7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**3**), 3-phenylmethyl-6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4] triazole (**4**), 3-(o-hydroxy-phenyl-)6-methyl-5,6-dihydro-thiazolo[2,3-c][1,2,4]triazole (**5**), 3-(pyridin-2-yl)-4-allyl--1,2,4-triazole-5-thione (**6**), 2N-cinnamyl-(5-methyl- [1,3,4] thiadiazol-2-yl-) amine (**7**), selected by the National Cancer Institute USA, were tested *in vivo* (animals) against the IP P 388 Leukemia Tumor Test System (3PS31). The bicyclic systems (**2 - 5**) were selected by the National Cancer Institute USA and were evaluated *in vitro* for their anti-HIV activity.

The anti-HIV activity test and the IP P 388 Leukemia Tumor Test System (3PS31) were carried out in the National Cancer Institute, USA, Bethesda, Maryland. The procedure, as well as the Graphics Results Summary Section regarding the anti-HIV investigations are in the Supplementary material.

RESULTS AND DISCUSSION

Chemistry

The heating of 5H-3,7-diphenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**1**) [21] in a sealed tube in the presence of aqueous HBr leads to 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**2**) – (Fig. 1).

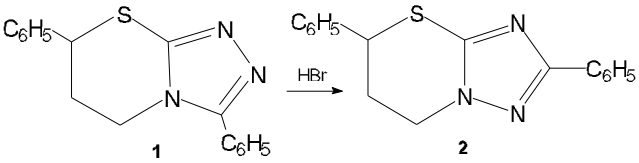

Fig. 1. The izomerization of 5H-3,7-diphenyl-6,7-dihydro-1,2,4-triazole[3,4-b] 1,3-thiazine (**1**) to 7H-2,5-diphenyl-5,6-dihydro-1,2,4-triazole[3,2-b]1,3-thiazine (**2**) in a sealed tube, in the presence of aqueous HBr

Compound (**2**) was identical with the reaction product of 3-phenyl-4-cinnamyl-1,2,4-triazole-5-thione with the HBr solution as described earlier [22]. The products obtained in two different ways showed identical melting points, without any depression in the melting point of their mixture, and had also the same ^{1}H NMR spectra. The analytical data are given in the experimental detail.

The synthesis of 5H-3-phenylmethyl-7-phenyl-6,7-dihydro-1,2,4-triazole[3,4-b]1,3-thiazine (**3**) [21], 3-phenylmethyl-6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4]triazole (**4**), 3-(o-hydroxy-phenyl-)6-methyl-5,6-dihydrothiazolo[2,3-c][1,2,4] triazole (**5**) [23], 3-(pyridin-2-yl)-4-allyl-1,2,4-triazole-5-thione (**6**) [24], 2N-cinnamyl-(5-methyl-[1,3,4]thiadiazol-2-yl-) amine (**7**) [24] were described earlier. The chemical structures of the investigated compounds are shown on Figs 1 and 2. The biological activity of these compounds has not been the subject of the studies.

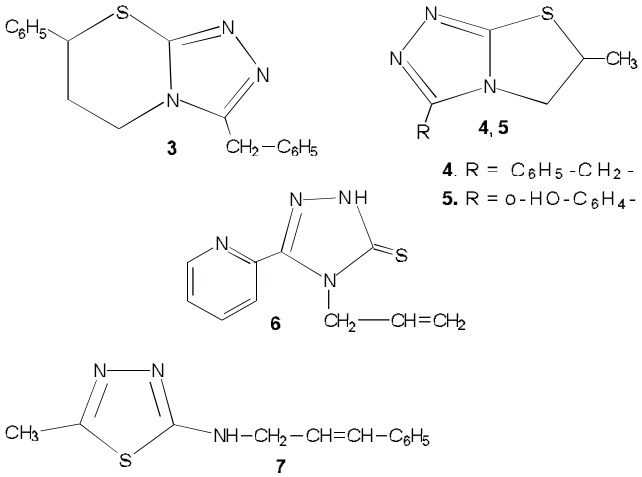

Fig. 2. The chemical structure of the studied compounds

Biological activity

Anti-HIV activity

The bicyclic systems (**2-5**) were selected by the National Cancer Institute USA, and were evaluated for their *in vitro* anti-HIV activity. For compounds (**2-5**), the inhibitory concentration (IC_{50}) was obtained. According to the data (Table 1) procured, the compounds (**2-5**) had activity against HIV-1 with IC_{50} values from 46.2–61.5 μg/mL and the maximal protection (%) in the range 1.87–20.36. Compound (**2**) showed the highest activity against HIV-1, with the value of maximal protection of 1.87%, 2.0%. None of the evaluated compounds showed any specific activity against HIV-1 in CEM-IW cells.

Table 1. Anti-HIV activity of compounds (**2–5**) in CEM-IW cells

Compd.	Number assigned by NCI	Plate[a]	HIV-1	
			IC_{50} (μg/mL)	Max. protection (%)
4	609097	4877	46.2	16.32
		4852	46.2	20.36
5	609098	4852	46.7	11.23
		4877	46.7	10.00
3	609099	4853	61.5	13.96
		4878	61.5	10.90
2	609100	5803	58.7	1.87
		5828	58.7	2.00

a – the actual experiment number from which the results were taken*

* Supplementary material

Anti-tumor activity

The compounds (**2-7**), selected by the National Cancer Institute USA, were tested *in vivo (*animals) against the IP P 388 Leukemia Tumor Test System (3PS31). The results are given in Tables 2, 3 and 4**.** One type of report is referred to as the 'Leukemia Screen Test Results' (LSTR) (Table 2). A second, more detailed report, is known as the 'Screening Data Summary' (SDS) Report (Table 3). The presumptive activity was confirmed by first screener, Screener Code –

SCR 99 (Tables 2 and 3). The values -0.9 and T/C% = 127 for Tumor/Line/Code/Gen = A02/R/007 and -1.4, -1.2, T/C% = 127, 130 for Tumor/Line/Code/Gen = A05/R/006 (Table 3) confirmed the activity of the investigated compounds. Survival systems indicate a degree of success when T/C percents exceed 125. Tumor inhibition systems indicate a degree of success when the T/C percents do not exceed 42. Minus values (only occurring for tumor inhibition systems) reflect the percent of tumor regression between initial and final tumor volume. The Leukemia Screen Test Results (LSTR), Screener Code – SCR 09 (Table 4) shows further anti-tumor screening data for (**2-7**).

Table 2. Leukemia Screen (3PS31) Test Results (LSTR)

NSC	Doses tested	T/C %[b]	SCR[a]
999991	240.00 mg/kg 120.00 60.00	108 102 107	99
999992	240.00 mg/kg 120.00 60.00	TOX 105 108	99
999993	240.00 mg/kg 120.00 60.00	105 102 103	99
999994	240.00 mg/kg 120.00 60.00	TOX 102 107	99
999995	240.00 mg/kg 120.00 60.00	106 103 103	99

a – SCR = Screener Code
99 – NCI information only
b –T/C % = The test evaluation expressed as a percent of the control evaluation, providing a measure of effectiveness of the compound being tested.

Table 3. Screening Data Summary (SDS) Report

NSC		DOS/INJ/U	BWD[g]		T/C %[b]	SCR[a]
WD1/2:1/5[f]			TSC:22P[c]	SEX:M[e]		
999991		240.00 mg/kg 120.00 60.00	-0.9 1.2 1.5 HOST:06[d]	BWC = -1.4[h]	127 120 112	99
Tumor/Line/Code/Gen = A02/R/007						
WD1/2:1/5[f]			TSC:22P[c]	SEX:M[e]		
999991		480.00 mg/kg 240.00 120.00 60.00	-1.4 -1.2 - 0.3 - 0.6 HOST:06[d]	BWC = 1.0[h]	127 130 109 106	99
Tumor/Line/Code/Gen = A05/R/006						

a – SCR = Screener Code
99 – NCI information only
b – T/C % = The test evaluation expressed as a percent of the control evaluation, providing a measure of effectiveness of the compound being tested. Survival systems indicate a degree of success when T/C percents exceed 125. Tumor inhibition systems indicate a degree of success when the T/C percents do not exceed 42. Minus values (only occurring for tumor inhibition systems) reflect the percent of tumor regression between initial and final tumor volume.
c – "TSC:22P" = this item identifies the Test Status Code (TSC) and TSC suffix, P – Active Test, F- Inactive Test, or R – Erratic Test (unreliable data).
d – Host Code for all animal in the experiment (Test and Control)
Host Code in Vivo (Strain)
06 – CD2 F1 (CDF1)
e – SEX = M
M = Male
f – WD1/2:1/5, this item identifies initial (1) and final (2) animal weigh days. (In this example, the days are 1 and 5)
g – BWD = Animal Body Weight Differences which is computed by subtracting the Control Group body weight change from the Test Group body weight change
h – BWC = Body Weight Change calculated as final average body weight minus initial average body weight. This item is calculated for the Control Group only.
Accredited Animal Suppliers
Supplier: Camm Research Institute
Symbol: CRI
Code: 06
Host: Rats

Activity Thresholds of Common Systems

Model	Code	Drug RT/SCHED	Active T/C % Parameter	MC1
Prescreen				
IP P388 Leukemia	3PS31	IP/Q1DX5	Med survival time	≥ 127
			Confirming test	≥ 120

Screening Models
PS – P388 Leukemia

Table 4. Leukemia Screen (3PS31) Test Results (LSTR)

Compd.	Number assigned by NCI	Doses tested	T/C %	SCR[a]
6	609095	240.00 mg/kg 120.00 60.00	100 101 102	09
7	609096	240.00 mg/kg 120.00 60.00	95 96 100	09
4	609097	240.00 mg/kg 120.00 60.00	100 93 104	09
5	609098	240.00 mg/kg 120.00 60.00	101 100 96	09
3	609099	240.00 mg/kg 120.00 60.00	95 99 102	09
2	609100	240.00 mg/kg 120.00 60.00	87 89 92	09

a – SCR = Screener Code
09 – IIT Research Inst. – *in vivo*

The anti-HIV investigations showed the moderate activity of the studied bicyclic derivatives (**2-5**). From the structure-activity relationship, the lack of the substituent at the nitrogen atom N3 of the bicyclic system (**2**) increases its anti-HIV activity. Due to the planar structure of the benzene and 1,2,4-triazole rings of the compound (**2**) [22], a strong delocalization of electrons and a negative electrostatic potential on the 1,2,4-triazole ring appears.

Acknowledgement

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