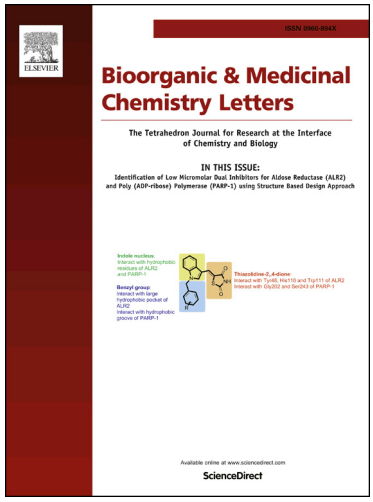

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Synthesis, SAR and molecular docking studies of benzo[*d*]thiazole-hydrazones as potential antibacterial and antifungal agents

Gao-Feng Zha[1], Jing Leng[1], N. Darshini[2], T. Shubhavathi[2], H. K. Vivek[3], Abdullah M. Asiri[4], Hadi M. Marwani[4], K. P. Rakesh[1*], N. Mallesha[2*] and Hua-Li Qin[1*]

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Abstract

A series of new benzo[*d*]thiazole-hydrazoes analogues were synthesized and screened for their *in vitro* antibacterial and antifungal activities. The results revealed that compounds **13**, **14**, **15**, **19**, **20**, **28** and **30** exhibited superior antibacterial potency compared to the reference drug chloramphenicol and rifampicin. Compounds **5**, **9**, **10**, **11**, **12**, **28** and **30** were found to be good antifungal activity compared to the standard drug ketoconazole.

A preliminary study of the structure-activity relationship (SAR) revealed that the antimicrobial activity depended on the effect of different substituents on the phenyl ring. The electron donating (OH and OCH_3) groups presented in the analogues, increase the antibacterial activity (except compound **12**), interestingly, while the electron withdrawing (Cl, NO_2, F and Br) groups increase the antifungal activity (except compound **19** and **20**). In addition, analogues containing thiophene (**28**) and indole (**30**) showed good antimicrobial activities. Whereas, aliphatic analogues (**24-26**) shown no activities in both bacterial and fungal stains even in high concentrations (100µg/mL). Molecular docking studies were performed for all the synthesized compounds of which compounds **11**, **19** and **20** showed the highest glide g-scores.

Key words: Benzo[*d*]thiaozole, SAR, Antimicrobial, Docking study

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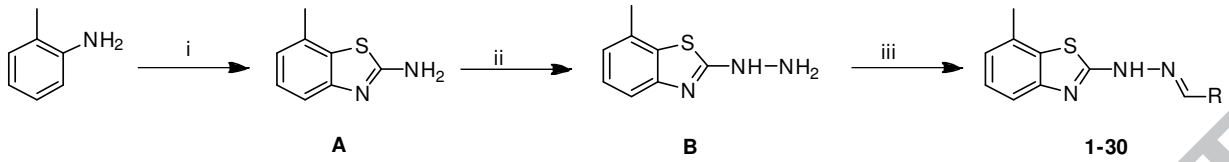
The damage of bacterial and fungal infections has increased hugely in recent years.[1, 2] Infectious diseases caused by bacterial pathogens have become a main public health problem due to the extensive occurrence of drug resistance. Resistance to antimicrobial agents has increased health concerns and resulted in mortality and morbidity from treatment failures.[3,4] The development of novel structure leads remains a key challenge for medicinal chemists to design new, effective and broad spectrum of antibacterial and antifungal drugs. The seek out of novel antimicrobial drugs is an area characterized by active analysis with the aim of overcoming the phenomenon of numerous drug resistance pathogens of bacteria and fungi.[5-7] Therefore, there is an urgent need to develop new antimicrobial agents, especially those with a new drug target or with the ability to overcome drug resistance.[8] That is why antibacterial and antifungal agent developments are very vital and should be always up-to-date.

Based on the above fact, the need and considerable interest in the discovery of new pilot structures and new chemical entities will act as antimicrobials. Regarding development of novel antimicrobial drugs, benzo[*d*]thiazole analogues are of ample interest due to their various functions and therapeutic agents in medicinal chemistry. Benzo[*d*]thiazole scaffold possesses a wide range of biological activities, including antibacterial,[9] antifungal,[10] anticancer,[11] anti-tumor,[12] antiviral,[13] cholinesterase inhibitors,[14] analgesic,[15] antioxidant[16] and anti-inflammatory[17]activities.

Motivated by the special features of benzo[*d*]thaiazole analogues and our ongoing research program,[18-23] we aimed towards the development of new heterocycles as therapeutic agents, herein we reported the synthesis of benzo[*d*]thaiazole-hydrazones and their antibacterial and antifungal activities. In addition, in this work, we have also carried out molecular docking studies of compounds in order to correlate their structural motif with their antibacterial and antifungal activities.

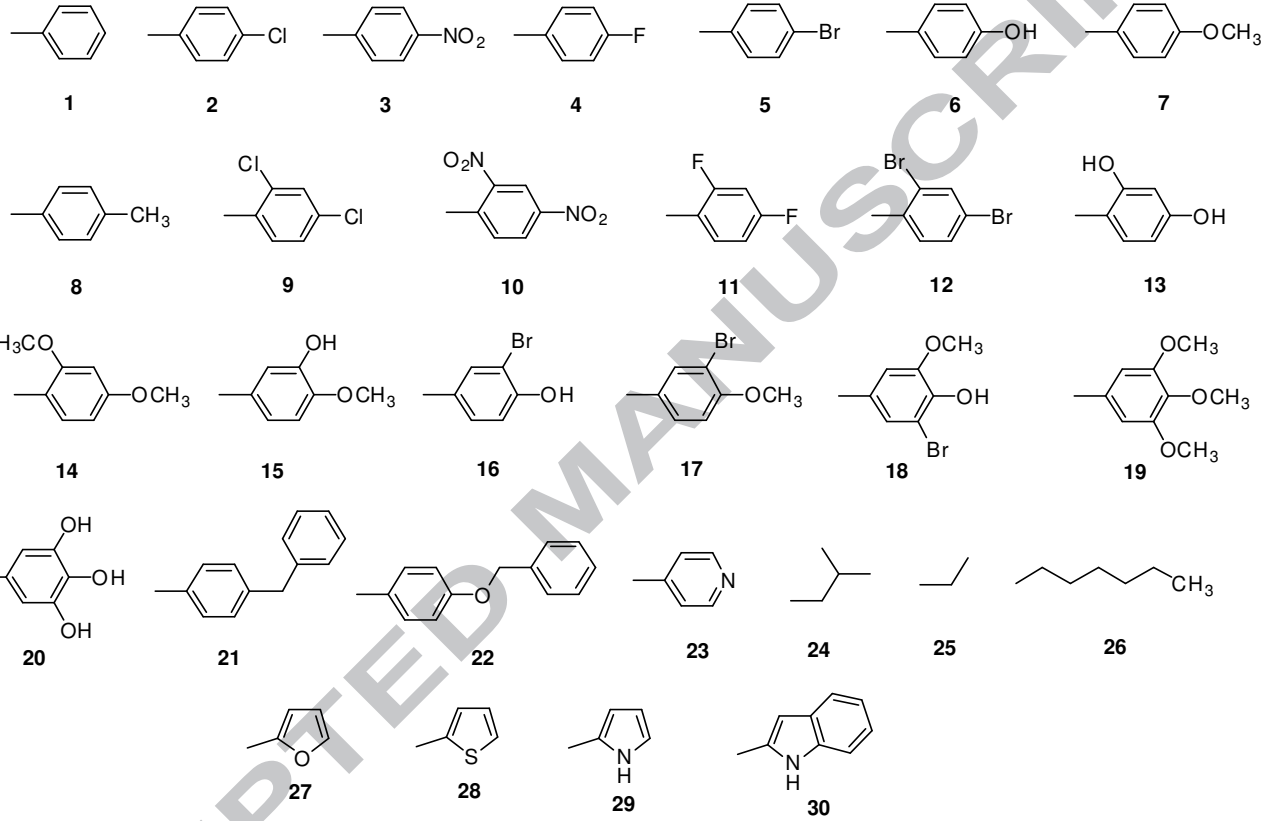
Syntheses of the benzo[*d*]thiaozole-hydrazones were achieved according to the procedures illustrated in **Scheme**. 7-Methylbenzo[*d*]thiazol-2-amine (**A**) was synthesized according to the literature reported method.[24-25] A mixture of compound **A**, hydrazine hydrate, catalytic amount of con. HCl and ethylene glycol was refluxed for 3-4 hr to afford the compound **B**. The benzo[*d*]thiaozole-hydrazones (**1-30**) were obtained by the reaction of **B** with different aldehydes in the presence of catalytic amount of glacial acetic acid. All the derivatives were obtained in good to excellent yield. The formation of the hydrazones was confirmed by the presence of absorption at 1601 to 1635 (-N=CH) in IR spectra and 7.80-8.02 δ singlet peak in ^{1}H NMR spectra. All the chemical structures were confirmed by ^{1}H NMR, ^{13}C NMR and mass spectra (See supplementary material).



A **B** **1-30**

Reagents and conditions: i. NH_4SCN, Br_2, glacial acetic acid, NH_3, ii. Hydrazine hydrate, Con. HCl, ethylene glycol, 3-4 hr, rt
iii. R-CHO, 3-4 drops of acetic acid, EtOH, reflux, 8-10 hr

Where R =



Scheme: Synthesis of target compounds (1-30)

Thirty analogues of benzo[*d*]thiazole-hydrazones (**1-30**) were screened for their *in vitro* antibacterial activity against two Gram-positive strains *Staphylococcus aureus*, *Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus* (MRSA090) and activity against two Gram-negative strains *Escherichia coli* and *Klebsiella pneumonia* by using the agar well diffusion method[26] as well as micro dilution method.[27] The results of antibacterial screening were summarized in **Table 1** and **2**. All assays were performed in triplicate and the results were expressed as the mean of the diameter of inhibition zone in milimeter (mm). Chloramphenicol and rifampicin was used as the reference drug for the antibacterial activity.

The results revealed that most of the compounds have shown moderate to excellent inhibitory activity against the four tested bacteria. The electronic property of the compounds has close relationship to their biological activity.[28-29] Compounds **19** and **20** showed good antibacterial activity against all four bacterial pathogens due to the presence of electron donating (OH and OCH_3) groups in the molecule.[30-31] Compound **28** showed good antibacterial activity against the Gram-positive strains *Staphylococcus aureus* and *B. subtilis*, we envision that the presence of thiophene moiety in the molecule could contribute significantly to the antibacterial activities since thiophenes were reported to have strong antibacterial activity against bacterial fatty acid synthetase (Type II).[32-33] Compound **14** and **15** showed good antibacterial activity against the *Staphylococcus aureus*, *B. subtilis* and *Escherichia coli* and less activity against the *Klebsiella pneumonia* due to the presence of electron donating groups in the molecule. Compound **30** has strong antibacterial activities against *Staphylococcus aureus* and *Escherichia coli* due to the presence of active indole moiety in the molecule.[34] Compounds **6**, **7** and **13** showed good antibacterial activity against all bacterial strains. Compound **12** showed good antibacterial activity against the *Staphylococcus aureus*, *B. subtilis* and *Klebsiella pneumonia*. Comparing to compounds **6** and **13**, compound **20** which possessed extra phenolic groups at 3, 4 and 5 positions on the

phenyl ring exhibited superior antibacterial activities against all the testing bacterial strains. These results provided clear evidence that the antibacterial activities of the compounds could be increased with the increasing number of phenolic groups on the phenyl ring. Increasing the number of methoxyl groups on the phenyl ring was also proved to be a factor affecting the activities of tested bacterial strains. Compound **19** showed superior antibacterial activity against all of the bacterial pathogens compared to compounds **7** and **14**. The presence of methoxy groups on the phenyl ring, plays an important role in increasing the antibacterial activity of these molecules. The activity of compound **15** with two electron donating (one OH and one OCH_3) groups on the phenyl ring is inferior to that of compounds **19** (three OCH_3 groups) and **20** (three OH groups), both of which possess three electron donating groups; however similar to that of compounds with two electron donating groups (**13** with two OH groups, **14** with two OH groups). The above observation indicated that antibacterial activity of the synthetic compounds enhanced along with the increasing of phenolic or methoxy groups on the phenyl ring to those molecules, and the trend is three OH > three OCH_3 > two OH > two OCH_3 ~ one OH, one OCH_3 > one OH > one OCH_3. Compounds **16**, **17** and **18** which contain both electrons donating and withdrawing groups on the phenyl ring are found to have lower antibacterial and antifungal activities. The observed activity against Gram-negative bacterial pathogens that compound **18** showed may be explained by the contribution of the phenolic and methoxy groups presented on the phenyl ring.

Rifampicin, rifapentine and rifabutin were reported to have extremely highly antibacterial activities[35], among these three antibacterial standard drugs, rifampicin was used as antibacterial standard to compare with the synthesized compounds (**1-30**) to examine their activities against methicillin-resistant *Staphylococcus aureus* (MRSA090) bacterial strain (**Table 2**). It is worthy to note, the compounds **19** (19 µg/mL) and **20** (18 µg/mL) showed excellent antibacterial activity against the MRSA090 stains with the MIC values lower than

the standard drug rifampicin (22 µg/mL) and chloramphenicol (25 µg/mL). The antibacterial MIC values of the compounds **13**, **14**, **19**, **20**, **28** and **30** suggest that their antibacterial activity are superior compared to that of the standard chloramphenicol and close to (if not better than) the activity of the standard rifampicin. Compared to the activities of the standard drug rifampicin and MIC values of Gram negative bacterial strains *Escherichia coli* and *Klebsiella pneumonia,*compounds **19** and **20** exhibited better antibacterial activity, while compounds **13**, **14**, **28** and **30** exhibited moderate antibacterial activity.

Interestingly, all the electron withdrawing (Cl, NO_2, F and Br) groups **2**, **3**, **4**, **5**, **9**, **10** and **11** (except **12**) on the benzene ring showed less or nil activities. This evidence confirmed that suitable functional groups on phenyl ring were necessary for better antibacterial activities in drug design.[36] Hence, these results implied that phenolic or methoxy groups play important roles in the antibacterial activities of these tested compounds.

Table 1: Antibacterial activity of synthesized compounds (1-30)

Entry	Zone of Inhibition (mm)[a]															
	Gram positive bacteria								Gram negative bacteria							
	Staphylococcus aureus				*Bacillus subtilis*				*Escherichia coli*				*Klebsiella pneumonia*			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
01	NA	NA	NA	NA	05±1	07±2	11±3	13±1	09±2	11±2	13±1	15±2	NA	NA	07±1	10±3
02	05±3	09±1	11±2	13±1	07±2	09±3	11±2	14±2	NA	NA	06±1	10±2	NA	NA	NA	NA
03	07±2	09±3	12±1	14±2	NA	04±2	08±2	11±3	04±1	18±2	11±1	14±3	07±2	11±2	14±0	16±2
04	09±3	12±1	16±0	18±2	06±1	09±3	12±2	14±3	05±1	09±1	14±2	17±2	06±1	11±3	16±1	19±3
05	NA	NA	05±1	09±3	04±2	07±3	11±3	13±5	03±1	07±1	11±2	14±3	06±1	11±3	14±2	17±3
06	10±3	16±4	20±0	23±2	11±1	14±3	18±1	21±3	17±3	19±0	22±1	25±0	14±1	17±3	22±1	26±1
07	12±0	14±2	19±3	24±1	13±0	18±2	20±1	26±2	10±0	12±2	15±1	10±0	14±3	16±1	20±0	22±4
08	NA	NA	NA	10±1	NA	NA	NA	NA	10±1	14±3	17±3	22±0	NA	NA	NA	NA
09	04±3	12±1	16±1	20±3	04±3	09±1	13±1	16±2	NA	NA	NA	NA	10±2	15±1	19±1	22±0
10	06±1	09±3	13±1	19±4	03±0	07±2	12±1	16±3	08±1	13±1	15±3	18±2	05±0	19±3	14±1	19±1
11	05±1	08±2	11±0	15±2	07±1	10±3	13±2	15±2	04±0	07±1	11±3	14±2	NA	NA	NA	NA
12	11±1	15±2	18±2	21±3	13±1	17±3	24±2	27±1	05±1	08±1	12±3	16±4	08±1	11±1	15±3	20±0
13	14±1	18±2	23±1	26±4	12±1	18±1	24±2	28±2	06±1	12±2	17±1	20±0	12±1	16±1	21±3	25±3
14	10±1	15±2	20±1	28±3	13±1	19±3	25±3	29±1	13±0	18±4	24±2	23±0	04±1	09±1	12±0	15±3
15	13±1	17±2	22±1	26±0	15±1	19±0	23±0	28±2	10±1	15±2	21±4	27±3	4±2	10±2	14±3	16±3
16	NA	NA	NA	NA	07±1	11±2	13±2	15±3	06±1	09±3	11±3	16±4	NA	NA	11±1	14±2
17	NA	NA	NA	NA	04±1	08±2	11±3	16±2	07±1	09±2	13±2	16±4	07±2	11±3	14±2	16±3
18	08±1	10±2	13±4	16±3	05±1	09±1	13±4	17±3	11±1	15±4	19±2	21±3	09±0	14±4	17±3	21±1
19	22±1	28±1	33±1	38±1	19±1	24±2	28±4	33±3	20±1	25±3	31±4	36±1	21±1	27±3	33±1	38±4
20	17±2	24±1	28±3	31±1	14±2	22±1	27±3	35±0	14±1	20±4	26±4	37±1	17±3	23±0	28±3	37±1
21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	04±1	18±2	11±3	15±1
22	07±1	11±0	14±6	18±0	NA	NA	NA	NA	NA	NA	NA	NA	07±1	12±0	14±2	17±3
23	NA	NA	NA	NA	NA	NA	NA	NA	07±2	14±2	16±3	19±3	05±1	09±1	14±3	18±3
24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	NA	NA	NA	NA	NA	NA	NA	NA	04±0	09±3	13±1	17±3	11±0	16±3	21±3	24±3
28	12±1	18±3	24±1	28±2	10±2	15±3	21±3	24±3	10±1	14±2	18±3	22±1	07±2	11±2	18±3	21±3
29	NA	NA	NA	NA	NA	NA	NA	NA	12±1	17±2	22±1	24±3	10±1	14±22	19±1	23±1
30	14±1	18±3	24±3	30±1	10±3	14±3	19±3	21±3	17±0	24±3	30±1	33±1	04±0	09±1	18±3	24±0
Std	**24±1**	**28±2**	**34±3**	**37±1**	**21±0**	**25±3**	**31±1**	**34±1**	**23±0**	**27±1**	**32±1**	**38±1**	**24±1**	**29±3**	**33±1**	**36±1**
Control DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.
Std: Chloramphenicol**, NA:** No activity**, (±)** Standard deviation**.**

Table 2: Antibacterial activity of synthesized compounds (**1-30**)

Entry	Zone of Inhibition (mm)[a]			
	MRSA090			
	25µg/mL	**50µg/mL**	**75µg/mL**	**100µg/mL**
01	NA	NA	NA	NA
02	04±1	09±1	12±2	15±1
03	03±1	07±1	10±2	16±2
04	08±1	12±1	16±1	19±1
05	09±1	11±2	15±2	19±1
06	14±1	17±2	21±1	24±1
07	10±1	13±1	16±2	21±2
08	06±1	10±1	14±2	17±1
09	05±1	08±1	13±1	16±2
10	08±1	13±2	16±2	18±2
11	10±1	13±2	16±1	19±1
12	08±1	12±1	15±1	17±2
13	12±1	16±1	20±2	24±2
14	15±1	18±1	22±2	27±2
15	12±1	16±1	19±1	24±1
16	07±1	11±1	14±2	17±2
17	08±1	12±0	16±2	18±2
18	07±1	13±3	19±1	20±2
19	19±1	25±2	31±1	34±2
20	20±1	26±1	33±1	37±1
21	NA	NA	NA	NA
22	05±1	10±3	12±1	14±2
23	07±1	10±2	14±1	17±0
24	NA	NA	NA	NA
25	NA	NA	NA	NA
26	NA	NA	NA	NA
27	05±1	09±1	13±2	16±1
28	13±1	16±1	22±4	28±0
29	04±1	08±1	11±2	17±1
30	16±1	23±1	27±0	30±1
Std A	19±1	22±2	27±1	31±1
Std B	23±1	27±2	30±2	33±2
Control DMSO	-	-	-	-

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.
Std A: Chloramphenicol**, Std B:** Rifampicin**, NA:** No activity**, (±)** Standard deviation.

All the synthesized benzo[*d*]thiazole-hydrazones (**1-30**) were screened for their *in vitro* fungal activity against three fungal strains such as *Aspergillus niger*, *Fusarium moniliforme* and *Fusarium oxysporum* by using agar well diffusion method[37] as well as microdilution method.[38] The results of antifungal screening were summarized in **Table 3.** All

assays were performed in triplicate and the results were presented as the mean of the diameter of inhibition zone in milimeter (mm). Ketoconazole was used as the reference drug for the antifungal activity.

Antifungal screening results revealed that some compounds showed excellent inhibition against the tested fungal strains compared to the result of using standard drug ketoconazole. Of all the synthesized compounds, analogues **9** and **12** showed potent activities against all the three tested fungal strains. It may be due to the presence of electron withdrawing (Cl and Br) groups on the phenyl ring.[39] Compounds **10** and **11** exhibited superior antifungal activity against the fungal pathogens such as *Aspergillus niger* and *Fusarium moniliforme*. Compounds **2** and **4** showed good antifungal activities against the *Fusarium moniliforme* and compound **5** showed good antifungal activities against all the three fungal strains. These facts may be explained by the presence of electrons withdrawing groups on the phenyl ring.[39] Compounds **19** showed good antifungal activity against *Fusarium moniliforme* and compound **20** showed good antifungal activity against *Fusarium moniliforme* and *Fusarium oxysporum* strains. It may be due to the presence of more number of electrons donating groups on the phenyl ring, but single substituted (**6** and **7**) or double substituted (**13**, **14** and **15**) number of electros donating groups on the phenyl ring do not improve the any antifungal activities. Compounds **28** and **30** showed good activity against the *Aspergillus niger* and *Fusarium oxysporum* fungal pathogens due to the presence of thiophene and active indole parts in the molecules respectively. Aliphatic containing compounds **24-26** were not efficient to inhibit the growth of bacteria, even at high concentration (100µg/mL).

Based on their promising antimicrobial activities, these synthetic compounds were further tested for their minimum inhibitory concentration (MIC) (**Table 4**). The results showed that, compounds **19** and **20** exhibited excellent MBC activity against the

Staphylococcus aureus, *Bacillus substilis* and *Escherichia coli* (MIC values are below the 20µg/mL) bacterial strains. Compounds **11** and **12** showed excellent MFC activity against the fungal pathogens of *Fusarium moniliforme* and *Fusarium oxysporum* (MIC values are below the 20µg/mL).

To analyse the SAR studies:

1. Effect of electrons donating groups on the phenyl ring: The increasing numbers of phenolic and methoxy groups on the phenyl ring, led to increased antibacterial activity (except compound **12**) and less or moderate antifungal activity. The increased order of antibacterial activity was three OH > three OCH_3 > two OH > two OCH_3 ~ one OH, one OCH_3 > one OH > one OCH_3.
2. Effect of electron withdrawing groups on the phenyl ring: The electron withdrawing (Cl, NO_2, F and Br) groups introduced to the phenyl ring, the antifungal activity increased (except compound **19** and **20**).
3. Effect of aromatic heterocyclic aldehydes: Compounds containing some heterocyclic moieties like thiophene (**28**) and indole (**30**) exhibited good antibacterial and antifungal activities.
4. Effect of aliphatic aldehydes: Compounds with aliphatic groups present in the molecule, there is no activities in both bacterial and fungal strains.

Table 3: Antifungal activity of synthesized compounds (**1-30**)

Entry	Zone of Inhibition (mm)[a]											
	Aspergillus niger				*Fusarium moniliforme*				*Fusarium oxysporum*			
	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL	25µg/mL	50µg/mL	75µg/mL	100µg/mL
01	NA	NA	NA	NA	NA	NA	NA	NA	04±1	08±1	13±2	16±3
02	08±1	13±4	16±3	19±1	11±1	14±2	20±4	26±1	071	11±3	14±2	17±2
03	06±1	13±1	17±2	21±3	NA	NA	04±1	08±3	06±1	10±4	14±2	21±3
04	10±1	13±1	18±2	22±1	14±1	18±2	21±3	30±1	10±1	13±4	18±3	21±0
05	14±1	18±2	23±2	28±1	12±1	17±2	24±3	28±1	13±1	18±2	23±1	29±0
06	NA	NA	NA	NA	08±1	10±1	13±2	17±3	NA	NA	NA	NA
07	08±1	11±1	14±2	16±2	NA	NA	NA	NA	NA	NA	NA	NA
08	NA	NA	NA	NA	NA	NA	NA	NA	07±1	11±2	14±2	17±3
09	20±1	24±1	29±1	33±4	17±1	21±2	28±0	32±1	18±1	22±3	27±1	31±1
10	21±1	24±2	29±1	32±1	20±1	24±1	30±0	36±3	13±1	19±1	25±1	28±3
11	17±1	24±3	29±1	30±1	18±1	23±1	28±1	33±2	10±1	13±1	16±0	20±0
12	24±1	29±1	36±1	40±2	21±1	27±1	32±1	41±1	16±1	24±2	30±1	38±1
13	08±1	14±2	17±2	21±1	NA	NA	10±1	14±0	NA	NA	NA	08±0
14	NA	NA	NA	04±1	NA	NA	NA	NA	07±1	12±1	14±2	17±2
15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	08±1	12±2
16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	08±1	11±0	14±2	18±2	07±1	11±2	13±2	17±2	NA	NA	NA	10±1
18	07±1	11±3	16±1	18±2	09±1	13±2	17±2	20±1	08±1	14±2	17±2	20±1
19	09±1	14±2	20±1	21±2	23±1	28±1	33±1	37±1	07±1	11±2	17±1	20±1
20	10±1	14±1	20±1	23±1	10±1	17±2	26±1	34±1	15±1	22±1	27±2	33±1
21	NA	NA	NA	NA	NA	NA	08±0	14±2	NA	NA	10±1	15±1
22	NA	NA	NA	NA	04±1	09±1	15±1	20±1	NA	05±1	09±1	14±2
23	06±1	11±2	13±1	16±0	NA	NA	NA	NA	NA	NA	NA	NA
24	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	10±1	13±1	17±2	22±1	10±1	13±1	18±1	21±1	04±2	10±4	14±0	18±1
28	11±1	15±1	21±4	26±0	07±1	09±1	12±1	15±0	21±1	24±1	29±0	30±1
29	NA	NA	NA	NA	04±1	09±1	14±2	18±1	NA	NA	10±1	15±0
30	12±1	19±1	24±0	30±1	08±1	14±2	09±1	22±1	17±1	24±0	31±0	36±1
Std	**24**±1	**28**±2	**34**±1	**37**±2	**21**±1	**25**±1	**31**±1	**34**±0	**23**±1	**22**±1	**32**±1	**38**±1
Control DMSO	-	-	-	-	-	-	-	-	-	-	-	-

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.

Std: Ketoconazole**, NA:** No activity**, (±)** Standard deviation

Table 4: Minimum inhibitory concentration (MIC) of the synthesized compounds

Entry	MIC (µg/mL) Values[a]							
	Antibacterial					Antifungal		
	S. aureus	***B. subtilis***	***E. Coli***	***K. pneumoniae***	**MRSA**	***A. niger***	***F. moniliforme***	***F. oxysporum***
6	28±1	26±1	25±2	-	30±1	-	-	-
7	30±2	28±1	27±2	-	32±1	-	-	-
9	-	-	-	-	-	25±0	28±0	26±2
10	-	-	-	-	-	25±0	25±2	22±5
11	23±1	25±2	24±50	22±2	-	26±5	20±3	18±2
12	28±2	28±2	24±0	20±2	-	20±5	18±5	16±1
13	24±5	29±1	33±1	24±1	20±1	-	25±5	27±5
14	26±2	24±1	25±2	22±1	22±2	-	-	-
19	19±15	18±2	20±4	22±5	19±1	30±5	26±4	23±5
20	17±2	15±3	19±6	24±5	18±0	28±5	25±3	22±5
28	32±1	26±4	35±7	26±2	20±1	30±2	28±0	23±2
30	24±3	25±1	24±2	25±5	22±2	25±2	24±5	22±5
Std (A)	**27**±2	**27**±1	**25±5**	**28±2**	**25**±1	**-**	**-**	**-**
Std (B)	**21**±1	**21**±2	**22**±1	**22**±1	**22**±2	**-**	**-**	**-**
Std (C)	**-**	**-**	**-**	**-**	**-**	**28±4**	**27±5**	**24±5**

[a] Values are mean of three determinations, the ranges of which are <5% of the mean in all cases.
Std (A): Chloramphenicol for antibacterial; **Std (B):** Rifampicin for antibacterial;
Std (C): Ketoconazole for antifungal,
(-): Not tested**, (±)** Standard deviation

Environmental changes alter gene expression in bacteria thereby affects its growth. Hfq is an important component in the regulation of gene expression in cooperation with sRNAs. Hfq in Gram-negative bacteria functions as a post-transcriptional regulator that acts by mediating interactions between many sRNAs and their mRNA targets[40]. To study the binding mode of new class of ligands against the YmaH of *Bacillus substilis*, molecular docking was subsequently performed. To our delight, the results of the docking study obtained are concordant with *in vitro* data; compound **19** was found to reside near the RNA binding site, which is crucial for the post-transciptional regulation of proteins and is also essential for

bacteria to perish in host (**Fig 1**), while compound **20** exhibited hydrophilic (-OH-H) interaction with native guanine of RNA (**Fig 2**). On the other hand, compounds **12** (**Fig 3**) and **30** had hydrogen bonding affinity with His57 through a interaction of –NH-O (**Fig 4**). However, compound **12**, **19** and **20** only have good docking scoring, whose value is -7.22, -8.48, and -8.92 respectively (**Table 5**). These results shows that newly synthesized compounds **19** and **20** display good docking scoring concordant result with *in vitro* data.

In the present investigation, a series of new benzo[*d*]thiazole-hydrazones were synthesized in good yield and tested for their preliminary *in vitro* antibacterial and antifungal activities. Here we found compounds (**13**, **14**, **15**, **19**, **20**, **28** and **30)** showing good antibacterial activity and compounds (**5**, **9**, **10**, **11**, **12**, **28** and **30)** showing good antifungal activities at the common antibiotic level. Further, SAR study analysis the effect of electrons donating (OH and OCH_3) and withdrawing (Cl, NO_2, F and Br) groups on the phenyl ring plays a major role in the antimicrobial activities. Based on these results, we could conduct that benzo[*d*]thiazole-hydrazones seem to be a promising compound for further research and development of novel antimicrobial agents. Molecular docking studies were performed for all the synthesized compounds, among them compounds **11**, **19** and **20** showed the highest glide g-scores.

Table 5: Molecular docking scores of all the synthesized compounds against YmaH from *B. subtilis* as obtained through Glide docking.

Compounds	**YmaH (PDB id-3HSB)**			
	RMSD OPLS-2005	**Docking Score**	**Glide Gscore**	**Glide Hbond**
1	0.039	-5.63	-5.81	0.00
2	0.035	-5.80	-5.86	0.00
3	0.043	-5.44	-5.64	0.00
4	0.025	-5.68	-5.76	-0.16
5	0.039	-5.54	-5.72	0.00
6	0.01	-5.93	-6.21	0.00
7	0.039	-6.30	-6.48	0.00
8	0.029	-5.42	-5.60	0.00

9	0.034	-5.49	-5.94	0.00
10	0.047	-5.46	-6.81	-0.32
11	0.034	-5.78	-6.13	0.00
12	0.008	-7.22	-6.53	0.00
13	0.025	-6.12	-6.43	0.00
14	0.015	-6.13	-6.30	-0.32
15	0.038	-6.39	-6.39	-0.32
16	0.024	-5.69	-5.83	0.00
17	0.023	-5.48	-5.65	0.00
18	0.022	-5.54	-5.72	0.00
19	0.034	-8.48	-7.57	0.00
20	0.039	-8.92	-7.41	-0.45
21	0.002	-5.40	-5.41	0.00
22	0.042	-5.31	-5.65	0.00
23	0.028	-5.28	-5.57	0.00
24	0.003	-5.26	-5.27	0.00
25	0.001	-5.23	-5.42	0.00
26	0.012	-5.15	-5.22	0.00
27	0.032	-5.90	-6.11	0.00
28	0.001	-5.08	-5.15	-0.11
29	0.022	-5.07	-5.90	0.00
30	0.015	-5.85	-6.03	0.00

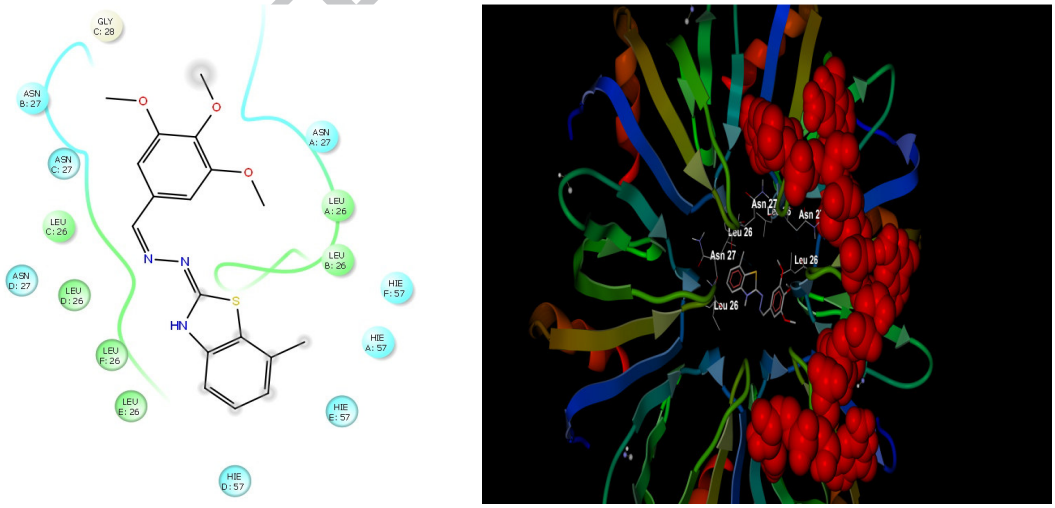


Fig. 1: Docking model structure of compound **19** into the YmaH binding pocket. Compound represented by stick form and coloured by element (CPK), protein as secondary structure, where as RNA is represented by space filled red coloured CPK form.

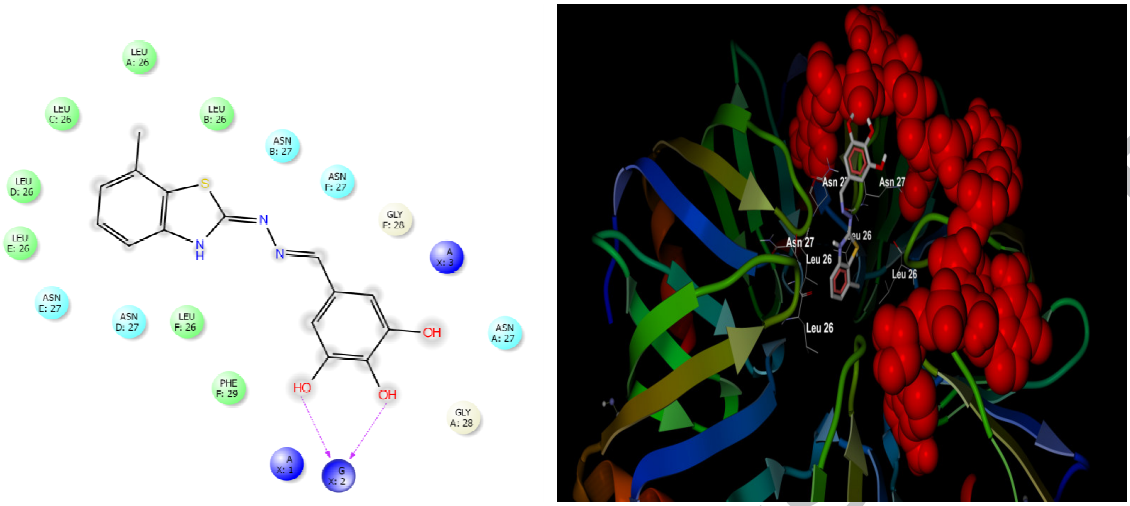


Fig 2: Docking model structure of compound **20** into the YmaH binding pocket. Compound represented by stick form and coloured by element (CPK), protein as secondary structure, where as RNA is represented by space filled red coloured CPK form.

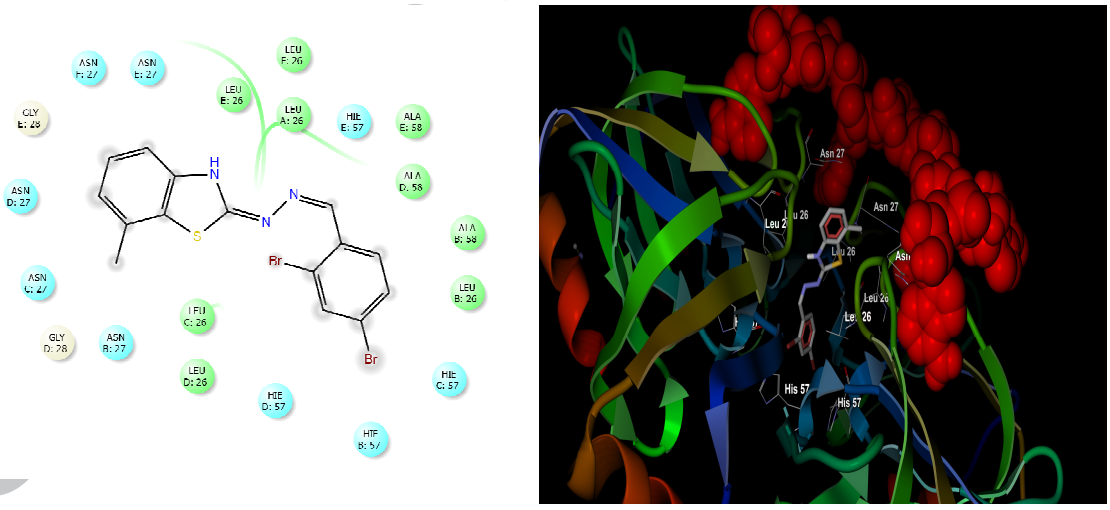


Fig 3: Docking model structure of compound **12** into the YmaH binding pocket. Compound represented by stick form and coloured by element (CPK), protein as secondary structure, where as RNA is represented by space filled red coloured CPK form.

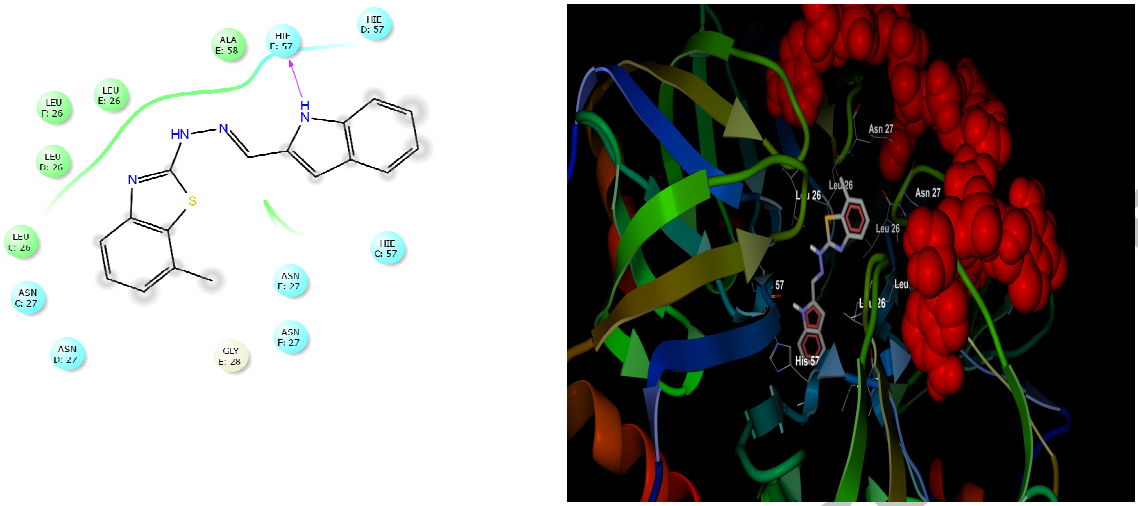


Fig 4: Docking model structure of compound **30** into the YmaH binding pocket. Compound represented by stick form and coloured by element (CPK), protein as secondary structure, where as RNA is represented by space filled red coloured CPK form.

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Synthesis, SAR and molecular docking studies of benzo[*d*]thiazole-hydrazones as potential antibacterial and antifungal agents

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