European Journal of Medicinal Chemistry 45 (2010) 3191–3195

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

New 6-amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-ones: Synthesis, characterization and antibacterial activity evaluation

Gabriela Laura Almajan [a,*], Stefania-Felicia Barbuceanu [a], Ioana Saramet [a], Constantin Draghici [b]

[a] Organic Chemistry Department, Faculty of Pharmacy, Traian Vuia Street 6, 020956 Bucharest, Romania
[b] C.D. Nenitescu Institute of Organic Chemistry, Romanian Academy, Splaiul Independentei 202B, 060023, Bucharest, Romania

ARTICLE INFO

Article history:
Received 16 December 2009
Received in revised form
24 February 2010
Accepted 25 February 2010
Available online 3 March 2010

Keywords:
Amino-triazolothiadiazines
Triazolothiadiazinones
Cyclodehydration
Antibacterial activity

ABSTRACT

(4-X-Phenylsulfonyl)phenyl] containing 6-amino-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines and [1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-ones were synthesized by intermolecular condensation of 2-chloro-N-phenylacetamide, 2-chloroacetic acid, oxalylchloride and bromo-diethylmalonate with 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiols (X = H, Cl, Br). The structures of newly synthesized compounds were confirmed by elemental analysis and IR, NMR spectral data. All the compounds were screened for their antibacterial activities. Some of them exhibited good activities against *Staphylococcus epidermidis* ATCC 14990, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922.

© 2010 Elsevier Masson SAS. All rights reserved.

1. Introduction

In the recent years, a special attention was given to 5-substituted-4-amino-1,2,4-triazole-3-thiols owing to their promising biological activities such as antimicrobial, anti-inflammatory, analgesic, hypoglycemic, anticancer [1–8]. In addition to these important biological applications, 4-amino-1,2,4-triazole-3-thiols are also of great utility in preparative organic chemistry. The amino and mercapto groups are ready-made nucleophilic centers for the synthesis of condensed heterocyclic rings such as triazolothiadiazoles, triazolothiadiazines, triazolotetrazines and triazolothiadiazepines [9–11]. 1,2,4-Triazoles fused with six-member ring systems (we refer here to triazoles fused with pyridine, pyridazine, pyrimidine, pyrazine and triazine) have diverse applications in the fields of medicine, agriculture and industry.

The recent literature survey revealed that a special attention was given to 1,2,4-triazolo[3,4-b][1,3,4]thiadiazine derivatives which proved to have promising biological activities: antimicrobial, antiviral, anti-HIV, CNS-stimulant, antifungal [12–17].

Designing new drugs is based on the development of hybrid molecules by combining different pharmacophore fragments in a single structure, which may lead to compounds with interesting biological profiles.

Our previous studies on the synthesis of biologically active compounds have demonstrated that the derivatives with 1,2,4-triazole nucleus have weaker antimicrobial activity than those which containing this nucleus condensed with one another heterocycle (thiadiazine, thiazole) [18,19].

For this reason, we considered interesting to synthesize new compounds containing [1,2,4]-triazole nucleus fused with a different substituted [1,3,4]-thiadiazine moiety, in order to study their antibacterial activity.

The present study describes the synthesis and characterization of novel amino-triazolothiadiazines and triazolothiadiazinones and evaluation of their antibacterial activities.

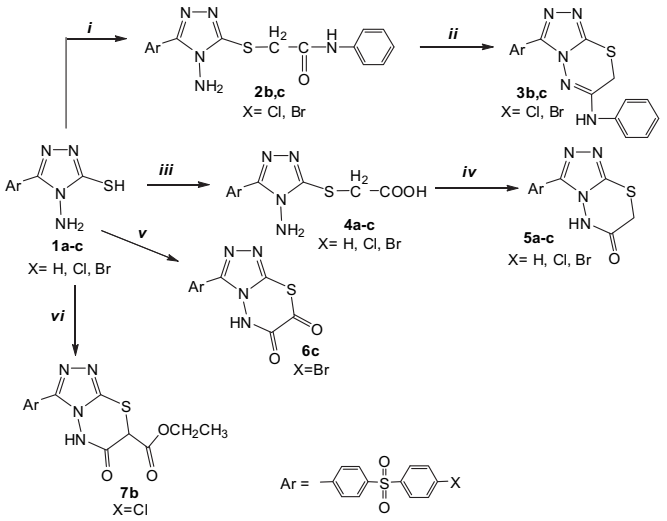
2. Chemistry

The reaction sequences employed for synthesis of title compounds are shown in Scheme 1. The key intermediates, 4-amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiols **1a-c**, (X = H, Cl, Br) were prepared from corresponding substituted benzoic acid hydrazides according to literature [20].

Triazoles **1b,c** (X = Cl, Br) were treated with 2-chloro-N-phenyl-acetamide in the presence of potassium hydroxide in ethanol and led to obtaining N-phenyl-2-{4-amino-5-[4-(4-X-phenylsulfonyl)

* Corresponding author. Tel.: +40 741 095490; fax: +40 21 3180729.
E-mail address: laura.almajan@gmail.com (G.L. Almajan).

0223-5234/$ – see front matter © 2010 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2010.02.057



i- $PhNHCOCH_2Cl$/KOH/reflux

ii- $POCl_3$/reflux

iii- $ClCH_2COOH/CH_3COONa$/EtOH/reflux

iv- $POCl_3$/reflux

v- ClCOCOCl/PhH anh./reflux

vi- $BrCH(COOEt)_2$/ CH_3COONa/EtOH/reflux

Scheme 1. Synthesis of the title compounds.

phenyl]-4*H*-1,2,4-triazol-3-yl}thioacetamides **2b,c** (X = Cl, Br). If X = H, S-alkylated derivative could not be properly isolated and characterized because a complex reaction mixture was obtained whose components could not be isolated in order to be characterized. Therefore the corresponding triazolothiadiazine was not synthesized. Compounds **2b,c** heating with phosphorus oxychloride underwent intramolecular ring closure with the formation of 6-phenylamino-3-[4-(4-X-phenylsulfonyl)phenyl]-7*H*-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazines **3b,c** (X = Cl, Br).

An attempt to transform the aminotriazoles **1a-c** into the corresponding triazolothiadiazinones **5a-c** by refluxing with ethylchloroacetate in basic medium [21] was unsuccessful. Therefore, this transformation was achieved in two steps. In the first step, S-alkyl derivatives **4a-c,** obtained by reaction of triazoles **1a-c** with chloroacetic acid in presence of sodium acetate, were isolated and characterized. Finally, we found that the respective triazolothiadiazinone **5a-c** was formed by cyclization of these intermediates via elimination of water in presence of phosphorus oxychloride.

Transformation of aminotriazoles **1a-c** in triazolothiadiazinone was successful only if X = halogen. Thus, treatment of the aminotriazole **1c** with oxalylchloride in dry benzene afforded the corresponding triazolothiadiazin-6,7-dione **6c**. Also, the interaction of **1b** with bromo-diethylmalonate led to the formation of the 6-oxotriazolothiadiazin-7-carboxylate **7b**.

3. Antibacterial activity

The synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive (*Enterococcus faecalis* ATCC 29212; *Staphylococcus aureus* ATCC 25923; *Staphylococcus epidermidis* ATCC 14990; *Bacillus cereus* ATCC 13061) and Gram-negative (*Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 27853; *Enterobacter clocae* ATCC 49141; *Escherichia coli* ATCC 25922; *Pseudomonas aeruginosa* ATCC 9027) bacteria by using the broth dilution method [22] for determination of MIC. Tetracycline and ampicillin were used as control drugs.

4. Results and discussions

4.1. Chemistry

Obtaining of compounds **2b,c**; **3b,c**; **4a-c**; **5a-c**; **6c** and **7b** was confirmed on the basis of elemental analysis and IR, NMR spectral data. The elemental analysis data along with some physical properties of these compounds are reported in Table 1.

Formation of S-alkyl derivatives **2b,c** and **4a-c** is supported by observed changes in IR and NMR spectra. Thus, depending on the nature of the fragment bound to the thiol sulphur atom, IR spectra show characteristic bands: an intense band at 1668–1677 cm^{-1} attributed to the amide carbonyl group for **2b,c** and a very strong band in 1712–1714 cm^{-1} region, assigned to carboxyl group in **4a-c**. Asymmetric and symmetric stretching frequencies of amino group appeared at 3370–3374 cm^{-1} and 3202 cm^{-1} for **2b,c**.

The ^1H NMR spectrum of **2b,c** showed a singlet at 4.40–4.25 ppm due to the presence of SCH_2 protons, while for **4a-c**, some protons signal appears at 4.20–4.23 ppm. Most important signals of ^{13}C NMR spectra are those attributed to SCH_2 atoms (~36 ppm for **2b,c** and ~34 ppm for **4a-c**) and *C*=O atoms (~164 ppm for **2b,c** and ~169 ppm for **4a-c**, respectively).

The structure of **3b,c** derivatives was confirmed by the absence of the carbonyl bands in its IR spectra, the appearance of the characteristic singlet signal at 4.66 ppm due to the methylene protons of thiadiazinic SCH_2 group and the appearance of a new positive signal at 156–158 ppm which corresponding to quaternary carbon of N=*C*H group formed by cyclodehydration.

Similarly, some changes occur in the spectra of compounds **5a-c** compared with those of corresponding S-alkyl derivatives **4a-c**. So, as a result of overall action of inductive effect and magnetic anisotropic effect of the aromatic rings, the chemical shift of the SCH_2 group in the thiadiazine moiety appears upfield (4.05–4.10 ppm in ^1H NMR spectra) in comparison with the obtained values for **4a-c**. ^{13}C NMR data of triazolothiadiazinones **5a-c** exhibit a new peak at 164–166 ppm which corresponding to cyclic carbonyl group obtained after intramolecular dehydration.

Transformation of triazoles **1b,c** into triazolothiadiazinones **6c** and **7b** is supported by the presence of the cyclic amide stretching frequencies in the IR spectra: 3213 and 1683 cm^{-1} for **6c** and 3116 and 1676 cm^{-1} for **7b**, respectively. In addition, each of these compounds presents in the IR spectrum, bands characteristic of different functional groups, which are attached on the thiadiazine ring. So, the intense band at 1750 cm^{-1} for **6c** indicating the presence of other carbonyl group and the band at 1744 cm^{-1}, together with the bands at 2977, 2941, 2909 cm^{-1} confirm the presence of ethylcarboxylate group in 7 position of **7b**. The signals recorded in the NMR spectra are characteristic of these functional groups. The amide NH proton appears as broadened singlet at 10.87 ppm in **6c** and 8.88 ppm in **7b**, respectively. ^{13}C NMR spectra exhibit peaks at 172.10 and 160.90 ppm corresponding to carbonyl groups for **6c** and at 168.64, 164.69 and 44.75 ppm, assigned to amide carbonyl, ester carbonyl and S*C*H carbons for **7b**. In addition, the ^1H NMR spectra of **7b** contains a two-proton quadruplet from OCH_2CH_3 group at 3.32 ppm and a three-proton triplet from OCH_2CH_3 group at 1.41 ppm. The signals corresponding to carbon-atoms of this group appear at 62.31 ppm (OCH_2CH_3) and 14.20 ppm (OCH_2CH_3), respectively.

4.2. Antibacterial activity

The newly synthesized compounds were tested for *in vitro* antibacterial activities compared with "parent" 4-amino-1,2,4-

Table 1
Characterization data of the synthesized compounds.

Compd.	X		Molecular formula	Molecular weight (g/mol)	M.p. (°C)	Yield (%)	Elemental analysis[a] calc. (found)		
							C	H	N
2b	Cl	–	$C_{22}H_{18}ClN_5O_3S_2$	499.9	217–219	68	52.85 (52.80)	3.63 (3.58)	14.01 (13.97)
2c	Br	–	$C_{22}H_{18}BrN_5O_3S_2$	544.4	226–228	65	48.53 (48.48)	3.33 (3.28)	12.86 (12.81)
3b	Cl	–	$C_{22}H_{16}ClN_5O_2S_2$	481.9	280–283	55	54.82 (54.78)	3.35 (3.30)	14.53 (14.47)
3c	Br	–	$C_{22}H_{16}BrN_5O_2S_2$	526.4	248–250	57	59.19 (50.13)	3.06 (3.02)	13.30 (13.25)
4a	H	–	$C_{16}H_{14}N_4O_4S_2$	390.4	218–220	71	49.22 (49.16)	3.61 (3.70)	14.35 (14.29)
4b	Cl	–	$C_{16}H_{13}ClN_4O_4S_2$	424.8	180–182	61	45.23 (45.17)	3.08 (3.14)	13.19 (13.11)
4c	Br	–	$C_{16}H_{13}BrN_4O_4S_2$	469.3	231–232	64	40.95 (40.87)	2.79 (2.87)	11.94 (11.86)
5a	H	–	$C_{16}H_{12}N_4O_3S_2$	372.4	203–205	78	51.60 (51.52)	3.25 (3.31)	15.04 (14.96)
5b	Cl	–	$C_{16}H_{11}ClN_4O_3S_2$	406.8	163–165	85	47.23 (47.17)	2.73 (2.80)	13.77 (13.69)
5c	Br	–	$C_{16}H_{11}BrN_4O_3S_2$	451.3	197–199	72	42.58 (42.49)	2.46 (2.53)	12.41 (12.34)
6c	Br	–	$C_{16}H_9BrN_4O_4S_2$	465.3	279–282	71	41.30 (41.24)	1.95 (1.90)	12.04 (11.98)
7b	Cl	–	$C_{19}H_{15}ClN_4O_5S_2$	478.9	187–189	78	47.65 (47.60)	3.16 (3.12)	11.70 (11.65)

[a] Elemental analysis value limit = ±0.4% of the theoretical value.

triazole **1a-c**. They were assessed against five Gram-negative and four Gram-positive bacterial strains and tetracycline and ampicillin were used as reference drug molecules.

The data generated from this study (Table 2) showed that compounds displayed low to moderate activity. The obtained results can be attributed to quite bulky structure of the tested compounds but they may be associated with the nature of tested bacterial species.

Thus, we can see that none of the tested compounds has inhibitory action against *A. baumanii*, *C. freundii, E. cloacae, E. faecalis, B. cereus*. We can also notice that the exerted action on Gram-negative bacteria *E. coli* and *P. aeruginosa* is better than on Gram-positive strains *S. aureus* and *S. epidermidis*.

4-Amino-5-[4-(4-X-phenylsulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiols **1a-c**, (X = H, Cl, Br) displayed low antibacterial activity, which is in contradiction with the purpose of Ghannoum and co-workers who believe that the both NH_2 and SH groups should be free for antibacterial activity [23].

According to our studies, preservation of the free NH_2 group and involvement of the SH group in an alkylation reaction (**2b,c** and **4a-c**) sometimes cause an increase of antibacterial activity. Thus, even if have a linear and bulky structure, **2b** showed excellent activity against *P. aeruginosa* (MIC = 32 μg/mL), **2c** and **4a-c** showed good activity against *S. aureus* (MIC = 64 μg/mL) and *E. coli* (MIC = 64 μg/mL), respectively, comparative with triazoles **1a-c**. In these cases the increased activity could be attributed to the presence of CONH group (for **2b,c**) and COOH group (for **4a-c**), respectively. Blocking the NH_2 group in S-alkylated derivatives **2b,c** and **4a-c**, by thiadiazine ring closure exerts a visible decrease of this action. If the tested microorganism is *A. baumanii* there is a slight increase of antibacterial activity. The most active compounds with triazolothiadiazine nucleus are **5a** (MIC = 32 μg/mL against *S. epidermidis*) and **6c** ((MIC = 64 μg/mL) against *P. aeruginosa* and *S. aureus*).

All the tested compounds, which are considered active, are less effective than drugs taken as a standard.

Table 2
Antibacterial activities of compounds **1a-c**; **2a,b**; **3a,b**; **4a-c**; **5a-c**; **6c**; **7b** as MIC values (μg/mL).

Compd.	X	Gram-negative bacteria[a]					Gram-positive bacteria[b]			
		Ab	*Cf*	*Ebc*	*Ec*	*Pa*	*Ef*	*Sa*	*Se*	*Bc*
1a	H	512	512	512	512	512	1024	512	128	1024
1b	Cl	128	512	512	1024	128	1024	512	512	1024
1c	Br	128	512	512	1024	512	1024	512	512	1024
2b	Cl	1024	512	512	128	32	1024	128	256	1024
2c	Br	1024	1024	256	256	128	1024	64	256	512
3b	Cl	512	128	1024	128	64	1024	256	1024	1024
3c	Br	512	512	512	256	256	1024	1024	1024	512
4a	H	1024	512	1024	64	256	512	256	128	1024
4b	Cl	1024	512	1024	64	256	256	128	256	1024
4c	Br	1024	512	1024	64	256	512	512	512	1024
5a	H	256	512	512	128	128	1024	512	32	1024
5b	Cl	256	512	512	128	128	512	256	256	1024
5c	Br	256	512	512	128	128	1024	1024	1024	1024
6c	Br	1024	512	512	512	64	1024	64	512	1024
7b	Cl	1024	512	512	512	128	1024	1024	1024	1024
Te		2	2	–	2	16	32	<2	32	<2
Am		–	2	–	2	–	<2	<2	<2	–

Te = tetracycline; **Am** = ampicillin.
– = No inhibitory action.
[a] *Ab* (*Acinetobacter baumanii* ATCC 19606); *Cf* (*Citrobacter freundii* ATCC 27853); *Ebc* (*Enterobacter cloacae* ATCC 49141); *Ec* (*Escherichia coli* ATCC 25922); *Pa* (*Pseudomonas aeruginosa* ATCC 9027).
[b] *Ef* (*Enterococcus faecalis* ATCC 29212); *Sa* (*Staphylococcus aureus* ATCC 25923); *Se* (*Staphylococcus epidermidis* ATCC 14990); *Bc* (*Bacillus cereus* ATCC 13061).

5. Conclusions

This study reports the synthesis, characterization and antibacterial activity evaluation of new triazolothiadiazines which present in 6, or 6 and 7 positions different functional groups (carbonyl, substituted-amino, or ester). The target compounds were obtained from 4-amino-5-[4-(4-X-phenyl sulfonyl)phenyl]-4*H*-1,2,4-triazole-3-thiols **1a-c**, (X = H, Cl, Br) directly, by intermolecular condensation with oxalylchloride and bromo-diethylmalonate, or in two steps, by intramolecular cyclization of intermediaries S-alkyl derivatives. The antibacterial data given for the compounds presented in this paper allowed us to state that the variation of antibacterial activity may be associated with the nature of tested bacterial strains and is due to the chemical structure of the tested compounds. The most active compound with triazolothiadiazine nucleus of this series is 3-[4-(phenylsulfonyl)phenyl]-5*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6(7*H*)-one **5a** (MIC = 32 μg/mL against *S. epidermidis*).

6. Experimental protocols

6.1. Chemistry

Melting points were determined with Boetius apparatus and are uncorrected. The IR spectra (in KBr pellets) were recorded on the Vertex 70 Bruker apparatus. The NMR spectra (in DMSO-d_6, at room temperature) were registered on a Varian Gemini 300 BB apparatus working at 300 MHz for ^{1}H and 75 MHz for ^{13}C, using TMS as internal standard. The content of C, H, and N were done with ECS-40-10-Costeh micro-dosimeter, after drying the compounds at 105 °C.

6.1.1. General procedure for synthesis of 2-{4-amino-5-[4-(4-X-phenylsulfonyl)phenyl}-4H-1,2,4-triazol-3-ylthio}-N-phenylacetamide ***2b,c***

2-Chloro-N-phenylacetamide (5 mmol), solution in ethanol (30 mL) was added to solution of compound **1** (5 mmol) in aqueous ethanol (10 mL) containing KOH (5 mmol). The reaction mixture was boiled for 45 min and cooled down and then water (50 mL) was added. The colorless precipitate was filtered off, washed with water, dried and purified by recrystallization from ethanol.

2b: IR (KBr, cm^{-1}): 3371, 3203 (NH, NH_2); 3089 (aromatic CH); 2963, 2923 (SCH_2); 1677 (C=O); 1603, 1580 (C=N + C=C_{aryl}); 1321, 1290, 1156 (SO_2); 1011 (N–N); 766 (C–Cl); 686 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 10.46 (s, 3H, NH, NH_2); 8.17 (d, 2H, J = 7.9 Hz, aromatic protons); 8.14 (d, 2H, J = 8.5 Hz, aromatic protons); 8.01 (d, 2H, J = 8.5; aromatic protons); 7.72 (dd, 2H, J = 7.4; 2.7 Hz, aromatic protons); 7.57 (d, 2H, J = 7.9 Hz, aromatic protons); 7.30 (t, 1H, J = 7.9 Hz, aromatic proton); 7.07 (t, 2H, J = 7.9 Hz, aromatic protons); 4.40 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 164.78 (C=O); 164.69 (C3-triazolic ring); 163.96 (C5-triazolic ring); 143.12, 139.34, 139.15, 138.62, 130.11, 129.60, 128.87, 128.53, 127.70, 127.60, 123.73, 119.20 (aromatic ring carbons); 36.89 (SCH_2).

2c: IR (KBr, cm^{-1}): 3374, 3202 (NH, NH_2); 3089 (aromatic CH); 2923, 2860 (SCH_2); 1668 (C=O); 1611, 1580 (C=N + C=C_{aryl}); 1323, 1290, 1156 (SO_2); 1003 (N–N); 686 (C–S–C); 582 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 10.41 (s, 3H, NH, NH_2); 8.16 (d, 2H, J = 7.2 Hz, aromatic protons); 8.13 (d, 2H, J = 7.2 Hz, aromatic protons); 8.02 (d, 2H, J = 8.1; aromatic protons); 7.71 (dd, 2H, J = 7.1; 2.7 Hz, aromatic protons); 7.54 (d, 2H, J = 8.1 Hz, aromatic protons); 7.28 (t, 2H, J = 7.1 Hz, aromatic proton); 7.04 (t, 1H, J = 7.1 Hz, aromatic protons); 4.25 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 164.96 (C=O); 164.62 (C3-triazolic ring); 163.88 (C5-triazolic ring); 143.13, 139.39, 139.15, 138.65, 130.12, 129.73, 128.88, 128.61, 127.72, 127.66, 123.73, 120.17 (aromatic ring carbons); 36.87 (SCH_2).

6.1.2. General procedure for synthesis of 3-[4-(4-X-phenylsulfonyl) phenyl]-N-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6-amine ***3b,c***

N-phenylacetamide **2** (2.7 mmol) in phosphorus oxychloride (8.5 mL) was refluxed for 2–3 h; the excess oxychloride was evaporated under vacuum to dryness. The oily residue was triturated with ether and then was neutralized with KOH 10%. The obtained precipitate was filtered off, washed with water until pH = 7, dried and recrystallized from ethanol.

3b: IR (KBr, cm^{-1}): 3316, 3142 (NH); 3087 (aromatic CH); 2922, 2852 (SCH_2); 1619, 1579, 1502 (C=N + C=C_{aryl}); 1324, 1157 (SO_2); 1010 (N–N); 766 (C–Cl); 689 (C–S–C); ^{1}H NMR (DMSO-d_6 + TFA, δ, ppm): 9.35 (s, 1H, NH); 8.13 (d, 2H, J = 8.8 Hz, aromatic protons); 8.08 (d, 2H, J = 8.5 Hz, aromatic protons); 7.99 (d, 2H, J = 8.5; aromatic protons); 7.68 (d, 2H, J = 8.8 Hz, aromatic protons); 7.60 (d, 2H, J = 7.7 Hz, aromatic protons); 7.33 (t, 1H, J = 7.7 Hz, aromatic proton); 7.00 (t, 2H, J = 7.7 Hz, aromatic protons); 4.66 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6 + TFA, δ, ppm): 159.85 (C3-triazolic ring); 158.44 (C5-triazolic ring); 156.92 (C=N thiadiazine ring); 142.01 139.37, 139.15, 138.35, 138.06, 129.96, 129.04, 128.48, 126.59, 122.14, 120.73, 120.40, 113.09 (aromatic ring carbons); 28.13 (SCH_2).

3c: IR (KBr, cm^{-1}): 3326, 3148 (NH); 3087 (aromatic CH); 2918, 2854 (SCH_2); 1614, 1579, 1502 (C=N + C=C_{aryl}); 1326, 1158 (SO_2); 1005 (N–N); 682 (C–S–C); 584 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 9.42 (s, 1H, NH); 8.15 (dd, 2H, J = 8.2, 3.2 Hz, aromatic protons); 8.08 (d, 2H, J = 8.5 Hz, aromatic protons); 7.99 (d, 2H, J = 7.9; aromatic protons); 7.33 (d, 2H, J = 8.2 Hz, aromatic protons); 7.63 (d, 2H, J = 7.5 Hz, aromatic protons); 7.40 (d, 2H, J = 7.5 Hz, aromatic proton); 7.03 (t, 1H, J = 7.5 Hz, aromatic proton); 4.66 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6 + TFA, δ, ppm): 160.51 (C3-triazolic ring); 158.63 (C5-triazolic ring); 158.13 (C=N thiadiazine ring); 142.93, 139.31, 138.36, 130.12, 138.06, 129.55, 129.18, 128.28, 1256.72, 121.25, 117.30, 115.94, 115.23 (aromatic ring carbons); 28.25 (SCH_2).

6.1.3. General procedure for synthesis of 2-{4-amino-5-[4-(4-X-phenylsulfonyl)phenyl}-4H-1,2,4-triazol-3-ylthio}acetic acid ***4a-c***

A mixture of **1** (1 mmol), sodium acetate (6.1 mmol) and chloroacetic acid (1.1 mmol) in ethanol (10 mL) was heated under reflux for 12 h. After cooling, the solvent was removed under reduced pressure, the residue was dissolved in water (50 mL) and the product was extracted with CH_2Cl_2 (3 × 50 mL). The organic layer was washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure and the solid product was recrystallized from ethanol.

4a: IR (KBr, cm^{-1}): 3398 (OH); 3294, 3148 (NH_2); 3089 (aromatic CH); 2956, 2875 (SCH_2); 1714 (C=O); 1618, 1583, 1517 (C=N + C=C_{aryl}); 1328, 1158 (SO_2); 1012 (N–N); 685 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.10 (s, 4H, aromatic protons); 8.05 (dd, 2H, J = 8.5, 2.1 Hz, aromatic protons); 7.51–7.85 (m, 3H, aromatic protons); 4.20 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 169.30 (COOH); 164.58 (C3-triazolic ring); 164.12 (C5-triazolic ring); 141.09, 140.59, 133.61, 131.15, 130.56, 129.59, 128.02, 127.58 (aromatic ring carbons); 34.68 (SCH_2).

4b: IR (KBr, cm^{-1}): 3418 (OH); 3301, 3160 (NH_2); 3097 (aromatic CH); 2938, 2840 (SCH_2); 1712 (C=O); 1623, 1597, 1529 (C=N + C=C_{aryl}); 1318, 1159 (SO_2); 1009 (N–N); 765 (C–Cl); 685 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.03 (s, 4H, aromatic protons); 7.95 (d, 2H, J = 8.5 Hz, aromatic protons); 7.55 (d, 2H, J = 8.5 Hz, aromatic protons); 4.23 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 169.34 (COOH); 163.33 (C3-triazolic ring); 162.91 (C5-triazolic ring); 140.83, 139.60, 139.21, 130.13, 129.59, 129.00, 128.26, 128.21 (aromatic ring carbons); 34.85 (SCH_2).

4c: IR (KBr, cm^{-1}): 3427 (OH); 3282, 3176 (NH_2); 3092 (aromatic CH); 2987, 2933 (SCH_2); 1717 (C=O); 1632, 1580, 1554 (C=N + C=C_{aryl}); 1324, 1158 (SO_2); 1011 (N–N); 685 (C–S–C); 579 (C–Br); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.15 (s, 4H, aromatic protons); 8.00 (d, 2H, J = 8.7 Hz, aromatic protons); 7.70 (d, 2H, J = 8.7 Hz, aromatic protons); 4.20 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 169.07 (COOH); 164.80 (C3-triazolic ring); 164.15 (C5-triazolic ring); 143.30, 139.58, 139.27, 130.31, 129.78, 128.75, 127.93, 127.73 (aromatic ring carbons); 34.48 (SCH_2).

6.1.4. General procedure for synthesis of 3-[4-(4-X-phenylsulfonyl) phenyl]-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6(7H)-one ***5a-c***

A mixture of **4** (1 mmol) and phosphoryl chloride (10 mL) was heated under reflux for 3 h. After cooling, the solvent was removed under reduced pressure and the formed solid residue was poured into ice-water and neutralized with cold ammonia solution. The obtained precipitate was filtered off and recrystallized from chloroform:petroleum ether 1:1 (v/v).

5a: IR (KBr, cm^{-1}): 3312 (NH); 3088 (aromatic CH); 2947, 2870 (SCH_2); 1768 (C=O); 1621, 1573, 1502 (C=N + C=C_{aryl}); 1324, 1287, 1159 (SO_2); 1012 (N–N); 685 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.27 (d, 2H, J = 8.5 Hz, aromatic protons); 8.11 (d, 2H, J = 7.8 Hz, aromatic protons); 8.00 (d, 2H, J = 8.5 Hz, aromatic protons); 7.70 (tt, 1H, J = 7.8, 1.5 Hz, aromatic proton); 7.63 (t, 2H, J = 7.8 Hz, aromatic protons); 4.07 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.61 (C=N thiadiazine ring); 162.12 (C3-triazolic ring); 158.70 (C5-triazolic ring); 140.18, 139.60, 133.28, 130.60, 130.24, 129.84, 127.98, 127.54 (aromatic ring carbons); 34.38 (SCH_2).

5b: IR (KBr, cm^{-1}): 3288 (NH); 3091 (aromatic CH); 2914, 2868 (SCH_2); 1775 (C=O); 1618, 1584, 1498 (C=N + C=C_{aryl}); 1320, 1283, 1157 (SO_2); 1010 (N–N); 763 (C–Cl); 688 (C–S–C); ^{1}H NMR (DMSO-d_6, δ, ppm): 8.21 (d, 2H, J = 8.6 Hz, aromatic protons); 8.11

(d, 2H, J = 8.6 Hz, aromatic protons); 7.91 (d, 2H, J = 8.5 Hz, aromatic protons); 7.55 (d, 2H, J = 8.5 Hz, aromatic protons); 4.05 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 166.18 (C=N thiadiazine ring); 160.48 (C3-triazolic ring); 158.78 (C5-triazolic ring); 140.75, 139.34, 138.04, 130.20, 129.14, 128.28, 128.18, 127.57 (aromatic ring carbons); 34.36 (SCH_2).

5c: IR (KBr, cm^{-1}): 3307 (NH); 3087 (aromatic CH); 2939, 2848 (SCH_2); 1772 (C=O); 1624, 1572, 1500 (C=N + C=C_{aryl}); 1325, 1291, 1159 (SO_2); 1009 (N–N); 688 (C–S–C); 578 (C–Br); ^1H NMR (DMSO-d_6, δ, ppm): 8.17 (d, 2H, J = 8.5 Hz, aromatic protons); 8.09 (d, 2H, J = 8.5 Hz, aromatic protons); 7.92 (d, 2H, J = 8.5 Hz, aromatic protons); 7.84 (d, 2H, J = 8.5 Hz, aromatic protons); 4.10 (s, 2H, SCH_2); ^{13}C NMR (DMSO-d_6, δ, ppm): 164.28 (C=N thiadiazine ring); 163.52 (C3-triazolic ring); 159.16 (C5-triazolic ring); 144.30, 139.48, 139.05, 133.08, 129.59, 128.68, 128.12, 127.98 (aromatic ring carbons); 34.42 (SCH_2).

6.1.5. General procedure for synthesis of 3-[4-(4-X-phenylsulfonyl) phenyl]-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-6,7-dione **6c**

A mixture of **1c** (1 mmol) and oxalylchloride (1 mmol) in dry benzene (10 mL) was heated under reflux for 8 h. The solvent was removed under reduced pressure, the formed solid product was filtered and recrystallized from chloroform:petroleum ether 1:1 (v/v).

6c: IR (KBr, cm^{-1}): 3213 (NH); 3090 (aromatic CH); 1750, 1683 (C=O); 1631, 1572, 1471 (C=N + C=C_{aryl}); 1326, 1160 (SO_2); 1010 (N–N); 686 (C–S–C); 581 (C–Br); ^1H NMR (DMSO-d_6, δ, ppm): 10.87 (s, 1H, NH); 8.14 (d, 2H, J = 8.5 Hz, aromatic protons); 8.07 (d, 2H, J = 8.5 Hz, aromatic protons); 7.95 (d, 2H, J = 8.5 Hz, aromatic protons); 7.89 (d, 2H, J = 8.5 Hz, aromatic protons); ^{13}C NMR (DMSO-d_6, δ, ppm): 172.10, 160.90 (C=O thiadiazine ring); 157.72 (C3-triazolic ring); 151.20 (C5-triazolic ring); 143.15, 142.89, 139.50, 133.08, 129.62, 128.49, 127.44, 127.20 (aromatic ring carbons).

6.1.6. General procedure for synthesis of ethyl 3-[4-(4-X-phenylsulfonyl)phenyl]-6-oxo-6,7-dihydro-5H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine-7-carboxylate **7b**

A mixture of **1b** (1 mmol), sodium acetate (6.1 mmol) and bromo-diethylmalonate (1 mmol) in ethanol (10 mL) was heated under reflux for 8 h. After cooling, the solvent was removed under reduced pressure and the residue was treated with cold water (50 mL). The obtained precipitate was filtered off and recrystallized from chloroform:petroleum ether 1:1 (v/v).

7b: IR (KBr, cm^{-1}): 3116 (NH); 3093 (aromatic CH); 2977, 2941, 2909 (CH, CH_2, CH_3); 1744 (COO), 1676 (C=O); 1638, 1613, 1582 (C=N + C] C_{aryl}); 1324, 1157 (SO_2); 1012 (N–N); 769 (C–Cl); 696 (C–S–C); ^1H NMR (DMSO-d_6, δ, ppm): 8.88 (s, 1H, NH); 8.04 (d, 2H, J = 8.5 Hz, aromatic protons); 7.93 (d, 2H, J = 8.8 Hz, aromatic protons); 7.86 (d, 2H, J = 8.5 Hz, aromatic protons); 7.83 (d, 2H, J = 8.8 Hz, aromatic protons); 4.89 (s, 1H, SCH); 3.32 (q, 2H, J = 7.2 Hz, CH_2); 1.41 (t, 3H, J = 7.0 Hz, CH_3); ^{13}C NMR (DMSO-d_6, δ, ppm): 168.64 (COO), 164.69 (C=O); 163.15 (C3-triazolic ring); 157.10 (C5-triazolic ring); 141.12, 139.62, 139.34, 130.12, 129.13, 128.88, 127.80, 127.62 (aromatic ring carbons); 62.35 (CH_2); 44.75 (CH); 14.20 (CH_3).

6.2. Antibacterial activity

Minimal inhibitory concentrations (MICs, μg/ml) were determined on different microorganisms using broth micro dilution procedure according to the recommendations of the National Committee for Clinical Laboratory Standards [22]. Minimum inhibitory concentrations (MIC) were defined as the lowest concentration of the compounds that inhibited visible growth of microorganisms after incubation at 35 °C for 24 h.

Gram-positive (*E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 14990, *B. cereus* ATCC 13061) and Gram-negative (*A. baumanii* ATCC 19606, *C. freundii* ATCC 27853, *Enterobacter cloacae* ATCC 49141, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027) bacteria were used as quality control strains. Bacterial strains were grown in Mueller-Hinton broth (Merck). The inoculums densities were 5×10^5 colony forming units (cfu) in 1 mL. Serial dilutions of the test compounds, previously dissolved in dimethylsulfoxide (DMSO), were prepared in test tubes to final concentrations of 1024; 512; 256; 128; 64; 32; 16; 8; 4; 2 μg/mL 100 μL of 24 h old inoculums was added to each tube. Tetracycline and ampicillin were used as standard antibiotic. Tests using DMSO as negative control were carried out in parallel and it was determined that the solvent had no antimicrobial activity against any of the test microorganisms. Because the MIC values are not spectacular, no statistical calculations were made.

Acknowledgements

This work is supported by project ID_226 no. 301/1.10.2007 from the Exploratory Research Projects of the National University Research Council (NURC-Romania).

References

[1] V. Padmavathi, A.V.N. Mohan, K. Mahnesh, A. Padmaja, Chem. Pharm. Bull. 56 (2008) 815–820.
[2] V. Padmavathi, G.S. Reddy, A. Padmaja, P. Kondaiah, A. Shazia, Eur. J. Med. Chem. 44 (2009) 2106–2112.
[3] R.M. Shaker, A.A. Aly, Phosphorus, Sulfur Silicon Relat. Elem. 181 (2006) 2577–2613.
[4] I. Küçükgüzel, S.G. Küçükgüzel, S. Rollas, M. Kiraz, Bioorg. Med. Chem. Lett. 11 (2001) 1703–1707.
[5] S.-H. Fang, V. Padmavathi, Y.K. Rao, D.R.C.V. Subbaiah, P. Thriveni, M. Geethangili, A. Padmaja, Y.-M. Tzeng, Int. Immunopharm 6 (2006) 1699–1705.
[6] B. Tozkoparan, E. Küpeli, E. Yeşilada, M. Ertan, Bioorg. Med. Chem. 15 (2005) 1808–1814.
[7] M. Balbaa, H. Mansour, H. El-Sawy, E.-S.H. El-Ashry, Nucleos. Nucleot. Nucleic Acids 21 (2001) 695–708.
[8] B.S. Holla, B.S. Rao, B.K. Sarojini, P.M. Akberali, N.S. Kumari, Eur. J. Med. Chem. 41 (2006) 657–663.
[9] N. Demirbas, A. Demirbas, S.A. Karaoglu, E. Çelik, Arkivoc i (2005) 75–91.
[10] O.V. Dyablo, A.F. Pozharskii, Chem. Heterocycl. Comp. 33 (1997) 1003–1027.
[11] P. Vainilavicius, R. Smicius, V. Jakubkiene, S. Tumkevicius, Monatsh. Chem. Chem. Mon. 132 (2001) 825–831.
[12] I.A. Al-Masoudi, Y.A. Al-Soud, N.J. Al-Salihi, N.A. Al-Masoudi, Chem. Heterocycl. Comp. 42 (2006) 1377–1403.
[13] G. Turan-Zitouni, Z.A. Kaplancikli, K. Erol, F.S. Kiliç, Il Farmaco 54 (1999) 218–223.
[14] N.D. Heindel, J.R. Reid, J. Heterocycl. Chem. 17 (1980) 1087–1088.
[15] T. Karabasanagouda, A.V. Adhikari, N.S. Shetty, Eur. J. Med. Chem. 42 (2007) 521–529.
[16] M.S. Karthikeyan, B.S. Holla, N.S. Kumari, Eur. J. Med. Chem. 43 (2008) 309–314.
[17] M. Bhalla, A. Hitkari, V.R. Gujrati, T.N. Bhalla, K. Shanker, Eur. J. Med. Chem. 29 (1994) 713–717.
[18] G.L. Almajan, I. Saramet, S.F. Barbuceanu, C. Draghici, G. Bancescu, Rev. Chim. (Bucharest) 60 (2009) 896–901.
[19] S.F. Barbuceanu, G.L. Almajan, I. Saramet, C. Draghici, A.I. Tarcomnicu, G. Bancescu, Eur. J. Med. Chem. 44 (2009) 4752–4757.
[20] G.L. Almajan, S.F. Barbuceanu, I. Saramet, C. Draghici, Rev. Chim. (Bucharest) 56 (2005) 1182–1187.
[21] A.R. Farghaly, E. De-Clercq, H. El-Kashef, Arkivoc x (2006) 137–151.
[22] National Committee for Clinical Laboratory Standards, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Approved Standard-Fifth Edition. M7-A5 Ed. NCCLS, Wayne, PA, 2000, 1–33.
[23] M.A. Ghannoum, N.F. Eweiss, A.A. Bahajaj, M.A. Qureshi, Microbios 37 (1983) 151–159.