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NOVEL TRIAZOLO – THIADIAZOLE FUSED HETEROCYCLIC COMPOUNDS AND THEIR EVALUATION OF ANTIMICROBIAL ACTIVITIES

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ABSTRACT:
2{(Furan-2-yl-methyl)carbomyl}benzoic acid,1(Trivial name Phthalamic acid) on condensation with hydrazine, CS_2, Ethanoic KOH afford 4 – amino [2-(furan-2-ylmethylaminocarbomyl)phenyl]-4H-1,2,4-triazole-3-thiol,2. 2 on further condensation with 5-substituted furoic acid **3a-f** yield 3-[2-(furan-2-yl methylamino carbonyl)]-6-(5-substituted fura-2-yl)]-[1,2,4]-triazol-[3,4-b][1,3,4]-thiadiazole **4a-f**. All the derivatives were characterized by spectral studies. The antimicrobial activities of all compounds have also been monitored.

KEYWORDS: Phthalamic acid, triazole-thiadiazole, fused heterocyclic compounds, furan derivatives, spectral studies, antibacterial and antifungal activities.

INTRODUCTION:

The designing new anti-infective derivatives becomes an urgent need for curing of microbial infections [i-v]. The 1,2,4 – thiazole and 1,3,4 – thiadiazole moieties have been introduced into various pharmaceutical agent [vi-xvi]. Hence the chemistry of [1,2,4] triazolo-[1,3,5] thiadiazole fused heterocycle system received remarkable attention. They display the effective pharmaceutical activity like anticancer, anti T.B., analgesic etc [xvii-xix]. For this reason, the present communication comprises the novel triazolo-thiadiazole fused heterocycles with introduced with furan ring. Only few reports found on furan ring containing triazolo-thiadiazole fused system [xx].

EXPERIMENTAL SECTION:

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and ^{1}H NMR spectra were acquired at 400 MHz on a Bruker NMR spectrometer using DMSO–d_6 as a solvent as well as TMS an internal reference standard. LC-MS of selected samples taken on LC-MSD-Trap-SL_01046.

GENERAL PROCEDURE:

Preparation of 2-((furan-2-ylmethyl)carbamoyl)benzoic acid [1]

It was prepared by method reported earlier [xxi].

Preparation of 4-amino-5-[2-(furan-2-ylmethylaminocarbonyl)phenyl]-4H-1,2,4-triazole-3-thiol [2]

This was synthesized by method reported by us [xxii].

Preparation of 3-[2-(furan-2-ylmethylaminocarbonyl)]-6-(5-substituted fura-2-yl)]-[1,2,4]-triazol-[3,4-b][1,3,4]-thiadiazole (4a-f)

A mixture of 4-amino-5-[2-(furan-2-ylmethylaminocarbonyl)phenyl]-4H-1,2,4-triazole-3-thiol [2] (0.01 mol) and furoic acid derivative [3(a-f)] (0.01mole) was mixed with dry $POCl_3$. The resulted slurry was refluxed for 7-9 hrs. Excess $POCl_3$ was then distilled off and the residue was gradually poured onto crushed ice with stirring. It was kept aside for overnight. The solid separated out was filtered, washed thoroughly with cold water, 20% $NaHCO_3$ solution and recrystallised from a mixture of dioxane : ethanol (50:50v/v) mixture.

N-(furan-2-ylmethyl)-2-(6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzamide (4a)

$C_{21}H_{15}N_5O_2S$(401),Yield 76%, M.P. 208 -209^oC Calcd.: %C,62.83; %H, 3.77; %N, 17.45; %S, 7.99. Found: %C, 62.8; %H,3.7; %N,17.4;%S,7.9,M^+: 403, IR (KBr,cm^{-1}): 3030 (Aromatic C-H), 2850, 2920, 1430(-CH_2) , 1680, 1580, 1610 (-CONH) ,1145 (C-O), 1675 (–C=N st.), 690 (–C–S–C– of thiadiazole), 1235 (–N–N=C– st.). ^{1}H NMR (400 MHz, DMSO) δ: 8.20 – 6.35 ppm (7H,m, aromatic-H), 5.25(2H,s,-CH_2) ,8.10 (1H, s,NH of CONH), (3a): 8.10-7.50 (5H, m, Ar-H).

2-(6-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-N-(furan-2-ylmethyl)benzamide (4b)

$C_{21}H_{14}N_5O_2SCl$ (435), Yield 72%, M.P. 216-218^oC Calcd.: %C,57.86; %H,3.24; %N, 16.07;%S,7.36. Found: %C, 57.8; %H,3.2; %N,16.0;%S, 7.3. IR (KBr) (cm^{-1}): 3030 (Aromatic C-H), 2850, 2920, 1430(-CH_2) , 1680, 1580, 1610 (-CONH) ,1145 (C-O), 1675 (–C=N st.), 690 (–C–S–C– of thiadiazole), 1235 (–N–N=C– st.), 3502-3504 (-NH of –CSNH), 1080,655 (C-Cl). ^{1}H NMR (400 MHz,DMSO) δ: 8.20 – 6.35 ppm (7H,m, aromatic-H), 5.25 (2H,s, -CH_2) ,8.10 (1H, s, NH of CONH), 8.10-7.65(4H,m,Ar-H).

2-(6-(4-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-N-(furan-2-ylmethyl)benzamide (4c)

$C_{21}H_{14}N_5O_2SBr$ (479),Yield 78%, M.P. 214-215^oC Calcd.: %C,52.51; %H,2.94; %N,14.58;%S,6.68. Found: %C, 52.5; %H,2.9; %N,14.5;%S, 6.6. IR (KBr) (cm^{-1}): 3030 (Aromatic C-H), 2850, 2920, 1430(-CH_2) , 1680, 1580, 1610 (-CONH) ,1145 (C-O), 1675 (–C=N st.), 690 (–C–S–C– of thiadiazole), 1235 (–N–N=C– st.),3502-3504 (-NH of –CSNH), 680(C-Br). ^{1}H NMR (400 MHz, DMSO)δ: 8.20 – 6.35 ppm (7H,m, aromatic-H), 5.25(2H,s,-CH_2) ,8.10 (1H,s, NH of CONH), 8.00-7.70(4H,m,Ar-H).

2-(6-(4-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-N-(furan-2-ylmethyl)benzamide (4d)

$C_{21}H_{14}N_5O_2SF$ (419), Yield 75%, M.P. 227-228^oC Calcd.: %C, 60.13; %H,3.36; %N,16.70;%S,7.64. Found: %C, 60.1; %H,3.3; %N,16.6;%S, 7.6. IR (KBr) (cm^{-1}): 3030 (Aromatic C-H), 2850, 2920, 1430(-CH_2) , 1680, 1580, 1610 (-CONH) ,1145 (C-O), 1675 (–

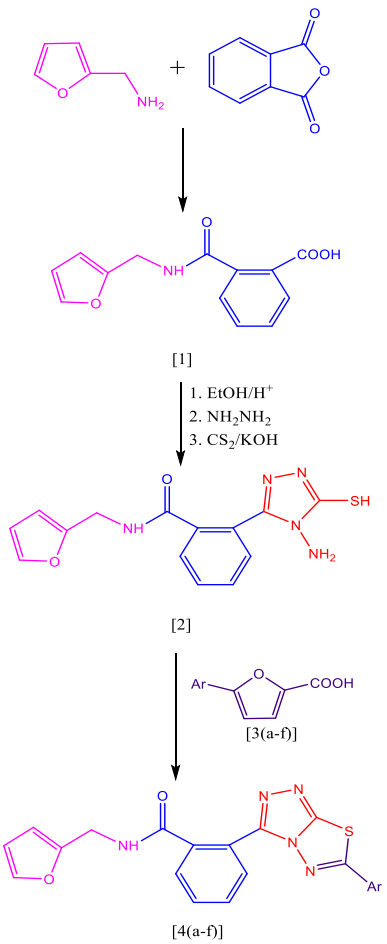
C=N st.), 690 (–C–S–C– of thiadiazole), 1235 (–N–N=C– st.),3502-3504 (-NH of –CSNH), 1360 (C-F).^{1}H NMR (400 MHz, DMSO) δ: 8.20 – 6.35 ppm (7H,m, aromatic-H), 5.25(2H,s,-CH_2) ,8.10(1H, s, NH of CONH), 7.90-7.25(4H,m,Ar-H).

2-(6-(2,4-dichlorophenyl)-[1,2,4]-triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-N-(furan-2ylmethyl)benzamide (4e)

$C_{21}H_{13}N_5O_2SCl_2$ (469),Yield 70%, M.P. 231-232^{o}C Calcd.: %C,53.63; %H,2.79; %N, 14.89;%S,6.82. Found: %C, 53.63; %H, 2.79; %N,14.89;%S,6.82,M^+: 472,IR (KBr) (cm^{-1}): 3030 (Aromatic C-H), 2850, 2920, 1430(-CH_2) , 1680, 1580, 1610(-CONH),1145 (C-O), 1675 (–C=N st.), 690 (–C–S–C– of thiadiazole), 1235 (–N–N=C– st.), 3502-3504 (-NH of –CSNH), 1085, 650 (C-Cl). ^{1}H NMR (400 MHz, DMSO)δ: 8.20 – 6.35 ppm (7H,m, aromatic-H), 5.25(2H,s,-CH_2) ,8.10(1H,s, NH of CONH), 7.85-7.50(3H,m,Ar-H.

N-(furan-2-ylmethyl)-2-(6-(4-nitrophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzamide (4f)

$C_{21}H_{14}N_6O_4S$ (446),Yield 74%, M.P. 223-224^{o}C Calcd.: %C,56.50; %H,3.16; %N,18.82;%S,7.18. Found: %C, 56.4; %H,3.1; %N,18.8;%S, 7.1.IR (KBr) (cm^{-1}): 3030 (Aromatic C-H), 2850,2920, 1430(-CH_2) , 1680, 1580, 1610 (-CONH) ,1145 (C-O), 1675 (–C=N st.), 690 (–C–S–C– of thiadiazole), 1235 (–N–N=C– st.),3502-3504 (-NH of –CSNH), 1550,1375(NO_2). ^{1}H NMR (400 MHz, DMSO)δ: 8.20 – 6.35 ppm (7H,m, aromatic-H), 5.25(2H,s,-CH_2) ,8.10 (1H,s, NH of CONH), 8.40-8.00(4H,m,Ar-H).



Where, Ar = (a) -C_6H_5 (b) 4-ClC_6H_4 (c) 4-BrC_6H_4 (d) 4-FC_6H_4 (e) 2,4-ClC_6H_3 (f) 4-$NO_2C_6H_4$

Scheme-1 Synthesis of 3-[2-(furan-2-yl methylamino carbonyl)]-6-(5-substituted fura-2-yl)]-[1,2,4]-triazol-[3,4-b][1,3,4]-thiadiazole (4a-f)

RESULT AND DISCUSSION:

It was observed that 3-[2-(furan-2-yl methylamino carbonyl)]-6-(5-substituted fura-2-yl)]-[1,2,4]-triazol-[3,4-b][1,3,4]-thiadiazole [3a-f] have been synthesized by the reaction of 4-amino-5-[2-(furan-2-yl methyl amino carbonyl)phenyl]-4H-1,2,4-triazole-3-thiol[2] on condensation with various furoic acid derivatives. The structures of all **4a-f** were confirmed by elemental content and IR spectral features. The data are given in the individual synthesis of each compound.

Antimicrobial Evaluation

Antibacterial activities

The antibacterial activities of all the compounds were studied against gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*) and gram-negative bacteria (*E. coli and Salmonella typhimurium)* at a concentration of 50μg/ML by agar diffusion assay [xviii,xix]. The wells were dug in the media with the help of a sterile metallic borer. Recommended concentration (100 μl) of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, ciprofloxacin was served as negative and positive controls, respectively. The plates were incubated immediately at 37˚C for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug ciprofloxacine. The percentage area of inhibition of zone measured. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains. Compounds 4e were found more toxic for microbes. Other compounds found to be less or moderate active shown in Table-1.

Table: -1 Antibacterial Activity of Compounds (4a-f)

Compounds	**Area of Inhibition Zone (mm)**			
	Gram +Ve		**Gram –Ve**	
	Bacillus subtilis	***Staphylococcus aureus***	***Salmonella typhimurium***	***E. coli***
4a	15	13	12	12
4b	17	15	15	14
4c	16	12	12	12
4d	16.5	13	16	15
4e	17	16	13	13
4f	16	14	14	13
ciprofloxacine	**18**	**18**	**17**	**17**

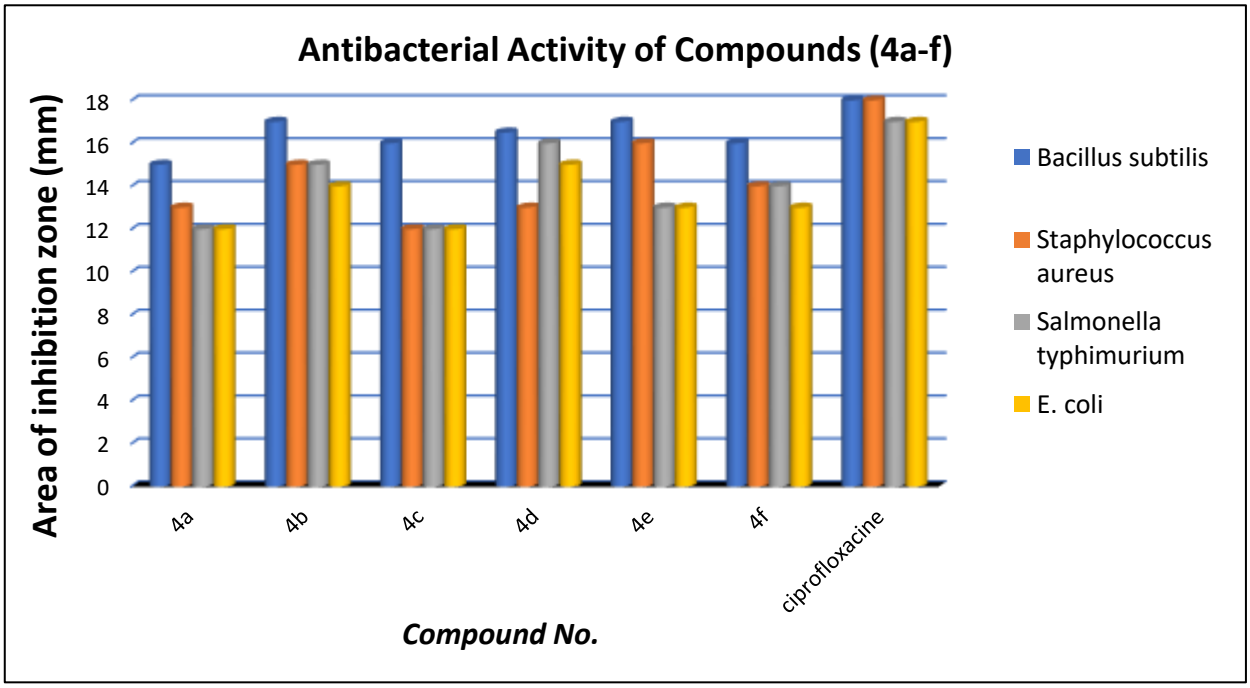


Figure: -1 Antibacterial Activity of Compounds (4a-f)

Antifungal Activities

The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Penicillium expansum*, *Botryodiplodia theobromae*, *Nigrospora sp.*, *Trichothesium sp.* The antifungal drug, ketoconazole was used as a positive control. Antifungal screening for compounds **3a-f** and positive control was performed at a recommended concentration. The fungal strains were grown and maintained on potato dextrose agar plates [xxiii,xxiv]. The cultures of the fungi were purified by single spore isolation technique. Each compound (4a–f) in DMSO solution was prepared for testing against spore germination of each fungus. The fungal culture plates were inoculated and incubated at 25± 2˚C for 48 h. The plates were then observed and the diameters of the zone of inhibition (in mm) were measured. The percentage inhibition for fungi was calculated after five days using the formula given below:

$$\text{Percentage of inhibition} = \frac{100(X - Y)}{X}$$

Where, X = Area of colony in control plate

Y = Area of colony in test plate

The fungicidal activity displayed by compounds (4a-f) is shown in Table-2.

Table: -2 Antifungal Activity of Compounds (4a-f)

	Zone of Inhibition at 1000 ppm (%)			
Compounds	***Penicillium expansum***	***Botryodiplodia theobromae***	***Nigrospora sp.***	***Trichothesium sp.***
4a	75	79	81	77
4b	78	82	84	81

4c	73	79	82	79
4d	77	77	83	81
4e	80	85	87	83
4f	74	80	81	79
ciprofloxacine	**89**	**88**	**83**	**85**

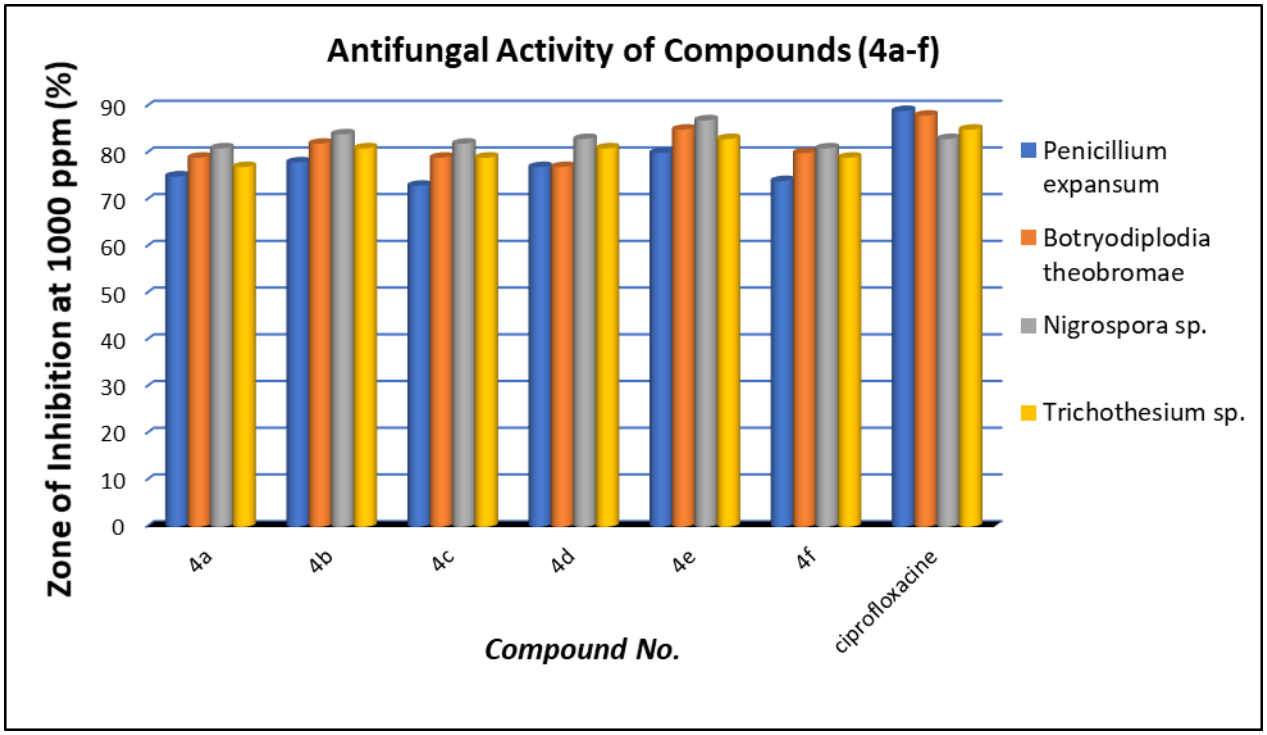


Figure: -2 Antifungal Activity of Compounds (4a-f)

CONCLUSION:

The novel fused heterocyclic ligand, 3-[2-(furan-2-ylmethylaminocarbonyl)]-6-(5-substituted fura-2-yl)]-[1,2,4]-triazol-[3,4-b][1,3,4]-thiadiazole **4a-f** synthesised by condensation of 4-amino-5-[2-(furan-2-ylmethylaminocarbonyl)phenyl]-4H-1,2,4-triazole-3-thiol [2] with various furoic acid derivatives **3a-f**. The structures of fused compounds **4a-f** were characterized by C, H, N, S analysis and spectral data, which are consistent with predicted structure. The antimicrobial activities of all the compounds were showed good activities.

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