

Solvent free synthesis of 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide derivatives as Novel antimicrobial and antitumor agents

ABSTRACT

In pursuit of designing novel chemical entities with antitumor and antimicrobial activities, 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide derivatives have been synthesized as a scaffold of a series of amine derivatives, these analogs were on reductive amination with solvent free condition. All the synthesized compounds were assessed for antibacterial, antifungal, and antitumor activities against standard strains. The compounds 3a, 3b and 3g showed highest degree of inhibition against *A. Flavus* and compounds 3a, 3b, 3d and 3h showed highest degree of inhibition against *C. albicans*, compounds 3a, 3e and 3g has shown the encouraging antibacterial activity results against *E. coli* and compounds 3a, 3b and 3h exhibited a promising activity against *B. subtilis*. Compounds 3a, 3b and 3c exhibited promising antitumor activity.

Keywords: Benzoxazole, Solvent free condition. Antitumor, antimicrobial.

1. INTRODUCTION

Benzoxazole skeleton have been found in numerous biological and pharmaceutical substances and which showed wide spectrum of pharmaceutical properties. In support for this study, substituted benzoxazoles showed various activities including antineoplastic, antitubercular, anthelmintic, and antimicrobial [34], anti-HIV, antifungal, antiprotozoal [1-6]. "The transformation of amines through different aldehydes is an important method in synthetic chemistry because of their benefit as intermediates for the preparation of agrochemicals and pharmaceuticals" [7-8]. It is thought worthwhile to carry out the synthesis of title compounds. In point of view to obtain some new chemical entities with together effective pharmacophores in a distinct molecular framework for the increased biological activities. The synthesis of benzoxazoles were made by many methods including Natural Sunlight Photocatalytic Synthesis [34] and solvent free methods [22].

"In account of the aforementioned information and with the extension of the former investigations on benzoxazole skeleton and its pharmacological activities" [9-15], the preparation of a new series of compounds (Figure-6) have been achieved. The present work sheds light on the cost effective solvent free synthetic route for the synthesis of sequence of 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide derivatives. The synthesized derivatives have been subjected to biological activities, which displayed significant activities against all the strains.

2. METHODOLOGY

By taking the sample of compounds in a glass capillary tube being sealed at one end, melting points were determined in an electrically heated apparatus, which are uncorrected. The purity and reaction progress of all the compounds were checked by TLC on silica gel plates using

n-Hexane, ethyl acetate solvent system and spots located by UV and iodine chamber. All the chemicals used in this were purchased from Sigma-Aldrich and SD Fine. IR spectra were recorded using KBr pellets on a Perkin Elmer Spectrophotometer. ¹H-NMR spectra on Agilent400 MHz Spectrophotometer and chemical shifts were expressed as ppm and TMS as internal standard. Mass spectra were recorded on Xevo G2-XS Qtof.

2.1.1 Synthesis of 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide (2)

Methyl-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetate (1 g, 0.0039 mol, 1eq) was added to ethanol (50 mL) and hydrazine hydrate (0.22 mL, 0.0047 mol, 1.2 eq) was added slowly. The reaction mixture was stirred under reflux in absolute alcohol for 4 hours. Progress of the reaction was confirmed by TLC (Chloroform: Methanol in 7:3 ratio). Reaction mixture was then poured to ice water to obtain compound 2. [16-20].

White solid; yield (90%), MP.(184-186°C); IR (KBr, cm^{-1}): 1592 (C=N), 3330 (N-H); ¹H NMR (400 MHz, DMSO) δ : 2.4 (s, 3H), 4.02 (s, 2H), 4.32 (br, 2H, NH₂), 7.07-7.48 (m, 3H), 9.4 (br, 1H, NH) (D₂O exchangeable); ¹³C NMR (DMSO): δ 21 (CH₃), 39 (CH₂), 110 – 135 (aromatic carbons), 146 (C=N), 166(C=O); MS (m/z): 237 (M+).

2.1.2 General procedure for the synthesis of 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide derivatives 3(a-h)

"A 2-[(5-Methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide amine (1mmol) was ground with a substituted aldehyde (1 mmol) for 15 to 20 minutes in an agate mortar and pestle at room temperature (25°C) under solvent free conditions. To the resulting mixture 1:1 ratio of Sodium borohydride and boric acid and then the mixture was added ground for 20-30 minutes until TLC showed complete disappearance of the aldehyde. The reaction mixture was washed with water and further purified by recrystallization with ethanol". [21-25]

2.1.2 General procedure for the synthesis of 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide derivatives 3(a-h)

A 2-[(5-Methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide amine (1mmol) was ground with a substituted aldehyde (1 mmol) for 15 to 20 minutes in an agate mortar and pestle at room temperature (25°C) under solvent free conditions. To the resulting mixture 1:1 ratio of Sodium borohydride and boric acid and then the mixture was added ground for 20-30 minutes until TLC showed complete disappearance of the aldehyde. The reaction mixture was washed with water and further purified by recrystallization with ethanol. [21-25]

2.1.3 2- [(5-methyl-1, 3-benzoxazol-2-yl)sulfanyl]-N'-(thiophen-2-ylmethyl)acetohydrazide (3a)

Grey colour solid, yield (75 %) MP (Melting Point) (235-236°C); IR(cm^{-1}): 1166 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ : 2.33 (s,3H), 4.22 (s,1H), 4.6 (s, 2H), 7.04 (s, 2H), 7.30 - 7.64 (Aromatic-H's),11.80 (s, 1H); ¹³C NMR (DMSO) δ : 21.3 (CH₃), 34.1 (CH₂), 35.00 (CH₂), 110-150 (Aromatic carbons), MS (m/z): 333 (M+)

2.1.4 N'-(furan-2-ylmethyl)-2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide (3b)

Grey colour solid, yield (73 %), MP (250-253°C); IR(cm^{-1}): 1156 (C-N str.); ¹H NMR (400 MHz, DMSO) δ : 2.33 (s,3H), 4.22 (s,1H), 4.6 (s, 2H), 7.04 (s, 2H), 7.30 - 7.64 (Aromatic-

H's), 11.80 (s, 1H); ¹³C NMR (DMSO) δ : 21.3 (CH₃), 34.1 (CH₂), 35.00 (CH₂), 110-150 (Aromatic carbons) MS (m/z): 317 (M⁺)

2.1.5 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]-N'-(2-nitrobenzyl)acetohydrazide (3c)

Grey colour solid, yield (75 %), MP (296-298 °C); IR (cm⁻¹): 1156 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ: 2.41 (s, 3H), 3.98 (s, 1H), 4.6 (s, 2H), 7.04 (s, 2H), 7.30 - 7.64 (Aromatic H's), 11.66 (s, 1H); ¹³C NMR (DMSO) δ : 21.42 (CH₃), 34.01 (CH₂), 35.00 (CH₂), 109-150 (Aromatic carbons) ; MS (m/z): 372 (M⁺)

2.1.6 N'-(4-chlorobenzyl)-2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide (3d)

Grey colour solid, yield (74 %), MP (280-282 °C); IR (cm⁻¹): 1152 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ: 2.45 (s, 3H), 3.97 (s, 1H), 4.62 (s, 2H), 7.02 (s, 2H), 7.30 - 7.64 (Aromatic-H's), 11.66 (s, 1H); ¹³C NMR (DMSO) δ : 21.42 (CH₃), 29.01 (CH₂), 34.00 (CH₂), 109-147 (Aromatic carbons), 183.72 (C=O); MS (m/z): 362 (M⁺).

2.1.7 N'-benzyl-2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide (3e)

Grey colour solid, yield (77 %), MP (230-232 °C); IR (cm⁻¹): 1142 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ: 2.41 (s, 3H), 3.97 (s, 1H), 4.64 (s, 2H), 7.01 (s, 2H), 7.11 - 7.71 (Aromatic-H's), 11.07 (s, 1H); ¹³C NMR (DMSO) δ : 21.55 (CH₃), 34.17 (CH₂), 34.57 (CH₂), 109-150 (Aromatic carbons), 169.37 (C=O); MS (m/z): 327 (M⁺).

2.1.8 N'-(2-chlorobenzyl)-2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide (3f)

Grey colour solid, yield (78 %), MP (285-287 °C) ; IR (cm⁻¹): 1164 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ: 2.39 (s, 3H), 4.25 (s, 1H), 4.68 (s, 2H), 7.10 (s, 2H), 7.11 - 7.71 (Aromatic-H's), 11.92 (s, 1H); ¹³C NMR (DMSO) δ : 21.38 (CH₃), 34.98 (CH₂), 35.17 (CH₂), 110-164 (Aromatic carbons), 168.69 (C=O); MS (m/z): 361 (M⁺).

2.1.9 N'-(2-bromobenzyl)-2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]acetohydrazide (3g)

Light brown colour solid, yield (80%), MP (298-299 °C); IR (cm⁻¹): 1144 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ: 2.45 (s, 3H), 3.92 (s, 1H), 4.61 (s, 2H), 6.98 (s, 2H), 7.12 - 8.0 (Aromatic-H's), 11.18 (s, 1H); ¹³C NMR (DMSO) δ : 21.39 (CH₃), 34.16 (CH₂), 34.27 (CH₂), 110-164 (Aromatic carbons), 169 (C=O); MS (m/z): 406 (M⁺).

2.1.10 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl]-N'-(4-nitrobenzyl)acetohydrazide (3h)

Grey colour solid, yield (71 %), MP (280-281 °C); IR (cm⁻¹): 1174 (C-N bond stretching); ¹H NMR (400 MHz, DMSO) δ: 2.31 (s, 3H), 4.12 (s, 1H), 4.68 (s, 2H), 6.74 (s, 2H), 7.08 - 8.0 (Aromatic-H's), 12.02 (s, 1H); ¹³C NMR (DMSO) δ : 21.37 (CH₃), 34.90 (CH₂), 39.36 (CH₂), 110-164 (Aromatic carbons), 168 (C=O); MS (m/z): 372 (M⁺).

2.2 Biological Activities

2.2.1 Antibacterial Activity

"The newly synthesized 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide derivatives were tested for antibacterial activity against bacterial strains, *Escherichia coli* (ATTC-8739), *Bacillus subtilis* (ATTC-6633) by agar well diffusion method" [26-27]. The 24 hr

old Mueller-Hinton broth culture of test bacteria were swabbed on sterile Mueller-Hint on agar plates using sterile cotton swab followed by punching wells of 6 mm with the help of sterile cork borer. The standard drug (Streptomycin 1mg/mL of sterile distilled water), compounds 3(a-h) (25mg/mL of 10% DMSO), and control (10% DMSO) were added to the respectively label dwells. The plates were allowed to stand for 30 minutes and were incubated at 37°C for 24 hr in upright position and the zone of inhibition was recorded and tabulated in Table 2. And compounds 3a,3e and 3g have shown the very good activity against *E.coli* and compounds 3a, 3b and 3h showed considerable activity against *B. subtilis*.

2.2.2 Antifungal Activity

“The Compounds3(a-h)were screened for anti-fungal activity against fungal *A. flavus* and *C. albicans* strain with fluconazole as a standard drug respectively. The compounds 3a, 3b and 3g Showed inhibition against *A. flavus* when compared with the standard drug fluconazole. Compounds 3a, 3b, 3d and 3h showed inhibition against *C. albicans* when compared with the standard drug fluconazole” [28-29].

2.2.3 Antitumor activity

“The antitumor activity against HepG2, human liver cancer cell lines were estimated using the 3-[4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, Cells were dispensed in a 96-well sterile microplate (5 × 10⁴ cells/well) and incubated at 37 °C with series of different concentrations in DMSO, of 3(a-h) compounds for 48 h in a serum-free medium prior to the MTT assay. After incubation, media were carefully removed, and 40 µL of MTT (2.5 mg/mL) was added to each well and then incubated for an additional 4 h. The purple formazan dye crystals were solubilised by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm using multi-Mode micro plate reader analysed using Megalen software. The relative cell viability was expressed as the mean percentage of viable cells compared to the untreated control cells. All experiments were conducted in triplicate and repeated in three different days. All the values were represented as mean ± SD and IC50s were determined” [30-32].

Table 1. Antibacterial activity

Compounds	Bacteria			
	<i>E. coli</i>		<i>B. subtilis</i>	
	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL
2	1.5±0.6	1.9±0.8	1.4±0.3	1.6±0.5
3a	1.8±0.1	2.1±0.30	1.9±0.24	2.4±0.29
3b	1.5±0.2	1.8±0.5	2.0±0.12	2.4±0.12
3c	1.4±0.12	1.7±0.6	1.3±0.21	1.8±0.6

3d	1.6±0.2	2.1±0.4	1.5±0.12	2.2±0.24
3e	1.7±0.23	2.2±0.5	1.6±0.2	2.1±0.21
3f	1.4±0.22	1.8±0.21	1.3±0.21	2.1±0.32
3g	1.8±0.25	2.2±0.20	1.6±0.31	2.2±0.31
3h	1.5±0.12	1.9±0.21	1.6±0.23	2.3±0.21
Streptomycin	1.8±0.25	2.6±0.28	2.1±0.32	2.5±0.34

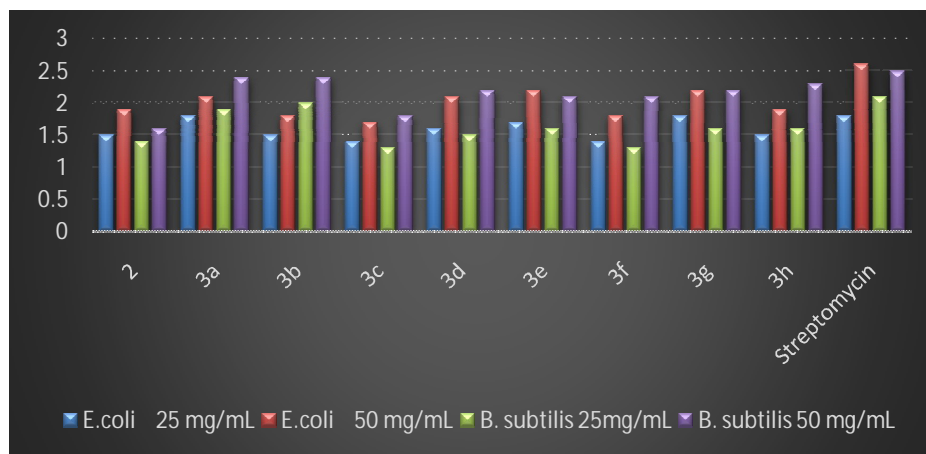


Fig. 1. Antibacterial activity of Compounds 2 &3(a-h)

Table 2. Antifungal activity

Compounds	Fungi			
	<i>A. flavus</i>		<i>C. albicans</i>	
	25 mg/mL	50 mg/mL	25 mg/mL	50 mg/mL
2	25	53	36	46
3a	44	74	38	78
3b	44	70	55	64
3c	24	69	35	71

3d	36	72	38	73
3e	37	69	39	71
3f	25	67	29	65
3g	38	70	37	69
3h	41	71	38	75
Fluconazole	38	77	39	81

*Values are represented as the mean \pm SEM.

*Values are significant for the standard at 0.005 level of significance.

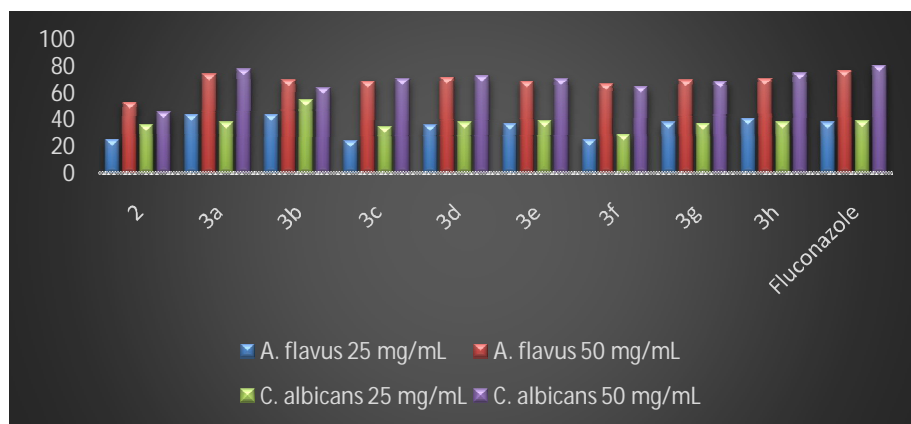


Fig. 2. Antifungal activity of Compounds 2 & 3(a-h)

Table 3. Antitumor activity

compounds	% of inhibition 25 mg/ml	% of inhibition 50 mg/ml	% of inhibition 100 mg/ml	IC 50 Value
2	31.4 %	52.2 %	59.4%	96
3a	32.6 %	56.4 %	62.8 %	109
3b	30.4 %	56.9 %	63.4 %	110

3c	34.4 %	60.1 %	67.3 %	112
3d	30.6 %	56.2%	64.2 %	106
3e	30.4 %	54.4 %	60.1 %	104
3f	29.6%	48.1 %	52.3 %	97
3g	30.4%	48.3 %	52.4 %	98
3h	30.6%	50.1 %	60.3 %	104
Std			76.4 %	136

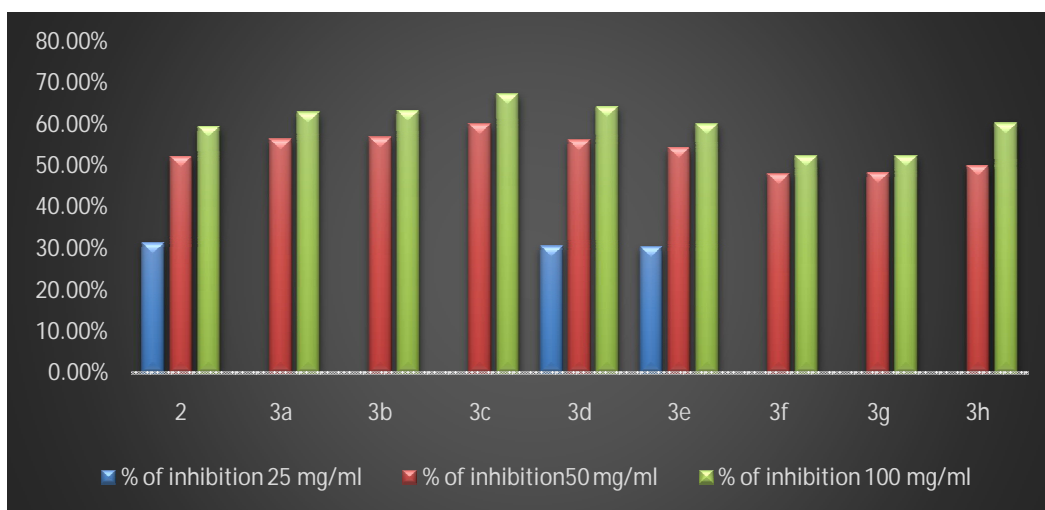


Fig. 3. Antitumor activity of Compounds 2 & 3(a-h)

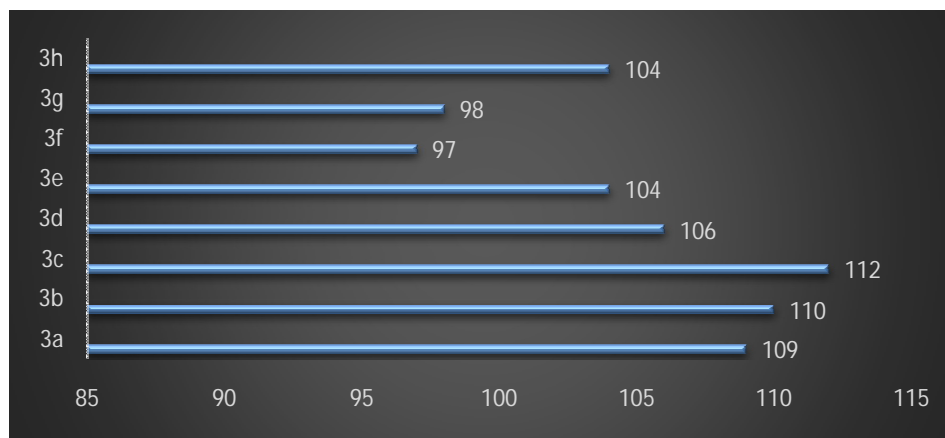


Fig. 4. IC 50 value of Compounds 2 & 3(a-h)

Table 4. Physical data of compounds 3(a-h)

Compound	Molecular formula	Molecular weight	M.P (°C)
3a	$C_{15}H_{15}N_3O_2S_2$	333.42	235-236

3b		$C_{15}H_{15}N_3O_3S$	317.36	250-253
3c		$C_{17}H_{16}N_4O_4S$	372.40	296-298
3d		$C_{17}H_{16}ClN_3O_2S$	361.84	280-282
3e		$C_{17}H_{17}N_3O_2S$	327.40	230-232
3f		$C_{17}H_{16}ClN_3O_2S$	361.84	285-287
3g		$C_{17}H_{16}BrN_3O_2S$	406.30	298-299
3h		$C_{17}H_{16}N_4O_4S$	372.40	280-281

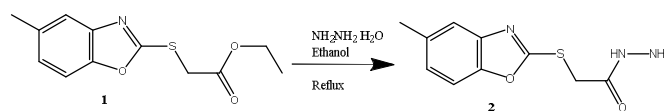


Fig. 5. Synthetic route for the synthesis of Compound 2

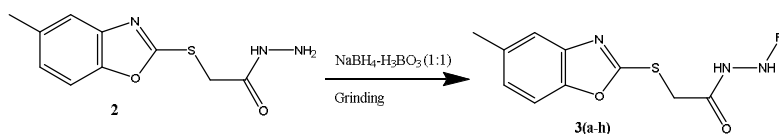


Fig. 6. Synthetic route for the synthesis of Compounds 3(a-h)

3. RESULTS AND DISCUSSION

3.1 Chemistry

2-[(5-Methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide derivatives were prepared from solvent free reductive amination reaction with different aldehydes. The compound Methyl-[(5-methyl-1,3-benzoxazol-2-yl) sulfanyl] acetate 1 was used to prepare [9], further the target molecules 2(a-h) were synthesized using the intermediate 2-[(5-methyl-1,3-benzoxazol-2-

yl)sulfanyl] acetohydrazide 2. The compound 1 was treated with hydrazine hydrate (0.22 mL, 0.0047 mol, 1.2 eq). The reaction mixture was stirred under reflux in absolute alcohol for 4 hour. Progress of the reaction was confirmed by TLC (Chloroform: Methanol in 7:3 ratio). Reaction mixture was then poured to ice water to obtain compound 2.

The compounds 2(a-h) were prepared by treating 2-[(5-methyl-1,3-benzoxazol-2-yl)sulfanyl] acetohydrazide amine (1mmol) with different substituted aldehydes (1 mmol) for 15 to 20 minutes in an agate mortar and pestle at room temperature (25°C) under solvent free conditions. To the resulting mixture was added 1:1 ratio of Sodium borohydride and boric acid was added and then the mixture was ground for 20-30 minutes until TLC showed complete disappearance of the aldehyde. The reaction mixture was washed with water and further purified by recrystallization.

In the ¹H NMR of 2a, the disappearance of -NH₂ proton and appearance of -NH supported the formation of product. The mass peak of compounds 3a showed at M+ 333 and for compound 3b M+ 317 which matches their molecular weights. The construction of compounds 3(c-h) followed a similar method of preparation.

The compounds 3(a-h) were screened for antimicrobial and antitumor activities. The compounds 3a, 3b and 3g Showed highest degree of inhibition against *A. flavus* and compounds 3a, 3b, 3d and 3h Showed highest degree of inhibition against *C. albicans*, compounds 3a,3e and 3g has shown the very good antibacterial activity against *E. coli* and compounds 3a, 3b and 3h shows remarkable activity against *B. subtilis*. Compounds 3a, 3b and 3c exhibited potential antitumor activity.

4. CONCLUSION

The overall study reports the synthesis of different compounds via solvent free methods in good yield. This method is functionally simple and is specially free from the use of toxic metals, solvents and other oxidants. The target molecules were characterized and confirmed by mass, IR, ¹H NMR and ¹³C NMR analysis and screened for antitumor and antimicrobial activities. The expected target compounds were prepared, structurally confirmed by IR, ¹H NMR, ¹³C NMR and mass spectral analysis and *in-vitro* screened for their biological activities. The data described here indicates that compound 3a, 3b and 3g has developed as potentially active compounds. These molecules have shown significant outcomes as compared to standard drug and considered as potential molecules for further study.

ACKNOWLEDGEMENTS

The authors are grateful to the Department of Chemistry, Kuvempu University and Principal, Sahyadri Science College, Shivamogga for providing the necessary research facilities. We are also grateful to Sophisticated Analytical Instruments and biological activities facility, Mysore University, Karnataka India, SAIF Dharwad for providing ¹H NMR, ¹³C NMR and Mass spectral facilities.

References

1. D. M. Livermore. Antibiotic resistance in staphylococci. International Journal of Antimicrobial Agents. 2000;16:S3-S10.
2. McKee M L, Kerwin S M. Synthesis, Metal Ion Binding, and Biological Evaluation of New Anticancer 2-(2'-Hydroxyphenyl)benzoxazole Analogs of UK-1. BioorgMedChem. 2008;16:1775-1783.

3. Mohamed A. Abdelgawad. New Benzoxazole Derivatives as Antiprotozoal Agents: In Silico Studies, Synthesis and Biological Evaluation. *Hindawi Journal of Chemistry*. 2021.
4. Dadmal T L, Appalanaidu, Killari, Kumbhare R M, Mondal T, Ramaiah M J & Bhadra, Manika Pal. Synthesis and biological evaluation of triazole and isoxazole tagged Benzothiazole/Benzoxazole derivatives as potent cytotoxic agents. *New J. Chem.* 2018;42:15546-15551.
5. Ravikumar P, Raoji G S B, Venkata Sastry K, Kalidasua S, Balaaraju T. Design, Synthesis, and Anticancer Evaluation of Tetrazole-Fused Benzoxazole Derivatives as Tubulin Binding Agents. *Russ J Gen Chem.* 2018;88(10):2183–218.
6. Maruthamuthu, Shameela Rajam. The chemistry and biological significance of imidazole, benzimidazole, benzoxazole, tetrazole and quinazolinone nucleus. *Journal of Chemical and Pharmaceutical Research.* 2016;8(5):505-526.
7. Chanda K, Rajasekhar S, Maiti B A. Decade Update on Benzoxazoles, a Privileged Scaffold in Synthetic Organic Chemistry. *Synlett.* 2017;28:521–541.
8. Taylor AP, Robinson RP, Fobian, YM Blakemore, DC Jones, L Hand Fadeyi O. Modern Advances in Heterocyclic Chemistry in Drug Discovery. *Organic & Biomolecular Chemistry.* 2016;14: 6611-6637.
9. Laliteshwar P Singh, Viney Chawla, Pooja Chawla and Shailendra K Saraf. Synthesis and antimicrobial activity of some 2-phenyl-benzoxazole derivatives. *Der Pharma Chemica.* 2010;2(4):206-212.
10. Lumenschein GR, Jr Smit EF, Planchard D. A randomized phase II study of the MEK1/MEK2 inhibitor trametinib (GSK1120212) compared with docetaxel in KRAS-mutant advanced non-small-cell lung cancer (NSCLC) dagger. *Ann Oncol.* 2015;26:894–901.
11. R Surendra Kumar, Ibrahim A Arif, Anis Ahamed, Akbar Idhayadhulla. Anti-inflammatory and antimicrobial activities of novel pyrazole analogues. *Saudi J Biol Sci.* 2016;23(5):614-20.
12. Gurmeet Kaur P, Balamurugan, Sahana Vasudevan, Saikiran Jadav and SA Princy. Antimicrobial and Antibiofilm Potential of Acyclic Amines and Diamines against Multi-Drug Resistant *Staphylococcus aureus*. *Fmicb.* 2017;8:1767.
13. Raghunath B. Toche, Ravindra A, Janaro. Synthesis, Characterization and antimicrobial evaluation of novel urea, sulfonamide and acetamide 3,4-dihydropyrazino[1,2- α]indol-1(2H)-one derivatives. *Arabian journal of chemistry.* 2015;12(8):1-11.
14. Azizian J, Torabi P, Noei J. Synthesis of benzimidazoles and benzoxazoles using $TiCl_3OTf$ in ethanol at room temperature. *Tetrahedron Lett.* 2016;57:185–188.
15. Jayanna ND, Vagdevi HM, Dharshan, JC, Prashith Kekuda TR, Hanumanthappa BC, Gowdarshivannanavar BC. Synthesis and Biological Evaluation of Novel 5,7-Dichloro-1,3-benzoxazole Derivatives. *Journal of Chemistry Hindawi.* 2013; 1-9.
16. Naruti Longkumer, Kikoleho Richa, Rituparna Karmaker, Visekhonuo Kuotsu. Green Synthesis of Bromo Organic Molecules and Investigations on Their Antibacterial Properties: An Experimental and Computational Approach. *Acta Chim. Slov.* 2019;66: 276–283.
17. RV Satyendra, KA Vishnumurthy, HM Vagdevi, KP Rajesh, H Manjunatha, A Shruthi. Synthesis, in vitro antioxidant, anthelmintic and molecular docking studies of novel dichloro substituted benzoxazole-triazolo-thione derivatives. *Eur J Med Chem.* 2011; 46: 3078-3084.
18. Mohd Amir, Kumar Shikha. Synthesis and anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities of some new 2-[(2,6-dichloroanilino) phenyl]acetic acid derivatives. *European Journal of Medicinal Chemistry.* 2004;39:535–545.
19. Dinesh R Godhani, Anand A Jogel, Anil M, Sanghani and Jignasu P Mehta. Thermal study of synthesized 1,2,4-triazole compounds and their kinetic parameter evaluation. *Journal of Chemical and Pharmaceutical Research,* 2014;6(6):1034-1041.

20. Abdelgawad M. A, Bakr R B, Ahmad W, Al-Sanea, M M, & Elshemy, HA H. New pyrimidine-benzoxazole/benzimidazole hybrids: Synthesis, Antioxidant, Cytotoxic Activity, in vitro Cyclooxygenase, and phospholipaseA2-V Inhibition. *Bioorganic Chemistry*, 2019;92:103218.
21. Mahbubur Rahman MD, Badrul Islam MD, Mohitosh Biswas, Khurshid Alam AHM. In vitro antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Research Notes*.2015;8:621
22. The Thai Nguyen, Xuan-Trang Thi Nguyen, Thuy-Linh Ho Nguyen, and Phuong Hoang Tran. Synthesis of Benzoxazoles, Benzimidazoles, and Benzothiazoles Using a Bronsted Acidic Ionic Liquid Gel as an Efficient Heterogeneous Catalyst under a Solvent-Free Condition. *ACS Omega* 2019;4;368–373.
23. Ingle V, Gorepati P, Mane Y. Samarium (III) Triflate as an Efficient and Reusable Catalyst for Facile Synthesis of Benzoxazoles and Benzothiazoles in Aqueous Medium. *Synlett*. 2013;24:2241–2244.
24. Chauhan PMS, Martins CJA, Horwell DC. Syntheses of novel heterocycles as anticancer agents. *Bioorg Med Chem*. 2005;13:3513–3518.
25. Therese Horsch, Evelyn M. Molloy, Florian Bredy, Veit G. Haensch, Kirstin Scherlach, Kyle L. Dunbar, Jonathan Franke, and Christian Hertweck. Alternative Benzoxazole Assembly Discovered in Anaerobic Bacteria Provides Access to Privileged Heterocyclic Scaffold. *Angew. Chem*. 2022;134:1521-3757.
26. Jiatao Yu and Ming Lu. [C12mim]Br: A Temperature-dependent Phase Transfer Catalyst and Its Application for Aerobic Oxidative Synthesis of 2-Aryl Benzimidazoles, Benzoxazoles or Benzothiazoles Catalyzed by TEMPO Based Ionic Liquid. *J Chin. Chem. Soc*. 2014;61:578-582.
27. Ajay BN, Govindasamy S. Synthesis of benzoxazoles by an efficient Ullmann-type intramolecular C(aryl)-O bond-forming coupling cyclization with a BINAM-copper(II) catalyst. *Synthesis*. 2010;4:579–586.
28. Easmon J, Purstinger G, Thies KS, Heinisch G, Hofmann J. Synthesis, structure-activity relationships, and antitumor studies of 2-benzoxazolyl hydrazones derived from alpha-(N)-acyl heteroaromatics. *J Med Chem*. 2006;49:6343–6350.
29. P K Jauhari, A. Bhavani, Subhash Varalwar, Kiran Singhal, Prem Raj. Synthesis of some novel 2-substituted benzoxazoles as anticancer, antifungal, and antimicrobial agents. *Med Chem Res*. 2008;17:412–424.
30. Vinsova J, Horak V, Buchta V, Kaustova J. Highly lipophilic benzoxazoles with potential antibacterial activity. *Molecules*. 2005;10:783–793.
31. Neelima D Tangellamudia, Suchita B Shindea, Venkatesh Pooladandab, Chandraiah Godugub, Sridhar Balasubramanian. Facile synthesis of 2-aryl 5-hydroxy benzo[d]oxazoles and their in vitro antiproliferative effects on various cancer cell lines. *Bioorganic & Medicinal Chemistry Letters*. 2018;28:3639–3647.
32. El Helby A A, Sakr H, Eissa I, H Abulkhair, H Al-Karmalawy, A A & El Adl K. Design, synthesis, molecular docking, and anticancer activity of benzoxazole derivatives as VEGFR-2 inhibitors. *Archiv Der Pharmazie*. 2019;10:352.
33. Cheng-Juan Wu, Xin-Yu Li, Ting-Rui Li, et al. Natural Sunlight Photocatalytic Synthesis of Benzoxazole-Bridged Covalent Organic Framework for Photocatalysis. *J. Am. Chem. Soc*. 2022;144:41;18750–18755.
34. Gajanan S. Shanbhan, Amit Bhargavagiridhar, Pal Singh Shrinivas D. Joshinarendra Chundawat, Synthesis, molecular simulation studies, in vitro biological assessment of 2-substituted benzoxazole derivatives as promising antimicrobial agents. *Turkish Journal of Chemistry*: 2023; 47:263-279.