SYNTHESIS OF NEW HETEROCYCLIC COMPOUNDS CONTAINING 1,3,4-THIADIAZOLE MOIETY AND THEIR ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

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Abstract

A new compounds of 2-mercapto-5-phenyl-4,6-dione-1,3,5-tiazino[6,5-b][1,3,4] thiadiazole (2), 2-[2-p-methoxyphenyl-4-oxo-1,3-thiazolidin-3-yl]-5-mercapto-1,3,4-thiadiazole (4), 1-[2-(2-amino-1,3,4-thiadiazol-5-ylthio)acetyl] -2,4,5-trihydropyridazin-3,6-dione (7) and 1-[2-(2-amino-1,3,4-thiadiazole-5-ylthio) acetyl]-3-methylpyrazol-5-one (8) were synthesized in good yield via condensation of 2-amino-5-mercapto-1,3,4-thiadiazole (1) with different organic reagents. All new compounds were tested against three strains of Gram negative bacteria (Escherichia coli, Klebsiella pneumoniae and Proteus vulgaris) and two strains of fungi (Candida albicans and Aspergillus niger), comparing these activities with that of the starting material. Compounds (2), (4), and (7) were highly active against all bacteria strains. Compound (1) was highly active against Candida albicans than others while none of the tested compounds were active against Aspergillus niger.

Keywords: 1,3,4-Thiadiazole, Biological activity, Pyrazole, Pyridazine

Introduction

Heterocyclic play an important role in biochemical processes because the side groups of the most typical and essential constituents of living cells are based on aromatic heterocycles. Between them, sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through the development of organic synthesis. Compounds possessing 1,3,4-thiadiazole ring system show antifungal, bacteriostatic as well as anthelmintic effects[1]. In the field of archaeological conservation, amino-mercapto-1,3,4-thiadiazole is the most widely and 2-amino-4-morphlino-*s*-triazine are used clinically due to their antitumor properties to treat lung, breast and ovarian cancer[5].

Used corrosion inhibiting compounds in the treatment of bronze artifacts[2]. On the other hand heterocycles containing 1,3,4-thiadiazole ring have been put to much use in the chemistry of disperse dyes[3].

1,3,5-Triazines (or *s*-triazines) are a class of compounds well known for a long time, and still continue the object of considerable interest, mainly due to their application in different fields, including the production of herbicides and polymer photostabilizers[4]. Some 1, 3,5-triazines display important biological properties; for example hexamethylmelamine

Thiazolidin-4-ones are important compounds due to their broad range of biological activity. 2,3-Diaryl-1,3-thiazolidin-4-one derivatives also possess anticonvulsant, hypnotic, or anticancer properties and have been reported as novel inhibitors of the bacterial enzyme Mur B which has a precursor acting during the biosynthesis of peptidoglycan[6].

Pyrazole chemistry has been the focus of high attention for more than three decades due to versatile biological activities of pyrazole derivatives. The pyrazol-5-one has been extensively studied, and several useful drugs and dyestuffs containing this ring are known[7]. Antipyrine (2,3-dimethyl-1-phenyl-5-pyrazolone), and its derivatives exhibit a wide variety of potentially useful applications including biological, clinical and pharmacological. Butazolidine, another pyrazolone, is a powerful anti-inflammatory drug for rheumatic conditions[8].

Pyridazine derivatives continue to be an object of interest for improving medicinal drugs for blood pressure control such as Hydralazine, which has been used for many years in the treatment of essential

hypertension[9]. Recently, it has been reported that a considerable number of pyridazinone derivatives bear analgesic activity as Emorfazone[10].

In view of the diverse applications associated with the title compounds, it is desirable to obtain facile methods for the synthesis of these ring systems.

Experimental

General:

All melting points were recorded on a hot stage Gallen Kamp melting point apparatus and were uncorrected. The IR spectra of the compounds were recorded on Shimadzu FTIR-8300 spectrometer as KBr disc. ^{1}H-NMR (δ, ppm) spectra were recorded in DMSO-d_6 on a Fourier transform Varian spectrometer (300MH_z) using TMS as the internal standard, measurement were made at the Chemistry Department of Georgia State University, USA. ^{13}C-NMR (δ, ppm) were recorded on a Fourier transform Bruker spectrometer (75MHz) in DMSO-d_6, measurement were made at Organic Chemical Technology Department, Technical University of Budapest, Hungary. Thin layer chromatography was carried out using Fertigfollen precoated sheets type Polygram SilG, and the plates were developed with iodine vapour.

2-Amino-5-mercapto-1,3,4-thiadiazole 1

A mixture of (13.5g, 0.1mol) of thiosemicarbazide and (9g, 0.1mol) of anhy drous sodium carbonate was dissolved in 70ml abs. ethanol, to this solution (18.3ml, 0.24mol) of carbon disulfide was added. The resulting mixture was heated under reflux for 7 hours.

The reaction mixture was then allowed to cool down to room temperature. Most of the solvent was removed under reduced pressure and the residue was poured on crushed ice, and carefully acidified with conc. hydrochloric acid to give pale yellow precipitate. The crude product was filtered and washed with cold water, recrystallized from ethanol to give the desired product as yellow needles, yield 70%, m.p. 232-234C°. **IR** (cm^{-1}): 3394-3274 (NH_2), 2600 (S-H), 1598 (C=N), 1361(C=S), 669 (C-S).

2-Mercapto-5-phenyl-4,6-dione-1,3,5-triazino [6,5-b][1,3,4] thiadiazole 2

2-Amino-5-mercapto-1,3,4-thiadiazole**1** (1.33g,0.01mol) and phenyl isocyanate (1.33g, 0.03mol) were heated under reflux in dry pyridine 15ml for 10 hours. Evaporation in vacuo gave the desired product. The crude product was recrystallized from chloroform, yield 87%, m.p. 193-195; **IR** (cm^{-1})**:** 3091(aromatic C-H), 1647 (C=O), 1596 (C=N), 1330 (C=S)**.** **^{1}H-NMR** δ**:** 7.00-7.50 (m, 5H, ph-H), 8.45 (s, 1H, -SH). **^{13}C-NMR** δ**:** 115.12-128.80 (aromatic carbons), 139.70, 152.56 (thiadiazole carbons), 173.32, 173.56 (carbonyl carbons).

2-[*p*-Methoxybenzylidine] amino-5-ercapto-1,3,4-thiadiazole 3

A mixture of 2-amino-5-mercapto-1,3,4-thiadiazole **1** (1.33g,0.01mol) and anisaldehyde (0.01mol) were refluxed in abs. ethanol (20ml) containing few drops of glacial acetic acid for (4-5) hours. After cooling to room temperature the precipitate was filtered and dried. The products were recrystallized from ethanol. Yield 75 %, m.p. 205-207; **IR** (cm^{-1})**:** 3100 (aromatic C-H), 2923 and 2868 (aliphatic C-H), 1602 (C=N), 823 (C-H *p*-substituted).

2[2-*p*-Methoxyphenyl-4-oxo-1,3-thiazolidin-3-yl]-5-mercapto-1,3,4-thiadiazole 4

Thioglycolic acid (0.69ml, 0.01mol) was added dropwise to (2.51g, 0.01 mol) of 2-[*p*-methoxybenzylidine]amino-5-mercapto-1,3,4-thiadiazole **3** in 15ml dry benzene with stirring. The reaction mixture was refluxed for 10 hours. Then the solvent was distilled off and the residue neutralized with sodium bicarbonate solution. The precipitate was filtered off and recrystallized from ethanol, yield 70%, m.p. 212-214C°; **IR** (cm^{-1})**:** 3100 (aromatic C-H), 2900 and 2846 (aliphatic C-H), 1705 (C=O), 833 (C-H *p*-substituted).

2-Amino-5-thioethylacetate-1,3,4-hiadiazole 5

Ethylchloroacetate (1.06ml, 0.01mol) was added dropwise to a solution of 2-amino-5-mercapto-1,3,4-thiadiazole **1** (1.33g,0.01mol), KOH (0.56g, 0.01mol) in 20ml abs. ethanol. The reaction mixture was refluxed for 8 hours, after that the mixture was filtered and the filtrate poured on crushed ice. The resulting product was recrystallized from chloroform,

yield 80%, m.p. 78-80C°; **IR** (cm^{-1})**:** 2918 and 2850 (aliphatic C-H), 1728 (C=O).

2-Amino-5-thioacetic acid hydrazide-1,3,4-thiadiazole 6

A mixture of thiadiazole ester **5** (2g, 0.01mol) and hydrazine hydrate (0.015mol) was refluxed for 4 hours, ethanol (15ml) was added and refluxed for 8 hours. The separated precipitate was filtered and washed with cold water, yield 75%, m.p 212-215C°. **IR** (cm^{-1})**:** 3300-3200 (NH_2), 1645 (C=O).

1-[2-(2-Amino-1,3,4-thiadiazol-5-ylthio) acetyl]-2,4,5-trihydropyridazin-3,6-dione7

Succinic anhydride (1.5g, 0.015mol) dissolved in 30ml acetic acid was added to carbohdrazide **6** (3.07g, 0.015mol) and the reaction mixture was refluxed for 7 hours. Then the mixture was poured on crushed ice, the formed solid product was filtered off and recrystallized from petroleum ether (40-60)C°, yield 65%, m.p. 76-78C°. **IR** (cm^{-1})**:** 3460 (O-H), 1726 (pyridazine C=O), 1625 (exocyclic carbonyl). **^{1}H-NMR** δ**:** 1.87-2.03 (t, 2H, -CH_2- of pyridazine ring), 2.78 (s, 2H, -SCH_2), 5.27 and 5.39 (2s, 3H, -NH_2 and –NH of tautomeric form) (D_2O exchange), 9.45 (b, 1H, -NH of pyridazine ring) (D_2O exchange). **^{13}C-NMR** δ**:** 21.10 (-CH_2- pyridazine), 28.96 (-S<u>C</u>H_2), 151.18, 152.62 (thiadiazole carbons), 168.49, 168.84, 174.27 (carbonyl carbons)

1-[2(2-Amino-1,3,4-thiadiazol-5-ylthio) acetyl]-3-methylpyrazol-5-one 8

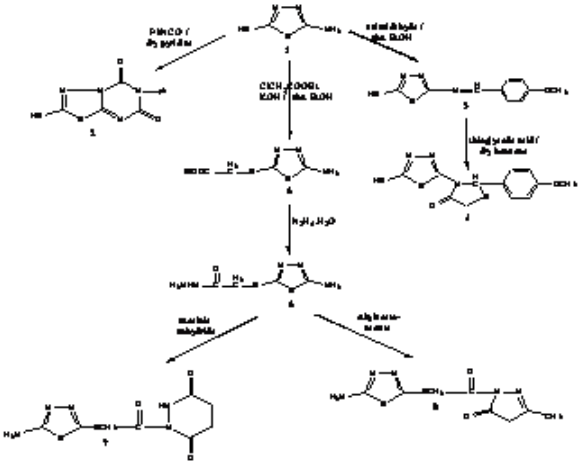
A mixture of (2.05g, 0.01mol) of the carbohdrazide **6** and ethyl acetoacetate (1.27ml, 0.01mol) in abs. ethanol (20ml) was heated under reflux for 7 hours. After concentration and cooling, the solid product that forms was filtered off and recrystallized from ethanol, yield 80%, m.p. 102-104C°. **IR** (cm^{-1})**:** 3550 (O-H), 1718 (pyrazole C=O), 1651 (exocyclic carbonyl). **^{1}H-NMR** δ**:** 1.24 (s, 3H,-CH_3), 1.99 (s, 2H, -CH_2 of pyrazolone), 2.03 (s, 1H, -CH of tautomeric form), 3.34 and 3.52 (2s, 3H, -NH_2 and –NH tautomer) (D_2O exchange), 4.11-4.20 (2d for each 1H, -SCH_2), 8.51(s, 1H, -OH) (D_2O exchange). **^{13}C-NMR** δ**:** 14.53 (-<u>C</u>H_3), 16.41 (-CH_2-pyrazole), 44.14 (<u>C</u>-CH_3 pyrazole), 60.95 (-S<u>C</u>H_2-), 149.93, 150.47 (thiadiazole carbons), 164.75, 170.12 (carbonyl carbons)

Biological Activity

The antimicrobial activity of the prepared compounds was tested by disc diffusion method. Compounds (**1**, **2**, **4**, **7**, **8**) were assayed for their antimicrobial activity in vitro against 3 strains of Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*) and 2 strains of fungi: Yeast-like fungi (*Candida albicans*) and moulds (*Aspergillus niger*). Prepared agar and petridishes were sterilized by autoclaving for 15min at 121C°. The agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In the solidified medium suitably spaced apart holes were made all 6mm in diameter. These holes were filled with 100µl of the prepared compounds (1mg of the compound dissolved in 1ml of DMSO solvent), DMSO was used as a solvent. These plates were incubated at 37C° for 24h for both bacteria and fungi, except the Aspergillus niger plate was incubated at 28Cº for 72h. The inhibition zones caused by the compounds then measured (mm).

Results and Discussion

The synthesis pathway leading to the title compounds is given in **Scheme 1**.2-Amino-5-mercapto-1, 3,4-thiadiazole **1** was reacted with



Scheme (1)

Cyclization of imine **3** was performed through its reaction with thioglycolic acid in dry benzene to produce 4-thiazolidine **4**,**Scheme (1).** The FT-IR spectrum of **4** showed the appearance of ν(C=O) at 1705cm^{-1} due to the thiazolidinone ring , this was consider as the most characteristic evidence for the success of cyclization step.

The 2-amino-5-thioacetic acid hydrazide-1,3,4-thiadiazole **6** was obtained by the reaction of compound **5** with hydrazine hydrate, **Scheme1**. The FT-IR spectrum of the hydrazide showed the disappearance of (C=O) stretching band attributed to ester group at 1728cm^{-1} with the appearance of bands at 1645cm^{-1}

phenyl isocyanate to obtained [1,3,5] triazino [6,5-b][1,3,4] thiadiazole **2**. The structure of compound **2** was elucidating by FT-IR, ^{1}H-NMR, ^{13}C-NMR

The FT-IR spectrum of **2** showed bands at 3282, 3091, 1647 and 1330 cm^{-1} due to the (N-H), (C-H aromatic), (C=O) and (C=S) stretching vibrations, respectively. ^{1}H-NMR spectrum exhibited signals at δ7.00, 7.30 and 7.50 due to the aromatic protons. Sulfhydryl proton (S-H) absorbed at δ8.45. ^{13}C-NMR spectrum shows the following chemical shifts:

δ (115.12, 118.22, 121.84, 128.80), (139. 70, 152.56) and 173.56 for the aromatic carbon atoms, two thiadiazole carbons and carbonyl carbon, respectively.

and 1595cm^{-1}due to the ν(C=O) (Amide I) and δ(NH) (Amide II).

Refluxing of the acid hydrazide **6** with succinic anhydride, ethyl acetoacetate afforded compounds **7** and **8**, respectively **(Scheme1)**. The structure of compound **7** and **8** were confirmed by their FT-IR, ^{1}H-NMR, and ^{13}C-NMR. The FT-IR spectrum of compound **7** showed two broad bands at 3460cm^{-1} and 3203cm^{-1} which were assignable to (O-H), (-NH_2) and (N-H) stretching vibrations. From this we can say that compound **7** can be exist in two tautomeric, keto and enol forms. ^{1}H-NMR spectrum of compound **7** exhibited triplet signal at 1.87-2.03 due to two methylene groups of pyridazine ring. The –SCH_2–absorption occurs at 2.78. Two single peaks at 5.27 and 5.39 are due to the –NH_2 and –NH (tautomeric form), respectively. The pyridazine (N-H) absorption appeared at 9.45. The latter absorptions were further proved by D_2O exchange. ^{13}C-NMR of compound **7** shows that the two methylene carbon atoms of pyridazine ring absorb at δ21.10. The –S<u>C</u>H_2- absorption appeared at 28.96 upfield than expected, this was due to the hydrogen bond between N-H of pyridazine ring and exocyclic C=O. Thiadiazole carbons absorbed at 151.18 and 152.62. The signals at 168.49 and 168.84 are assigned for the two carbonyl carbon of pyridazine ring. The exocyclic carbonyl carbon appeared at 174.27.

For compound **8,** the FT-IR spectrum showed the presence of bands at 3350 and 1718 cm^{-1} due to ν(O-H) and ν(C=O) moieties of pyrazole ring. From this we can say that compound **8** can exist in equilibrium between keto and enol forms. ^{1}H-NMR, **fig. 1**, showed that methyl protons appear at δ1.24. The methylene protons of pyrazolone ring together with –CH- (enol form) absorb at δ1.99 and δ2.03, respectively. The two single peaks at δ3.34 and δ3.52 were due to the absorption of –NH_2 and NH, respectively, which were further characterized by their disappearance due to the D_2O exchange, **fig. 2**. The signal of –SCH_2- protons was split into quartet and appeared in the region (4.11-4.20), this resembles (AB) system .The single peak at δ8.51 was attributed to O-H group of the tautomeric form, which was further assigned by D_2O exchange, **fig. 2**, this gives a good support for the results obtained from IR analysis. ^{13}C-NMR, **fig. 3**, showed the following characteristic chemical shifts: a signal at 14.53 is characteristic of methyl group carbon. Signal at δ16.41 is assignable to (CH_2) group of the pyrazolone ring. The (–<u>C</u>-CH_3) carbon of pyrazole ring appears at δ44.14. The –S<u>C</u>H_2- absorption occurs at δ60.95. Thiadiazole ring carbon atoms absorption appears at δ149.93 and δ150.47. The signals at δ164.75 and δ170.12 are due to the absorption of the two carbonyl carbon atoms. All the data mentioned above for compound **8** give a good support for the proposed structures.

Biological Activity Results

The results of antibacterial and antifungal activities are given in Table 1. It is clearly found that compounds **2**, **4** and **7** have the highest activity against *E.coli*, *Klebsiella* and *Proteus* than others. In case of fungi, compounds **1**, **2**, **4**, **7** and **8** show no activity against *Aspergillus niger* while against *Candida* compound **1** shows the higher activity.

Table (1)
Antimicrobial activity of the compounds against tested microorganism

Comp. No.	Gram negative bacteria			Fungi	
	E.coli	Kleb	Port.	Cand.	Asper.
1	–	+	++	++	–
2	++	++	++	+	–
4	+++	+++	+++	+	–
7	++	++	+++	–	–
8	–	+	–	+	–

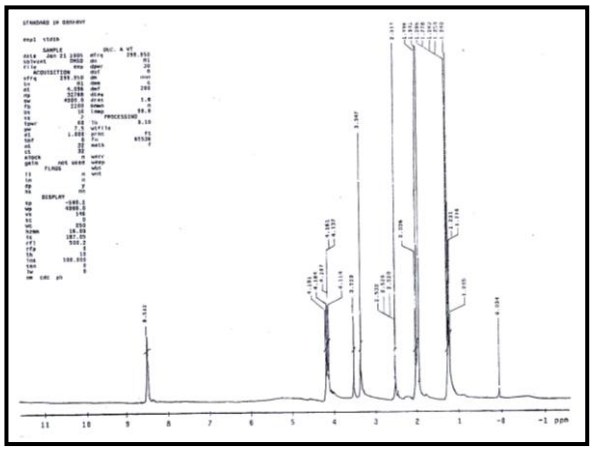


Fig. (1) : ^{1}H-NMR spectrum of compound 8

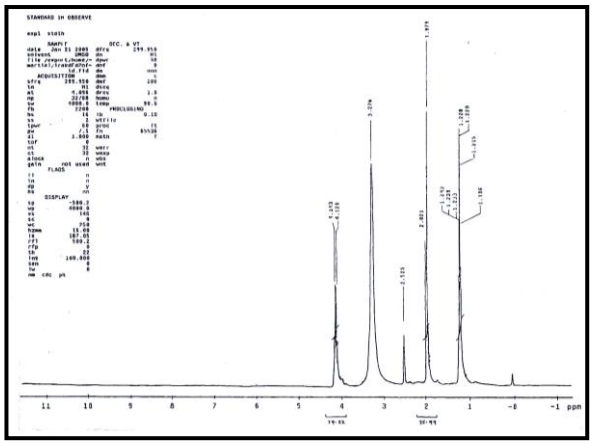


Fig. (2) : D_2O Exchange spectrum of compound 8

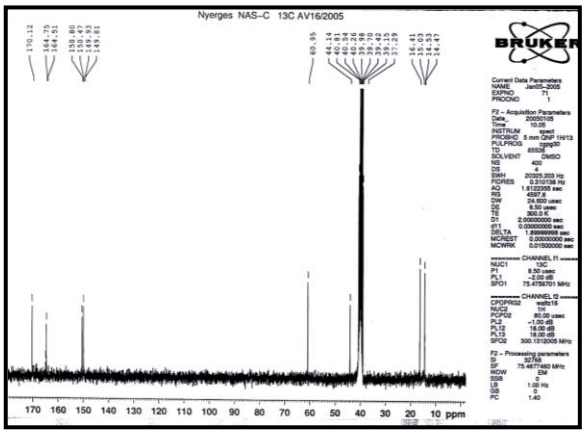


Fig. (3) : ^{13}C-NMR spectrum of compound 8

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االخلاصة

تم تحضير مركبات 5-فنيل- 6،4-دايون- ,3,1،5- ترايزينو [,5,6b][4,3,1] ثاياديازول (2) ، 2-[2- بارا ميثوكسي فـنيل-4- أوكسو-3،1- ثايازوليدين-3- يل]- 5-مركبتو-4,3,1- ثاياديازول (4) ، 1-[2-(2-أمينو- ,4,،3,1- ثاياديازول-5- يل ثايو) أستيل]-,42،5- ترايهايدرو بايريدازين- 6،3-دايون (7) ،1-[2- أمينو- 4,3,1- ثاياديازول-5- يل ثايو) أستيل]-3- مثيل بايرازول -5- أون (8) الجديدة وبكميات جيدة من تكاثف 2- أمينو-5- مركبتو-1,3,4- ثاياديازول (1) مع كواشف عضوية مختلفة. لقد تم اختبار الفعالية البايولوجية لجميع هذة المركبات ضد ثلاثة أنواع من البكتريا ونوعان من الفطريات ومقارنة هذة النتائج مع المادة الأساس . لقد دلت النتائج المستحصلة بان المركبات (2) ، (4)، (7) تمتلك أعلى فعالية ضد جميع أنواع البكتريا و المركب (1) يمتلك أعلى فعالية ضد *Candida albicans* بينما لا تمتلك هذة المركبات فعالية ضد *Aspergillus niger* .