Synthesis, Structure Elucidation and Antimicrobial Evaluation of some Novel Triazolo[3,4-b]thiadiazole and Triazolo[3,4-b]thiadiazine Derivatives

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Síntesis, elucidación estructural y evaluación antimicrobiana de algunos nuevos derivados de triazolo[3,4-b]tiadiazol y triazolo[3,4-b]tiadiazina

Síntesi, elucidació estructural i avaluació antimicrobiana d'alguns nous derivats de triazolo[3,4-b]tiadiazole i triazolo[3,4-b]tiadiazine

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RESUMEN

Se preparan derivados de triazoles condensados por reacción del producto de partida **4** con diversos reactivos electrófilos. Las estructuras de todos los nuevos compuestos se establecen mediante análisis elemental y datos espectroscópicos. Todos los compuestos sintetizados se criban en busca de su actividad antibacteriana. Los derivados de 1,2,4-triazol **4**, **14** y **15** exhiben una buena actividad antibacteriana respecto al antibiótico estándar Gentamicina. Se estudia el equilibrio tautomérico entre las formas tiona y tiol de los compuestos **4** y **15** mediante cálculos mecanocuánticos semiempíricos (AM1, MNDO y PM3) y *ab initio* (RHF/3-21G* y RHF/6-31G*) en fase gas.

Palabras clave: 1,2,4-triazoles, triazolo[3,4-*b*]tiadiazoles, triazolo[3,4-*b*]tiadiazines, triazolo[3,4-*b*]tiadiazepina, actividad antibacteriana.

SUMMARY

Fused triazoles derivatives have been prepared from the reaction of the starting material **4** with some electrophilic reagents. The structures of all new compounds have been established on the basis of elemental analysis and spectroscopic data. All the synthesized compounds have been screened for their antibacterial activity. 1,2,4-Triazole derivatives **4**, **14** and **15** exhibited a good antibacterial activity compared with the standard antibiotic Gentamycin. The tautomeric equilibria of the compounds **4** and **15** thione and thiol form were studied by quantum chemical calculations semi-empirical (AM1, MNDO and PM3) and ab initio level (RHF/3-21G* and RHF/6-31G*) in the gas phase.

Keywords: 1,2,4-triazoles, triazolo[3,4-b]thiadiazoles, triazolo[3,4-b]thiadiazines, triazolo[3,4-b]thiadiazepine, antibacterial activity.

RESUM

Es preparen derivats de triazoles condensades per reacció del producte de partida **4** amb diversos reactius electròfils. Les estructures de tots els nous compostos s'estableixen mitjançant anàlisi elemental i dades espectroscòpiques. Tots els compostos sintetitzats es cribren cercant la seva activitat antibacteriana. Els derivats d'1,2,4-triazole **4**, **14** i **15** exhibeixen una bona activitat antibacteriana respecte a l'antibiòtic estàndard Gentamicina. S'estudia l'equilibri tautomèric entre les formes tiona i tiol dels compostos **4** i **15** mitjançant càlculs mecanoquàntics semiempírics (AM1, MNDO i PM3) i *ab initio* (RHF/3-21G* i RHF/6-31G*) en fase gas.

Mots clau: 1,2,4-triazoles, triazolo[3,4-*b*]tiadiazoles, triazolo[3,4-*b*]tiadiazines, triazolo[3,4-*b*]tiadiazepina, activitat antibacteriana.

INTRODUCTION

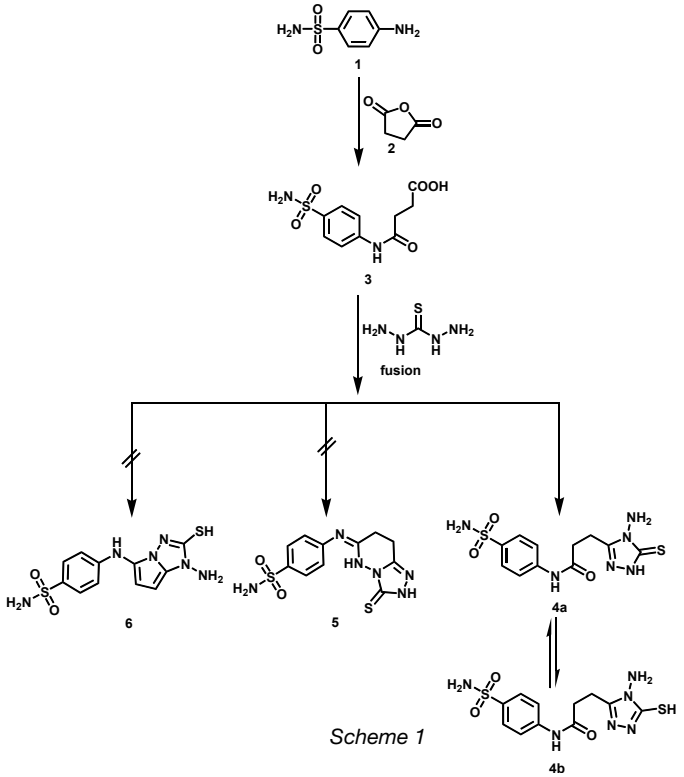
Fused 1,2,4-triazoles express antifungal, bactericidal,[1-3] anxiolytic,[4,5] anticonvulsant[6] and herbicidal[7] activities or can act as antidepressants.[8,9] The sulfonamide $-SO_2NH-$ group occurs in numerous biologically active compounds, which include antimicrobial drugs, saluretics, carbonic anhydrase inhibitors, insulin-releasing, antithyroid agents, antitumour drugs and number of other biological activities.[10–17] Sulfonamides are among the most widely used antibacterial agents in the world, chiefly because of their low cost, low toxicity and excellent activity against common bacterial diseases[18]. Heterocyclic compounds containing an amide group are used in the synthesis of biologically substances[19]. This promoted us to synthesize a series of fused triazoles containing both the sulfonamide and the amide groups and characterized their structure by IR, ^{1}H-NMR, and MS techniques to investigate their antibacterial activities.

RESULTS AND DISCUSSION

Sulfanilamide **1** was allowed to react with the succinic anhydride **2** under reflux the corresponding succinamic acid derivative **3** was afforded. Its IR spectrum showed bands at 3330-2750 (broad-COOH), 3320, 3280 (NH, NH_2), 1750, 1690 cm^{-1} (2 C=O). ^{1}HNMR Spectrum revealed the following signals at 2.4-2.7 (m, 4H, CH_2CH_2), 7.2 (s, 2H, NH_2), 10.2 (s, 1H, NH), 12.1ppm (br, 1H, OH). When compound **3** was subjected to react with thiocarbohydrazide, three possible structures **4**, **5** and **6** can be formulated (Scheme1). The structures **5** and **6** were discarded on the basis of analytical and spectral data. Infrared spectrum revealed the presence of absorption bands characteristic for NH, NH_2 (3390, 3260, 3220) and C=O (1700 cm^{-1}). Mass spectrum of **4** showed a molecular ion peak at m/z 342 (M^+; 13.03%) with a base peak at 56 (100%) and other significant peaks appeared at m/z 343 (M+1, 33.45%), 254 (13.03%), 185 (41.55%) and other significant peaks appeared at m/z, 133 (41.20%), 125 (88.87%), 101 (58.15%), 68 (44.01%).

The thiadiazole nucleus which incorporates N=C=S linkage exhibits a large number of biological activities[20]. The reactivity of compound **4** towards carboxylic acid derivatives was also investigated. Thus, refluxing of compound **4** in formic acid caused cyclization by elimination of two moles of water to give the triazolo[3,4-*b*]thiadiazole derivative **7**. The IR spectrum exhibited absorption bands at 3278, 3104 (NH, NH_2) and 1694 cm^{-1} (C=O). Its mass spectrum revealed a molecular ion peak at m/z 352 (M^+, 18.15%), with a base peak at m/z 88 (100%) and other significant peaks appeared at m/z 311 (28.57%), 262 (34.04%), 239 (7.28%), 185 (3.34%), 134 (38.21%), 94 (82.82%).

Three possible structures **8**, **9** and **10** can be formulated when compound **4** was refluxed in acetic anhydride. Structure **10** was established on the basis of analytical and spectral data. Its IR spectrum of **10** showed absorption bands at 3200 (NH), 1740, 1720, 1710, 1690 cm^{-1} (4 C=O).



Scheme 1

While, ^{1}HNMR revealed signals at δ 2.6, 2.75, 2.82 (3s, 9H, $3COCH_3$) and 14.2 (broad, 2H, 2 NH).

Compound **4** may exist in two tautomeric forms, that is, as thione (NH) and the thiol (SH) forms, the first with a C=S double bond **4a**, the latter having the endocyclic double bond C=N and the hydrogen atom transfer to the sulphur atom **4b** (see Scheme 1). Molecular models of possible tautomeric forms were calculated in the gas phase, using semi-empirical AM1, PM3 and MNDO methods using HyperChem 7.5 software. The initial geometries of the molecules were built means of standard parameters and then optimized by Polak-Ribiere geometrical optimization. The heat of formation and relative stabilities of the two tautomeric forms **4a** and **4b** are listed in Table 1.

Table 1. Heat of formation (ΔH^o_f) and relative stabilities ($\Delta\Delta H^o_f$ kcal.mol-1) of tautomeric forms of 4a and 4b.

Method	ΔH^o_f		$\Delta\Delta H^o_f$
	4a(NH)	**4b(SH)**	
MNDO	172.81	159.59	-13.22
AM1	22.66	17.49	-5.17
PM3	26.20	18.03	-8.17

$\Delta\Delta H^o_f = \Delta H^o_f$ **(SH) -** ΔH^o_f **(NH)**

All semiempirical calculations lead to the same result that the **thione**-form has more heat of formation value than the **thiol**-form. The negative sign of the $\Delta\Delta H^o_f$ indicates that the **thiol**-form is more stable by all semi-empirical computational methods.

The equilibrium geometries have been calculated also by *Ab initio* calculations in the gas phase at HF/3-21G* and HF/6-31G* basis set level, were carried out using the GAUSSIAN 98 program.[21] The optimized structures information was shown in Fig. **1** along with their total energies at 3-21G* and 6-31G* (Hartree) and relative stability (in kcal mol^{-1}). The data in Fig. 1 show that the relative energies of tantomers **4a** and **4b** obtained in HF/6-31G* and HF/3-21G* calculation in gas phase differ significantly. Thus, while the HF/3-21G* data indicate preference for the thiol form. The HF/6-31G* data, on the other hand, indicate preference for the thione form.

The behavior of compound **4** towards some halogenated reagents was discussed herein. Thus, reaction of **4** with ethyl chloroacetate yielded the triazolo[3,4-b]thiadiazine derivative **11**. The IR spectrum of **11** revealed absorption bands at 3362, 3260 (NH, NH_2), 1722, 1704 cm^{-1}(2 C=O). ^{1}HNMR spectrum exhibited signals at 2.8 (m, 4H, 2 CH_2), 3.7 (s, 2H, SCH_2), 7.5-7.8 (m, 6H, Ar-H + NH_2) and 8.0 ppm (2s, 2H, 2NH).

On the other hand reaction of **4** with phenacyl bromide yielded the thioacetylbenzoyl derivative **12** rather than the triazolothiadiazine **13**. Its IR spectrum showed the presence of absorption bands at 3362, 3260 (NH, NH_2) and 1720, 1702 cm^{-1} (2 C=O). ^{1}HNMR spectrum exhibited the following signals at δ 2.7 (m, 4H, 2 CH_2), 4.3 (s, 2H, SCH_2), 7.1-7.9 (m, 13H, Ar-H +NH_2), 10.3 ppm (s, 1H, NH).

Compound **4** was treated with 2,3-dichloronaphthoquinone caused to furnish the corresponding triazolothiadi-

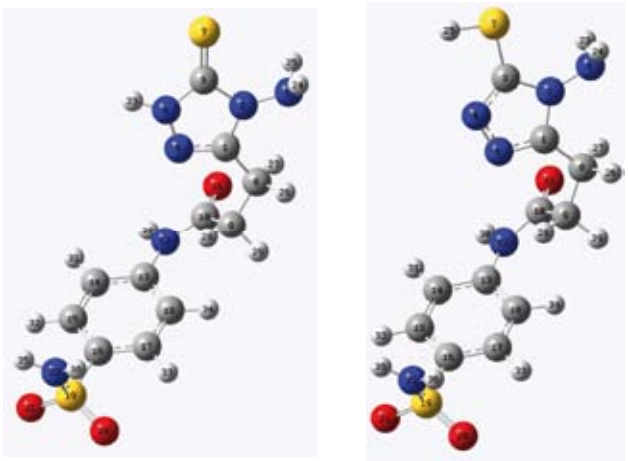


Fig. 1. HF/6-31G* optimized geometries of thione –thiol tautomers

azine derivative **14** through elimination of two moles of HCl. Its IR spectrum showed absorption bands at 3412, 3370, 3250 (NH, NH_2), and 1690, 1666, 1654 cm^{-1} (3 C=O). Mass spectrum revealed a molecular ion peak m/z 496 (M^+, 9.81%), with a base peak at 199 (100%) and other significant peaks were appeared at m/z 395 (8.05%), 341 (25.25%), 265 (18.22%), 114 (52.14%), 92 (42.58%), 77 (9.30%).

Also, the reactivity of the starting material **4** towards some carbonyl compounds was studied. Interaction of compound **4** with p-chlorobenzaldehyde in acetic acid afforded the Schiff's base **15**. IR spectrum revealed absorption bands at 3410, 3330, 3220 (NH, NH_2), 1680 cm^{-1} (C=O). ^{1}HNMR spectrum of **15** showed signals at δ 7.1-8.0 (, 11H, Ar-H + NH_2 +N=CH), 10.0 (s, 1H, NH), 10.5, 13.9 (s, 1H, NH: SH-tautomer).

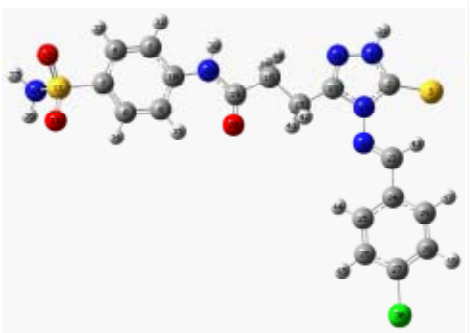
The thiol/thione tautomeric equilibrium were also carried out using semiempirical and *ab initio* molecular orbital methods. Thus, the tautomeric equilibrium involving thiol (SH) **15a** or thione (NH) **15b** forms. Similar trends were observed to the calculation above, which semiempirical calculations in gas phase indicate that the thiol form more stable (Table 2), whereas the HF/6-31G* calculation preference for thione form (Fig. 2).

Table 2. Heat of formation (ΔH^o_f) and relative stabilities ($\Delta\Delta H^o_f$ kcal.mol-1) of tautomeric forms of 15a and 15b.

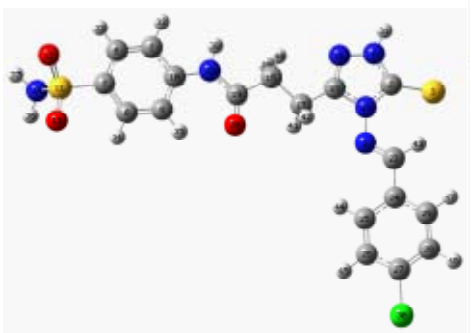
Method	ΔH^o_f		$\Delta\Delta H^o_f$
	4a(NH)	4b(SH)	
MNDO	162.10	149.96	-12.14
AM1	218.25	207.09	-11.16
PM3	43.56	31.64	-11.92

$$\Delta\Delta H^o_f = \Delta H^o_f \text{ (SH)} - \Delta H^o_f \text{ (NH)}$$

While, reaction of **4** with acetylacetone yielded triazole **17** rather than the thiadiazole **16** on the basis of elemental analysis and spectral data. Also, reaction of **4** with cyclohexanone or 1,3-cyclohexanedione and/or demidone furnished the corresponding spiro compounds triazolothiadiazole derivatives **18-20**, respectively (Scheme 4). The IR spectrum of **17** showed absorption bands at 3362, 3258 cm^{-1} (NH, NH_2), 2924, 1716, 1700 cm^{-1} (2 C=O). ^{1}HNMR spectrum revealed signals at 2.2, 2.6 (s, 6H, CH_3, $COCH_3$), 2.8 (m, 4H, 2 CH_2), 4.3 ppm (s, 2H, CH_2COCH_3). Mass spectrum of **17** exhibited a molecular ion peak at



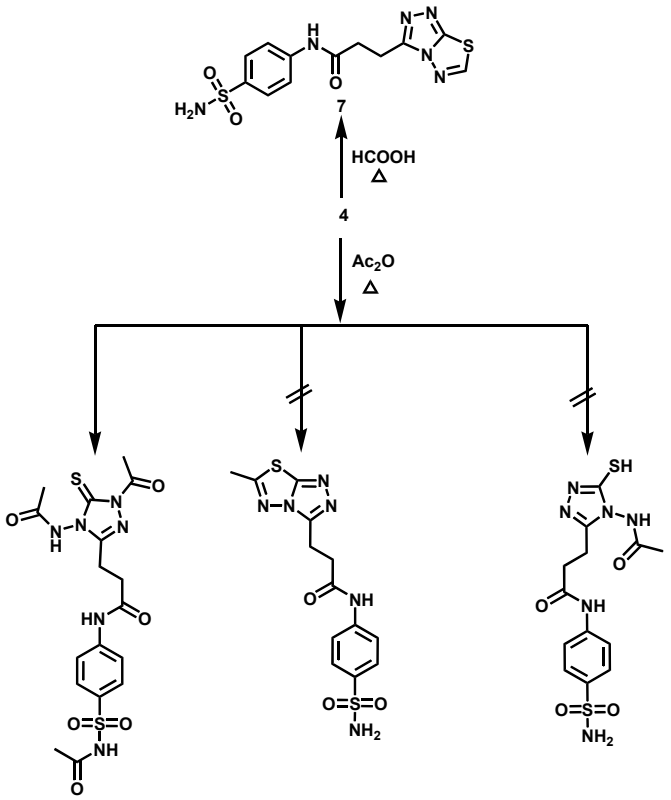
Thione
E= -2497.2260028 a.u.



Thiol
E= -2497.1977051 a.u.

Fig. 2 Optimized structures and atom numbering for the tautomric forms of thione (15a) and thiol (15b), proess obtained at the HF/6-31G* level

$$\Delta E = E(SH) - E(NH) = 17.75 \text{ kcal.mol}^{-1}$$



Scheme 2

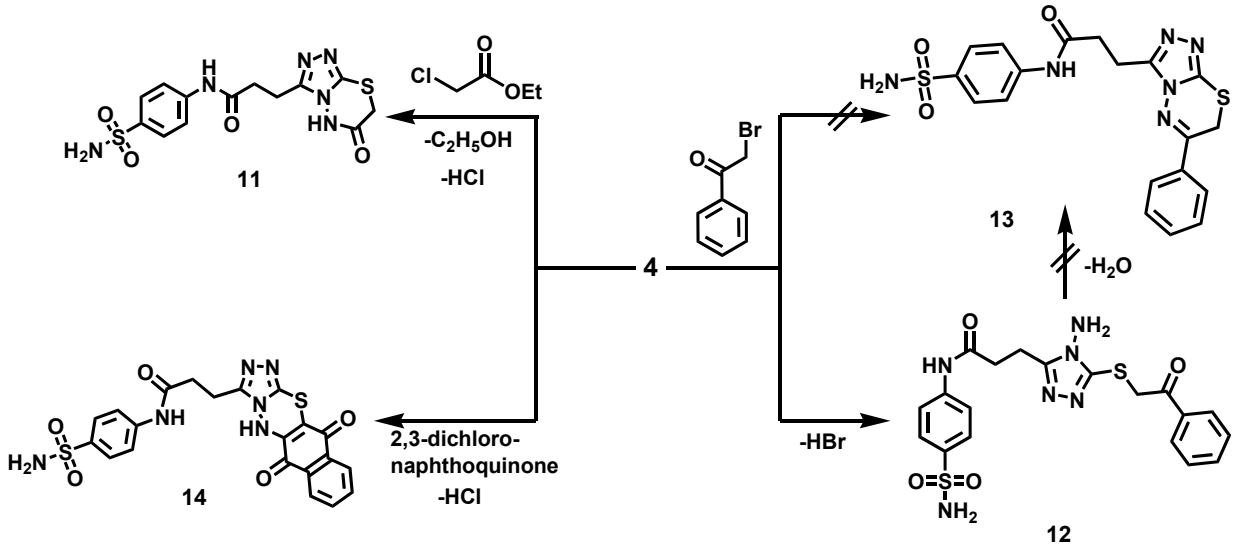
m/z 424 (M^+; 16.88%) with a base peak at 99 (100%) and other significant peaks were observed at 362 (7.15%), 314 (28.21%), 288 (8.38%), 217 (72.28%), 155 (70.18%), 111 (63.78%), 73 (31.30%). The IR spectrum of **18** revealed bands at 3310, 3280 (NH, NH_2), 1710 cm^{-1} (C=O). ^{1}H-NMR spectrum of **18** exhibited signals at 1.9-2.2 (m, 10H, $5CH_2$-cyclo), 5.4 (s, 2H, NH_2), 7.0-7.8, 10.1, 10.2 (2s, 2H, 2NH). The IR spectrum of **19** showed bands at 3402, 3360 (NH, NH_2), 1710, 1656 cm^{-1}(2C=O). Mass spectrum of compound **19** revealed a molecular ion peak at m/z 436 (M^+, 0.38) with a base peak at 62 (100%) and other significant peaks appeared at 354 (3.58%), 320 (5.18%), 256 (6.48%), 203 (5.56%), 181 (8.32%), 125 (8.88%), 84 (10.88%), 55 (8.85%). IR spectrum of **20** showed bands at 3424, 3390 (NH, NH_2), 1693, 1650 cm^{-1} (2C=O). Mass spectrum of **20** exhibited a molecular ion peak at m/z 464 (M^+; 1.78%) with a base peak at 126 (100%) and other significant peaks were appeared at m/z 421 (4.14%), 369 (7.64%), 359 (5.28%), 269 (3.56%), 254 (54.71%), 172 (9.94%), 64 (38.33%).

The dicyanotriazolothiadiazole derivative **21** was obtained by reaction of **4** with [bis(methylsulfanyl)methylidene]malononitrile, via the elimination of two moles of methyl mercaptan. The IR spectrum of **21** revealed bands at 3340, 3186 (NH, NH_2), 2202(C≡N), 1706 cm^{-1} (C=O). Its ^{1}H-NMR spectrum exhibited signals at 2.7-2.9 (m, 4H, $2CH_2$), 7.2-8.1 ppm (m, 8H, Ar-H+ NH_2 + 2NH). Mass spectrum of compound **21** showed a molecular ion peak at m/z 416 (M^+ 5.23%), with a base peak at 73 (100%), and other significant peaks were appeared at m/z 368 (17.48%), 288 (18.80%), 238 (1.81%), 211 (18.88%), 149 (55.78%), 128 (15.08%), 95 (28.03%), 58 (20.00%).

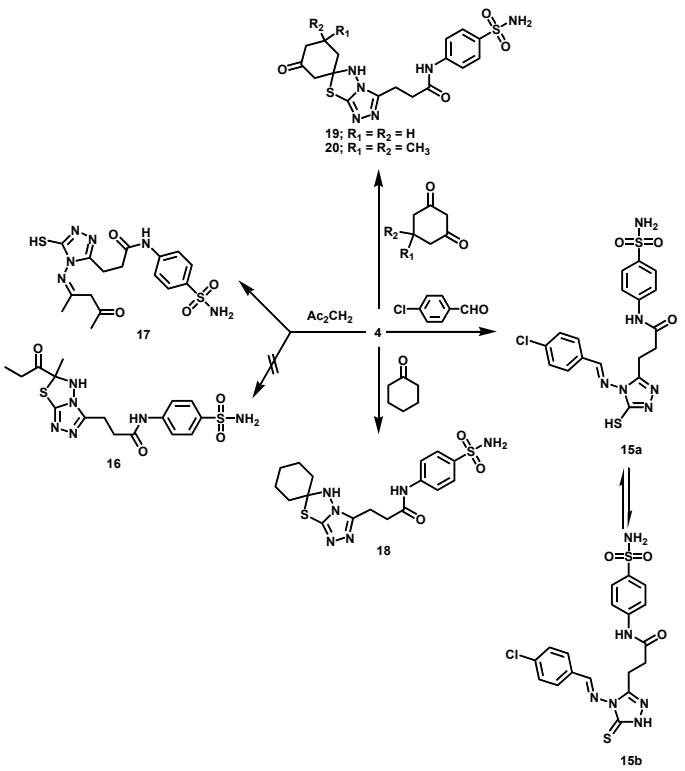
Finally, the triazolothiadiazepine derivative (**22)** was obtained in good yield through reaction of **4** with ethoxymethylenemalononitrile. Its IR spectrum showed bands at 3350, 3270 (NH, NH_2), 2210 (C≡N) and 1705 cm^{-1} (C=O). Mass spectrum of **22** revealed a molecular ion peak at m/z 419 (M^+; 8.47%) with base peak at 254 (100%) and other significant peaks were appeared at m/z 327 (11.50%), 288 (1.34%), 199 (28.87%), 146 (47.88%), 118 (53.88%), 90 (57.58%), 77 (13.50%).

BIOLOGICAL ACTIVITY

The results of the antibacterial studies are presented in Table 1 revealed that the synthesized compound **4** containing mercapto, sulfonamide moieties and compound **14** which contains naphthoquinone and sulfonamide moieties, compound **15** having mercapto, 4-chlorophenyl, sulfonamide moieties showed a promising activity equal that Gentamycin. On the other hand, the tested compounds exhibited moderate antibacterial activities. From these results it can be concluded that biologically active compounds **4, 14** and **15** (MIC values were 50 µg/mL) are nearly as active as the standard antibiotic Gentamycin.



Scheme 3



Scheme 4

EXPERIMENTAL

All m.p.s are uncorrected. Elemental analyses were carried out at the microanalytical laboratories of the faculty of Science, Cairo University. The IR spectra (KBr) were measured on a Shimadzu IR 110 spectrometer, ^{1}H-NMR spectra were obtained on a BRUKER proton NMR-Avance 300 (300 MHz), in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as internal standard. Mass spectra were run on HP Model MS.5988.

N-(4-sulfamoyl-phenyl)-succinamic acid 3.
A mixture of sulfanilamide (0.01 mol) and succinic anhydride (0.01 mol) in ethanol (30 ml) containing 3 drops of triethylamine was refluxed for 3hr. the obtained solid was recrystallized from ethanol to give (**3**, Table 2).

3-(4-Amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)-*N*-(4-sulfamoyl-phenyl)-propion- amide 4.
A mixture of **3** (0.01 mol) and thiocarbohydrazide (0.01 mol) was fused at 220ºC in an oil bath for 15 min. After cooling the reaction mixture was triturated with ethanol to give (**4**, Table 2).

***N*-(4-Sulfamoyl-phenyl)-3-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazol-3-yl-propionamide 7.**
A solution of **4** (0.01 mol) in formic acid (10 ml) was refluxed for 6hr. the obtained solid was recrystallized from dioxane to give (**7**, Table 2).

3-(1-Acetyl-4-acetylamino-5-thioxo-4,5-dihydro-1*H*-[1,2,4]triazol-3-yl)-*N*-(4-acetyl- sulfamoyl-phenyl)-propionamide 10
A solution of **4** (0.01 mol) in acetic anhydride (20 ml) was heated under reflux for 10 hr. After the solution had cooled, the excess acetic anhydride was removed under reduced pressure. The obtained solid was recrystallized from ethanol to give (**10**, Table 2).

3-(6-Oxo-6,7-dihydro-5*H*-[1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-3-yl)-*N*-(4 sulfamoyl -phenyl)-propionamide 11.
To a solution of **4** (0.01 mol) in dioxane (50 ml) and triethylamine (0.01 mol), ethyl chloroacetate (0.01 mol) was added. The reaction mixture was refluxed for 2 hrs. The precipitate that had formed was filtered off, washed with water, dried and recrystallized from dioxane to give (**11**, Table 2).

3-[4-Amino-5-(2-oxo-2-phenyl-ethylsulfanyl)-4*H*-[1,2,4]triazol-3-yl]-*N*-(4-sulfamoyl-phenyl)-propionamide 12.
A mixture of **4** (0.01 mol) and phenacyl bromide (0.01 mol) in dimethylformamide (10 ml) containing triethylamine (0.01 mol) was refluxed for 8 hrs. The solid that had

Compd. No.	Escherichia coli (NCTC 5933)		Serratia marcescens (IMRU-70)		Staphylococcus aureus (NCTC-7447)		Bacillus cereus (ATCC-14579)	
	Diameter (m m)	MIC (µg/mL^{-1})	Diameter (m m)	MIC (µg/mL^{-1})	Diameter (m m)	MIC (µg/mL^{-1})	Diameter (m m)	MIC (µg/mL^{-1})
4	26	60	24	70	28	50	26	60
7	20	80	20	80	24	70	18	90
10	18	90	20	80	20	80	18	90
11	18	90	18	90	20	80	14	100
12	14	100	18	90	18	90	14	100
14	28	50	26	60	28	50	24	70
15	26	60	26	60	28	50	24	70
17	20	80	14	100	24	70	14	100
18	20	80	20	80	24	70	18	90
19	20	80	18	90	22	65	20	80
20	18	90	20	80	20	80	20	80
21	18	90	20	80	20	80	20	80
22	14	100	18	90	20	80	18	90
DMF	-	-	-	-	-	-	-	-
Gentamycin	**28**	-	**27**	-	**30**	-	**25**	-

TABLE 1: Antibacterial of the synthesized compounds.

Compd. No.	M.P. (°C)	Yield (%)	Mol. Formula (M.wt)	Required (found) C	H	N
3	208-10	81	$C_{10}H_{12}N_2O_5S$ (272)	44.12 44.40	4.41 4.60	10.29 10.40
4	258-60	84	$C_{11}H_{14}N_6O_3S_2$ (342)	38.60 38.30	4.59 4.30	24.56 24.80
7	>300	75	$C_{12}H_{12}N_6O_3S_2$ (352)	40.91 40.60	3.41 3.10	23.86 23.60
10	176-78	68	$C_{17}H_{20}N_6O_6S_2$ (468)	43.59 43.20	4.27 4.40	17.95 17.70
11	291-93	79	$C_{13}H_{14}N_6O_4S_2$ (382)	40.84 40.50	3.66 3.80	21.99 21.70
12	226-28	71	$C_19H_{20}N_6O_4S_2$ (460)	49.57 49.70	4.35 4.60	18.26 18.50
14	>300	65	$C_{21}H_{16}N_6O_5S_2$ (496)	50.81 50.60	3.23 3.50	16.94 16.70
15	253-55	62	$C_{18}H_{17}N_6O_3S_2Cl$ (464.5)	46.50 46.30	3.66 3.40	18.08 18.30
17	290-92	73	$C_{16}H_{20}N_6O_4S_2$ (424)	45.28 45.40	4.72 4.50	19.81 19.60
18	>300	68	$C_{17}H_{20}N_6O_3S_2$ (420)	48.57 48.30	4.76 4.90	20.00 20.20
19	>300	63	$C_{17}H_{20}N_6O_4S_2$ (436)	46.79 46.90	4.59 4.70	19.27 19.50
20	>300	61	$C_{19}H_{24}N_6O_4S_2$ (464)	46.15 46.30	5.17 5.30	18.10 18.30
21	270-72	76	$C_{15}H_{12}N_8O_3S_2$ (416)	43.27 43.50	2.88 3.10	26.92 26.70
22	292-94	70	$C_{15}H_{14}N_8O_3S_2$ (418)	43.06 43.20	3.35 3.10	26.79 26.60

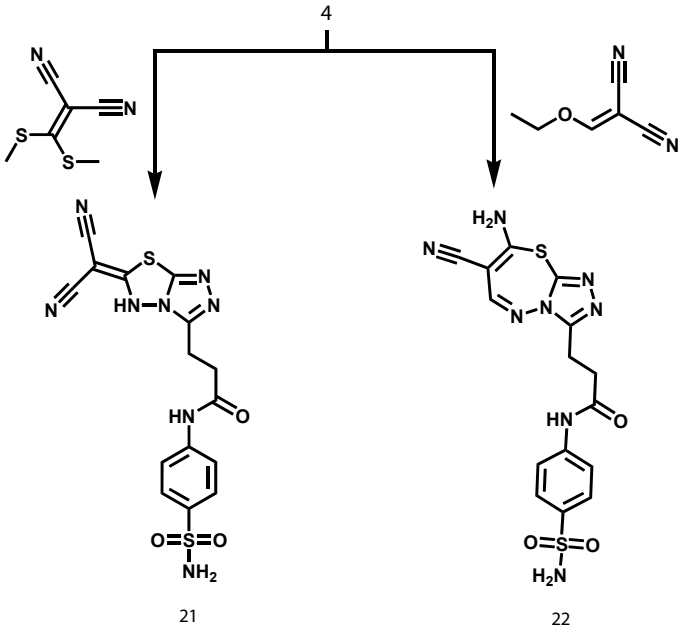
Table 2: Characterization data of newly synthesized compounds.

formed was collected and crystallized from ethanol to give (**12**, table 2).

3-(5,10-Dioxo-5,10-dihydro-4H-11-thia-1,2,3a,4-tetraaza-cyclopenta[b]anthracen-3-yl)-N-(4-sulfamoyl-phenyl)-propionamide 14.

A mixture of **4** (0.01 mol) and 2,3-dichloronaphthoquinoine (0.01 mol) in dimethylformamide (10 ml) containing (0.01 mol) triethylamine was refluxed for 12 hr. the solid obtained was recrystallized from dioxane to give (**14**, Table 2).

3.9. Synthesis of 3-{4-[(4-Chloro-benzylidene)-amino]-5-mercapto-4H-[1,2,4]triazol-3-yl}-N-(4-sulfamoyl-phenyl)-propionamide 15.



Scheme 5

A mixture of **4** (0.01 mol) and 4-chlorobenzaldehyde (0.01 mol) in glacial acetic acid (10 ml) was refluxed for 3hr. the solvent was evaporated under reduced pressure, and the solid product was collected and recrystallized from acetic acid to give (**15**, Table 2).

3-[5-Mercapto-4-(1-methyl-3-oxo-butylideneamino)-4*H*-[1,2,4]triazol-3-yl]-*N*-(4sulfa moyl-phenyl)-propionamide 17, 18, 19, 20, 3-(6-Dicyanomethylene-5,6-dihydro-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)-*N*-(4-sulfamoyl-phenyl)-propion- amide 21, 3-(8-Amino-7-cyano-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazepin-3-yl)-*N*-(4-sulfamoyl-phenyl)-propionamide 22.

A mixture of **4** (0.01 mol) acetylacetone or cyclohexanone or 1,3-cyclohexanedione or demidone or [bis(methylsulfanyl) methylidene]-malononitrile and / or ethoxymethylenemalononitrile (0.01 mol) and triethyl-amine (0.01 mol) in dimethylformamide (20 ml) was refluxed for 12 hr. the obtained solid was recrystallized from DMF/H_2O to give compounds **17**,**18-22**, respectively (Table 2).

MICROBIOLOGY

All the test compounds were assayed *in vitro* for antibacterial activity against two different strains of Gram-negative [*Escherichia coli* (NCTC 5933) and *Staphylococcus aureus* (NCTC 7447)] and Gram-positive [*Serratia marcescens* (IMRU 70) and *Bacillus subtilis* (ATCC 14579)] .The antibacterial activity of the compounds was determined by the agar diffusion technique[22]. A 1 mg mL^{-1} solution in dimethylformamide was used. The bacteria was maintained on nutrient agar media. DMF showed no inhibition zones. The agar media was incoubated with different microorganism culture tested. After 24h of incubation at 30°C for bacteria, the diameter of the inhibition zone (m m) was measured. Gentamycin in a concentration of 0.2 mg/mL was used as

Compd. No.	IR (KBr, cm^{-1})	^{1}HNMR (ppm, δ) (DMSO-d_6)
3	3330-2750(br-COOH), 3320, 3280 (NH,NH_2), 2940 (CH-aliph), 1750, 1690 (2C=O),	2.7(m, 4H, 2xCH_2), 7.2(s, 2H, NH_2), 7.4-7.9(m, 4H, Ar-H), 10.2(s, 1H, NH), 12.1(br, 1H, COOH)
4	3390, 3260, 3220 (NH, NH_2), 2940 (CH-aliph.), 1700 (C=O), 1620 (C=N).	2.8 (m, 4H, 2 CH_2), 7.3-7.6 (m, 4H, Ar-H), 7.8 (br, 4H, 2 NH_2), 8.3 (s, 2H, 2 NH).
7	3278, 3104 cm^{-1} (NH, NH_2), 3054 (CH-arom.), 2924, 2854 (CH-aliph.), 1694 (C=O), 1582 cm^{-1} (C=N).	
10	3200(NH), 2926 (CH-aliph.), 1740, 1720, 1710, 1690 (4 C=O), 1630 cm^{-1} (C=N).	2.2 (m, 4H, 2 CH_2), 2.6, 2.75, 2.82 (3s, 9H, 3$COCH_3$), 7.2-8.5 (m, 4H, Ar-H), 14.2 (s, 2H, 2 NH).
11	3362, 3260 (NH, NH_2), 3094 (CH-arom.), 2944 (CH-aliph.), 1722,1704 (2C=O),1598 (C=N).	2.7 (m, 4H, 2 CH_2), 4.3 (s, 2H, SCH_2), 7.1-7.9 (m, 13H, Ar-H +NH_2), 10.3 (s, 1H, NH).
12	3362, 3260 (NH, NH_2), 2926, 2854 (CH-aliph.), 1720, 1702 cm^{-1} (2 C=O).	
14	3412, 3370, 3250 (NH, NH_2), 2924 (CH-aliph.), 1690, 1666, 1654 (3 C=O), 1600 cm^{-1} (C=N).	2.4 (m, 4H, 2 CH_2), 7.1-8.0 (, 11H, Ar-H+ NH_2+N=CH), 10.0 (s, 1H, NH), 10.5, 13.9 (s, 1H, NH:SH-tautomer).
15	3410, 3330, 3220 (NH, NH_2), 2950 (CH-aliph.), 1680 (C=O), 1620 cm^{-1} (C=N)	2.6 (s, 3H, $COCH_3$), 2.8 (m, 4H, 2 CH_2), 4.3 (s, 2H, CH-$_2$$COCH_3$), 7.2-8.0 (m, 8H, Ar-H + NH_2 + 2NH)
17	3362, 3258 (NH, NH_2), 2924, 2854 (CH-aliph.), 1716, 1700 (2 C=O), 1598 cm^{-1} (C=N).	1.9-2.2 (m, 10H, 5CH_2-cyclo), 2.6 (m, 2CH_2), 5.4 (s, 2H, NH_2), 7.0-7.8 (m, 4H, Ar-H), 10.1,10.2 (2s, 2H, 2NH).
18	3310, 3280 (NH, NH_2), 2930 (CH-aliph.), 1710 (C=O), 1620 cm^{-1} (C=N).	
19	3402, 3360 (NH, NH_2), 2940 (CH-aliph.),1710, 1656 (2C=O), 1592 cm^{-1} (C=N).	
20	3424, 3390 (NH, NH_2), 2968 (CH-aliph.),1693, 1650 (2C=O), 1590 cm^{-1} (C=N)	
21	3340, 3186(NH, NH_2), 2924 (CH-aliph.), 2202 (C=N),1706 (C=O), 1620 cm^{-1} (C=N).	2.7-2.9 (m, 4H, 2CH_2), 7.2-8.1 (m, 8H, Ar-H+ NH_2 + 2NH).
22	3350, 3270 (NH,NH_2),2930 (CH-aliph.),2210(C=N), 1705 cm^{-1} (C=O).	

Table 3: Spectroscopic data for the synthesized compounds

a reference for antibacterial Activity. The minimal inhibitory concentration (MIC) of some of the test compounds was measured by a twofold serial dilution method[23].

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