Full Paper

In-Vitro Anti-HIV and Antitumor Activity of New 3,6-Disubstituted [1,2,4]Triazolo[3,4-*b*][1,3,4]thiadiazoles and Thiadiazine Analogues

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A series of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles (**7–15**) and the thiadiazine analogues **16–18** have been synthesized under microwave irradiation (MWI). All synthesized compounds are evaluated for their antiviral activity against the replication of HIV-1 and HIV-2 activity in MT-4. However, compounds **12** and **18** showed EC_{50} = 2.11 and 1.97 μg/mL. The results suggest that these compounds can be considered as a new lead in the development of antiviral agents. Compounds **4–18** were tested *in vitro* against a panel of tumor cell lines. All compounds are inactive against all the tumor sub-lines, except **10** which exhibited activity against $CD4^+$ human acute T-lymphoblastic leukaemia of CC_{50} = 64 μM.

Keywords: Anti-HIV activity / Antitumor activity / Microwave-assisted synthesis / Triazolothiadiazoles / Thiadiazines

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Introduction

A number of [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles and [1,2,4]triazolo[1,3,4]thiadiazines possess diverse pharmacological activities [1–7] such as antimicrobial [8], antitumor [9, 10], antiviral [3, 11], antibacterial [3, 12], herbicidal [13], anti-HIV-1 [14], CNS stimulant [15], antifungal [16, 17], and antihelminitic [18] activities. Holla *et. al*. [9] reported that the various biological activities of [1,3,4]thiadiazoles are possibly due to the presence of the =N-C-S moiety. Recently, Shaker [19] has reviewed the synthesis of biological activity of various triazoles, triazolothiadiazoles and triazolothiadiazines. Additionally, Al-Masoudi *et al*. [20] have reviewed recently the synthetic approaches of 1,2,4-triazoles and their pharmacological importance.

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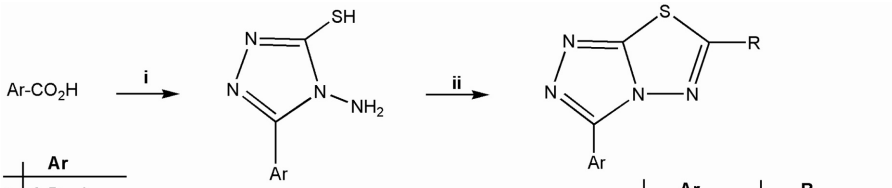
Abbreviation: microwave irradiation (MWI)

In view of these findings and in continuation of our previous work [21] on the synthesis of acyclic C-nucleosides of triazolothiadiazoles and thiadiazines, herein, we report the synthesis of some [1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazoles, and the evaluation of triazolothiadiazine analogues, which could be favorable candidates for achieving some pharmacological specificity regarding the development of effective clinical anticancer and anti-HIV drugs.

Chemistry

Condensation of the appropriate substituted benzoic acids (3-bromo-, 4-amino, and 3,4-dichlorobenzoic acids) **1–3** with thiocarbohydrazide in pyridine under microwave irradiation (MWI) for 15 min afforded, after purification, the 4-amino-5-aryl-2,4-dihydro-[1,2,4]triazole-3-thions **4–6** in 75, 73, and 91% yield, respectively.

Treatment of **4–6** with the appropriate substituted benzoic acids in DMF at 140°C for 145 min gave the 3,6-disubstituted 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazole derivatives **7–15** in 26–97% yield. The structures of **4–6** and **7–15** were confirmed by the ^{1}H-, ^{13}C-NMR, and mass spectra. Compound **4–6** and **7–15** showed a similar NMR



	Ar
1	3-Br-ph
2	4-NH_2-Ph
3	3,4-Cl_2-Ph

	Ar
4	3-Br-ph
5	4-NH_2-Ph
6	3,4-Cl_2-Ph

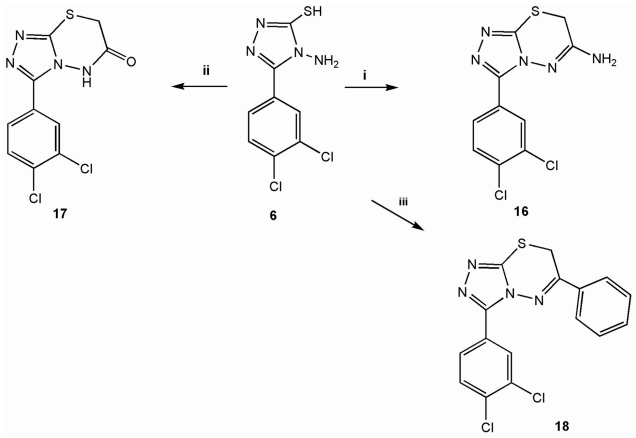
	Ar	R
7	3-Br-ph	3,4-Cl_2-ph
8	4-NH_2-Ph	3-coumarin
9	3,4-Cl_2-Ph	3,4-Cl_2-Ph
10	3,4-Cl_2-Ph	4-NH_2-Ph
11	3,4-Cl_2-Ph	3-pyridine
12	3,4-Cl_2-Ph	2-pyrazine
13	3,4-Cl_2-Ph	2-pyrrole
14	3,4-Cl_2-Ph	2-thiophene
15	3,4-Cl_2-Ph	2-furan

Conditions and reagents: (i) $(NH_2NH)_2C=S$, pyridine, MWI, 15 min; (ii) RCO_2H; DMF, MWI, 145 min, 140°C.

Scheme 1. Synthesis route of compounds **1–15**.

spectral pattern. In the ^{13}C-NMR spectra of **4–6**, the resonances at the region δ 165.2–166.2 and δ 148.0–148.2 were assigned to the carbons of *C*-SH and C=N groups, respectively. The protons of **7–15** were fully analyzed, while the ^{13}C-NMR spectra showed two high-field signals at the regions δ 165.2–167.8 ppm and δ 162.1–164.8 ppm which are attributed to N=*C*-S and *C*-6, respectively. C-3 (C=N) displayed signals at δ 143.1–147.8 ppm. The carbons of coumarin, pyridine, pyrazine, pyrrole, thiophene, and furan were assigned. The synthesis route of compounds **1–15** is depicted in Scheme 1.

Next, our attention focused on synthesis of new triazolo-thiadiazine as potentially active compounds. Thus, treatment of **6** with 2-chloracetonitrile in the presence of 3% H_2SO_4/SiO_2 under MWI at 85°C for 30 min afforded **16** (65%). Similar treatment of **6** with 2-chloroacetic acid containing NaOAc furnished **17** (68%). Further, heating of **6** with phenacyl chloride under MWI gave **18** (61%) (Scheme 2). Structures of the newly synthesized compounds **16–18** were assigned by the ^{1}H, ^{13}C-NMR and mass spectra. The ^{1}H-NMR spectra showed rather similar patterns for the phenyl, while the singlets at δ 3.97–4.39 ppm were attributed to SCH_2 group. In the ^{13}C-NMR spectra of **16–18**, C=N (*C*-3) resonated at δ 147.7, 148.8, and 147.5 ppm, respectively, while C-6 (C=O at compound **17**) appeared at higher field δ 164.2, 171.5, 164.0 ppm, respectively. CH_2S carbons resonated at δ 37.8, 34.2, and 36.0 ppm, respectively.



Reagent and conditions: (i) 2-chloroacetonitril, 3% H_2SO_4/SiO_2, MWI, 30 min; (ii), 2-chloroacetic acid, NaOAc, EtOH, MWI, 30 min; (iii) $PhCOCH_2Cl$, EtOH, MWI, 30 min.

Scheme 2. Synthesis route of compounds **16–18**.

Biology

In-vitro anti-HIV and other virus assays

Compounds **4–18** were tested for their *in-vitro* anti-HIV-1 (strain IIIB) and HIV-2 (strain ROD) activity in human T-lymphocyte (MT-4) cells using the MT-4/MTT assay [22], as well as their activity against other viruses, such as human T-cells containing an integrated HTLV-1 genome, MDBK (Madin-Darby bovine (normal) kidney cells) with BVDV (bovine viral diarrhea virus), BHK (hamster normal kidney fibroblast), BHK (kidney fibroblast) cells from the YFV (yellow fever virus) and Reo (reovirus 1), mock-infected VERO-76 (monkey normal kidney), HSV-1 (herpesvirus 1), VV (vaccinia virus), VSV (vesicular stomatitis virus), CVB-2 (coxsackievirus B2), Sb-1(poliovirus 1) and RSV (respiratory syncytial virus). The anti-HIV activity is summarized in Table 1, in which the data for efavirenz [23] and capravirine [24] are included for comparison. All the compounds are inactive except for **12** and **18** which showed EC_{50} = 2.11 and 1.97 μg/mL, respectively for which the data can be discussed. However, none of our com-

Table 1. *In-vitro* anti-HIV-1[a] and HIV-2[b] of some new triazolo-thiadiazoles and thiadiazines.

Compound	Virus strain	av. EC_{50} (μg/mL)	av. CC_{50} (μg/mL) ± SD	SI
4	III_B	>75.4	75.4 ± 6.71	<1
	ROD	>75.4	75.4 ± 6.71	<1
5	III_B	>12.00	12.00 ± 1.13	<1
	ROD	>12.00	12.00 ± 1.13	<1
6	III_B	>72.83	72.83 ± 3.36	<1
	ROD	>72.83	72.83 ± 3.36	<1
7	III_B	>4.12	4.12 ± 2.08	<1
	III_B	>4.12	4.12 ± 2.08	<1
8	III_B	>108.3	108.3 ± 8.86	<1
	ROD	>108.3	108.3 ± 8.86	<1
9	III_B	>67.8	67.8 ± 13.86	<1
	ROD	>67.8	67.8 ± 13.86	<1
10	III_B	>19.61	19.61 ± 20.84	<1
	ROD	>19.61	19.61 ± 20.84	<1
11	III_B	>80.05	80.05 ± 11.17	4
	ROD	>80.05	80.05 ± 11.17	<1
12	III_B	>2.11	2.11 ± 1.13	<1
	ROD	>2.11	2.11 ± 1.13	<1
13	III_B	>68.48	68.48 ± 5.63	<1
	ROD	>68.48	68.48 ± 5.63	<1
14	III_B	>92.70	92.70 ± 15.2	<1
	ROD	>92.70	92.70 ± 15.2	<1
15	III_B	>27.51	27.20 ± 7.79	<1
	ROD	>27.20	27.20 ± 7.71	<1
16	III_B	>2.70	2.70 ± 5.30	<1
	ROD	>2.70	2.70 ± 5.30	<1
17	III_B	>35.23	35.23 ± 6.20	<1
	ROD	>35.23	35.23 ± 6.20	<1
18	III_B	>1.97	1.97 ± 0.40	<1
	ROD	>1.97	1.97 ± 0.40	<1
Efavirenz	III_B	>0.003	40	13333
Capravirine	III_B	>0.0014	11	7857

[a] Anti-HIV-1 activity measured with strain III_B.
[b] Anti-HIV-2 activity measured with strain ROD.

pounds approached the activity level of the reference compounds.

Compounds **12** and **18** are equipotent against HIV-1 and HIV-2 replication *in vitro* and, therefore, most probably they are no NNRTI's (Non-Nucleoside Reverse Transcriptase Inhibitors). The anti-HIV activity and the selectivity of these compounds are however too limited to perform extensive mode-of-action studies. The synthesis of new analogues of this triazolothiadiazines derivative may lead to the discovery of more potent and selective analogues that will allow the elucidation of their molecular mode-of-action.

Regarding other viruses, the Coxsackie virus B (CVB-2) was the only virus inhibited by compounds **9**, **10**, **11**, and **14** with EC_{50} = 12, 20, 31, and 22 μg/mL, respectively (CC_{50} > 100 μg/mL), meanwhile, all other compounds demonstrated inactivity against the other screened viruses.

In-vitro antitumor assay

Compounds **4 – 18** were tested *in vitro* against a panel of tumor cell lines, consisting of $CD4^+$ human T-cells with integrated leukaemia (CCRF-CEM), human acute T-lymphoblastic leukaemia (WIL-2NS), human splenic B-lymphoblastoid cells (CCRF-SB), human acute B-lymphoblastic leukaemia (SK-MEL-28), human skin melanoma (SK-MEL-28), human breast adenocarcinoma (MCF-7), human lung squamous carcinoma (SK-MES-1), human hepatocellular carcinoma (HepG2), human prostate carcinoma (DU-145), human foreskin fibroblast (CRL7065), and human lung fibroblast (MRC-5), using the microculture tetrazolium assay (MTT) method for estimation of the *in-vitro* tumor-inhibiting activity. All compounds are inactive against all the tumor sub-lines, except **10** which exhibited activity against $CD4^+$ human acute T-lymphoblastic leukaemia of CC_{50} = 64 μM.

Experimental

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario Elementar apparatus (Shimadzu, Japan). NMR spectra were recorded on 300 and 600 MHz (^1H) and at 62.9 MHz (^{13}C) spectrometers (Bruker, Germany) with TMS as internal standard and on δ scale in ppm. Heteronuclear assignments were verified by ^1H- / ^{13}C-HMBC experiment. Mass spectra were recorded at 70 eV on EI and FAB mass spectra were measured on a MAT 8200 spectrometer (Finnigan MAT, USA) using 3-nitrobenzyl alcohol (NBOH) or glycerol as matrices. Some molecular ions were detected by doping the sample with Na^+ ion.

General preparation of 4-amino-5-aryl-1,2,4-triazole-3-thiols *4 – 6*

A mixture of **1 – 3** (1.00 mmol) and thiocarbohydrazide (106 mg, 1.00 mmol) in pyridine (5 mL) was heated at 140°C under MWI for 15 min. After cooling, the solution was evaporated to dryness and the residue was washed with water, acidified with conc. HCl, filtered, washed with water, and dried. The solid was recrystallized from EtOH to give the desired products **4 – 6**.

*4-Amino-5-(3-bromophenyl)-1,2,4-triazole-3-thiols **4** [25]*

From 3-bromo-benzoic acid (201 mg). Yield: 203 mg (75%), mp. 155 – 157°C. ^1H-NMR (DMSO-d_6): δ 8.05 (s, 1H, SH), 7.94 (d, 2H, *J* = 7.7 Hz, ArH), 7.84 (d, 1H, *J* = 7.9 Hz, ArH), 7.50 (t, 1H, *J* = 7.8 Hz, ArH), 3.61 (br s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6): δ 165.9 (C-SH), 148.0 (C=N), 135.5, 132.9, 131.7, 130.8, 128.2 (Ar), 121.6 (Ar-C-Br). Anal. calc. for $C_8H_7BrN_4S$ (271.14): C, 35.44; H, 2.60; N, 20.66. Found: C, 35.19; H, 2.52; N, 20.44. MS: *m/z* (FAB) 271/273 $[M + H]^+$.

*4-Amino-5-(4-aminophenyl)-1,2,4-triazole-3-thiols **5** [25]*

From 4-amino-benzoic acid (137 mg). Yield: 198 mg (73%), mp. 162 – 163°C. ^1H-NMR (DMSO-d_6): δ 8.25 (s, 1H, SH), 7.90 (d, 2H, *J* = 7.7 Hz, ArH), 6.80 (d, 2H, *J* = 7.8 Hz, ArH), 5.80 (br s, 2H, NH_2), 4.31 (br s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6): δ 166.5 (C-SH), 147.5 (C=N),

147.2 (Ar-C-NH_2), 130.1, 128.5, 119.6 (ArC), Anal. calc. for $C_8H_9N_5S$ (207.26): C, 46.36; H, 4.38; N, 33.79. Found: C, 46.19; H, 4.29; N, 33.58. MS: *m/z* (FAB) 208 $[M + H]^+$.

4-Amino-5-(3,4-dichlorophenyl)-1,2,4-triazole-3-thiols ***6***

From 4-amino-benzoic acid (191 mg). Yield: 238 mg (91%), mp. 197–198°C. ^{1}H-NMR (DMSO-d_6): δ 8.07(dd, 1H, *J* = 7.8 Hz, 1.9 Hz, ArH), 7.90 (d, 1H, *J* = 2.0 Hz, ArH), 7.78 (d, 1H, *J* = 1.9 Hz ArH), 5.85 (br s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6): δ 165.4 (C-SH), 148.0 (C=N), 131.4, 131.3 (A-C-Cl), 131.0, 130.9, 127.0, 129.2 (Ar). Anal. calc. for $C_8H_6Cl_2N_4S$ (261.13): C, 36.80; H, 2.32; N, 21.46. Found: C, 36.61; H, 2.26; N, 21.19. MS: *m/z* (FAB) 261/263 $[M + H]^+$.

General procedure of 3,6-diaryl-6-[1,2,4]]triazolo[3,4-*b*][1,3,4]thiadiazoles *7–15*

A mixture of **4–6** (0.25 mmol) and aryl or alkyl benzoic acid (0.25 mmol) in DMF (5 mL) was heated at 140°C in the microwave for 145 min. After cooling, the solution was evaporated to dryness to give solid or semi-solid products. The solid product was recrystallized from EtOH to give the desired products.

*3-(3-Bromophenyl)-6-(3,4-dichlorophenyl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***7***

From **4** (68 mg) and 3,4-dichloro-benzoic acid (48 mg). Yield: 88 mg (83%), mp. 142-144°C. ^{1}H-NMR (DMSO-d_6): δ 8.06 (dd, 1H, *J* = 7.8 Hz, *J* = 2.0 Hz, ArH), 7.95–7.76 (m, 5H, ArH), 7.48 (t, 1H, *J* = 7.8 Hz, ArH). ^{13}C-NMR (DMSO-d_6): δ 165.8 (N=C-S), 163.3 (C-6), 143.1 (C=N), 135.5, 135.4 (AC-Cl), 133.2, 132.9, 131.6, 131.4, 130.9, 130.7, 129.2, 128.1, 126.8 (Ar), 121.6 (ArC-Br). Anal. calc. for $C_{15}H_7BrCl_2N_4S$ (426.12): C, 42.28; H, 1.66; N, 13.15. Found: C, 42.48; H, 1.56; N, 13.37. MS: *m/z* (FAB) 426 $[M + H]^+$.

*3-(4-Aminophenyl)-6-(coumarin-3-yl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***8***

From **5** (52 mg) and coumarin-3-carboxylic acid (48 mg). Yield: 42 mg (47%), mp. 100–103°C. ^{1}H-NMR (DMSO-d_6): δ 7.60 (d, 2H, *J* = 7.0 Hz, ArH), 7.39 (s, 1H, coumarin-H), 7.32–6.77 (m, 4H, coumarin-H), 6.53 (d, 2H, *J* = 7.0 Hz, ArH), 4.68 (br s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6): δ 165.5 (N=C-S), 163.5 (C-6), 158.8 (C=O), 150.7 (coumarin-C-8a), 148.5 (ArC-NH_2), 146.0 (C=N), 131.2, 128.1, 128.0, 127.0, 124.6, 121.1, 118.9, 116.8, (ArC + coumarin-C). Anal. calc. for $C_{18}H_{11}N_5O_2S$ (361.38): C, 59.82; H, 3.07; N, 19.38. Found: C, 59.68; H, 2.98; N, 19.03. MS: *m/z* (FAB) 362 $[M + H]^+$.

*3,6-Bis-(3,4-dichlorophenyl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]-thiadiazole* ***9***

From **6** (65 mg) and 3,4-dichloro-benzoic acid (48 mg). Yield: 101 mg (97%), mp. 203–205°C. ^{1}H-NMR (DMSO-d_6): δ 8.04 (dd, 1H, *J* = 7.5 Hz, *J* = 1.9 Hz, ArH), 7.88 (dd, 1H, *J* = 7.8 Hz, *J* = 2.0 Hz, ArH), 7.85 (d, 1H, *J* = 2.0 Hz, ArH), 7.77 (d, 1H, *J* = 1.9 Hz, ArH), 7.74 (s, 1H, ArH). ^{13}C-NMR (DMSO-d_6): δ 165.4 (N=C-S), 164.8 (C-6), 144.7 (C=N), 135.6, 131.5 (ArC-Cl), 131.4, 130.9, 130.0, 129.3, 129.0 (Ar). Anal. calc. for $C_{15}H_6Cl_4N_4S$ (416.11): C, 43.30; H, 1.45; N, 13.46. Found: C, 43.02; H, 1.38; N, 13.19. MS: *m/z* (FAB) 416/418 $[M + H]^+$.

*6-(4-Aminophenyl)-3-(3,4-dichlorophenyl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***10***

From **6** (65 mg) and 4-amino-benzoic acid (34 mg).Yield: 77 mg (85%), mp. 198–200°C. ^{1}H-NMR (DMSO-d_6): δ 8.05 (dd, 1H, *J* = 7.8 Hz, *J* = 2.1 Hz, ArH), 7.89 (d, 1H, *J* = 2.1 Hz, ArH), 7.78 (s, 1H, ArH), 7.61 (d, 2H, *J* = 7.0 Hz, ArH), 6.53 (d, 2H, *J* = 7.0 Hz, AH), 3.99 (br s, 2H, NH_2). ^{13}C-NMR (DMSO-d_6): δ 166.1 (N=C-S), 162.4 (C-6), 148.0 (ArC-NH_2), 145.0 (C=N), 131.4, 131.3 (AC-Cl), 130.9, 130.8, 129.2, 128.2, 122.8, 120.1 (ArC). Anal. calc. for $C_{15}H_9Cl_2N_5S$ (362.24): C, 49.74; H, 2.50; N, 19.33. Found: C, 49.52; H, 2.40; N, 19.02. MS: *m/z* (FAB) 362/364 $[M + H]^+$.

*3-(3,4-Dichlorophenyl)-6-(pyridin-3-yl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***11***

From **6** (65 mg), picolinic acid (31 mg) and $POCl_3$ (15 mL) at 95°C. Yield: 23 mg (26%), mp. 80–82°C. ^{1}H-NMR ($CDCl_3$): δ 9.19–8.21 (m, 4H, pyridine-H), 8.05 (dd, 1H, *J* = 7.7 Hz, *J* = 1.9 Hz, ArH), 7.83 (d, 1H, *J* = 1.9 Hz, ArH), 7.72 (s, 1H, ArH). ^{13}C-NMR (DMSO-d_6): δ 165.9 (N=C-S), 163.0 (C-6), 148.9 (pyridine-C), 145.9 (C=N), 136.2 (pyridine-C), 131.7, 131.5 (ArC-Cl), 131.4, 131.7, 129.5 (ArC), 127.2, 123.7 (pyridine-C). Anal. calc. for $C_{14}H_7Cl_2N_5S$ (348.21): C, 48.29; H, 2.03; N, 20.11. Found: C, 48.02; H, 1.96; N, 19.93. MS: *m/z* (FAB) 348/350 $[M + H]^+$.

*3-(3,4-Dichlorophenyl)-6-(pyrazin-2-yl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***12***

From **6** (65 mg) and pyrazine-2-carboxylic acid (31 mg). Yield: 24.4 mg (28%), mp. 122–126°C. ^{1}H-NMR (DMSO-d_6): δ 8.51–8.32 (m, 3H, pyrazine-H), 8.05 (dd, 1H, *J* = 7.8 Hz, *J* = 1.8 Hz, ArH), 7.88 (d, 1H, *J* = 1.8 Hz, ArH), 7.75 (s, 1H, ArH). ^{13}C-NMR (DMSO-d_6): δ 167.8 (N=C-S), 165.3 (C-6), 147.2, 146.2, 145.9, 145.0 (pyrazine-C), 144.1 (C=N), 133.4, 133.0 (ArC-Cl), 131.1, 130.9, 129.4 (ArC). Anal. calc. for $C_{13}H_6Cl_2N_6S$ (349.20): C, 44.71; H, 1.73; N, 24.07. Found: C, 44.50; H, 1.66; N, 23.85. MS: *m/z* (FAB) 349/351 $[M + H]^+$.

*3-(3,4-Dichlorophenyl)-6-(pyrrol-2-yl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***13***

From **6** (65 mg) and pyrrole-2-carboxylic acid (28 mg). Yield: 30 mg (36%), semi-solid. ^{1}H-NMR (DMSO-d_6): δ 11.0 (br s, 1H, NH), 8.17 (dd, 1H, *J* = 7.8 Hz, *J* = 2.2 Hz, ArH), 7.95 (d, 1H, *J* = 2.2 Hz, ArH), 7.61 (s, 1H, ArH), 6.85-6.68 (m, 3H, pyrrole-H). ^{13}C-NMR (DMSO-d_6): δ 166.5 (N=C-S), 163.0 (C-6), 145.5 (C=N), 131.8, 131.7 (AC-Cl), 131.0, 130.9, 130.0, 129.4 (ArC), 119.5, 118.5, 111.0, 110.0 (pyrrole-C). MS: *m/z* (FAB) 359/361 $[M + Na]^+$.

*3-(3,4-Dichlorophenyl)-6-(thiophen-2-yl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole* ***14***

From **6** (65 mg) and thiophene-2-carboylic acid (32 mg). Yield: 48 mg (54%), semi-solid. ^{1}H-NMR (DMSO-d_6): δ 8.09 (dd, 1H, *J* = 7.7 Hz *J* = 2.0 Hz, ArH), 7.84 (d, H, *J* = 2.0 Hz, ArH), 7.49 (s, 1H, ArH), 7.00-7.25 (m, 3H, thiophen-H). ^{13}C-NMR (DMSO-d_6): δ 165.2 (N=C-S), 163.1 (C-6), 147.8 (C=N), 133.0 (C_2-thiophene), 132.9 (ArC-Cl), 131.5, 130.5, 129.9, 128.6 (ArC), 127.9, 127.7, 125.0 (thiophene-C). MS: *m/z* (FAB) 353/355 $[M + H]^+$.

*3-(3,4-Dichlorophenyl)-6-(furan-2-yl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazole 15*

From **6** (65 mg) and furan-2-carboxylic acid (28 mg). Yield: 40 mg (48%), semi-solid. ^{1}H-NMR (DMSO-d_6): δ 8.07 (dd, 1H, *J* = 7.8 Hz *J* =

2.0 Hz, ArH), 7.82 (d, H, *J* = 2.0 Hz, ArH), 7.46 (s, 1H, ArH), 7.35 (dd, 1H, *J* = 7.9 Hz *J* = 2.5 Hz furan-H), 6.56 – 6.46 (m, 2H, furan-H). ^{13}C-NMR (DMSO-d_6): δ 165.2 (N=C-S), 162.1 (C-6), 147.6 (C=N), 143.0 (C_2-furan), 133.1, 132.8 (ArC-Cl), 131.5, 130.5, 129.9, 128.6 (Ar), 112.0, 111.2 (furan-C). Anal. calc. for $C_{13}H_6Cl_2N_4OS$ MS: *m/z* (FAB) 360/362 $[M + Na]^+$.

*3-(3,4-Dichlorophenyl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]-thiadiazin-6-amine **16***

From **6** (250 mg, 0.96 mmol), 2-chloracetonitrile (76 mg, 1.00 mmol), and 3% H_2SO_4/SiO_2 (20 mg). The mixture was heated under MWI at 85°C for 30 min. After cooling, the residue was partitioned between $CHCl_3$ (3 × 20 mL) and water (20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified on a short column of silica gel (5 g) and eluted with an gradient, with MeOH (0 to 5%) and $CHCl_3$ to give **16** (187 mg, 65%), mp. 137 – 141°C. ^{1}H-NMR (DMSO-d_6): δ 8.02 (dd, 1H, *J* = 7.6 Hz *J* = 2.0 Hz, ArH), 7.80 (d, H, *J* = 2.0 Hz, ArH), 7.44 (s, 1H, ArH), 6.31 (br s, 2H, NH_2), 2.97 (s, 2H, SCH_2). ^{13}C-NMR (DMSO-d_6): δ 164.2 (C_6-NH_2), 147.7, 147.9 (C=N), 133.3, 133.0 (Ar-C-Cl), 130.6, 129.2, 128.1 (Ar), 37.8 (SCH_2). Anal. calc. for $C_{10}H_7Cl_2N_5S$ (300.17): C, 40.01; H, 2.35; N, 23.33. Found: C, 39.89; H, 2.26; N, 23.02. MS: *m/z* (FAB) 300/302 $[M + H]^+$.

*3-(3,4-Dichlorophenyl)-[1,2,4]]triazolo[3,4-**b**][1,3,4]-thiadiazin-6-one **17***

From **6** (250 mg, 0.96 mmol) and 2-chloroacetic acid (94 mg, 1.00 mmol) in EtOH (15 mL) containing NaOAc (30 mg). The mixture was heated under MWI at 85°C for 30 min. After cooling, the residue was partitioned between $CHCl_3$ (3 × 20 mL) and water (20 mL). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified on a short column of silica gel (5 g) and eluted with an gradient, with MeOH (0 to 5%) and $CHCl_3$ to give **17** (205 mg, 68%), mp. 111 – 114°C. ^{1}H-NMR (DMSO-d_6): δ 8.60 (s, 1H, NH), 7.92 (dd, 1H, *J* = 7.7 Hz *J* = 1.9 Hz, ArH), 7.77 (d, H, *J* = 1.9 Hz, ArH), 7.41 (s, 1H, ArH), 3.78 (s, 2H, SCH_2). ^{13}C-NMR (DMSO-d_6): δ 171.5 (C=O), 147.8 (C=N), 133.7, 133.2 (Ar-C-Cl), 130.4, 129.0, 128.2 (Ar), 34.2 (SCH_2). Anal. calc. for $C_{10}H_6Cl_2N_4OS$ (301.15): C, 39.88; H, 2.01; N, 18.60. Found: C, 39.62; H, 1.95; N, 18.41. MS: *m/z* (FAB) 301/303 $[M + H]^+$.

*3-(3,4-Dichlorophenyl)-6-phenyl-[1,2,4]]triazolo[3,4-**b**][1,3,4]thiadiazine **18***

From **6** (350 mg, 1.34 mmol) and phenacyl chloride (216 mg, 1.40 mmol) in EtOH (15 mL). The mixture was heated at 95°C under MWI for 30 min. After cooling, the residue was partitioned between $CHCl_3$ (3 × 20 mL) and water (20 mL). The combined organic extracts was dried (Na_2SO_4), filtered, and evaporated to dryness. The residue was purified on a short column of silica gel (7 g) and eluted with an gradient, with MeOH (0 to 5%) and $CHCl_3$ to give **18** (295 mg, 61%), mp. 137-141°C. ^{1}H-NMR (DMSO-d_6): δ 7.96 (dd, 1H, *J* = 7.5 Hz *J* = 1.9 Hz, ArH), 7.91 (m, 2H, ArH), 7.75 (d, H, *J* = 1.9 Hz, ArH), 7.53 – 7.47 (m, 3H, ArH), 7.43 (s, 1H, ArH), 4.39 (s, 2H, SCH_2). ^{13}C-NMR (DMSO-d_6): δ 164.0 (C-6), 147.5 (C=N), 133.8 (Ar), 133.5, 133.2 (Ar-C-Cl), 131.0, 130.6, 129.2, 128.9 (Ar), 36.0 (SCH_2). Anal. calc. for $C_{16}H_{10}Cl_2N_4S$ (361.25): C, 53.20; H, 2.79; N, 19.63. Found: C, 52.98; H, 2.70; N, 15.30. MS: *m/z* (FAB) 361/363 $[M + H]^+$.

References

[1] J. Mohan, G. S. R. Anjaneyulug, *Curr. Sci*. **1989**, *58*, 1028 – 1030.

[2] M. M. Ghorab, A. M. Sh. El-Sharief, Y. A. Ammar, Sh. I. Mohamed, *Phosphorus Sulfur Silicon Relat. Elem.* **2001**, *173*, 223 – 233.

[3] B. S. Holla, P. M. Akberali, M. K. Shivananda, *Farmaco* **2001**, *56*, 919 – 927.

[4] E. Palaska, G. Sahin, P. Kelicen, N. T. Durlu, G. Altinok, *Farmaco* **2002**, *57*, 101 – 107.

[5] L. Labanauskas, V. Kalcas, E. Udrenaite, P. Gaidelis, *et al*., *Pharmazie* **2001**, *56*, 617 – 619.

[6] A. Foroumadi, M. Mirzaei, A. Shafiee, *Pharmazie* **2001**, *56*, 610 – 612.

[7] V. K. Chadha, N. S. Ranwa, P. K. Dadheech, *J. Phytol. Res.* **1998**, *11*, 201 – 202.

[8] N. Demirbas, A. Demirbas, S. A. Karaoglu, E. Çelik, *ARKIVOC* **2005**, *i*, 75 – 91.

[9] B. S. Holla, K. N. Poojary, B. S. Rao, M. K. Shivananda, *Eur. J. Med. Chem.* **2002**, *37*, 511 – 517.

[10] K. S. Bhat, D. J. Prasad, B. Poojary, S. Holla, *Phosphorus Sulfur Silicon Relat. Elem.* **2004**, *179*, 1595 – 1603.

[11] K. Marina, M. Anastssia, M. Panagiotis, P. Nicole, *et al*., *Farmaco* **2002**, *57*, 253 – 257.

[12] M. Sakata, Y. Shirakawa, N. Kamata, Y. S. Hiroshino, O. Y. Jie, *J. Heterocycl. Chem.* **2000**, *37*, 269 – 271.

[13] G. M. Nizamuddin, M. H. Khan, M. K. Srivastava, *J. Sci. Ind. Res*. **1999**, *58*, 538 – 542.

[14] F. P. Invidiata, D. Simoni, F. Scintu, N. Pinna, *Farmaco* **1996**, *51*, 659 – 664.

[15] N. D. Heindel, J. R. Reid, *J. Heterocycl. Chem.* **1980**, *17*, 1087 – 1088.

[16] B. S. Goswami, J. C. S. Kataky, J. N. Baruah, *J. Heterocycl. Chem.* **1986**, *23*, 1439 – 1442.

[17] K. S. Rao, C. V. R. Sastry, *Ind. J. Pharm. Sci.* **1993**, *55*, 108 – 110.

[18] B. A. Nadkarni, V. R. Kamat, B. G. Khadse, *Arzneimittelforschung* **2001**, *51*, 569 – 573.

[19] R. M. Shaker, *ARKIVOC* **2006**, *ix*, 59 – 112.

[20] I. A. Al-Masoudi, Y. A. Al-Soud, N. J. Al-Salihi, N. A. Al-Masoudi, *Chem. Heterocycl. Comp.* **2006**, *467*, 1635 – 1646.

[21] Y. A. Al-Soud, N. A. Al-Masoudi, T. Schuppler, E. De Clercq, C. Pannecouque, (Part 1), *Nucleos. Nucleot. Nucleic Acids*, **2008**, *27(5)*, accepted; N. A. Al-Masoudi, Y. A. Al-Soud, (Part 2), in press.

[22] R. Pauwels, J. Balzarini, M. Baba, R. Snoeck, *et al*., *J. Virol. Methods* **1988**, *20*, 309 – 321.

[23] S. D. Young, S. F. Britcher, L. O. Tran, L. S. Payne, *et al*., *Antimicrob. Agents Chemother* **1995**, *39*, 2602 – 2605.

[24] T. Fujiwara, A. Sato, M. El-Farrash, S. Miki, *et al*., *Antimicrob. Agents Chemother*. **1998**, *42*, 1340 – 1345.

[25] N. F. Eweiss, A. A. Bahajaj, E. A. Elsherbini, *J. Heterocycl. Chem.* **1986**, *23*, 1451 – 1458.

