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LETTERS

Synthesis, characterization and antimicrobial evaluation of benzimidazole clubbed benzothiazole derivatives

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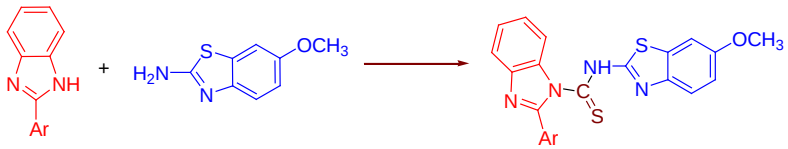
ABSTRACT



A new series of *N*-(6-methoxybenzo[*d*]thiazol-2-yl)-2-substituted phenyl-1*H*-benz[*d*]imidazole-1-carbothioamide derivatives **(5a-5h)** has been synthesized and evaluated for antibacterial, antifungal and antimalarial effects. All these compounds were characterized and screened for their *in vitro* antimicrobial activity against selected bacterial and fungal strains. Titled compounds were also evaluated for their antimalarial activity against *P. falciparum*. Antimicrobial activity screening results showed that some compounds namely **5b** against *P. aeruginosa*, **5c** against *S. aureus* and *E. coli*, **5d** against *E. coli* and *P. aeruginosa* and **5g** against *E. coli* from the series have emerged as prospective antibacterial leads endowed with excellent activity (MIC 12.5-62.5 µg/ml). While only one fungal strain *C. albicans* was susceptible towards synthesized compounds. On the other hand, compounds **5c** and **5h** exhibited noteworthy antimalarial activity with IC_{50} values of 0.18 & 0.11 µg/ml as compared to standard drugs chloroquine (IC_{50} 0.020 µg/ml) and quinine (IC_{50} 0.268 µg/ml).

Keywords: Benzothiazole, benzimidazole, antibacterial activity, antifungal activity and antimalarial activity.

INTRODUCTION

Infectious microbes have accompanied humanity for centuries and continue to represent leading causes of morbidity and mortality worldwide.[1,2] Infections acquired in health care settings have increased dramatically in the past few years. Even though deaths from bacterial and fungal infections have dropped in the developed world, but the treatment of these microbial infections in immune compromised patients with AIDS or undergoing anticancer therapy and organ transplants still remains an important and challenging problem to current chemotherapy.[3,5] Despite the fact that a large number of antibiotics and chemotherapeutics are available for treatment of infection; microbial resistance is still grave concern in the medical fraternity.[6,7] The antibiotic-resistant bacterial infections are widely recognized as public health threats, much less is known about the burden and consequences of drug-resistant fungal and malarial infections.[8,12] Since artemisinin-based combination therapies are frontline treatment against *P. falciparum* in many parts of world, still the developing resistance against antimalarial drugs is major concern worldwide.[13]

Hence, identification and discovery of novel medicinal agents with effective treatment of infectious diseases are highly warranted. Today more than 60% drugs used in clinical practice are synthesized derivatives and day-by-day the scope of synthetic medicinal chemistry is broadening.[14,15] Chemistry of heterocycles lies at the heart of drug discovery. Heterocyclic compounds are frequently found in nature and play a vital role in life. A number of pharmacologically active heterocyclic compounds are in regular clinical use. In the family of heterocyclic compounds, the nitrogen and sulphur containing heterocyclic ring system are gaining the exceptional devotion during past few decades. Benzothiazole and benzimidazole scaffolds are versatile heterocyclic nuclei having a broad

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spectrum of pharmacological activities.[16-19] The importance of benzothiazole, benzimidazole and their derivatives as potential antimicrobial agents such as taralazole (skin diseases), dimazole (antifungal), frentiazole (antiviral), albendazole (parasitic infection), cyclobendazole (anthelmintic), fuberidazole (antifungal),[20-25] etc., has been well established **(Figure 1)**. Considering these facts, and in continuation to our research work of dual heterocyclic moieties[26,27] it can be anticipated that combining two such heterocyclic molecules such as benzothiazole and benzimidazole in a single chemical entity exhibits superior antimicrobial activity as compared to compounds having individual benzothiazole and benzimidazole structural fragments.

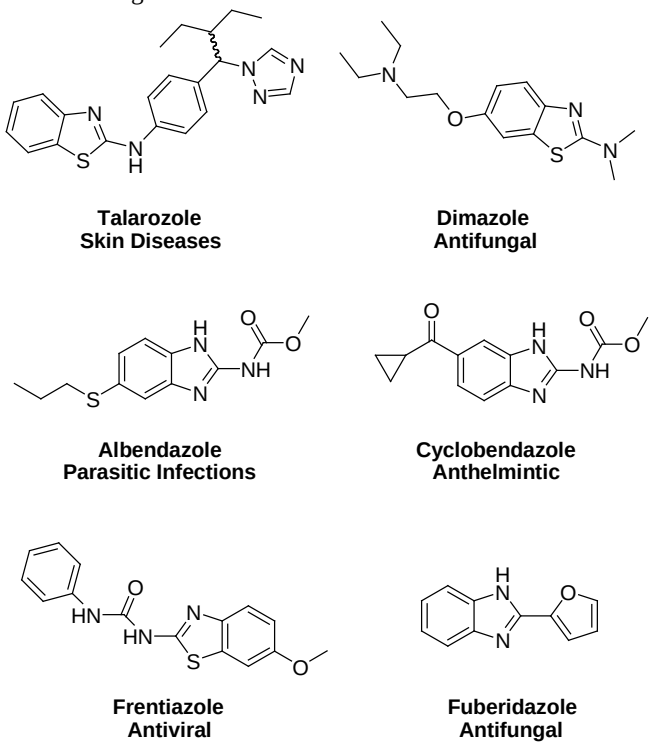


Figure 1. Some successful benzothiazole and benzimidazole based clinically available drugs.

EXPERIMENTAL

Materials and methods

Chemicals and all solvents were purchased from the commercial firms, SD Fines, Sigma Aldrich, Hi-Media and Rankem. Melting points were determined in open capillaries on a Perfit India melting point apparatus and are uncorrected. Thin layer chromatography (TLC) were performed to monitor the reaction and to determine the purity of the products using TLC plates pre-coated with silica gel G employing petroleum ether: ethyl acetate (2:3) as eluent and spots were visualized under iodine vapors and Ultraviolet (UV) irradiation. IR spectra (4000-400 cm^{-1}) were recorded on a Perkin Elmer IR spectrophotometer using KBr Pellets. ^{1}H-NMR spectra were recorded on BrukerAvance II 400 MHz model spectrophotometer with DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard with ^{1}H resonant frequency of 400 MHz and chemical shifts are expressed as δ (ppm). The splitting patterns are designed as follows; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The IR and ^{1}H-NMR spectral analysis were performed by Sophisticated Analytical Instrumentation Faculty (SAIF), Panjab University, Chandigarh. Antimicrobial screening was carried out in Microcare Laboratory Ltd., Surat, Gujrat, India.

Chemistry

General procedure for synthesis of benzimidazole derivatives (3):

A mixture of 0.03 mol of *o*-phenylenediamine (**1**), 20 ml of 4 N hydrochloric acid and 0.09 mol of aromatic acids (**2**) was heated together under reflux for 45 minutes. The reaction mixture was cooled to room temperature and made distinctly basic by gradual addition of concentrated ammonia solution. The precipitates obtained were collected and recrystallized from 10% ethanol to afford compound (**3**).[28]

General procedure for synthesis of 2-amino- 6 methoxybenzothiazole (4):

2-Amino 6-methoxy benzothiazole was prepared according to the procedure reported.[29]

General procedure for synthesis of benzimidazole clubbed benzothiazole derivatives (5a-5h):

A mixture of 2-amino 6-methoxy benzothiazole (0.05 mmol), benzimidazoles derivatives (0.05 mmol) and carbon disulfide (0.05mmol) in basic medium sodium bicarbonate (0.05 mmol) and N, N-dimethylformide (10 ml) was refluxed for 10 h. After the reactants consumed, the reaction mixture was poured into ice water and filtered the product. The product was dried and recrystallized from DMF: EtOH (1:1). The purity of final compounds was ascertained by thin layer chromatography.[30] The spectral data of synthesized compounds is given below:

2-(3-Aminophenyl)-N-(6-methoxybenzo[*d*]thiazol-2-yl)-1*H*-benz[*d*]imidazole-1-carbothiamide **(5a)**: Yield-24.12 %. M.P. 164-166˚C. IR (KBr) υ (cm^{-1}): 3371 (N-H); 3050 (Ar C-H); 2925 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.32-7.87 (m, 11H, Ar-H); 3.73 (s, 3H, OCH_3); 3.1 (s, 2H, NH_2).

2-(2-Chlorophenyl)-N-(6-methoxybenzo[*d*]thiazol-2-yl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5b)**: Yield-29.46%. M.P. 160-162˚C. IR (KBr) υ (cm^{-1}): 3318 (N-H); 2956 (Ar C-H); 2915 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.30-7.81 (m, 11H, Ar-H); 3.73 (s, 3H, OCH_3).

N-(6-methoxybenzo[*d*]thiazol-2-yl)-2-(3,4,5-trimethoxyphenyl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5c)**:Yield-47.08%. M.P. 237-239˚C. IR (KBr) υ (cm^{-1}): 3240 (N-H); 2948 (Ar C-H); 2850 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.05-7.96 (m, 9H, Ar-H); 3.84 (s, 12H, 4-OCH_3).

N-(6-methoxybenzo[*d*]thiazol-2-yl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5d)**: Yield-34%. M.P. 224-226˚C. IR (KBr) υ (cm^{-1}): 3300 (N-H); 2994 (Ar C-H); 2865 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.15-7.68 (m, 12H, Ar-H); 3.73 (s, 9H, OCH_3).

N-(6-methoxybenzo[*d*]thiazol-2-yl)-2-(3,4-dimethoxyphenyl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5e)**: Yield-33%. M.P.

232-234˚C. IR (KBr) υ (cm^{-1}): 3411 (N-H); 3099 (Ar C-H); 2829 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.20-7.77 (m, 10H, Ar-H); 3.80 (s, 3H, OCH_3).

2-(4-Chlorophenyl)-N-(6-methoxybenzo[*d*]thiazol-2-yl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5f)**: Yield-45.04%. M.P. 160-182˚C. IR (KBr) υ (cm^{-1}): 3390 (N-H); 3081 (Ar C-H); 2895 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.40-7.96 (m, 11H, Ar-H); 3.73 (s, 3H, OCH_3).

2-(4-Aminophenyl)-N-(6-methoxybenzo[*d*]thiazol-2-yl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5g)**: Yield-34.56%. M.P. 164-166˚C. IR (KBr) υ (cm^{-1}): 3448 (N-H); 3094 (Ar C-H); 2910 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.09-7.58 (m, 11H, Ar-H); 3.73 (s, 3H, OCH_3); 3.12 (s, 2H, NH_2).

N-(6-methoxybenzo[*d*]thiazol-2-yl)-2-(3,4-dinitrophenyl)-1*H*-benz[*d*]imidazole-1-carbothioamide **(5h)**: Yield-43.74%. M.P. 184-187˚C. IR (KBr) υ (cm^{-1}): 3486 (N-H); 2978 (Ar C-H); 2910 (O-CH_3). ^{1}HNMR (DMSO-d_6) δ, ppm: 8.1 (s, 1H, NH); 7.45-7.96 (m, 10H, Ar-H); 3.73 (s, 3H, OCH_3).

In vitro antimicrobial activity assay

All the newly synthesized compounds were screened for their *in vitro* antimicrobial activity against two Gram positive bacteria *viz. S. aureus* MTCC 96, *S. pyogenes* MTCC 442, two Gram negative strains *viz. E. coli* MTCC 443, *P. aeruginosa* MTCC 1688 and against the fungal strains namely *C. albicans* MTCC 227, *A. niger* MTCC 282, *A. clavatus* MTCC 1323 using serial broth dilution method. Human pathogenic and fungal microorganisms were procured from Microbial Type Culture Collection (MTCC), Microcare Laboratory Ltd., Surat, Gujrat. Preparation of nutrient broth, stock solutions and subculture were done following standard procedures. DMSO was used as diluent/vehicle to get desired concentration of drugs to test on standard bacterial and fungal strains. Inoculum size for test strain was adjusted to 108 Cfu [Colony Forming Unit] per millilitre by comparing the turbidity. Minimum inhibitory concentration (MIC, µg/ml) was measured for all the synthesized compounds and compared with that of standard drugs such as ampicillin (for antibacterial activity), nystatin and griseofulvin (for antifungal activity).[31]

In vitro antimalarial assay

The *in vitro* antimalarial assay was carried out in 96 well microtitre plates according to the microassay protocol of Rieckmann and co-workers with minor modifications. The cultures of *P. falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µL of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly maintained with 50% RBCs (O+). A stock solution of 5 mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µl volume were added to the test wells so as to obtain final concentrations (at fivefold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37ºC in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were observed microscopically to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Chloroquine and quinine were used as the reference drugs. The mean number of rings, trophozoites and schizonts recorded per 100 parasites from duplicate wells after incubation for 38 h and percent maturation inhibition with respect to control group.[32-34]

RESULT S AND DISCUSSION

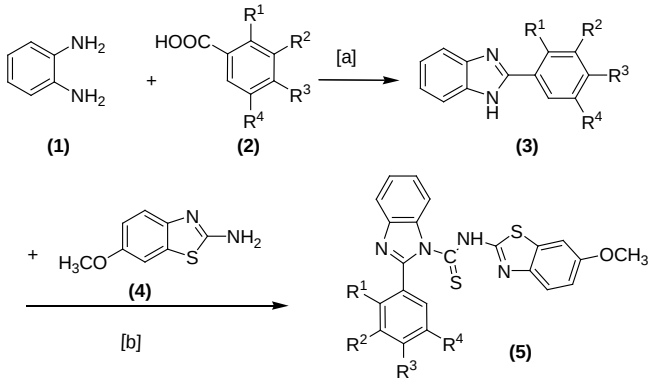
The target compounds have been synthesized by the method outlined in **Scheme-1**. The preparation of 2-substitutedphenyl-1*H*-benz[*d*]imidazole (3) was achieved by reacting *o*-phenylenediamine (1) with various substituted aromatic acids (2), under reflux. Sidewise synthesis of 6-methoxybenzo[*d*]thiazol-2-amine (4) was accomplished as per the reported procedure. The target compounds N-(6-methoxy-benzo[*d*]thiazol-2-yl)-2-substitutedphenyl-1*H*-benz[*d*]imidazole-1-carbothioamide (5) were obtained by refluxing appropriate benzimidazoles (3) with aminobenzothiazole (4) in carbon disulfide, sodium bicarbonate and N, N-dimethylformide in basic medium. Progress of reaction was monitored on the TLC plate. The newly synthesized compounds were characterized by IR, ^{1}H-NMR spectral studies. The IR spectrum of compounds revealed the absorption bands in the range of 3240-3448 cm^{-1}, 2948-3099 cm^{-1} corresponding to –NH stretching of amide and aromatic -CH stretch respectively. Singlets observed at around 8.0-8.1 ppm and 3.73-3.84 ppm was due to –NH amide group, and -OCH_3 of benzothiazole respectively. The peaks of aromatic protons were observed at δ 7.05-7.96 ppm and were found to be in accordance with substitution pattern on phenyl ring.

Antimicrobial Screening

In vitro antibacterial and antifungal activities

The titled compounds were screened for their *in vitro* antimicrobial activity against two Gram positive bacteria *viz. S. aureus* MTCC 96, *S. pyogenes* MTCC 442, Gram negative strains *viz. E. coli* MTCC 443, *P. aeruginosa* MTCC 1688 and against the fungal strains comprising *C. albicans* MTCC 227, *A. niger* MTCC 282, *A. clavatus* MTCC 1323 using serial broth dilution method. Minimum inhibitory concentration (MIC, µg/mL) was measured for all the synthesized compounds and compared with that of standard drugs such as ampicillin (for antibacterial activity), nystatin and griseofulvin (for antifungal activity). Positive controls produced significant MIC's against the tested bacteria and fungi. However, negative control did not

show observable inhibitory effect against any of the test organisms.



Compd.	R^1	R^2	R^3	R^4
5a;	H	NH_2	H	H
5b;	Cl	H	H	H
5c;	H	OCH_3	OCH_3	OCH_3
5d;	H	H	OCH_3	OCH_3
5e;	H	H	Cl	H
5f;	F	H	H	H
5g;	H	H	NH_2	H
5h;	H	H	NO_2	NO_2

Reagents and conditions; (a) Heated at 100ºC. (b) CS_2, $NaHCO_3$, DMF, reflux for 10 h.

Scheme 1. Synthesis of N-(6-methoxybenzo[*d*]thiazol-2-yl)-2-substituted phenyl-1*H*-benz[*d*]imidazole-1-carbothioamide derivatives (5a-5h).

Antibacterial data suggested that all the tested compounds exhibited significant and varying degree of activity against all the strains. Generally, the Gram negative bacterial strains were slightly more susceptible to tested compounds as compared to Gram positive. Biological evaluation revealed that compounds 5c substituted with 3,4,5-tri-methoxy and 5d substituted with 4,5-di-methoxy functional groups on phenyl ring were the most potent compounds (MIC range 25-250 µg/ml) in the series overall. However one compound 5g (substitution with 4-amino group) against *E. coli* was the most potent showing the MIC value 12.5 µg/ml which was higher than the standard drug ampicillin. Compound 5b (substitution with 2-chloro) against *E. coli*, *P. aeruginosa* and *S. aureus* showed promising activity with MIC's values 62.5 and 125 µg/ml. Rest of all the compounds exerted moderate to good antibacterial activity (**Table. 1**).

Antifungal activity results indicated that almost all the compounds were highly potent against *C. albicans* when compared with standard drug griseofulvin at MIC value 500 µg/mL. Out of tested, compound 5g (substitution with 4-amino group) was highly potent with MIC 125 µg/ml. Rest of all the compounds exhibited moderate to poor antifungal activity against *A. niger* and *A. clavatus* (**Table 2**).

Table 1. Antibacterial activity (MIC) of synthesized compounds (5a-5h).

Compd. No.	Antibacterial Activity Minimum Inhibitory Concentration (MIC µg/ml)			
	S. aureus (MTCC 96)	***S. pyrogenes*** (MTCC 442)	***E. coli*** (MTCC 443)	***P. aeruginosa*** (MTCC 441)
5a	125	100	125	200
5b	125	250	100	62.5
5c	25	125	50	100
5d	125	250	25	50
5e	250	100	125	100
5f	500	250	250	500
5g	250	500	12.5	500
5h	250	200	500	250
Negative control*	0	0	0	0
Ampicillin	250	100	100	ND

***** DMSO

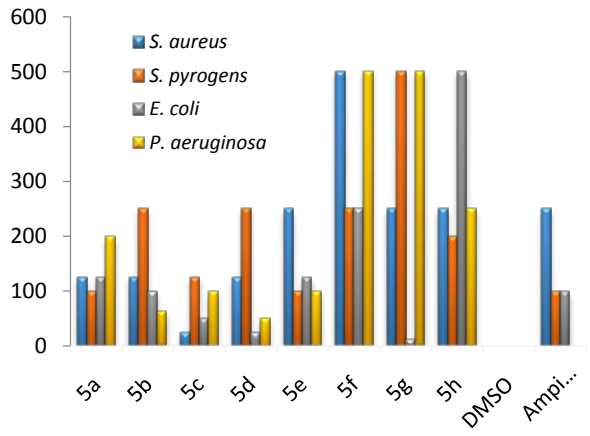


Figure 2. Antibacterial activity of synthesized compounds.

In vitro antimalarial activity

All the newly synthesized compounds (5a-5h) were screened for their *in vitro* antimalarial activity against the human malarial causative strain *Plasmodium falciparum* and were compared with standard drugs chloroquine (IC_{50} 0.020 µg/ml) and quinine (IC_{50} 0.268 µg/ml). The antimalarial activity data suggested that compounds 5c (3,4,5-tri-methoxy) and 5h (4,5-di-nitro) exhibited maximum inhibition against *P. falciparum* with IC_{50} values 0.18 and 0.11 µg/ml respectively among all in comparison to quinine. Compound 5d was significantly active while rest of the compounds were moderate in antimalarial activity (**Table 3**). The antibacterial, antifungal and antimalarial activities of each compound in term of MIC are depicted in figure 2, figure 3 and figure 4 respectively indicating the concentration (µg/ml) along the Y-axis and compound numbers

Table 2. Antifungal activity (MIC) of synthesized compounds (5a-5h).

Compd. No.	Antifungal Activity Minimum Inhibitory Concentration (MIC µg/ml)		
	C. albicans (MTCC 227)	***A. niger*** (MTCC 282)	***A. clavatus*** (MTCC 1323)
5a	250	500	500
5b	250	500	1000
5c	250	>1000	>1000
5d	250	1000	1000
5e	250	500	1000
5f	500	1000	500
5g	125	500	500
5h	500	1000	500
Negative control*	0	0	0
Nystatin	100	100	100
Griseofulvin	500	100	100

***** DMSO

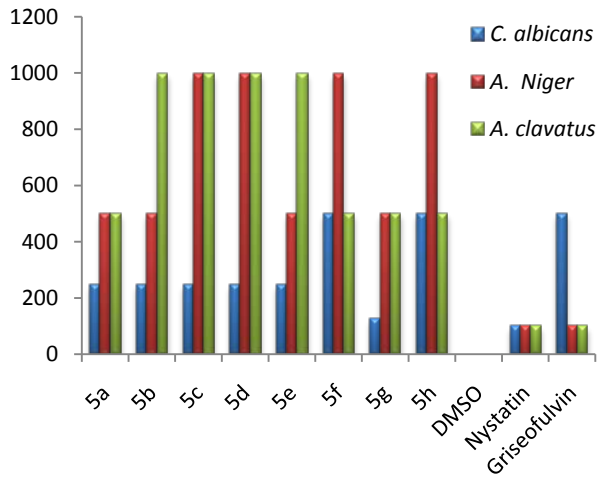


Figure 3. Antifungal activity of synthesized compounds.

along the X-axis. From SAR point of view it can be concluded that substitution with an electron donating group like tri-OCH_3 and di-OCH_3 resulted in enhanced antibacterial activity, while substitution with an amino group enhanced antifungal activity. On the other hand, substitution either with electron donating or electron withdrawing groups such as methoxy and nitro groups imparts a strong antimalarial activity against malarial strain.

Table 3. Antimalarial evaluation of the synthesized compounds (5a-5h).

Antimalarial Activity Against *Plasmodium Falciparum*	
Sr. No	**Mean IC_{50} Values (µg/ml)**
5a	1.95
5b	1.40
5c	0.18
5d	0.72
5e	0.56
5f	1.42
5g	1.10
5h	0.11
Negative Control*	0
Chloroquine	0.020
Quinine	0.268

***** DMSO

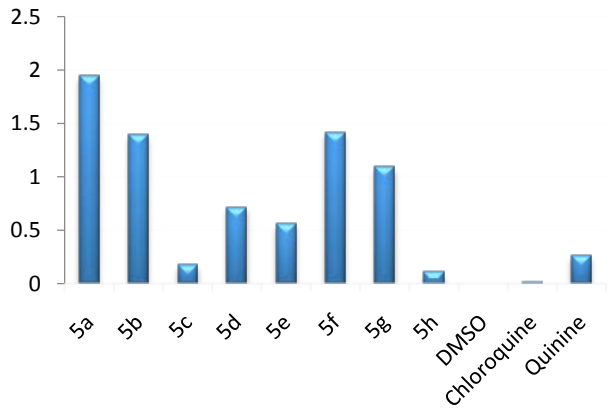


Figure 4. Antimalarial activity of synthesized compounds.

CONCLUSION

In the present work we have described synthesis, characterization and biological evaluation of benzimidazole clubbed benzothiazole derivatives. A total of eight new compounds were synthesized adopting standard procedure. All the newly synthesized compounds were characterized by IR and ^{1}H NMR spectral data. The biological findings inferred that compounds possessed significant activity profile. Antimicrobial activity data evidenced that compounds **5c** and **5d** were most potent against bacterial strains as compared to the standard drug ampicillin, while compound **5g** was the most potent against *C. albicans* as compared to standard drug griseofulvin. Rest of compounds were moderate to good in antimicrobial activity. On the other hand, compounds **5c** and **5h** emerged as potent antimalarial agents as compared to standard drugs chloroquine

and quinine. Thus, the current investigation revealed that synthesized compounds are of pharmacological interest and needs further scientific exploration for the development of novel antimicrobial agents.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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