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Synthesis, Biological evaluation, *insilico* Metabolism and Toxicity prediction of some Novel Benzimidazole-2-thione derivatives

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Abstract: Organic synthesis under solvent free condition and microwave irradiation has now become an area of interest for the synthesis of o-phenylenediamine condensation with thiourea give the known a wide variety of compounds. 1, 3 dihydrobenzimidazol-2-thione and its derivatives were synthesized by microwave irradiation techniques which results in excellent yields. All the synthesized compounds were also screened for antibacterial and antifungal activity. *Insilico* metabolism and toxicity prediction studies indicate that, Compound 2c, 3a and 3b are free from toxicity.

Keywords:- Microwave assisted synthesis, benzimidazole-2-thione, antibacterial agents *insilico* metabolism, toxicity prediction, spectroscopy.

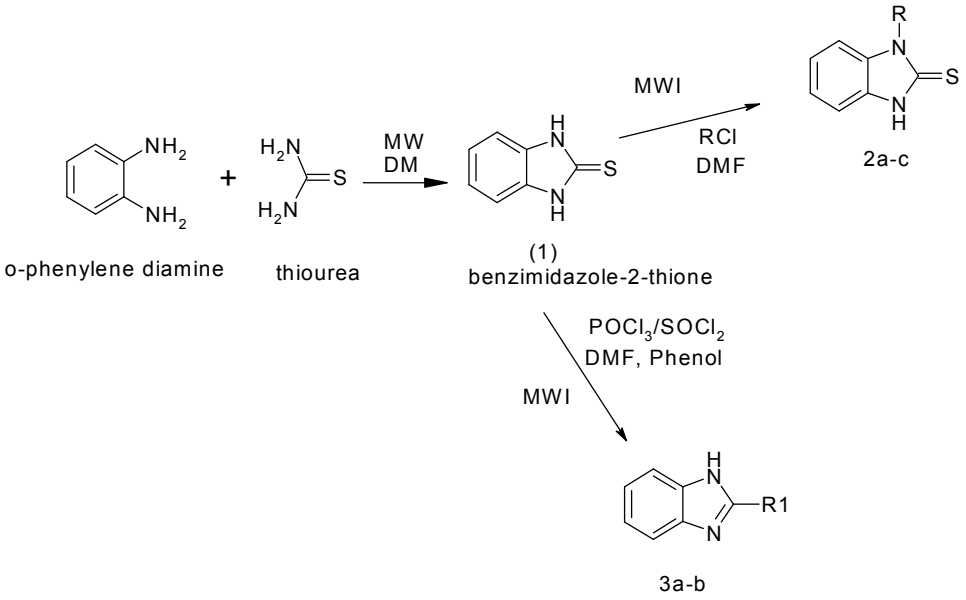
Introduction

Microwave-Induced Organic Reaction Environment (MORE) offers a simple, non conventional technique for the synthesis of wide variety of compounds having medicinal, pharmaceutical and commercial importance. To be creation microwave-associated organic synthesis can be faster as much 1000 fold and provide a clean and cheap alternative to conventional oil bath and sand bath[2]. Chemists have observed increase yield, reduce side reactions and decrease the amount of solvent required as energy transfer medium. In dry media synthesis, the reactions are carried out in solid state, solvent are not required (organic synthesis under solvent free conditions). Since the reaction time is significantly decreased. Result in lesser evaporation of solvents which prevent pollution[3].The development of drug resistance to correct anti bacterial, antifungal therapy continues to drive the search for more effective agents. In addition, primary and opportunistic bacterial and fungal infections human cell and fungi forms a handicap for sensitive activity. Several derivatives of benzimidazole are known to posses diverse type of biological activity[4]. Work on benzimidazole. it was considered worthwhile to prepare new benzimidazole-2-thione derivatives [5-10] from benzimidazole substituted in 1,2 and 3 positions. Different substitutions of benzimidazole-2-thione at the position of 1,2 and 3 have been reported to posses analgesic, anti-inflammatory, anti tubercular antiviral, antiherbicidal and sedative properties[11-16]. Since the sites of action of derivative of benzimidazole-2-thione compounds have posses additive action of their therapeutic value. The activity of benzimidazole-2-thione against microbes were found significantly.The synthesis, characterization,result and discussion of antibacterial antifungal activity screening studies of the newly synthesized compounds are reported in this article[17]. *Insilico* metabolism and toxicity prediction studies by ADME/Toxicity Property Calculator(in-silico screening based on known ADME/Toxicity knowledge base) software.[18]

Experimentation Work

1, 3 dihydrobenzimidazol-2-thione and its derivatives were synthesized in two steps according to the following scheme (fig. 1.)

Fig 1: Scheme for synthesis of benzimidazole-2-thione derivatives



2a-c (R=CH_3CO-,$ClCOCH_2$-,SO_3H-) ; **3a-b**, (R^1= Cl)

Synthesis of 1,3 Dihydro-Benzimidazol-2-thione (1) :-

O-phenylenediamine (0.046 mol) was taken and dissolved in sufficient amount of DMF, then thiourea (0.092 mol) was added and mixed well in clean and dried Erlenmeyer flask. The flask was capped with cotton, plugged funnel and heated in microwave irradiation at 40% intensity for 6 minutes 10 seconds till the colour changed to brown. A beaker containing water was placed at applicator in microwave oven. Beaker water was served as "Heating Sink" to the reaction vessel. When the reaction was completed, the brown coloured mixture was cooled at room temperature. The solid product formed was separated and excess of DMF was removed in vacuolar , the separated solid was dissolved in 10% NaOH solution. The aqueous alkaline solution was filtered and neutralized with aquous HCl (35%). The separated product was filtered, washed, dried and recystallize with methanol.

Synthesis of acetylchloride derivative of 1,3, dihydro benzimidazol -2-thione(2a) :-To a solution of 1,3 dihydrobenzimadazole - 2 - thione. (0.007 mol) in DMF, acetyl chloride (0.007 mol) was added in Erlenmeyer flask. The flask was capped with cotton plugged funnel and heated in microwave irradiation at 20% intensity for 10 second. A beaker containing water was placed at applicator in microwave oven. When reaction was completed, liquid was cooled at a room temperature. DMF was removed in vacuolar, the residue was diluted with water, and neutralized with 10% of NaOH. After washed with water it was dried. The final product obtained was recrystallized in methanol.

Synthesis of chloroacetyl chloride derivative of 1,3, dihydrobenzimidazol-2-thione(2b) :-To solution of 1,3 dihydrobenzimidazol- 2-thione (0.007 mol) in DMF, chloroacetylchloride (0.007 mol) was added in a Erlenmeyer flask. The flask was capped with cotton

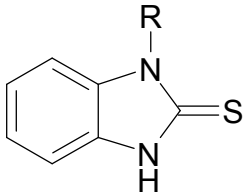
plugged funnel was heated in microwave irradiation at 20% intensity for 5 second. A beaker containing water was placed at applicator in microwave oven. When reaction was completed, reaction mixture was cooled at room temperature. DMF was removed in vacuo, the residue was diluted with water, and neutralized with 10% NaOH. After washed with water, the final product obtained was recrystallized in methanol, dried and weighed.

Synthesis of chlorosulphonic derivative of 1,3, dihydrobenzimidazol-2-thione(2c) :-
Take 1,3 dihydrobenzimidazol-2-thione (0.007 mol) in DMF, chlorosulphonic acid (0.007 mol) was added in Erlenmeyer flask. The flask was capped with cotton plugged; funnel was heated in microwave irradiation at 20% intensity for 8 second. A beaker containing water was placed at applicator in microwave oven. When reaction was completed reaction mixture was cooled at room temperature. DMF was removed in vacuo, the residue was diluted with water, and neutralized with 10% NaOH. Then after washed with water and dried. The final product obtained was recrystallized in methanol, dried and weighed

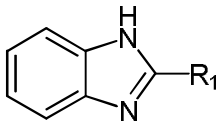
Synthesis of 2-chloro benzimidazole(3a):-
Take1,3, dihydrobenzimidazol-2-thione 1 gm (0.007mol), (0.14 mol) of phophorousoxochloride was added and a catalytic amount of phenol was taken in a Erlenmeyer flask. The flask was capped with cotton plugged funnel and a beaker containing water placed in the oven and subjected to microwave irradiation at 20% for 1 minutes 50 seconds. The reaction was monitored by TLC. After completion of the reaction the mixture was cooled in ice bath and neutralized with 40% NaOH to pH~10. The separated residue was recryslatallized in methanol and the product obtained was dried & weighed.

Synthesis of 2-chlorobenzimidazole(3b):-
A mixture of 1,3, dihydrobenzimidazol-2-thione 1 gm (0.007 mol) in DMF (0.014mol) of thionyl Chloride was added and catalytic amount of phenol was taken in a Erlenmeyer flask. The flask was capped with funnel which is plugged by cotton and a beaker containing water placed in the oven and subjected to microwave irradiation at 20% intensity in 6 seconds. When the reaction was completed mixture was cooled in ice bath and neutralized with 40% NaOH to pH~10. Then the obtained crude material was recrystallized dried & weighed.

Table 1: physicochemical parameters of compounds.



2a-c (R=CH_3CO-,$ClCOCH_2$-,SO_3H-)



3a-b (R_1=Cl)

Compound No	Reaction time	% yield	M.P. 0^0C	TLC Mobile Phase	R_f value
1	6 minute 10 seconds	91	259	Ethanol :Ethyl acetate (1:1)	0.45
2a	10 second	86	195	Ethanol :Ethyl acetate (1:1)	0.77
2b	5 second	89	165	Ethanol :Ethyl acetate (1:1)	0.68
2c	8 second	77	298	Ethanol :Ethyl acetate (1:1)	0.59
3a	1 minute 50 seconds	92	309	Ethanol :Ethyl acetate (1:1)	0.73
3b	6 second	85	309	Ethanol :Ethyl-acetate (1:1)	0.63

Physicochemical Properties of the Synthesized title compounds:

Log(p)..... .: 1.27
St..deviation. : 0.47
Estimation of Molar Refractivity
MR..... : 52.74 [cm.cm.cm/mol]
St..deviation.: 1.27
Normal Boiling Point [p=1atm]: 658.00 [K]
Standard Error: 20.400 [K]
Freezing Point [p=1atm]: Property estimation failed.

Estimation of the Thermodynamics properties

Heat of Formation [T=298.15K, p=1atm]:
Gibbs Energy [T=298.15K, p=1atm]:

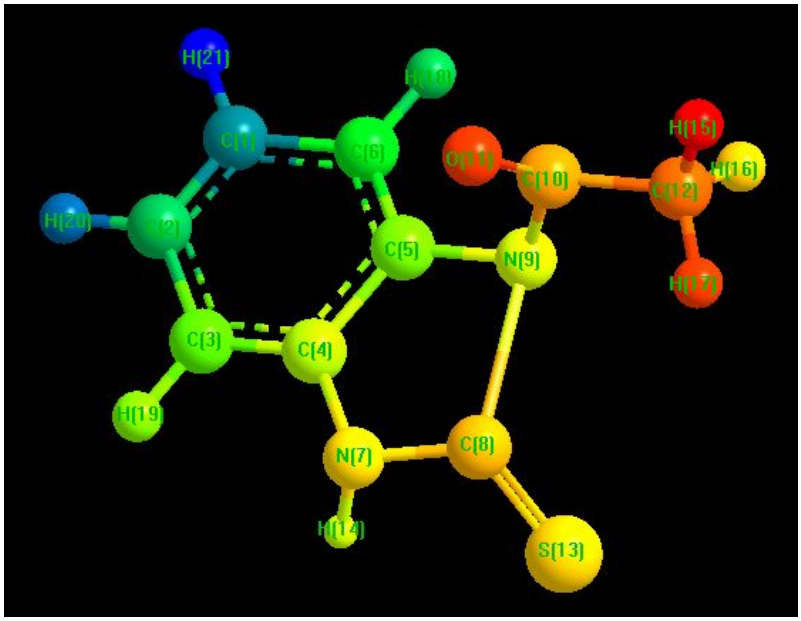
Characterization of the synthesized compounds

The benzimidazole-2-thione derivatives are synthesized by the reaction between substituted of Chloride and benzimidazole-2-thione. All melting points (m.p.) were determined in open capillary method using Jindal melting point apparatus and were uncorrected. The purity of the compounds was routinely checked by thin layer chromatography (TLC) using silica gel G (Merck). The instruments used for spectroscopic data are IR: Jasco FTIR-470 spectrophotometer (KBr) with diffuse reflectance method; MS-JEOL SX102 Mass spectroscopy by using Argon/Xenone (6Kv, 10mA) as the FAB gas and m-nitro benzyl alcohol (NBA) as the matrix. H^1NMR: JEOL GSX-400, 60MHz spectrometer in $CDCl_3$, TMS (tetra methyl saline) as an internal standard. H^1NMR, and IR spectra were consistent with the assigned structure. The results obtained which are shown in table 1 indicates, 1, 3, hydro benzimidazol-2-thione and its derivatives were synthesized under microwave irradiation. All compounds were in conformity with the structures envisaged. The structures were proved on the basis of spectral data.

Table 2: Spectroscopic data of the synthesized compounds

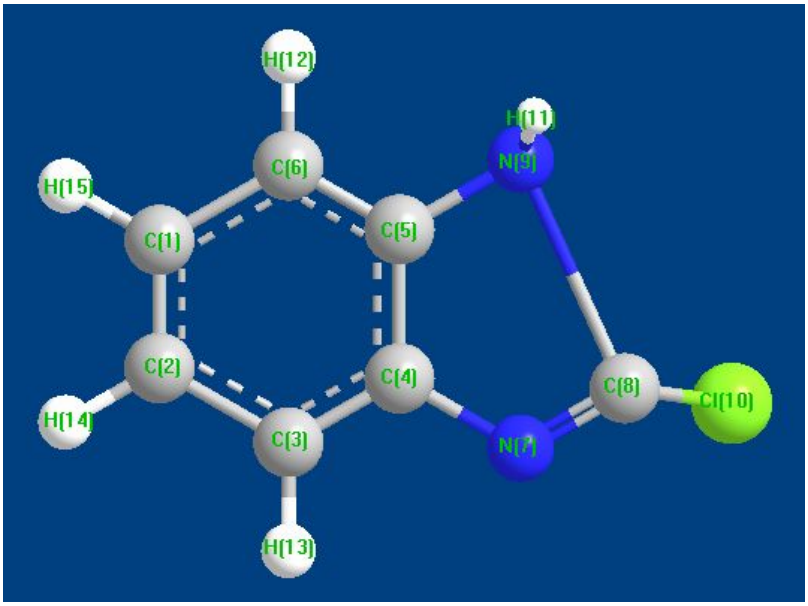
Com no	Analytical data
1	**IR(KBr/cm^{-1})**=3360(N-H), 1511-1179(C=S), 2822 (C-H); **Mass(m/e)**= MS(m/z 150), MS (m/z 150-32) 118, (m/z 150-59) 91
2a	**IR(KBr/cm^{-1})**=3356 (N-H), 1515-1365(C=S), 2997 (C-H), 1177 (C=O); **Mass(m/e)**= MS (m/z 192), (m/z192-43)149, (m/z 192-75)117;
2b	**IR(KBr/cm^{-1})**=3365(N-H), 1509-1308 (C=S), 2922(C-H), 1742(-C=O); **H^1 NMR δ/ppm in $CDCl_3$**= δ3.33- 3.74 (-NH), δ 4.56 - 4.88(CH_2), δ 7.34-7.93 [m4H(-CH)],
2c	**IR(KBr/cm^{-1})**=3359 (N-H), 1513-1267(C=S), 2881(C-H), 1365-1178(-SH) **H^1 NMR δ/ppm in $CDCl_3$**= δ3.3-7.4 (S-OH) δ4.1-4.8 (-NH) δ 7.2-7.5 (-CH)
3a	**IR(KBr/cm^{-1})**=3368 (N-H), 1624(C=N), 2882(C-H), 808(C-Cl)
3b	**IR(KBr/cm^{-1})**=3463 (N-H), 1623-1512(C=N), 3159 (C-H), 116 (C-Cl)

Fig.2-Three dimension Structure of synthesized title Compound with Energy minima-(2a-c)



1-(2-thioxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl) ethanone.
Chemical Formula: C9H8N2OS
Exact Mass: 192.04
Molecular Weight: 192.24
m/z: 192.04 (100.0%), 193.04 (10.7%), 194.03 (4.5%)
Elemental Analysis: C, 56.23; H, 4.19; N, 14.57; O, 8.32; S, 16.68

Fig:3 Three dimension Structure of synthesized title Compound with Energy minima (3a-b).



2-chloro-1H-benzo[d]imidazole
Chemical Formula: C7H5ClN2
Exact Mass: 152.01
Molecular Weight: 152.58
m/z: 152.01 (100.0%), 154.01 (32.0%), 153.02 (7.6%), 155.01 (2.7%)
Elemental Analysis: C, 55.10; H, 3.30; Cl, 23.24; N, 18.36

Biological evaluation:
All the synthesized benzimidazole derivatives (2a-c &3a-b) were evaluated for *in-vitro* anti bacterial activity against *staphylococcus aureus, Shiegella sonnei, Shiegella dysenteriae, Salmonellatyphimuriun, Vibriocholerae a*nd *Escherichia coli* at concentration of 25, 50 100 and 200 µ/ml by agar dilution method (spot inoculation method) in sterile nutrient agar

media.Norfloxacin was used as standard reference drug.[19]

Preservation of bacterial cultures:
All the strains of S*taphylococci, Streptococii, E.coli, Salmonella, Shigella, and Vibrious* were preserved as slab-slant cultures at a temperature of 4°C and also in freeze-dried state. Routine subculturing of the gram-positive bacteria was carried out on nutrient agar and the gram-negative strains on bromothymol blue lactose agar.

The agar dilution technique (spot inoculation method) for assessment of antibacterial activity:
The minimum inhibitory concentration (MIC) of the various synthetic compounds against the bacterial strains was determined by the agar dilution technique.[20]

Preparation of stock solutions of the synthetic compounds:
Desired amount of each of the synthetic compounds dissolved separately in 25% sterile dimethyl sulfoxide (DMSO) to prepare the stock solutions.

Preparation of norfloxacin solution :-
A stock solution of 10 µg/ml reference standard of norfloxacin was prepared with the help of sterile distilled water to prepare of 25, 50, 100 and 200 µg/ml used during agar dilution study.

Preparation of nutrient agar plates containing different concentration of the synthetic compound required for determination of minimum inhibitory concentrations (MIC) of the synthetic compounds with respect to different bacteria:[21]
Measured volumes of stock solutions of the synthetic compounds individually added aseptically to molten nutrient agar (oxoid) in the following concentration (µg/ml) : 0 (control), 25, 50, 100 and 200 and poured into sterile petri dishes. The pH of the media was adjusted to 7.2 - 7.4.
For uniform diffusion of the synthetic compound throughout the medium, the agar plates containing synthetic compounds were refrigerated overnight and subsequently dried for 2 hours at 37°C before use. Small squares were demarcated at the back of the agar containing portions of the plates with a marker to specify the actual location for each test organism.

Inoculum :
The inoculum for determination of the sensitivity pattern consisted of one loopful of an overnight grown culture of the test organism. The average size of the inoculum was about 10^5 cells contained in a 2 mm diameter standard loop.

Spot inoculation method (Agar dilution Method) :
When the nutrient agar plates containing the synthetic compounds and also the control plates having equal volumes of solvent were made ready, the overnight grown broth culture of each test organism was spot inoculated by Checker-board technique on the marked area of the plates. These were then incubated for 72 hours at 37°C. No growth of the organism on the test plate along with growth on the control plate was taken as an indication of antimicrobial activity of the drug. Minimum inhibitory concentration (MIC) was indicated by the lowest concentration of the synthetic drug, which inhibited the bacterial growth.

Table 3: Antibacterial biological evaluation of synthesized compounds

S. No.	Zone of Inhibition (mm)						
	Strain I	**Strain II**	**Strain III**	**Strain IV**	**Strain V**	**Strain VI**	**Strain VII**
1	12	13	10	12.5	12.5	10	10.5
2a	13	14	11.5	12	8	12.5	9
2b	12.5	6	11	7	9	7.5	7
2c	11.5	11	7.5	10	10	11	10
3a	11	11	12	20	10	10	8
3b	8	8.5	9	11.5	7.5	9	9
Ethanol	5	5	6.5	5.5	5	4.5	4.5
Ciprofloxacin	20	-	-	-	20	24	-
Ofloxacin	27	23	23	22	26	23	-
Tetracyclin	-	22	24	20	-	-	-
Miconazole	-	-	-	-	-	-	22
Amphotericin	-	-	-	-	-	-	17

Strain-I : Staphylococcus aureus (NCIM 2079) - *Concentration 50 µg*
Strain-II : *Staphylococcus epidermitis* (NCIM 2493) - *Concentration 50 µg*
Strain-III : *Bacillus subtilis* (NCIM 2063) - *Concentration 50 µg*
Strain-IV : *E.coli* (NCIM 2931) - *Concentration 50 µg*
Strain-V : *K. pneumoniae* (NCIM 2957) - *Concentration 50 µg*
Strain-VI : *P. vulgaries* (NCIM 2027) - *Concentration 50 µg*
Strain-VII : *C. albicans* (NCIM 347) - *Concentration 40 µg*

***In silico* metabolites and toxicity prediction:**

The metabolites and the toxicity of the compounds were predicted by computational method using Pallas version 3.1 ADME-Tox prediction software and pentium IV processor.

Table 4. *Insilico* predicted toxicity of the synthesized compounds (by ADME-Tox)

Com no	Toxicity	Ovt	Onco	Mut	Tertox	Irrit	Sent	Imtox	Neutox
2a	Probable	53	53	29	29	0	0	0	0
2b	HighProbable	79	5	79	29	0	0	0	0
2c	Not probable	0	0	0	0	0	0	0	0
3a	Not probable	0	0	0	0	0	0	0	0
3b	Not probable	0	0	0	0	0	0	0	0

Ovt= Over all toxicity, Onco= Oncogene, Mut= Mutagen, Tertox= Teretogen,
Irrit= Irritation, Sent= Sensitization, Imtox= Immunotoxicity, Neu tox= Neurotoxicity

Table 5. Metabolites of the synthesized compounds (by Metabolite Max-Alert)

Com Code	Alert	Count	Reaction
2a	Probable	6	Para-hydroxylation Amide hydrolysis
2b	Probable	7	Para-hydroxylation Amide hydrolysis Acetyl cystein conjugation
2c	Probable	2	Para-hydroxylation
3a	Probable	2	Para-hydroxylation
3b	Probable	2	Para-hydroxylation

Result and Discussion

Antimicrobial Activity -
The result obtained as per procedure described in table section 3, 4 & 5 is discussed below:

From table 3 the following conclusion is drawn

In case of *Staphylococcus aureus* (NCIM 2079), the most potent compound was found to be monomer-2, followed by 3, 1, 4, 5, 6, D_2 and D_1.

In case of *Staphylococcus epidermitis* (NCIM 2493), the most potent compound was found to be monomer-2, followed by 1. Monomer 4 and 5 was found to be equal potency. This was followed by 6, D_2, D_1 and 3.

In case of *Bacillus subtilis* (NCIM 2063) the most potent compound was found to be 5, followed by 2, 3 and 1. The Dimer D_2 was found to be equipotent with monomers 6. This was followed by 4 and D_1

In case of *E.coli* (NCIM 2931) the most potent compound was found to be monomer 5, followed by 1, 2, 6, 4, D, 3 and D_1.

In case of *K. pneumoniae* (NCIM 2957) the most potent compound was found to be 1. Monomer 4 and 5 was of equal potency. This was followed by 5, 3, 2 and 6.

In case of *P. vulgaries,* monomer-2 was found to be most potent followed by 4. Monomer- 4 and 5 was found to be equipotent. This was followed by 6 and 3.

In case of *C. albicans,* compound 1 was found to be most potent followed by 4. Monomer- 2 and monomer 6 was found to be of equal potency. And this was followed by 5 which has same potency as that of D-2. This was followed by D 1 which has same potency as that of monomer-3

In all the compounds and strains, monomer 5 was found to be most potent for *E.coli* and monomer-3, D-1 was found to be least potent fo*r Staphylococcus epidermitis,* Staphylococcus aureus and *E.coli* respectively.

One thing that is found common is that the synthesis of dimer caused decreased in activity than corresponding monomer and did not cause a significant increase in activity as compared to some monomers synthesized by bioisosteric principles. However, in all the tests, an increase in activity is seen as the spacer (methylene) is increased in the dimers. Compound-II, i.e., 2-(4-aminophenyl) benzothiazole was found to have good potency for all the strains.

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