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SYNTHESIS AND BIOLOGICAL STUDIES OF OXADIAZOLO-THIADIAZINES.

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Abstract:
Equimolar mixture of substituted triazole **(1)** and substituted aromatic aldehyde were refluxed in presence of alcoholic KOH to yield 4- substitutedbanzylideneamino-5-substituted-4H-1,2,4-triazole-3-thiol **(2)**, which cyclized to 2H,3H,4H,2-Carbethoxy, 3-substituted, Phenyl,5-substituted-[1,2,4]-triazole [3,4-b] [1,3,4] thiadiazine **(3)** using ethylchloro acetate and K_2CO_3. **(3)** undergoes nucleophilic addition reaction with hydrazine hydrate to form carbohydrazide 2H,3H,4H,2-Carboxy hydrazino, 3-substituted Phenyl, 5-substituted-[1,2,4]-triazole [3,4-b] [1,3,4] thiadiazine **(4),** which further on reaction with Carbon disulfide to achieved 2H,3H,4H-[2-5'-mercapto 1'-3'-4'-oxadiazol]-2'yl-3-substituted, Phenyl,5-substituted-[1,2,4]-triazole [3,4-b] [1,3,4] thiadiazine **(5)**.The structures of the compounds were confirmed by elemental as well as spectral techniques like IR, NMR. Representative samples were investigated for their antibacterial activities against gram positive and gram negative bacteria's and they showed promising activity.

Keywords: Triazole, Oxadiazole , Thiadiazine and Antibacterial.

Introduction:

In recent years, interest in 1, 3, 4-thiadiazines has increased in connection with a high biological activity and broad applications. Thiadiazine nucleus is a versatile pharmacophore, which exhibits a wide variety of biological activity such as antimicrobial[I-II], anti-irradiation[III-IV] and anti-parasitic[V].
In addition, 1,3,4-oxadiazole is a potential lead molecule for designing potent bioactive agents [VI]. They possesses diverse biological activity such as antimicrobial [VII-VIII], anti-inflammatory [IX], antitubercular [X], anticonvulsant [XI], anticancer, anti-HIV , hypoglycemic [XII] and genotoxic [XIII] activities. While considering their wide range of applications, we aim to synthesize a library of 1,3,4-Thiadiazine derivatives bearing Oxadiazole moiety and also, study their antibacterial potential.

Results and Discussion:

Equimolar mixture of substituted triazole **(1)** and substituted aromatic aldehyde were refluxed in presence of alcoholic KOH to yield 4- substitutedbanzylideneamino-5-substituted-4H-1,2,4-triazole-3-thiol **(2)**, which cyclized into Ethyl 6,7-dihydro-6-substitutedbanzylidene-3-substituted-5H-[1,2,4]Triazolo[3,4-b][1,3,4]thiadiazine-7-carboxylate**(3)** using ethylchloro acetate and K_2CO_3. **(3)** undergoes nucleophilic addition reaction with hydrazine hydrate to form 6,7-dihydro-6-substitutedbanzylidene-3-substituted-5H-[1,2,4]Triazolo[3,4-b][1,3,4]thiadiazine-7-carbohydrazide **(4),** which further on reaction with Carbon disulfide to achieved 5-(6,7-dihydro-6-substitutedbanzylidene-3-substituted-5H-[1,2,4]Triazolo[3,4-b][1,3,4]thiadiazine-7yl)-1,3,4-oxadiazole-2-thiol**(5)**. The formation of the compound (**4)** which was confirmed on the basis of IR bands at 3350, 3310 and 1630 due to NH-NH_2 and appearance of signal at 4.2 ppm and 9.08 ppm due to NH_2 and -NH respectively in ^{1}H-NMR. Compound (**5)** shows an additional peak at 9-10 ppm in ^{1}H-NMR due to –SH and disappearance of Signals due to –NH-NH_2 in IR and ^{1}H-NMR, cpnfirms the formation of desired product.

Experimental

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The progress of reaction was monitored by thin layer chromatography on silica gel coated aluminum plates (Merck) as adsorbent and UV light as visualizing agent. ^{1}H NMR spectra were recorded on Varian 500 MHz NMR spectrophotometer using $CDCl_3$/DMSO-d_6 as solvent and TMS as an internal standard (chemical shifts in δ ppm). C, H, N estimation was recorded on Carlo Erba 1108 (CHN) Elemental Analyzer.

General Procedure:

Synthesis of 4- substitutedbanzylideneamino-5-substituted-4H-1,2,4-triazole-3-thiol (2).

An equimolar mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**1)** (0.01 mol) and substituted aromatic aldehyde (0.01 mol) were refluxed for about 4-5 hrs in alcohol (20 ml) as a solvent and KOH (0.01 mol) as a catalyst. The progress of the reaction was monitored on TLC. Upon completion 3 hrs, the reaction was quenched onto crushed ice. The separated solid was filtered, washed with cold water and crystallized from alcohol, to yield respected (**2)**.

Synthesis of 2H,3H,4H-2-Carbethoxy-3-substitutedphenyl-5-substituted-[1,2,4]-triazole [3,4-b] [1,3,4] thiadiazine (3).

A mixture of **2** (0.01 mol), ethyl chloro acetate (0.01 mol), N, N- Dimethyl formamide (15 ml) and potassium carbonate (0.02 mol) were stirred for 30 mins at room temperature. Then the content was refluxed for 3-4 hrs. The progress of the reaction was monitored on TLC. Upon completion, the content was poured into cold water. Solid thus obtained was filtered, washed with cold water and crystallized from alcohol, to yield respected (**3).**

Synthesis of 2H,3H,4H-2-Carboxyhydrazino-3-substitutedphenyl-5-substituted-[1,2,4]-triazole [3,4-b] [1,3,4] thiadiazine (4).

Compound **(3)** (0.01 mol), hydrazine hydrate (2 gm, 0.02 mol) and ethanol (20 ml) were taken in 100 ml round bottom flask and refluxed for 2 hrs . After completion of the reaction (monitored by TLC) cooled, poured on crushed ice, solid thus obtained was washed with water and recrystallized from ethanol to get (**4)**.

Synthesis of 2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-substitutedphenyl,5-substituted-[1,2,4]-triazole [3,4-b] [1,3,4] thiadiazine (5).
A mixture of (4) (0.01 mol), Carbon disulfide (0.82 ml, 0.01mole), and Potassium hydroxide (0.768 gm, 0.01 mole) was refluxed in ethanol (10 ml) on water bath for 5-6 hrs. The reaction is then allowed to cool. The red solid so obtained is then filtered and washed with ethanol and finally recrystallized from hot ethanol to targeted (**5)**.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-methoxyphenyl)-5-methyl-[1,2,4]-triazole[3,4-b][1,3,4] thiadiazine (5a).**
Yield: 83 %; m.p. =144-146°C: IR (cm^{-1}): 1578 (C=N), 3356 (NH)**,**
^{1}H NMR(DMSO-d_6,δ/ ppm): 2.71 (s,3H, CH_3), 3.75 (s,3H, OCH_3),4.16 (s,1H, CH), 4.56 (s,1H, CH), 7.84-8.13 (m, 4H, Ar- H), 8.30 (s, 1H, NH) ,9.28 (s,1H, SH).
^{13}C NMR (DMSO-d_6, δ/ ppm): 18.45 (CH_3), 51.47 (CH), 58.73 (CH), 60.35 (OCH3), 122.46-131.50 (Ar-C),168.51 (C=N), 174.79 (C=N) 177.89 (C=N) 182.34 (C=N) .
Anal.Calcd for $C_{14}H_{14}N_6O_2S_2$: C,46.40;H,3.86;N,23.20%.Found: C,46.38;H,3.84,N,23.17%.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-hydroxyphenyl)-5-methyl-[1,2,4]-triazole[3,4-b][1,3,4] thiadiazine (5b).**
Yield: 80 %; m.p. =138-140°C: IR (cm^{-1}): 1544 (C=N), 3245 (NH), 3467 (-OH),
^{1}H NMR (DMSO-d_6, δ/ ppm): 2.11 (s, 3H, CH_3), 4.39 (s, 1H, CH), 4.51 (s,1H, CH), 5.03 (s,1H, OH) 7.41-8.44 (m, 4H, Ar- H), 8.13 (s, 1H, NH) ,9.21 (s, 1H, SH).
^{13}C NMR (DMSO-d_6, δ/ ppm): 18.45 (CH_3), 51.47 (CH), 58.73 (CH), 122.46-131.50 (Ar-C),168.51 (C=N), 174.79 (C=N) 177.89 (C=N) 182.34 (C=N).
Anal.Calcd for $C_{13}H_{12}N_6O_2S_2$: C,44.82;H,3.44;N,23.59%.Found: C,44.78;H,3.43,N,23.57%.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-methoxy,3-hydroxy phenyl)-5-methyl-[1,2,4]-triazole[3,4-b][1,3,4] thiadiazine (5c).**
Yield: 79 %; m.p. =134-137°C: IR (cm^{-1}): 1533 (C=N), 3390 (NH), 3488 (-OH),
^{1}H NMR(DMSO-d_6,δ/ ppm): 2.11 (s,3H, CH_3), 3.10 (s,3H, OCH_3),4.49 (s,1H, CH), 4.20 (s,1H, CH), 5.02 (s,1H, OH) 7.13-8.64 (m, 3H, Ar- H), 8.01 (s, 1H, NH), 9.45 (s,1H, SH).
^{13}C NMR (DMSO-d_6,δ/ ppm): 20.43 (CH_3), 52.22 (CH), 56.57 (CH), 61.32 (OCH3) 124.16-131.18, (Ar-C),171.33 (C=N), 173.70 (C=N) 177.89 (C=N) 187.51 (C=N).
Anal.Calcd for $C_{14}H_{14}N_6O_3S_2$: C,44.44;H,3.70;N,22.22%.Found: C,44.42;H,3.69,N,22.18%.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-chlorophenyl)5-methyl-[1,2,4]-triazole[3,4-b][1,3,4]thiadiazine (5d).**
Yield: 75 %; m.p.=148-150°C: IR (cm^{-1}): 1520 (C=N**),** 3211 (NH)**,**
^{1}H NMR(DMSO-d_6,δ/ ppm): 2.66 (s,3H, CH_3), 4.29 (s,1H, CH), 4.40 (s,1H, CH), 7.33-8.54 (m, 4H, Ar- H), 8.61 (s, 1H, NH), 9.13 (s,1H, SH).
^{13}C NMR (DMSO-d_6,δ/ ppm): 22.81 (CH_3), 54.33 (CH), 55.20 (CH), 125.50-135.60 (Ar-C),177.85 (C=N), 179.63 (C=N) 181.39 (C=N) 185.71 (C=N) .
Anal.Calcd for $C_{13}H_{11}ClN_6O_2S_2$: C,40.78;H,2.87;N,21.96%.Found: C,40.75;H,2.81,N,21.94%.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-Phenyl-5-methyl-[1,2,4]-triazole [3,4-b][1,3,4]thiadiazine (5e).**
Yield: 78%; m.p.=141-143°C: IR (cm^{-1}): 1566 (C=N**),** 3375 (NH)**,**

^{1}H NMR (DMSO-d_6,δ/ ppm): 2.23 (s,3H, CH_3), 4.19 (s,1H, CH), 4.69 (s,1H, CH), 7.02-8.12 (m, 5H, Ar- H), 8.81 (s, 1H, NH), 9.43 (s,1H, SH).
^{13}C NMR (DMSO-d_6,δ/ ppm): 21.70 (CH_3), 52.45 (CH), 55.57 (CH), 121.59-134.97 (Ar-C),161.90 (C=N), 164.33 (C=N) 166.58 (C=N) 170.11 (C=N) .
Anal.Calcd for $C_{13}H_{12}N_6OS_2$: C,46.99;H,3.61;N,25.30%.Found: C,46.96;H,3.59,N,25.27%.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-methoxyphenyl)-5-ethyl-[1,2,4]-triazole [3,4-b][1,3,4]thiadiazine (5f).**
Yield: 81 %; m.p. =140-142°C: IR (cm^{-1}): 1543 (C=N), 3396 (NH),
^{1}H NMR(DMSO-d_6,δ/ ppm): 2.20 (s,3H, CH_3), 3.42 (s,3H, OCH_3),4.22 (s,1H, CH), 4.37 (s,1H, CH), 5.12 (s,1H, OH) 7.33-8.71 (m, 3H, Ar- H), 8.44 (s, 1H, NH) ,9.31 (s,1H, SH).
^{13}C NMR (DMSO-d_6,δ/ ppm): 25.43 (CH_3), 59.32 (CH), 60.98 (CH), 67.55 (OCH3) 125.11-134.66, (Ar-C),154.03 (C=N), 161.31 (C=N) 165.90 (C=N) 169.11 (C=N) .
Anal.Calcd for $C_{15}H_{16}N_6O_2S_2$: C,47.87;H,4.25;N,22.34%.Found: C,47.83;H,4.22,N,22.31%.

Spectral Data of Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-hydroxyphenyl)-5-ethyl-[1,2,4]-triazole[3,4-b][1,3,4]thiadiazine (5g).**
Yield: 82 %; m.p. =136-138°C: IR (cm^{-1}): 1537 (C=N), 3357 (NH)**,**
^{1}H NMR(DMSO-d_6,δ/ ppm): 1.81 (s,3H, CH_3), 2.45 (s,2H, CH_2), 3.65 (s,3H, OCH_3),4.26 (s,1H, CH), 4.41 (s,1H, CH), 7.32-8.19 (m, 4H, Ar- H), 8.20 (s, 1H, NH),9.48 (s,1H, SH).
^{13}C NMR (DMSO-d_6,δ/ ppm): 19.13 (CH_2), 23.67 (CH_3), 53.29 (CH), 56.94 (CH), 60.32 (OCH3), 121.11-131.01 (Ar-C),157.09 (C=N), 158.80 (C=N), 161.76 (C=N),165.63 (C=N)
Anal.Calcd for $C_{14}H_{14}N_6O_2S_2$: C,46.40;H,3.86;N,23.20%.Found: C,46.37;H,3.84,N,23.16%.

Spectral Data of **2H,3H,4H-[2-5'-mercapto-1'-3'-4'-oxadiazol]-2'yl-3-(4-methoxy,3-hydroxy phenyl)-5-ethyl-[1,2,4]-triazole [3,4-b][1,3,4] thiadiazine (5h).**
Yield: 79 %; m.p. =132-134°C: IR (cm^{-1}): 1537 (C=N), 3357 (NH)**,**
^{1}H NMR(DMSO-d_6,δ/ ppm): 2.08 (s,3H, CH_3), 2.32 (s,2H, CH_2), 3.05(s,3H, OCH_3),4.21(s,1H, CH), 4.09 (s,1H, CH), 5.01 (s,1H, OH) ,7.01-8.23 (m, 3H, Ar- H), 7.89 (s, 1H, NH), 9.31 (s,1H, SH),.
^{13}C NMR(DMSO-d_6,δ/ ppm): 19.91 (CH_2), 20.34 (CH_3), 52.14 (CH), 55.98 (CH), 60.12 (OCH3) 123.87-130.72, (Ar-C),170.12 (C=N), 172.95 (C=N) 177.21 (C=N) 186.49 (C=N).
Anal.Calcd for $C_{15}H_{16}N_6O_3S_2$: C,45.91H,4.08;N,21.42%.Found: C,45.87;H,4.05,N,21.39%.

Table 1.Characterization of the synthesized compounds.

Compound	R	R'	Molecular formula*	Melting point (°C)	Yield (%)
5a	$-CH_3$	4-OCH_3	$C_{14}H_{14}N_6O_2S_2$	144-146	83
5b	$-CH_3$	4-OH	$C_{13}H_{12}N_6O_2S_2$	138-140	80
5c	$-CH_3$	4-OH, 3-OCH_3	$C_{14}H_{14}N_6O_3S_2$	134-137	79
5d	$-CH_3$	4-Cl	$C_{13}H_{11}ClN_6O_2S_2$	148-150	75
5e	$-CH_3$	4-H	$C_{13}H_{12}N_6OS_2$	141-143	78
5f	$-C_2H_5$	4-OCH_3	$C_{15}H_{16}N_6O_2S_2$	140-142	81
5g	$-C_2H_5$	4-OH	$C_{14}H_{14}N_6O_2S_2$	136-138	82
5h	$-C_2H_5$	4-OH, 3-OCH_3	$C_{15}H_{16}N_6O_3S_2$	132-134	79
5i	$-C_2H_5$	4-Cl	$C_{14}H_{13}ClN_6O_2S_2$	146-149	78
5j	$-C_2H_5$	4-H	$C_{14}H_{14}N_6OS_2$	139-143	76

*All the compounds gave satisfactory elemental analysis.

Table 2. Antimicrobial activities of some newly synthesized compounds.

Comound	Inhibition Zone (mm)			
	Gram-negative		Gram-positive	
	E.coli	*P.Putide*	*B.Subtilis*	*S.lactis*
5a	10	8	16	16
5b	8	8	14	14
5c	12	10	17	15
5d	7	5	12	11
5e	5	0	10	8
Ampicilin®	24	20	19	22

E.coli. = Escherichia coli; P.Putide = Pseudomonas Putide; B. Subtilis = Bacillus Subtilis; S. lactis = Sterptococcus lactis.

The sensitivity of microorganisms to the tested compounds is identified in the following manner*;
Highly Sensitive = Inhibition zone: 15-20 mm
Moderately Sensitive = Inhibition zone: 10-15 mm
Slightly Sensitive = Inhibition zone: 5-10 mm
Not Sensitive = Inhibition zone: 0 mm
* Each result represents the average of triplicate readings.

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General Scheme:-