BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

3,6- disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives having inhibitory activity against multidrug resistant bacteria

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Abstract

In the present investigation 36, synthesized compounds of 3,6- disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives were investigated for *in-vitro* antimicrobial activity against four bacterial and two fungal strains. Also, anti-microbial studies of the 3 compounds (having maximum antimicrobial activity) against multi drug resistant bacteria from clinical isolates were reported. Multidrug resistant bacteria studies showed that some of the compounds are active against both gram positive and gram-negative bacteria. Compounds P20, P19 and P23 showed the promising inhibitory effect against tested bacteria.

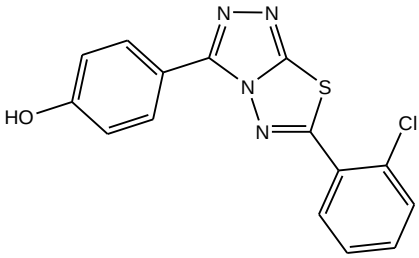
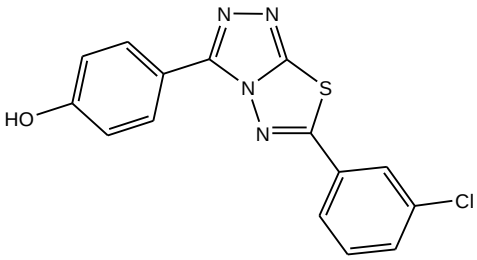
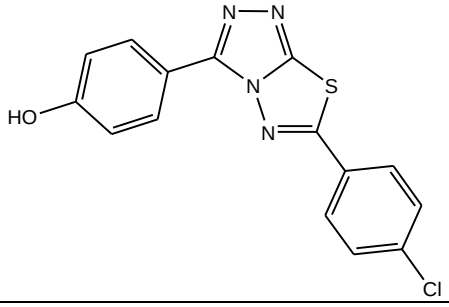
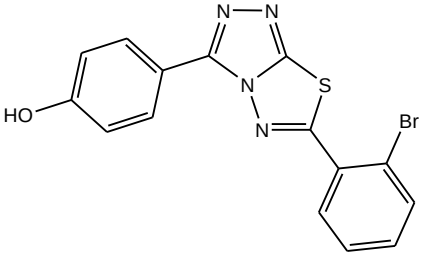
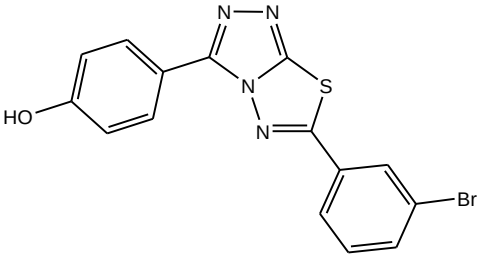
Key-words: Triazole, MDR, Anti-microbial

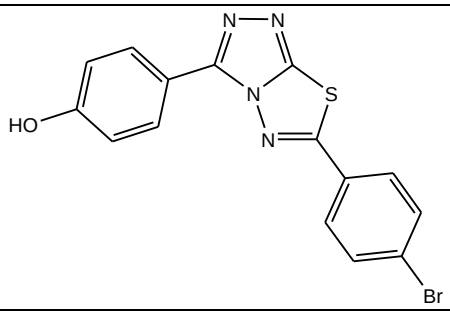
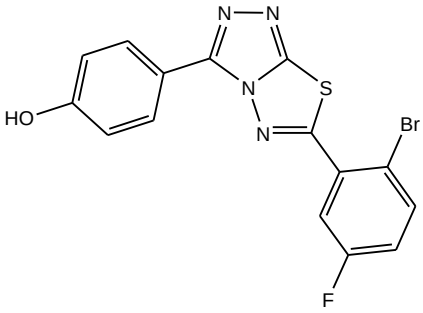
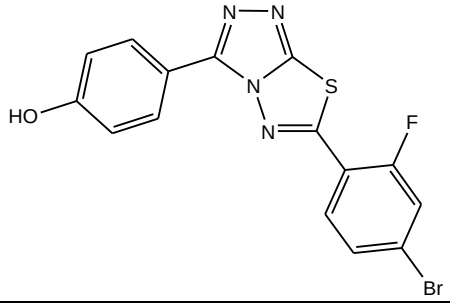
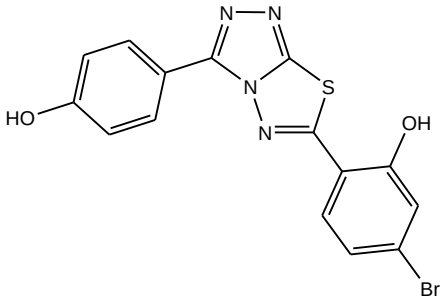
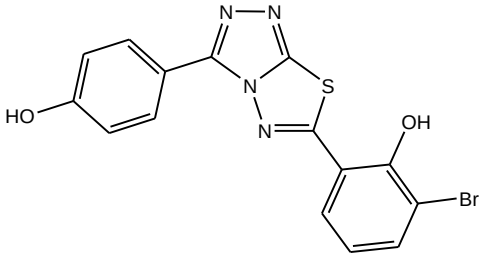
Introduction

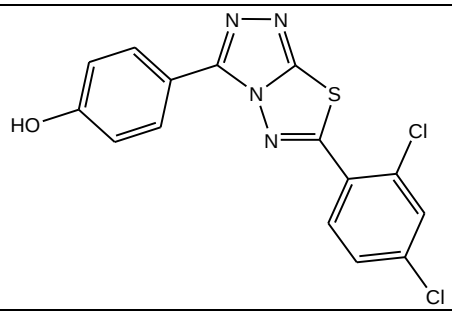
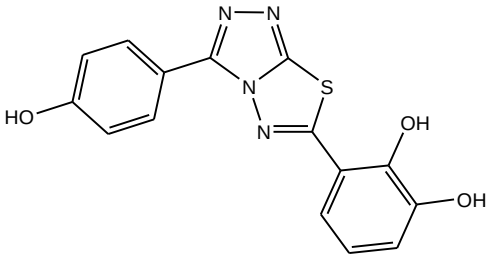
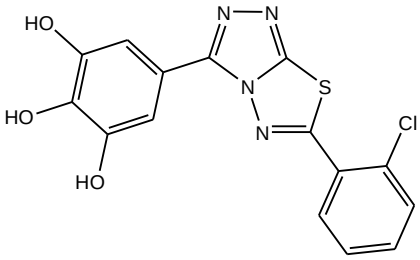
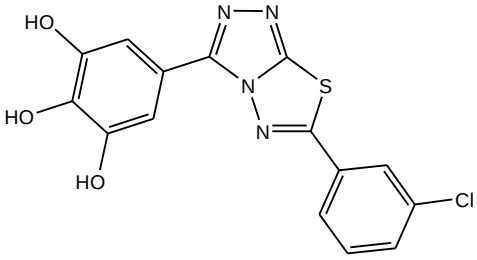
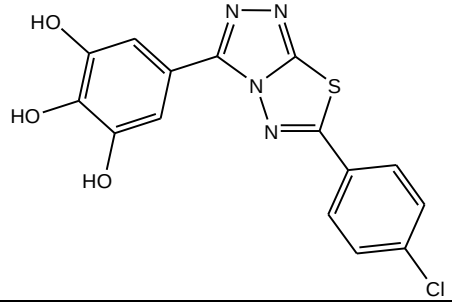
Antibiotics have been utilized in large quantities for human remedy, cattle, or even aquaculture fish, resulting in the choice of dangerous microorganism resistant to several drugs. Considered one of approaches can motive multi-drug resistance in microorganism. First, within an unmarried cell, these microorganisms may additionally collect many genes, each coding fordrug resistance to a single treatment. On resistance (R) plasmids, this buildup is most not unusual. Secondly, expanded expression of genes that code for multidrug efflux pumps, which extrude a wide spectrum of drugs, can cause multidrug resistance. [1-2].The fused triazole and thiadiazole ring device suggests diverse organic outcomes and it is viewed as cyclic analogues of two very vital factor thiosemicarbazide and biguanide which frequently show diverse biological sports. Triazolo-[3,4-b]-1, three,4-thiadiazole earrings have acquired lots interest all through latest years attributable to their prominent utilization as, antiviral, anthelmentic and antimicrobial activities. Literature survey discovered that fused triazolo-thiadiazole rings have received a great deal attention at some stage in latest years due to their prominent usage as antiviral, anthelmintic and antimicrobial sports. Triazolo-thiadiazole substituted in 3rdand 6th role through aryl, alkyl or hetero alkyl group enriched with more biological activities. In view of vast importance of these two nucleus triazolo-thiadiazole by-product were synthesized from aromatic esters. [3] inside the present work 36, synthesized compounds of three,6- disubstituted-1,2,four-triazolo-[3,4-b]-1,three,4-thiadiazole derivatives had been investigated for in-vitro antimicrobial pastime against four bacterial and two fungal strains. Additionally, anti-microbial studies of the 3 compounds (having maximum antimicrobial activity) against multi drug resistant micro organism from medical isolates had been said.

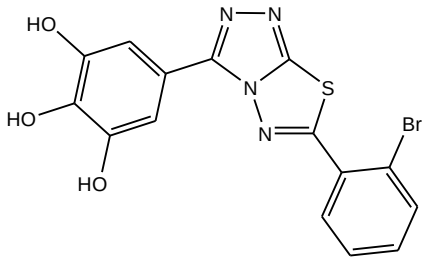
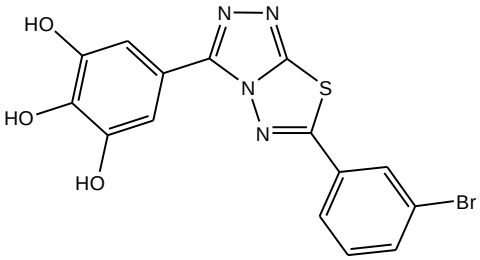
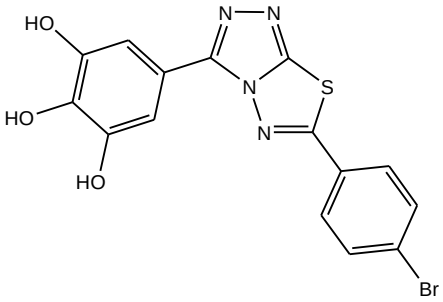
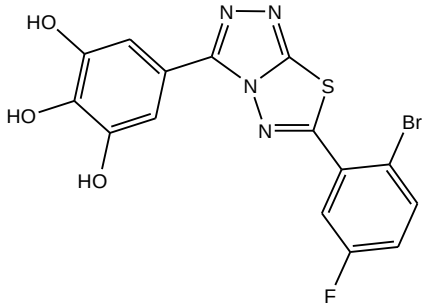
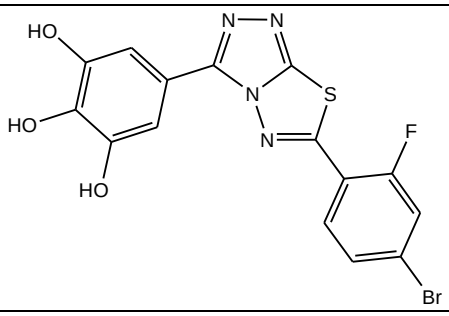
Methodology

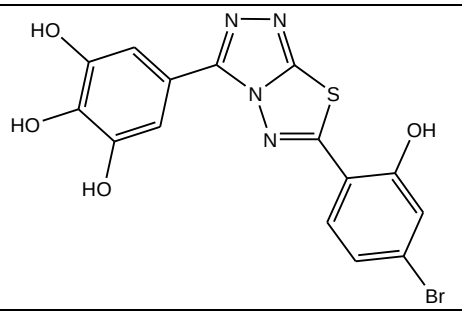
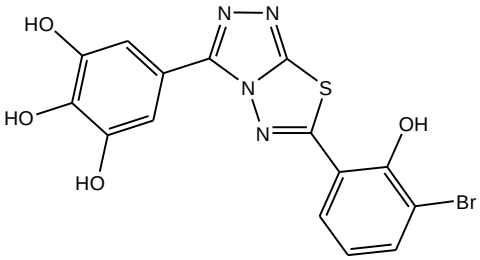
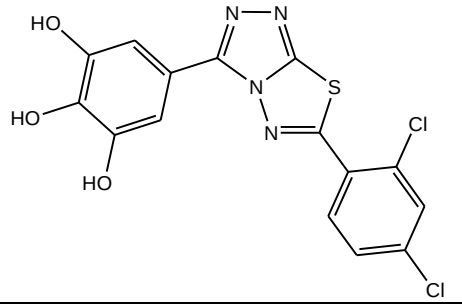
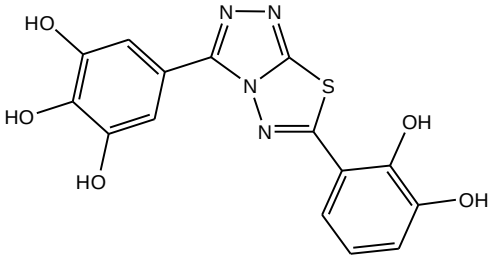
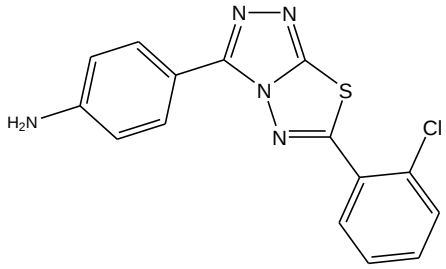
Table 1: List of compounds with their structure and IUPAC name

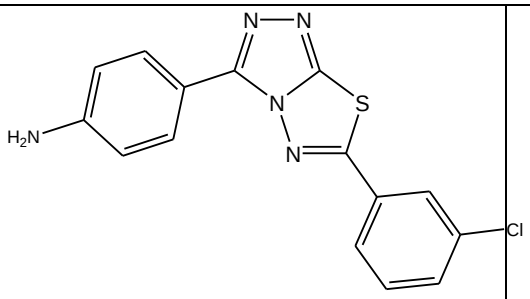
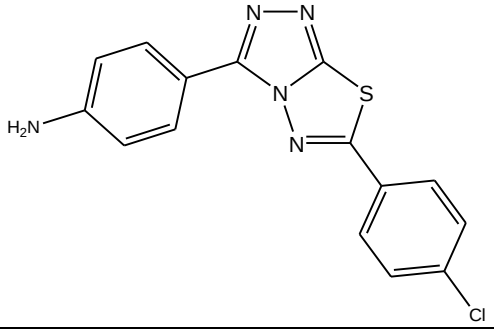
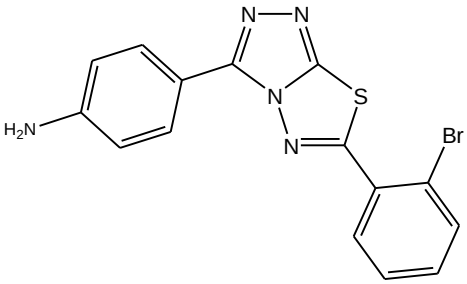
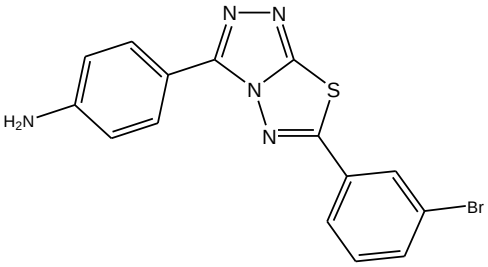
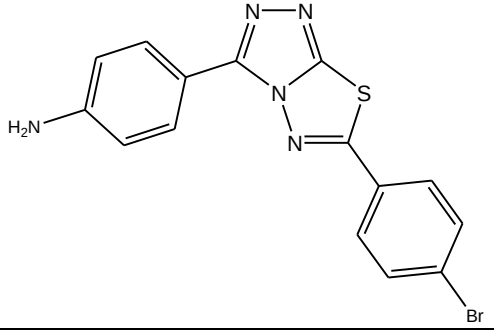
S. NO.	COMPOUNDS	STRUCTURE	IUPAC NAMES
1.	P1		4-(6-(2-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
2.	P2		4-(6-(3-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
3.	P3		4-(6-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
4.	P4		4-(6-(2-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
5.	P5		4-(6-(3-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol

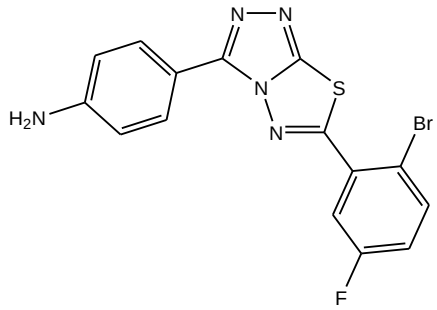
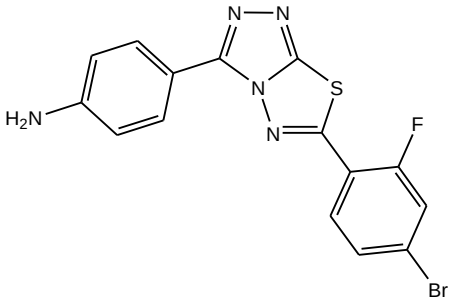
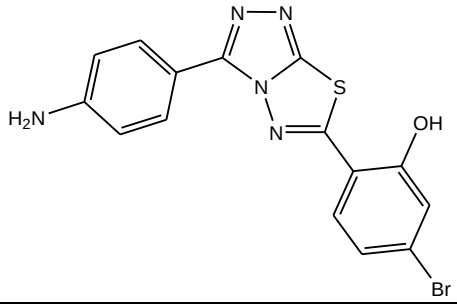
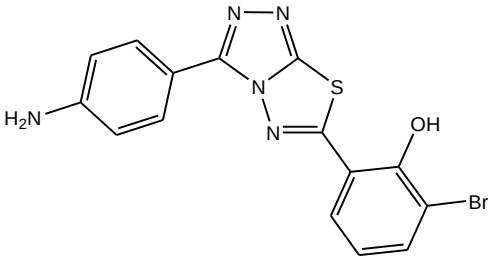
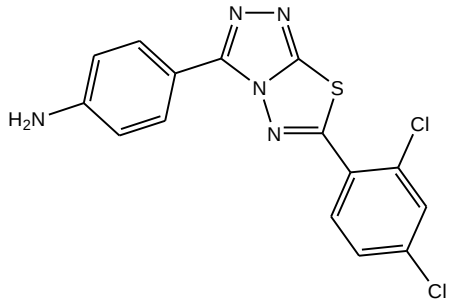
6.	P6		4-(6-(4-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
7.	P7		4-(6-(2-bromo-5-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
8.	P8		4-(6-(4-bromo-2-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
9.	P9		5-bromo-2-(3-(4-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)phenol
10.	P10		2-bromo-6-(3-(4-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)phenol

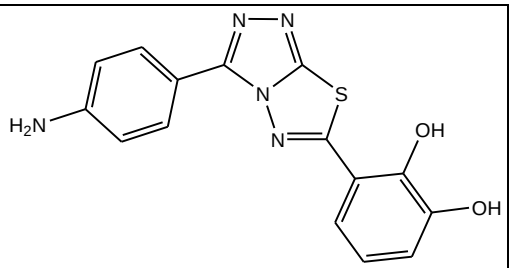
11.	P11		4-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)phenol
12.	P12		3-(3-(4-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzene-1,2-diol
13.	P13		5-(6-(2-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
14.	P14		5-(6-(3-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
15.	P15		5-(6-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol

16.	P16		5-(6-(2-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
17.	P17		5-(6-(3-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
18.	P18		5-(6-(4-bromophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
19.	P19		5-(6-(2-bromo-5-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
20.	P20		5-(6-(4-bromo-2-fluorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol

21.	P21		5-(6-(4-bromo-2-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
22.	P22		5-(6-(3-bromo-2-hydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
23.	P23		5-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
24.	P24		5-(6-(2,4-dihydroxyphenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzene-1,2,3-triol
25.	P25		4-(6-(2-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzenamine

26.	P26		4-(6-(3-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzenamine
27.	P27		4-(6-(4-chlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)benzenamine
28.	P28		4-(6-(2-bromophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzenamine
29.	P29		4-(6-(3-bromophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzenamine
30.	P30		4-(6-(4-bromophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl)benzenamine

31.	P31		4-(6-(2-bromo-5-fluorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)benzenamine
32.	P32		4-(6-(4-bromo-2-fluorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)benzenamine
33.	P33		2-(3-(4-aminophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-6-yl)-5-bromophenol
34.	P34		2-(3-(4-aminophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-6-yl)-6-bromophenol
35.	P35		4-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)benzenamine

36.	P36		4-(6-(2,4-dihydroxyphenyl)-[1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazol-3-yl)benzenamine

***In-vitro* antimicrobial activity**

The in-vitro antimicrobial activity of all the compounds (36) and trendy capsules have been assessed towards two representatives of Gram-fine bacteria viz. S. aureus (ATCC 23235), B. subtilis aureus (ATCC 6633), two Gram-negative microorganisms viz. E. coli (ATCC 25922), P. aeruginosa aureus (ATCC 27853) and two fungi viz. C. albicans (ATCC 10231), A. niger (ATCC 16880) via the broth micro dilution MIC method. Mueller Hinton broth and Sabouraud dextrose broth have been used as a nutrient medium to grow and dilute the compound suspension for the test microorganism and fungi, respectively. Ampicillin, Norfloxacin became used as trendy antibacterial capsules, while fluconazole become used as widespread antifungaldrug. Number one inoculation of microorganism turned into performed into Mueller- Hinton agar for overnight to provide some of colonies, which were then without delay suspended in saline solution until the turbidity matched the turbidity of the McFarland fashionable (10 CFU/ ml), i.e., inoculum length for test stress turned into adjusted to 108 colony forming unit (CFU)/ml per nicely by evaluating the turbidity (turbidimetric technique). Comparable technique changed into followed for fungi with Salbouraud dextrose broth. Dimethyl sulfoxide (DMSO) changed into used as diluents to get desired concentration of the synthesized compounds and popular tablets. every compound and fashionable tablets have been diluted to acquire 1000 µg/ml concentrations, as a inventory solution. Stock solution became further step by step diluted with the check medium and required concentrations have been received for number one and secondary screening. In primary screening (400, 200, a hundre, three.12 µg/ml) concentrations of the synthesized compounds had been examined. The energetic compounds discovered on this number one screening had been similarly diluted and tested in opposition to the corresponding microorganism. Every test tube changed into then put for incubation at 37° for 24 h for bacteria and forty-eight h for fungi. Boom or a lack of boom in the tubes containing the antimicrobial agent became decided through assessment with the increase manipulate, indicated by way of turbidity. The bottom concentration that absolutely inhibited visible boom of the organism became recorded because the MIC (µg/ml). a fixed of tubes containing only seeded broth and the DMSO controls were maintained under same situations to make sure that the solvent had no have an impact on on strain growth. The interpretation of the consequences was based totally on fluconazole for the fungi and ampicillin, norfloxacin for bacterial pathogens. [4]

Anti-microbial studies of the compounds using MDR bacteria

MDR scientific isolates of S. aureus (3 isolates), S. aureus (MRSA), Escherichia coli (three isolates), Klebsiella pneumoniae and Proteus mirabilis with their antibiotic resistance profiles have been received from Ennoble studies, Bhopal, (M.P.), India, popular traces S. aureus (ATCC 25923) and E. coli (ATCC 25922) changed into used for excellent control. all the take a look at strains had been maintained on nutrient agar slants (hi Media Laboratories Pvt. restrained, Mumbai, India) at four° and culture directly to nutrient broth for 24 hours prior to checking out. That microorganism served as take a look at pathogens for antibacterial pastime assay. [5]

Table 2: Antibiotic Resistance Profile of Bacterial Isolates Used

Antibiotics	**Test bacteria**									
	***Kp*1**	***Kp*2**	***Ec*1**	**Ec2**	***Ec*3**	***Pm***	***Sa*1**	***Sa*2**	***Sa* MRSA**	***Sa*3**
AK	S	R	S	S	R	S	S	S	S	R
AC	R	R	R	R	R	R	S	S	R	R
CFX	R	R	R	R	R	R	R	R	R	R
CS	R	R	S	R	R	S	S	S	S	R
CE	R	R	R	R	R	R	R	S	R	R
CI	R	R	R	S	R	S	R	R	R	R
CF	R	R	R	R	R	S	S	R	R	R
GF	S	R	S	R	R	S	S	S	S	R
G	S	R	R	S	R	R	S	S	R	R
I	S	S	S	S	S	S	R	R	S	R
LE	S	R	R	R	R	S	S	S	S	R
MR	S	R	S	S	S	S	S	S	R	R
OF	R	R	R	R	R	S	S	R	R	R
PT	S	R	S	S	R	R	S	S	R	R
VA	-	-	-	-	-	-	S	S	S	S
LZ	-	-	-	-	-	-	S	S	S	S

Note: AK=Amikacin, AC=Amoxycillin/Clavulanic acid, CFX=Cefixime, CS=Cefoperazone+Sulbactum, CE=Cefotaxime, CI=Ceftriaxone, CF=Ciprofloxacin, GF=Gatifloxacin, G=Gentamicin, I=Imipenem, LE=Levofloxacin, MR=Meropenem, OF=Ofloxacin, PT=Piperacillin/tazobactam, VA=Vancomycin, LZ=Linezolid, R=Resistant, S=Sensitive, Kp=Klebsiella pneumoniae, Ec=Escherichia coli, Sa=Staphylococcus aureus, Pm= Proteus mirabilis.

Assay of Antibacterial screening

Antibacterial interest of compounds P19, P20 and P23 changed into determined by means of agar properly diffusion approach in keeping with national Committee for medical Laboratory requirements (NCCLS). Inoculum containing 106 cfu/ml of every bacterial culture to be tested changed into spread on nutrient agar plates with a sterile swab moistened with the bacterial suspension. Subsequently, wells of 8 mm diameter have been punched into the agar medium and full of 100 µl (25 mg/ml) of Compounds and allowed to diffuse at room temperature for two h. The plates had been then incubated in the upright role at 37° for twenty-four h. Wells containing the same extent of DMSO (10%), and distilled water served as poor controls whilst widespread antibiotic discs of Imepenem (10 µg) and vancomycin (30 µg) were used as the high quality controls. After incubation, the diameters of the boom inhibition zones were measured in mm. 3 replicates have been achieved for each extract towards each of the check organism. Data were expressed as mean±deviation.

Results and Discussion

The in-vitro antimicrobial screening of all of the compounds and standard drug had been assessed towards representatives of Gram-positive bacteria viz. S. aureus, B. subtilis, Gram-bad microorganism viz. E. coli, P. aerugenosa and two fungi viz. C. albicans, A. niger via the broth micro-dilution MIC approach. Ampicillin changed into used as wellknown antibacterial pills, whereas fluconazole was used as widespread antifungal drug.

The results which were given in table 3Table demonstrate that all of the synthesized compounds having antibacterial activities when compared in both gram-positive bacteria. The compounds P20 have more potent activity followed by P19, P23. Activities of P-8, P-32, P-7, P-31, P-11, P-35 is also found to be potent but less than P-19, P-23. Compound P20 has antibacterial activity equivalent to standard drug. It may inhibit growth of microbes due to following reasons

- 3-OH groups
- More electronegativity
- Meta position of substituent's
- Maximum inhibitory activity

The obtained results for antifungal activities revealed that most of compounds could inhibit the growth of the tested fungal strains, however, none of them shown to be superior to the reference drug fluconazole.

Multidrug resistant bacterial studies showed that some of the compounds are active against both gram positive and gram-negative bacteria. Compounds P20, P19 and P23 showed the promising inhibitory effect against tested bacteria. *Sa1* bacteria is resistant to Cefixime, Cefotaxime and Ceftriaxone but zone of inhibition of compound P20 is more as compared to standard drug Vancomycin for *Sa* 1 strain. *Sa2* bacteria is resistant to Ciprofloxacin, Ceftriaxone, Ciprofloxacin but zone of inhibition of compound P19, P20 and P23 is more as compared to standard drug Vancomycin for *Sa* 2 strain. *Sa3* bacteria is resistant to Amikacin, Amoxycillin, Cefixime, Cefoperazone, Cefotaxime, Ceftriaxone, Ciprofloxacin, Gatifloxacin, Gentamicin, Imipenem, Levofloxacin, Meropenem, Ofloxacin, Piperacillin but zone of inhibition of compound P19 is more as compared to standard drug Vancomycin for *Sa3*bacteria.

Table 3: MICin µg/ml of synthesized compounds

Compound	MIC, µg/ml					
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***	***A. niger***	***C. albicans***
P1	50	50	50	50	50	25
P2	100	50	100	50	50	25
P3	50	100	50	100	100	100
P4	50	25	50	25	25	25
P5	50	25	50	50	25	25
P6	100	50	100	20	12.5	25
P7	25	25	25	50	12.5	50
P8	25	25	12.5	25	25	50
P9	50	25	100	200	12.5	50
P10	100	200	200	200	100	25
P11	25	50	12.5	25	25	25
P12	200	100	200	200	25	50
P13	50	50	50	50	25	25
P14	50	25	50	100	50	25
P15	100	50	200	200	50	100
P16	200	200	200	100	50	25

P17	50	100	25	100	25	25
P18	50	12.5	50	50	25	25
P19	12.5	12.5	12.5	6.5	50	50
P20	6.25	6.25	12.5	12.5	25	25
P21	50	50	50	50	25	25
P22	100	25	100	100	12.5	25
P23	12.5	25	12.5	50	12.5	12.5
P24	25	50	25	50	25	12.5
P25	25	25	25	50	25	25
P26	50	25	50	100	25	12.5
P27	200	25	200	25	50	100
P28	200	50	200	25	12.5	25
P29	100	100	100	50	25	25
P30	50	12.5	50	100	12.5	25
P31	25	25	25	25	12.5	50
P32	25	25	25	200	25	50
P33	50	50	50	50	25	50
P34	25	200	25	100	25	25
P35	25	12.5	25	12.5	50	25
P36	25	12.5	25	12.5	12.5	12.5
Ampicillin	6.25	6.25	6.25	6.25		
Norfloxacin	6.25	6.25	6.25	6.25		
Fluconazole					6.25	6.25

Table 4: Antibacterial screening of compounds using agar well diffusion techniques

Compounds	**Test Bacteria Zone of Inhibition (mm)**								
	Sa 1	***Sa*** 2	***Sa*** 3	***Sa* MRSA**	***Ec*1**	***Ec2***	***Ec3***	***Pm***	***Kp*1**
P19	17.32±0.20	21.62±0.12	17.06±0.11	--	19.10±0.23	10.42±0.23	--	13.10±0.01	14.10±0.28
P20	19.40±0.13	22.31±0.31	15.46±0.15	--	18.00±0.32	7.60±0.36	--	11.00±0.05	13.00±0.22
P23	17.30±0.15	23.01±0.24	12.81±0.02	--	22.10±012	9.10±021	--	15.10±0.25	6.09±0.67
I	NT	NT	NT	NT	28.00±0.45	18.50±0.50	17.20±0.44	19.00±0.15	17.00±0.35
V	18.40±0.15	21.60±0.37	16.60±0.11	18.72±0.1	NT	NT	NT	NT	NT

Note: Sa=Staphylococcus aureus, Ec=Escherichia coli, Pm=Proteus mirabilis, Kp=Klebsiella pneumoniae, --=No inhibition, NT=Not tested, I=Imepenem, V=Vancomycin. Values expressed as mean±standard deviation of three replicates.

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