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Research Article

MICROWAVE ASSISTED SYNTHESIS OF BENZOTHIAZOLE-SPIROOXINDOLE DERIVATIVES AS AN ANTIBACTERIAL, ANTIFUNGAL AND ANTIMALARIAL AGENT

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ABSTRACT

Microwave-assisted cyclization reaction between schiff's base of isatin and thioglycolic acid under *p*-TSA catalysis to afford benzothiazole-spirooxindole derivatives are reported. They were screened for their *in vitro* antibacterial activity against *E.coli* (MTCC 443), *P. Aeruginosa* (MTCC 1688) *S. Aureus* (MTCC 96) and *S. Pyogenus* (MTCC 442). Antifungal activity against *C. albicans* (MTCC 227), *A. niger* (MTCC 282), *A. clavatus* (MTCC 1323) and antimalarial activity against *P. falciparum.* Microbiological results showed that some of the compounds exhibited promising activity against test microorganisms are of interest with good hope to get more selective antibacterial, antifungal and antimalarial agents.

KEYWORDS

Benzothiazole-spirooxindole, Spiro[indole-thiazolidines], Antimycobacterial activity, Antibacterial activity, Antifungal activity, Antimalarial activity

INTRODUCTION

An antimicrobial agent kills microorganisms or inhibits their growth. [1] Antimicrobial medicines can be gathered according to the microorganisms like, antibacterial are used against bacteria and antifungals are used against fungi. They can also be categorized according to their function. Antimicrobial agents that merely inhibit their growth are called biostatic. A variety of microorganisms were elucidated to cause infectious diseases. Antimicrobial chemotherapy made remarkable advances during the 20th century. In an optimistic view that infectious diseases would be conquered in the near future. However, in response to the development of antimicrobial agents, microorganisms that have acquired resistance to drugs through a variety of mechanisms have emerged and continue to plague human beings. Infectious diseases caused by drug resistant bacteria are one of the most important problems in daily life. In the current situation, where multidrug-resistant bacteria have spread widely and options for treatment with antimicrobial agents are limited. Drug-resistant bacteria have been selected to minimize the transmission and spread of infection by using antimicrobial drugs control. Heterocyclic compounds would be the first step in resolving the issue of resistant organisms. [2] A heterocyclic compound is one which possesses a cyclic structure with at least two different kinds of hetero atoms like Nitrogen, Sulphur etc., are widely distributed in nature and have intrinsic affinity in the field of organic chemistry. The operation of these molecules enables the medicinal chemist to rapidly ascertain biologically active compounds across a wide range of therapeutic areas, over a viable time period. Among them, 2-aminobenzothiazoles involve a class of therapeutic compounds. The broad spectrum of pharmacological activity in individual benzothiazole derivatives shows that, this series of compounds have an undoubted interest because of their varied biological activities. [3] The grounds of significance for their unique structures and biological activities led to several applications in different areas of pharmaceutical and agrochemical research. [4] Additionally, spiro cyclic scaffolds are being gradually utilized in drug discovery. The construction of a spiro

heterocyclic framework has always been a challenging attempt for synthetic organic chemists as it frequently requires synthetic design based on specific strategies. Spirooxindoles are an important class of the heterocyclic compound likewise benzothiazole for synthesizing potential bioactive agents. They are present in many natural products and exhibits highly noticeable biological activities. [5,6]

Looking at the importance of these compounds and inspiration from our previous studies [7] the present work aim to continue the synthesis of novel benzothiazole-spirooxindole drivatives that should combine promising structural properties, here we have synthesized more seven compounds to establish a new antimicrobial agent. All the derivatives were synthesized and well characterized with various spectroscopic techniques. The synthesized compounds were screened for their *in vitro* antibacterial, antifungal and antimalarial activity.

EXPERIMENTAL

Synthesis of compounds 5a-5n

Synthesis of compound **5a-5g** have been synthesized in our previous work. [7] Further synthesis of compound **5h-5n** were prepared according to the reported method of our work.

***N*-(6-bromobenzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl) amino)acetamide (5h):** Pink colour; IR (KBr) (υmax, cm^{-1}): 3070 (C–H Aromatic), 3384 (N–H), 1650 (C=O amide), 1346 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 14.11 (s, 1H), 9.62 (s, 1H), 8.35 (d, *J* = 1.6 Hz, 1H), 7.91 (d, *J* = 7.5 Hz, 1H), 7.60 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.40-7.07 (m, 4H), 6.01 (s, 1H), 3.65-3.50 (m, 4H); ^{13}C NMR (100 MHz, DMSO) δ 186.82, 176.29, 168.07, 155.17, 153.43, 140.65, 133.82, 132.14, 126.24, 123.46, 120.60, 119.83, 118.38, 116.42, 110.66, 52.19, 35.43; ESI-MS: m/z Calculated 504.38, found $[M+H]^+$ 505.3; Anal. Calcd for $C_{19}H_{14}BrN_5O_3S_2$: C, 45.24; H, 2.80; Br, 15.84; N, 13.89; O, 9.52; S, 12.71 %; found: C, 45.90; H, 2.74; N, 13.65; S, 12.45 %.

2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)-N-(6-(trifluoromethoxy)benzo[d]thiazol-2-yl)aceta mide (5i): Brown colour; IR (KBr) (υmax, cm^{-1}): 3086 (C–H Aromatic), 3374 (N–H), 1525 (C=O amide), 1450 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 10.70 (s, 1H), 10.05 (s, 1H), 8.00 (d, *J* = 7.5 Hz, 1H), 7.86 (d, *J* = 1.4 Hz, 1H), 7.43-7.13 (m, 4H), 7.08 (dd, *J* = 7.5, 1.4 Hz, 1H), 3.82 (s, 1H), 3.55 (dd, *J* = 16.7, 11.7 Hz, 3H), 2.61 (s, 1H); ^{13}C NMR (100 MHz, DMSO) δ 186.82, 176.29, 168.07, 153.43, 150.57, 147.86, 140.65, 133.82, 129.75, 125.81, 123.55, 123.19, 120.89, 120.58 , 120.26, 119.83, 118.41, 117.95, 110.66, 52.19, 35.43; ESI-MS: m/z Calculated 509.48, found $[M+H]^+$ 510.4; Anal. Calcd for $C_{20}H_{14}F_3N_5O_4S_2$: C, 47.15; H, 2.77; F, 11.19; N, 13.75; O, 12.56; S, 12.59 %; found: C, 47.60; H, 2.35; N, 13.60; S, 12.12 %.

2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)-N-(6-hydroxybenzo[d]thi azol-2-yl)acetamide (5j): Dark yellow colour; IR (KBr) (υmax, cm^{-1}): 3090 (C–H Aromatic), 3361 (N–H), 1664 (C=O amide), 1400 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 11.95 (s, 1H), 10.72 (s, 1H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.64 (d, *J* = 1.4 Hz, 1H), 7.69-6.89 (m, 6H), 4.89 (s, 1H), 4.37 (s, 1H), 3.81 (s, 1H), 3.61 (s, 1H), 3.58 -3.46 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ 186.82, 176.29, 168.07, 154.09, 153.43, 146.53, 140.65, 134.99, 133.82, 123.55, 120.60, 119.83, 119.54, 115.36, 110.66, 109.32, 52.19, 35.43; ESI-MS: m/z Calculated 441.48, found $[M+H]^+$ 442.4; Anal. Calcd for $C_{19}H_{15}N_5O_4S_2$: C, 51.69; H, 3.42; N, 15.86; O, 14.50; S, 14.53 %; found: C, 51.92; H, 3.74; N, 15.25; S, 14.20 %.

2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)-N-(4-methoxybenzo[d]thia zol-2-yl)acetamide (5k): Brown colour; IR (KBr) (υmax, cm^{-1}): 3050 (C–H Aromatic), 3383 (N–H), 1572 (C=O amide), 1257 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 11.59 (s, 1H), 10.67 (s, 1H), 7.68 (dd, *J* = 7.5, 1.4 Hz, 1H), 7.43-7.28 (m, 4H), 7.28-7.03 (m, 1H), 6.99 (dd, *J* = 7.5, 1.6 Hz, 1H), 4.03 (s, 1H), 3.88 (s, 3H), 3.79 (s, 1H), 3.61 (s, 1H), 3.57-3.45 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ 186.82, 176.29, 168.07, 160.19, 151.86, 140.65, 140.43, 135.07, 133.82, 123.55, 121.38, 120.60, 119.83, 115.78, 112.98,

110.66, 56.78, 52.19, 35.4; ESI-MS: m/z Calculated 455.51, found $[M+H]^+$ 456.5; Anal. Calcd for $C_{20}H_{17}N_5O_4S_2$: C, 52.74; H, 3.76; N, 15.37; O, 14.05; S, 14.08 %; found: C, 52.32; H, 3.27; N, 15.65; S, 14.40 %.

***N*-(4-chlorobenzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)acetamide (5l):** Brown colour; IR (KBr) (υmax, cm^{-1}): 3074 (C–H Aromatic), 3245 (N–H), 1642 (C=O amide), 1496 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 11.71 (s, 1H), 10.63 (s, 1H), 7.95 (dd, *J* = 7.4, 1.5 Hz, 1H), 7.46-7.24 (m, 5H), 7.17 (td, *J* = 7.5, 1.6 Hz, 1H), 4.12 (s, 1H), 3.77 (s, 1H), 3.61 (s, 1H), 3.57-3.45 (m, 2H); ^{13}C NMR (100 MHz, DMSO) δ 186.83, 176.29, 168.07, 160.47, 147.60, 140.65, 133.83, 133.35, 130.01, 126.74, 123.55, 122.43, 122.09, 120.60, 119.83, 110.67, 52.20, 40.17-39.96, 35.44; ESI-MS: m/z Calculated 459.93, found $[M+H]^+$ 460.9; Anal. Calcd for $C_{19}H_{14}ClN_5O_3S_2$: C, 49.62; H, 3.07; Cl, 7.71; N, 15.23; O, 10.44; S, 13.94 %; found: C, 49.90; H, 3.30; N, 15.65; S, 13.41 %.

2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)-N-(4-methylbenzo[d]thiazol-2-yl)acetamide (5m): Shaddle brown colour; IR (KBr) (υmax, cm^{-1}): 3041 (C–H Aromatic), 3485 (N–H), 1542 (C=O amide), 1400 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 11.71 (s, 1H), 10.66 (s, 1H), 7.91 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.43-7.31 (m, 3H), 7.27 (dd, *J* = 7.5, 1.4 Hz, 2H), 7.17 (td, *J* = 7.4, 1.6 Hz, 1H), 4.16 (s, 1H), 3.78 (s, 1H), 3.61 (s, 1H), 3.58-3.45 (m, 2H), 2.51 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, DMSO) δ 186.82, 176.29, 168.07, 160.49, 151.81, 140.65, 136.44, 133.82, 132.95, 124.44, 123.55, 120.60, 119.83, 118.70, 118.07, 110.66, 52.19, 35.43, 19.19; ESI-MS: m/z Calculated 439.51, found $[M+H]^+$ 440.5; Anal. Calcd for $C_{20}H_{17}N_5O_3S_2$: C, 54.65; H, 3.90; N, 15.93; O, 10.92; S, 14.59 %; found: C, 54.95; H, 3.30; N, 15.60; S, 14.41 %.

***N*-(5,6-dimethylbenzo[d]thiazol-2-yl)-2-((2,4'-dioxospiro[indoline-3,2'-thiazolidin]-3'-yl)amino)acetamide(5n):** Light brown colour; IR (KBr) (υmax, cm^{-1}): 3175 (C–H Aromatic), 3275 (N–H), 1682 (C=O amide), 1476 (C–S); ^{1}H NMR (400 MHz, DMSO) δ 10.70 (s, 1H), 9.70 (s, 1H), 7.98 (d, *J*

= 11.7 Hz, 2H), 7.44-7.07 (m, 4H), 3.91 (s, 1H), 3.80 (s, 1H), 3.54 (dd, *J* = 18.2, 13.3 Hz, 3H), 2.30 (d, *J* = 13.6 Hz, 6H); ^{13}C NMR (100 MHz, DMSO) δ 186.82, 176.29, 168.07, 154.70, 154.29, 140.65, 134.83, 133.82, 129.95, 123.55, 120.60, 119.83, 119.33, 115.89, 110.66, 52.19, 35.43, 20.36; ESI-MS: m/z Calculated 453.54, found $[M+H]^+$ 454.5; Anal. Calcd for $C_{21}H_{19}N_5O_3S_2$: C, 55.61; H, 4.22; N, 15.44; O, 10.58; S, 14.14 %; found: C, 55.91; H, 4.74; N, 15.75; S, 14.40 %.

Determination of Antibacterial Activity

All the compounds were screened for their antibacterial activity by using Broth Dilution Method.

[8] Mueller Hinton Broth Was Used as Nutrient Medium to grow and dilute the Drug Suspension for the Test bacteria. The strains were procured from Institute of Microbial Technology, Chandigarh. DMSO was used as diluents to get desired concentration of drugs to test upon Standard bacterial strains. Primary 1000, 500, 250 and secondary 200, 100, 62.5, 50, 25, 12.5, 6.25, 3.25 μg mL^{-1} dilutions was done of each test compounds. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The lowest concentration inhibiting growth of the organism is recorded as the MIC. Compound **5a-5n** was screened against *E.coli* (MTCC 443), *P. Aeruginosa* (MTCC 1688) *S. Aureus* (MTCC 96) and *S. Pyogenus* (MTCC 442).

Determination of Antifungal Activity

Compound 5a-5n was tested for their antifungal activity against *C. albicans* (MTCC 227), *A. niger* (MTCC 282) and *A. clavatus* (MTCC 1323) by same method as mentioned in antibacterial activity.

Determination of Antimalarial Activity

In vitro antimalarial screening of Synthesized compounds against *P. falciparum* strain. According to the protocol of Rieckmann et al. [9] assay was carried out in 96 well microtitre plates with minor changes. The cultures of the strain were maintained in medium RPMI1640 supplemented with 25 mMHEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat in activated 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% haematocritina 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^{+ve}). 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 to100 µg/mL in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37º C in a candle jar. After 36 to 40 hour incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed 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).

RESULTS AND DISCUSSION

Chemistry

The synthetic pathway for the derivatives of *N*-(benzo[d]thiazol-2-yl)-2-((2,4'-dioxo spiro[indoline-3,2'-thiazolidin]-3'yl)amino)acetamide is shown in **Scheme 1**. To explore the scope of benzothiazole-spirooxindole derivatives, we extended our studies to synthesize compound **5h-5n**. Reaction time, yield and melting point of compound **5h-5n** are illustrated in **Table 1**.

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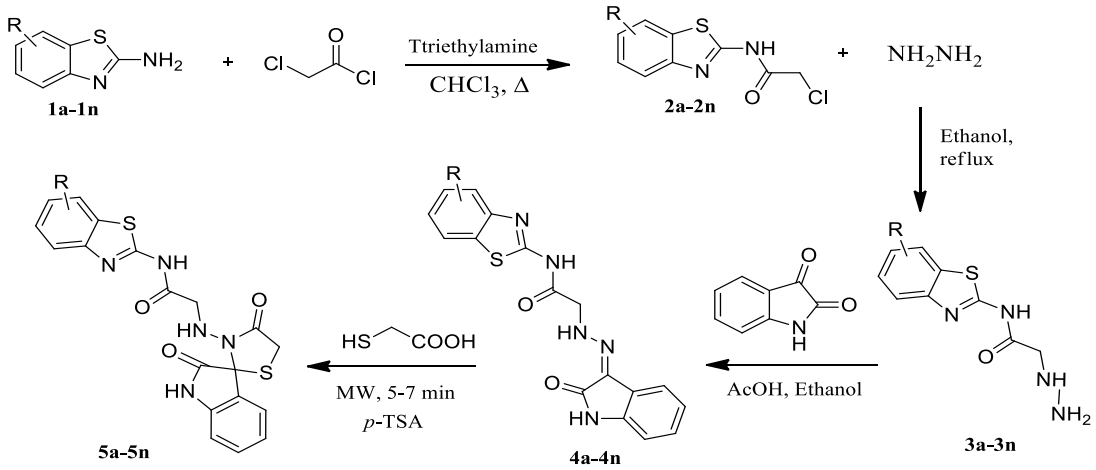

Scheme 1 Synthesis of targeted compound **5a-5n**

Table 1 Comparison of yield and reaction time for the synthesis of 5a-5n

Compound code	-R	Time (min)	Yield (%)	M.P˚C
5a	6-H	5	81.8	160-165 [7]
5b	6- CH_3	6	74.1	168-170 [7]
5c	6-F	5	77.8	165-167 [7]
5d	6-OCH_3	5	80.4	162-168 [7]
5e	6-NO_2	7	70.2	166-169 [7]
5f	6-Cl	5	75.2	165-168 [7]
5g	6-OC_2H_5	5	78.7	164-168 [7]
5h	6-Br	6	75.9	174-178
5i	6-OCF_3	5	72.3	158-160
5j	6-OH	6	80.9	167-170
5k	4- OCH_3	6	75.6	172-175
5l	4-Cl	6	72.2	168-170
5m	4- CH_3	7	75.1	170-172
5n	5,6-di CH_3	5	76.0	178-180

Evolution of Antibacterial Activity

Antibacterial activity of **5a-5n** was carried Broth dilution method against test cultures. Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin were used as standard drugs. Compound **5a-5n** were screened against two Gram negative bacteria, 1) *Escherichia coli* (MTCC 443), 2) *Pseudomonas Aeruginosa* (MTCC 1688) and two Gram positive bacteria 1) *Staphylococcus Aureus* (MTCC 96), 2) *Streptococcus Pyogenus* (MTCC 442). As shown in **Table 2**, all the compounds established promising antibacterial activity against Gram negative bacteria and Gram positive bacteria as

compared to standard drugs. All compounds showed moderate activity against *E. coli*. Compound **5k** can better inhibit by MIC value 25 µg/mL compare to Ampicillin and have similar value of Ciprofloxacin. Compound **5d**, **5e**, **5f**, **5m** and **5n** have similar MIC value (100 µg/mL) of Ampicillin. Compound **5b** and **5n** displayed better activity with MIC value 25 µg/mL having better inhibition than chloramphenicol and ciprofloxacin against S. Aureus and of course, comparatively excellent MIC value than Ampicillin. Compound **5g** has even better activity (MIC value 62.5 µg/mL) compare to Ampicillin against S. Pyogenus.

Table 2 Antibacterial Activity of Compound 5a-5n

	Minimal Inhibition Concentration [µg/mL]			
Compound code & Std. drugs	***E.Coli* MTCC 443**	***P.Aeruginosa* MTCC 1688**	***S.Aureus* MTCC 96**	***S.Pyogenus* MTCC 442**
5a	250	250	100	125
5b	250	250	25	125
5c	250	250	250	200
5d	250	100	100	100
5e	250	100	250	125
5f	250	100	125	100
5g	250	250	50	62.5
5h	250	200	100	100
5i	250	125	500	250
5j	250	250	100	250
5k	250	25	250	500
5l	250	250	125	250
5m	250	100	200	125
5n	250	100	25	100
Gentamycin	**0.05**	**1**	**0.25**	**0.5**
Ampicillin	**100**	**100**	**250**	**100**
Chloramphenicol	**50**	**50**	**50**	**50**
Ciprofloxacin	**25**	**25**	**50**	**50**
Norfloxacin	**10**	**10**	**10**	**10**

Evolution of Antifungal Activity

Antifungal activity of **5a-5n** was carried out against test cultures, *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323). Nystatin and Greseofulvin were used as standard drugs as shown in **Table 3**. In case of *C.albicans*, compound **5h** showed better MIC value (250 µg/mL) than greseofulvin and compounds **5e**, **5f**, **5i**, **5k**, **5m** have same MIC value as of

greseofulvin. Compounds **5a**, **5f** and **5l** have good activity against *A.Niger*. Furthermore, compound **5e** and **5k** also exhibited moderate activity against *A.Clavatus* in the comparison of both the standard drugs.

Table 3 Antifungal Activity of Compound 5a-5n

Minimal Inhibition Concentration [µg/mL]			
Compound code & Std. drugs	***C.Albicans* MTCC 227**	***A.Niger* MTCC 282**	***A.Clavatus* MTCC 1323**
5a	>1000	200	1000
5b	>1000	250	1000
5c	1000	500	1000
5d	1000	500	1000
5e	500	250	250
5f	500	200	500
5g	>1000	>1000	>1000
5h	250	500	>1000
5i	500	1000	500
5j	1000	500	500
5k	500	500	250
5l	1000	200	1000
5m	500	500	1000
5n	1000	500	500
Nystatin	**100**	**100**	**100**
Greseofulvin	**500**	**100**	**100**

Evolution of Antimalarial Activity

All the synthesized compounds **5a-5n** were screened for their antimalarial activity against *Plasmodium falciparum* **(Table 4)**. Choloroquine and Quinine were used as standard drugs. Almost all compounds demonstrated good antimalarial activity. Among them, compound **5d** and **5e** exhibit very good inhibition (MIC value 0.082 and 0.049 µg/mL) almost similar to the value of standard drug Choloroquine. Compound **5h** and **5j** showed better MIC value (0.32 and 0.38 µg/mL) than Quinine. Other compounds like **5a**, **5g**, **5k**, **5m** and **5n** also displayed good antimalarial inhibition in the range of MIC value 0.5-0.8 µg/mL, compare to Quinine.

Table 4 Antimalarial activity of compound 5a-5n

Comp. code	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**	**5l**	**5m**	**5n**
MIC[µg/mL]	0.82	1.36	1.88	0.082	0.049	1.54	0.97	0.32	1.69	0.38	0.89	0.52	0.61	0.54

Where, **MIC** (Minimal Inhibition Concentration)

MIC of standard drugs are **(1) Choloroquine** = 0.020 µg/mL **(2) Quinine** = 0.268 µg/mL

CONCLUSION

In the present research study, 2-aminobenzothiazoles and spirooxindoles are important classes of bioactive organic compound were to be linkage to synthesize of a series of benzothiazole-spirooxindole drivatives under MW irradiation within a very short time and with good yield. The combination of two structurally different moieties revealed that all the compounds give good bioactivity against four bacterial strains, three fungal strains and malarial strain. These observations conclude that benzothiazole-spirooxindole drivatives would help for the future development of novel antimicrobial drug designing.

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