Original Research Article

Synthesis of novel 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine derivatives and *In-Vitro* evaluation of their antitumor and antibacterial activities.

ABSTRACT

Benzoxazole is one of the biologically important heterocyclic compounds, which shows remarkable pharmacological activities. In this work, 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine derivatives have been synthesized under solvent free condition. Structures of the synthesized compounds have been confirmed through IR, ^{1}H NMR, ^{13}C NMR and Mass spectral methods. All the compounds were assessed for their antitumor and antibacterial activities. 6h has found remarkable activity on both antitumor and antibacterial studies and 6g and 6a exhibited promising antibacterial and antitumor activity.

Keywords: Benzoxazole; Solvent free condition; Antitumor; Antibacterial.

1.INTRODUCTION

Benzoxazoles are found in important classes of bioactive compounds like antibacterial antibiotic, antistress, antitumor, anticancer, antioxidant, cyclooxygenase inhibitory, antifungal, 5HT 3 receptor antagonists, anticonvulsant, antiviral, antidepressant, antiulcer, anti-HIV-1, antitubercular, insecticidal and anti-inflammatory [1-6]. On the other hand, amine derivatives have wide range of biological activities such as antiviral, anti-inflammatory, antitumorandanti- bacterial [7].

Among many diseases, Cancer is the leading disease cause number of deaths worldwide [14]. Scientists develop more selective, effective, and safe drugs every day [15]. By reviewing the literature, we found that benzoxazole derivatives have important biological activities mainly antitumor [8–10] and antibacterial [11].

Based on the structure ofbenzoxazoles and reported derivatives [12-16], we designed the reactions and evaluated their antitumor activity against HepG2 and antimicrobial activity against *S. aureus, Bacillus subtilis*, Gram-negative *E. coli* and *Salmonella typhi* and*Shigella.*

2.METHODOLOGY

2.1 Materials and Methods

Melting points were recorded on electro thermal melting point apparatus and 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 andspots located by UV and iodine chamber. All the chemicals used in this were purchased from Sigma-Aldrich and SD Fine. IRspectra were recorded using KBr pellets on a Perkin Elmer Spectrophotometer. ^{1}H-

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 5-methyl-1,3-benzoxazole-2-thiol (2)

2-Amino-4-methylphenol (1 g, 0.008mol, 1 eq) was reacted with KOH (0.54 g, 0.009 mol, 1.2 eq) in presence of methanol (50 mL) and stirred for 10 minute followed by the slow addition of CS_2 (0.64 mL, 0.009 mol, 1.2 eq) at room temperature. The reaction mass was refluxed for 6 h on water bath. The completion of the reaction was confirmed by TLC (Hexane: Ethyl acetate 9:1). Reaction mass was poured onto ice cold water and acidified with glacial acetic acid (pH 6). The obtained solid was filtered, dried, and recrystallized using ethanol.
Brown colour solid, yield (90%) MP (214-216˚C); MS (m/z): 166 (M+), 167 $(M+1)^+$; IR (KBr, ucm^{-1}): 3380; ^{1}H NMR (400 MH, DMSO) δ: 2.52 (s, 3H), 7.1-7.4 (m, 3H), 13.8 (br, SH); ^{13}C NMR (DMSO): δ 21 (CH_3), 110 – 135 (aromatic carbons), 146 (C=N).

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

5-Methyl-1,3-benzoxazole-2-thiol 2 (2 g, 0.012 mol, 1 eq) was refluxed for 5 hours with ethyl chloroacetate (1.48 g, 0.012 mol, 1 eq) in presence of anhydrous potassium carbonate (6.02 g, 0.036 mol, 3 eq) in dry acetone, the progress of the reaction was monitored by TLC (Hexane: Ethyl acetate in 9:1 ratio). Reaction mixture was then poured onto ice water to get the pure crystals of 3.
Dark brown colour solid, yield (85%), MP (85-88 ˚C); IR (KBr, ucm^{-1}): 1591 (C=N), 1710 (C=O); ^{1}H NMR (400 MH, DMSO) δ: 1.2 (t, 3H), 2.415-2.519 (s, 3H,), 3.3 (s, 2H), 4.3 (q, 2H).7.1-7.5 (m, 3H); ^{13}C NMR (DMSO): δ 14 (CH3), 21 (CH3), 34 (-O-CH2), 62 (S-CH2), 110 – 141 (aromatic carbons), 150 (CH_2CO), 168 (-C=O); MS (m/z): 252 (M+), 253 $(M+1)^+$;

2.1.3 Synthesis of 2-hydrazinyl-5-methyl-1,3-benzoxazole (4)

Methyl-[(5-methyl-1,3-benzoxazol-2-yl) sulfanyl] acetate 3 (1 g, 0.0039 mol, 1eq) was added to ethanol (50 mL) with stirring to dissolve and hydrazine hydrate (0.22 mL, 0.0047 mol, 1.2 eq). The reaction mixture was kept stirring for 3 hours and progress of the reaction was confirmed by TLC (Chloroform: Methanol in 7: 3 ratio). Reaction mixture was then poured into ice water to obtain compound 4.
White solid; yield (92%), MP (166-168 ˚C), 167 $(M+1)^+$; IR (KBr, ucm^{-1}): 1592 (C=N), 3330 (N-H); 1H NMR (400 MH, DMSO) δ: 2.4 (s, 3H), 4.03 (br, 2H, NH2), 7.07-7.48 (m, 3H), 9.4 (br, 1H, NH) (D_2O exchangeable); ^{13}C NMR (DMSO): δ 21 (CH_3), 110 – 135 (aromatic carbons), 146 (C=N);MS (m/z): 166 (M+)

2.1.4 Synthesis of 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine (5)

The reaction of 2-hydrazinyl-5-methyl-1,3-benzoxazole 4 (1g, 0.006 mol, 1 eq) with 3-aminocrotonitrile (0.503 g, 0.006 mol, 1eq) was taken in a refluxing ethanol (50 mL) in the presence of sodium acetate monohydrate (0.50g, 0.006 mol, 1eq), the progress of the reaction was confirmed by TLC (Hexane: Ethyl acetate 7: 3 ratio), the reaction mixture was then poured onto ice water, filtered and washed with cold water. The product 5 was recrystallized by ethanol.
Orange colour solid, yield (90%); IR (KBr, ucm^{-1}):3440, 3350(-NH str.), 1610 (-NH bend), 1280 (C-N str.), 760 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ: 2.44-2.52 (s, 3H), 3.32 (s, 3H), 5.3 (s, 1H), 6.1 (br, 2H, NH_2 D_2O exchangeable) 7.16-7.62 (m, 3H), ^{13}C NMR (DMSO): 14 (CH_3), 21 (CH_3), 88 (-C- NH_2), δ 110 – 155 (Aromatic carbons) ;MS (m/z): 229 (M^+), 230 $(M+1)^+$.

2.1.5 General procedure for the synthesis of 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine derivatives 6(a-h).

A 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine 5 (1mmol) was ground with substituted aldehyde (1 mmol) for 15 to 20 minutes in an agate mortar and pestle at room temperature under solvent free conditions.The resulting mixture was added p-toluene sulfonic acid(1mmol). 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.

2.1.6 3-Methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-*N*-(thiophen-2-ylmethyl)-1*H*-pyrazol-5-amine (6a)

Light pink colour solid, yield (91%); IR (cm^{-1}): 1176 (C-N str.), 621 (-NH wag.);^{1}H NMR (400 MH, DMSO) δ: 2.10 (s, 3H), 2.38 (s, 3H), 5.6 (s, 1H), 6.10 (s, 2H), 7.01-7.58 (Ar, 6H); ^{13}C NMR (DMSO): δ 13.42 (CH_3), 21.46 (CH_3), 30.15(CH_2), 100-154 (Aromatic carbons); MS (m/z): 324(M^+);

2.1.7 4-({[3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1*H*-pyrazol-5-yl] amino} methyl) phenol (6b)

Orange colour solid, yield (93%); IR (cm^{-1}):1153 (C-N str.), 622 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ: 2.22 (s, 3H), 2.43 (s, 3H), 5.1 (s, 1H), 5.55 (s, 2H), 6.84 - 7.39(Ar, 7H);^{13}C NMR (DMSO): δ 16.83 (1C, CH_3-C), 25.72 (1C, CH_3-C), 33.92(1C, CH_2-C),104-159 (16C-Ar-C); MS (m/z): 334(M^+)

***2.1.8 N*-(furan-2-ylmethyl)-3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1*H*-pyrazol-5-amine (6c)**

Brown colour solid, yield (85%); IR (cm^{-1}): 1153 (C-N str.), 622 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ: 2.39 (s, 3H), 2.47 (s, 3H), 5.38 (s, 1H), 6.11 (s, 2H), 7.10-7.66 (Ar-6H);^{13}C NMR (DMSO) δ: 13.15 (1C, CH_3-C), 21.46 (1C, CH_3-C), 28.94 (1C, CH_2-C), 98-154 (Aromatic carbons); MS (m/z): 308 (M^+).

***2.1.9 N*-(4-chlorobenzyl)-3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1*H*-pyrazol-5-amine (6d)**

Light brown colour solid, yield (87%); IR (cm^{-1}): 1150 (C-N str.), 622 (-NH wag.);^{1}H NMR (400 MH, DMSO) δ: 2.41 (s, 3H), 2.42 (s, 3H), 5.12(s, 1H), 5.58(s, 2H), 6.84 (Ar-2H), 7.06-7.15 (Ar-3H), 7.17-7.33(Ar-6H);^{13}C NMR (DMSO) δ :12.86 (1C, CH_3-C), 21.60 (1C, CH_3-C), 34.11 (1C, CH_2-C), 100-154 (Aromatic carbons); MS (m/z): 352 (M^+).

***2.1.10 N*-benzyl-3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1*H*-pyrazol-5-amine (6e)**

Pink colour solid, yield (81%); IR (cm^{-1}): 1161 (C-N str.), 622 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ: 2.42 (s, 6H), 5.17(s, 1H), 5.98(s, 2H), 6.84 (dd, Ar-2H), 7.24-7.28 (dd, Ar-1H), 7.27-7.38(dd, Ar-7H);^{13}C NMR (DMSO)δ :12.90 (1C, CH_3-C), 21.60 (1C, CH_3-C), 34.49 (1C, CH_2-C), 100-154 (Aromatic carbons); MS (m/z): 318 (M^+).

***2.1.11 N*-(3,4,5-trimethoxybenzyl)-3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1*H*-pyrazol-5-amine(6f)**

White colour solid, yield (88%);IR (cm^{-1}): 1161 (C-N str.), 622 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ:2.29 (s, 3H), 2.35(s, 3H), 3.71 (s, 3H), 3.78 (s, 3H), 3.93 (s, 3H) 5.01(s, 1H), 5.61(s, 2H), 6.61 (s, Ar-2H), 7.06-7.08 (dd, Ar-1H), 7.12 (s, Ar-1H), 7.410(s, Ar-1H);^{13}C NMR (DMSO)δ :12.65 (1C, CH_3-C), 21.47 (1C, CH_3-C), 35.32 (1C, CH_2-C), 56.00 (2C, O-CH_3), 60.00 (1C, O-CH_3) 100-154 (Aromatic carbons); MS (m/z):408 (M^+).

2.1.12 3-Methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-*N*-(2-nitrobenzyl)-1*H*-pyrazol-5-amine (6g)

Yellow color solid, yield (83%); IR (cm^{-1}): 1161 (C-N str.), 622 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ: 2.41 (s, 3H), 2.42 (s, 3H), 5.12(s, 1H), 5.58(s, 2H), 6.84 (dd, Ar-2H), 7.06-7.15 (dd, Ar-3H), 7.17-7.33(dd, Ar-6H); ^{13}C NMR (DMSO) δ :12.86 (1C, CH_3-C), 21.60 (1C, CH_3-C), 34.11 (1C, CH_2-C), 100-154 (Aromatic carbons); MS (m/z): 363 (M^+).

2.1.13 3-Methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-*N*-(3-nitrobenzyl)-1*H*-pyrazol-5-amine (6h)

Yellow colour solid, yield (82%);IR (cm^{-1}): 1161 (C-N str.), 622 (-NH wag.); ^{1}H NMR (400 MH, DMSO) δ: 2.41 (s, 3H), 2.42 (s, 3H), 5.12(s, 1H), 5.58(s, 2H), 6.84 (dd, Ar-2H), 7.06-7.15 (dd, Ar-3H), 7.17-7.33(dd, Ar-6H); ^{13}C NMR (DMSO) δ :12.86 (1C, CH_3-C), 21.60 (1C, CH_3-C), 34.11 (1C, CH_2-C), 100-154 (Aromatic carbons);MS (m/z): 363 (M^+).

2.2 Biological Activities

2.2.1 Antibacterial Activity

Test bacterial cultures were procured from Microbial Type Culture Collection (MTCC) of Institute of Microbial Technology, Chandigarh. Cultures of Gram-positive bacteria *S. aureus* (MTCC 7443), *subtilis*(MTCC 121) and Gram-negative *E. coli* (MTCC 7410) and *Salmonella typhi* (MTCC 733) *Shigella*(MTCC 1457) were grown on nutrient agar media used for antibacterial activity assay [17-20]**.** Antibacterial activity of chemical compounds 5 and 6(a – g) was tested for dual-culture agar diffusion assay (Nostro *et al*. 2000)with some modifications. Petri dishes were prepared by pouring 20 ml of sterilized Nutrient agar media under aseptic condition and allowed to solidify. After solidification of the media, 100 µl of standardized test microbial inoculum of Gram-positive bacteria *S. aureus*, *B. subtilis* and Gram-negative bacteria *E. coli, Shigella* and*. typhi*, were spread uniformly using glass loop. Different concentration 1 mg/ml and 0.5 mg/ml were tested diluting the sample with DMSO were added to plates for diffusion of antibacterial compounds, thereafter plates were incubated at 37 ºC for 24 h. The diameter of the inhibition zone around the discs is measured in millimeters (mm) and the average of three repeated agar discs were taken to assess the strength of antibacterial activity.

Comment [a1]: ??????

Comment [a2]: ??????

Comment [a3]: Unify the method of referring to scientific sources.

Comment [a4]: ?????

2.2.2 Antitumor Activity

The antitumor activities 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 (5×10^4 cells/well) and incubated at 37 °C with series of different concentrations, in DMSO, of 5 and 6(a –h) compounds for 48 h in a serum-free medium prior to the MTT assay[20-22]. 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 solubilized by the addition of 200 µL of DMSO. The absorbance was measured at 570 nm using a multi-Mode micro plate reader analyzed 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. IC50s were determined.

Table 1. Antibacterial activity (Zone of Inhibition at 1 mg/ ml)

Sl no	Compounds	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*	*Shigella*
1	5	4 mm	5 mm	6 mm	8 mm	6 mm
2	6a	13 mm	15 mm	16 mm	16 mm	16 mm
3	6b	-	-	-	-	-
4	6c	-	-	-	-	-
5	6d	-	-	-	-	-
6	6e	4 mm	5 mm	6 mm	8 mm	6 mm
7	6f	-	-	-	-	-
8	6g	13 mm	15 mm	16 mm	16 mm	16 mm
9	6h	13 mm	14 mm	16 mm	16 mm	16 mm

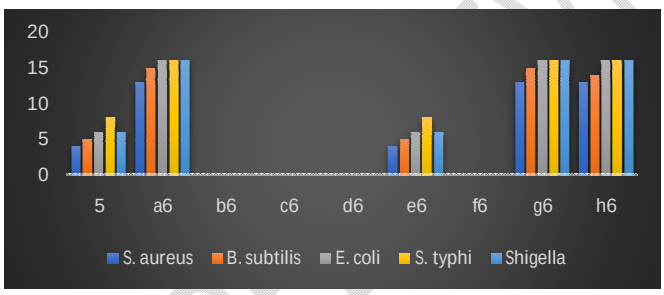


Fig. 1. Antibacterial activity (Zone of Inhibition at 1 mg/ ml)

Table 2. Antibacterial activity (Zone of Inhibition at 0.5 mg/ ml)

Sl no	Compounds	*S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*	*Shigella*
1	5	-	-	-	-	-
2	6a	9 mm	9 mm	12 mm	12 mm	12 mm
3	6b	-	-	-	-	-
4	6c	-	-	-	-	-
5	6d	-	-	-	-	-
6	6e	-	-	-	-	-
7	6f	-	-	-	-	-
8	6g	9 mm	9 mm	12 mm	12 mm	12 mm
9	6h	9 mm	9 mm	12 mm	12 mm	12 mm

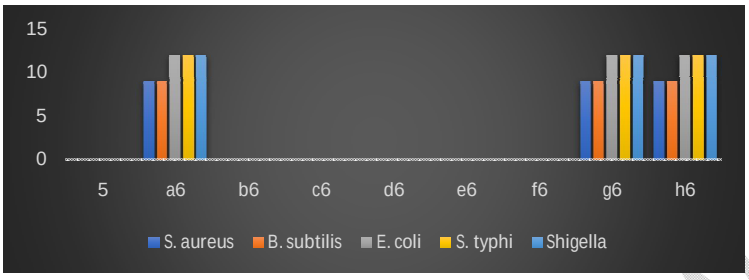


Fig. 2. Antibacterial activity (Zone of Inhibition at 0.5 mg/ ml)

Table 3. Antitumor activity

Sl No.	Compound Name	% Of inhibition	IC 50 Value
1	5	35.6 %	72.6
2	6a	52.20%	99.10
3	6b	19.98 %	41.44
4	6c	22.96 %	43.73
5	6d	24.62 %	48.14
6	6e	25.69%	55.67
7	6f	33.14 %	61.32
8	6g	68. 26%	114.24
9	6h	72. 39 %	124.61

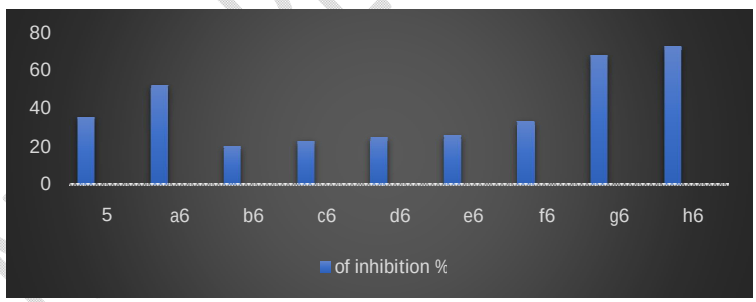


Fig. 3. Antitumor activity

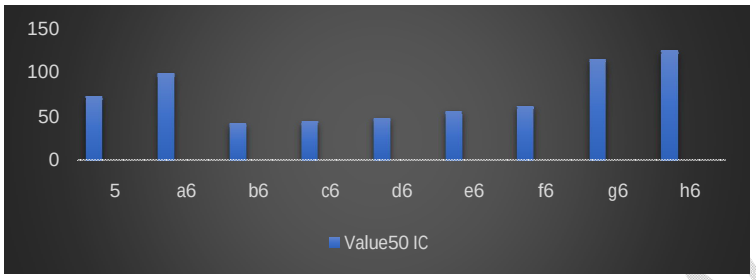
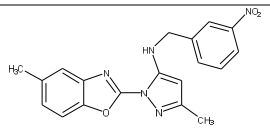


Fig. 4. IC 50 Value of compounds 5 & 6(a-h)

Table 4. Physical data of compounds 6 (a-h)

Compound	Molecular formula	Structure	Molecular weight	M.P (°C)	Yield (%)
6a	$C_{17}H_{16}N_4OS$		324.10	238-240	91
6b	$C_{19}H_{18}N_4O_2$		334.14	250-252	93
6c	$C_{17}H_{16}N_4O_2$		308.13	241-243	85
6d	$C_{19}H_{17}ClN_4O$		352.82	247-248	87
6e	$C_{19}H_{18}N_4O$		318.38	210-212	81
6f	$C_{22}H_{24}N_4O_4$		408.18	280-282	88
6g	$C_{19}H_{17}N_5O_3$		363.38	239-240	83

6h	$C_{19}H_{17}N_5O_3$		363.38	242-243	82

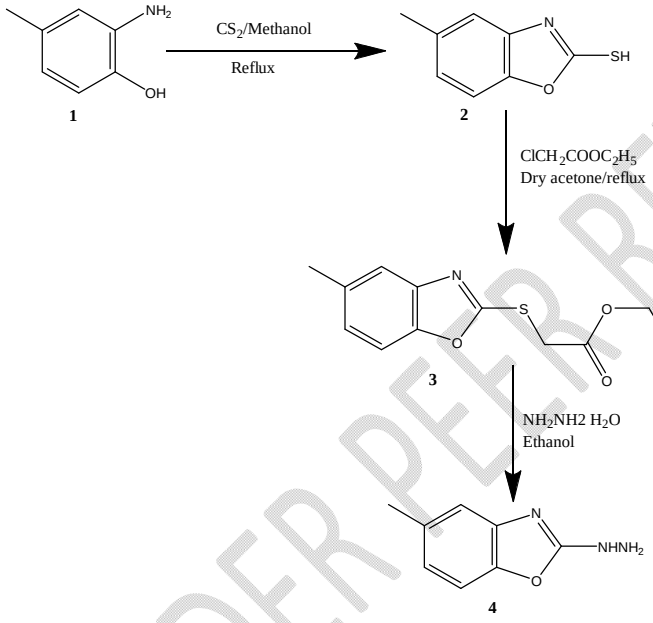


Fig. 5. Synthetic route for the synthesis of Compound 4

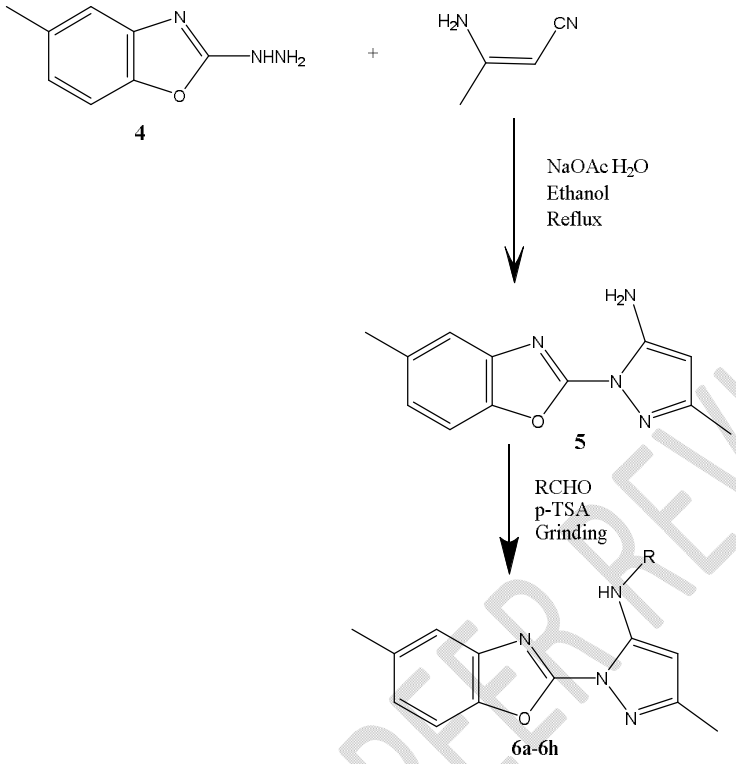


Fig. 6. Synthetic route for the synthesis of Compounds 6(a-h)

3. RESULTS AND DISCUSSIONS

3.1 Chemistry

Eight3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine derivatives were prepared from solvent free reductive amination reaction with selected aromatic aldehydes. The compound 2-hydrazinyl-5-methyl-1,3-benzoxazole 1 was prepared [9], further the target molecules 6(a-h) were synthesized using the intermediate 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine2. The compound 1 was treated with 3-aminocrotonitrile (0.503 g, 0.006 mol, 1eq) was carried out in refluxing ethanol (50 mL) in the presence of sodium acetate monohydrate (0.50g, 0.006 mol, 1eq) to get the intermediate compound 2. It is characterized by IR, 1H NMR and Mass analysis.

The compound 2 was treated with different aldehydes to get target molecules 6a-6h and confirmed by spectral analysis. The compound 2 was reacted with NaBH4/p-TSA to form respective compounds 6a and 6h. In the 1H NMR of 6a, the disappearance of -NH2 proton and appearance of -NH supported the formation of product. The mass peak of compounds 6a shows at M+ 324 and for compound 6b M+ 334 which is matching with their molecular weights. The construction of compounds 6(a-h)followed a similar method of preparation.

3.2 Biological Activity

The compounds 6(a-h)were screened for antibacterial and antitumor. In the antibacterial study, the compounds have shown inhibition of tested bacteria (Table 1). Among the synthesized compounds 6a, 6g and 6h showed very high zone of inhibition, while less activity was observed to the compounds 6e. The compound 6h is very good compound having potential of both antitumor and antibacterial activity and 6g and 6a exhibited promising antitumor activity.

4. CONCLUSION

This overall study reports the synthesis of different compoundsvia solvent free methods in good yield. The target molecule were characterized and confirmed by mass, IR, ^{1}HNMR and ^{13}CNMR analysis and screened for antitumor and antibacterial activities. Compound 6h is very good compound have potential of both antitumor and antibacterial activity and compound 6g and 6a are having promising activity, so the derivatives of 3-methyl-1-(5-methyl-1,3-benzoxazol-2-yl)-1H-pyrazol-5-amine were found to be biologically potent heterocyclic compound.

References

1. Mohamed A. Abdelgawad.New Benzoxazole Derivatives as Antiprotozoal Agents: In Silico Studies, Synthesisand Biological Evaluation. Hindawi Journal of Chemistry. 2021.
2. VirendraRMishra, ChaitannyaW.Ghanavatkara, Suraj NMali, Shahnawaz I, Qureshi, Hemchandra KChaudhari, NagaiyanSekara. Design, synthesis, antimicrobial activity and computational studies of novel azo linked substituted benzimidazole, benzoxazole and benzothiazole derivatives.2019;78:330-337.
3. 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.
4. Taylor AP, Robinson RP, Fobian, YM Blakemore, DC Jones, LHand Fadeyi O. Modern Advances in Heterocyclic Chemistry in Drug Discovery. Organic & Biomolecular Chemistry. 2016;14: 6611-6637.
5. R Surendra Kumar, Ibrahim A Arif , AnisAhamed , Akbar Idhayadhulla.Anti-inflammatory and antimicrobial activities of novel pyrazole analogues.Saudi J Biol Sci. 2016;23(5):614-20.
6. AielloS, Wells G, Stone EL, Kadri H, BazziR, Bell DR, Stevens MFG, MatthewsCS, Bradshaw TD, Westwell AD. Synthesis and Biological Properties of Benzothiazole, Benzoxazole, and Chromen-4-one Analogues of the Potent AntitumorAgent 2- (3, 4-Dimethoxyphenyl) -5-fluorobenzothiazole.J. Med. Chem. 2008;51:5135–5139.
7. GurmeetKaur P,Balamurugan, SahanaVasudevan, SaikiranJadav and SA PrincyAntimicrobial and Antibiofilm Potential of Acyclic Amines and Diamines against Multi-Drug Resistant Staphylococcus aureus.Fmicb.2017;8:1767.
8. 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.
9. Jayanna ND, Vagdevi HM, Dharshan, JC, PrashithKekuda 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.
10. Wang Y, Wang Y, Tao T, Qian H, Huang W. Structural and spectral comparisons between isomeric benzisothiazole and benzothiazole based aromatic heterocyclic dyes. J. Mol. Struct. 2015;1095:42–50.
11. 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.
12. 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.
13. Liu H, Gao W, Kumar V, Tangadanchu R, Zhou C, Geng R, Novel aminopyrimidinylbenzimidazoles as potentially antimicrobial agents: Design, synthesis andbiological evaluation. Eur. J. Med. Chem. 2018;143:66–84.
14. Demmer, CS, BunchL. European Journal of Medicinal Chemistry Benzoxazoles and oxazolopyridines in medicinal chemistry studies. Eur. J. Med. Chem.2015; 97:778–785.
15. SuramDurgamma, AkkarapalliMuralikrishna, VenkatapuramPadmavathi, AdivireddyPadmaja.Synthesis and antioxidant activity of amido-linked benzoxazolyl/benzothiazolyl/benzimidazolyl-pyrroles and pyrazoles. Med Chem Res. 2014;23:2916–2929.
16. Ajay BN, Govindasamy S. Synthesis of benzoxazoles by an efficient Ullmann-type intramolecular C(aryl)–O bond-formingcoupling cyclization with a BINAM-copper(II) catalyst. Synthesis. 2010;4:579–586.
17. 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)-acylheteroaromatics. J Med Chem.2006;49:6343–6350.
18. Temiz-Arpaci O, Ozdemir A, Yalcin I, Yildiz I, Aki-SxenerE,Altanlar N . Synthesis and antimicrobial activity of some 5-[2-(morpholin-4-yl)acetamido] and/or 5-[2-(4-substituted piperazin-1-yl)acetamido]-2-(p-substitutedphenyl)benzoxazoles.Arch Pharm Chem Life Sci.2005;338:105–111.
19. Mahbubur Rahman MD, Badrul Islam MD ,MohitoshBiswas, KhurshidAlam AHM. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC ResearchNotes.2015;8:621
20. The Thai Nguyen, Xuan-Trang Thi Nguyen, Thuy-Linh Ho Nguyen, and Phuong Hoang Tran.Synthesis of Benzoxazoles, Benzimidazoles, and BenzothiazolesUsing a Bronsted Acidic Ionic Liquid Gel as an EfficientHeterogeneous Catalyst under a Solvent-Free Condition.ACS Omega 2019;4;368−373.
21. Ravikumar P, Raolji G S B, VenkataSastry K, Kalidasua S, Balaaraju T Design, Synthesis, and Anticancer Evaluation of Tetrazole-Fused BenzoxazoleDerivatives as Tubulin Binding Agents. Russ J Gen Chem.2018;88(10):2183–218.
22. P K Jauhari,A. Bhavani,SubhashVaralwar, KiranSinghal, Prem Raj.Synthesis of some novel 2-substituted benzoxazoles asanticancer, antifungal, and antimicrobial agents.Med Chem Res. 2008;17:412–424.