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Synthesis, Characterization and Antimicrobial Evaluation of Novel 2-(1,3-Substituted-1*H*-Pyrazol-4-yl)-1*H*-Benzo[*d*]thiazoles

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ABSTRACT

In this paper we reported the synthesis and spectroscopic characterization of 2-(1,3-substituted-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazoles in excellent yield from 2-aminobenzenethiol with a preference of substituted pyrazol aldehydes as a starting material, using ceric ammonium nitrate (CAN) as a catalyst in presence of hydrogen peroxide. The *in vitro* study of antimicrobial activity of newly synthesized 1*H*-benzo[*d*]thiazole molecules by using seven organisms *viz.*, *E.Coli*, *P.Aeruginosa*, *S.Aureus*, *S.Pyogenus* (Bacterial strains), *C.Albicans*, *A.Niger* and *A.Clavatus* (Fungicidal strains) showed specific activity in inhibiting the growth of two Gram negative bacteria (*E.Coli* and *P.Aeruginosa*), two Gram positive bacteria (*S.Aureus* and *S.Pyogenus*) and three fungal strains. The results of antimicrobial studies stripped that the compounds were active against most of the bacterial strains whereas in fungicidal activity the compounds were more active only against *C.Albicans*.

Keywords: 1*H*-Benzo[*d*]thiazoles, Pyrazole aldehydes, 2-aminobenzenethiol, Antibacterial and antifungal activity.

1. INTRODUCTION

The synthesis of compounds containing benzothiazole moiety increased considerable interest because it has great pharmaceutical importance due to the momentous and effective biological activities *viz.*, antitumor, antitubercular, antimalarial, anticonvulsant, anthelmintic, analgesic, anti-inflammatory, antifungal, a topical carbonic anhydrase inhibitor and an antihypoxic [1-4]. The substitutions at C-2 position of benzothiazole sculpt universally results the change of its bioactivity therefore, the 2-substitued benzothiazole compounds involved in research aimed as evaluating new products possessing interesting versatile pharmaceutical activities. All this things regarding the benzothiazole molecules motivated us for synthesis, characterization and antimicrobial screening the novel 2-(1,3-substituted pyrazole)-1*H*-benzo[*d*]thaizole compounds.

2. Experimental

2.1. Materials and Methods

All Reagents and solvents were purchased from Spectrochem and Merck used as received. All melting points were determined in open capillary tube and are uncorrected. Infrared spectra were recorded in KBr on Shimadzu FT-IR 8400 spectrophotometer. The ^{1}H NMR spectra were measured in $CDCl_3$ solutions on a Bruker Av spectrophotometer (400 MHz) using TMS as an internal reference (chemical shifts in δ ppm). The mass spectra were recorded on Shimadzu GC-MS QP2010 Gas Chromatograph. All the synthesized compounds were micro analyzed satisfactorily for C, H and N on a Euro EA Elemental Analyzer, EA-3000, RS-232. TLC was performed on silica gel-G using hexane : ethylacetate (4:1) solvent system.

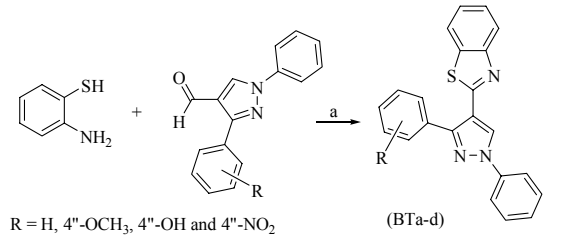
2.2. Method

2.2.1. Synthesis of 1,3-substituted-1*H*-pyrazole-4-carbaldehydes

Syntheses of 1,3-substituted-1*H*-pyrazole-4-carbaldehydes was achieved using previously published method [5-8].

2.2.2. General synthetic procedure for the 2-(1,3-substituted-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazoles

A mixture of 2-aminobenzenethiol (0.01 mol), pyrazole aldehyde (0.01 mol), Ceric ammonium nitrate (CAN) (0.001 mol) and hydrogen peroxide (30 %, 10 ml) in methanol was thoroughly mixed in 100 mL flat bottom flask, which was then refluxed on water bath for 6-8 hours (Scheme 1). The completion of reaction was monitored by TLC (solvent system, ethyl acetate : hexane 1:4). The reaction mixture was cooled at room temperature and poured into crushed ice, separated solid product was collected by suction and washed with cold saturated sodium bisulphite solution and recrystallized with methanol. 69-81 % yield (BTa-d).



[a]Reagents and conditins: Methanol, Hydrogen peroxide, Ceric ammonium nitrate, reflux for 6-8 hours

Scheme 1: Reaction scheme of 2-substituted 1*H*-benzo[*d*]thiazoles (BTa-d)

2.2.2.1. 2-(1′3′-diphenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazole (BTa)

Yield: 81 %, m.p. 180 °C, IR (ν cm^{-1}: KBr): 3147, 3057, 2997, 1629, 1593, 1554, 1496, 1354, 1311 and 702. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 7.34-7.37 (dd, 2H, Ar-H of benzothiazole), 7.45-7.52 (dd, 6H, Ar-H of 1-phenyl pyrazole and 3-phenyl pyrazole), 7.73-7.77 (dd, 4H, Ar-H of 1-phenyl pyrazole and 3-phenyl pyrazole), 7.83-7.85 (d, 1H, Ar-H of benzothiazole), 7.03-7.05 (d, 1H, Ar-H of benzothiazole), 8.78 (s, 1H, pyrazole H). Mass *m/z*: 352. Anal. Cacld. for $C_{22}H_{15}N_3S$; Cacld.: C, 74.76; H, 4.28; N, 11.89; S, 9.07; Found: C, 74.60; H, 4.06; N, 11.74; S, 8.91 %.

2.2.2.2. 2-(3′-(4″-Methoxyphenyl)-1′-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazole (BTb)

Yield: 78%, m.p. 155 °C, IR (ν cm^{-1}, KBr): 3234, 3197, 3093, 3058, 2942, 2847, 1672, 1663, 1614, 1550, 1369, 1312, 1260, 1233, 1052 and 713. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 3.89 (s, 3H, -OCH_3), 7.01-7.04 (d, 2H, Ar-H of 3-phenyl pyrazole), 7.34-7.38 (dd, 2H, Ar-H of 1-phenyl pyrazole), 7.47-7.52 (t, 3H, Ar-H of 1-phenyl pyrazole), 7.64-7.67 (d, 2H, Ar-H of 3-phenyl pyrazole), 7.76-7.78 (d, 1H, Ar-H of benzothiazole), 7.84-7.86 (dd, 2H, Ar-H of benzothiazole), 8.07-8.09 (d, 1H, Ar-H of benzothiazole), 8.95 (s, 1H, pyrazole H). Mass *m/z*: 383. Anal. Cacld. for $C_{23}H_{17}N_3OS$; Cacld.: C, 72.04; H, 4.47; N, 10.96; O, 4.17; S, 8.36; Found: C, 71.88; H, 4.29; N, 10.83; O, 4.03; S, 8.22 %.

2.2.2.3. 2-(3′-(4″-Hydroxy)-1′-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazole (BTc)

Yield: 69 %, m.p.: 130 °C, IR (ν cm^{-1}, KBr): 3456, 3375, 3078, 3057, 1680, 1620, 1587, 1518, 1350, 1298, 1462, 1404 and 692. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 4.32 (s, 1H, -OH), 7.42 & 7.43 (m, 3H, Ar-H of 1-phenyl pyrazole), 7.51-7.55 (m, 2H, Ar-H of 3-phenyl pyrazole), 7.68-7.73 (d, 1H, Ar-H of 3-phenyl pyrazole), 7.79-7.81 (d, 1H, Ar-H of benzothiazole), 7.82-7.89 (dd, 2H, Ar-H of 1-phenyl pyrazole), 8.04-8.08 (dd, 2H, Ar-H of benzothiaozle), 8.15-8.18 (d, 1H, Ar-H of benzothiazole), 8.29-8.35 (t, 1H, Ar-H of 3-phenyl pyrazole), 8.56 (s, 1H, pyrazole H). Mass *m/z*: 369. Anal. Cacld. for $C_{22}H_{15}N_3OS$; Cacld.: C, 71.52; H, 4.09; N, 11.37; O, 4.33; S, 8.68; Found: C, 71.39; H, 3.88; N, 11.22; O, 4.19; S, 8.50 %.

2.2.2.4. 2-(3′-(4″-Nitrophenyl)-1′-phenyl-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazole (BTd)

Yield: 73 %, m.p.: 170 °C, IR (ν cm^{-1}, KBr): 3124, 3090, 3063, 1683, 1597, 1531, 1348, 1313, 1502, 1454 and 684. ^{1}H NMR (400 MHz, $CDCl_3$) δ in ppm: 6.70-7.72 (d, 1H, Ar-H of benzothiazole), 6.80-6.85 (t, 2H, Ar-H of 1-phenyl pyrazole), 6.95-6.98 (t, 2H, 3-phenyl pyrazole), 7.09-7.11 (d, 1H, Ar-H of benzothiazole), 7.43-7.47 (dd, 2H, Ar-H of benzothiazole), 7.57-7.64 (dd, 3H, 1-phenyl pyrazole), 7.71-7.74 (dd, 2H, 3-phenyl pyrazole), 8.31 (s, 1H, pyrazole H). Mass *m/z*: 398. Anal. Cacld. for $C_{22}H_{14}N_4O_2S$; Cacld.: C, 66.32; H, 3.54; N, 14.06; O, 8.03; S, 8.05; Found: C, 66.16; H, 3.39; N, 13.89; O, 7.94; S, 7.90 %.

3. BIOLOGICAL ACTIVITY

Seven microbial strains were selected for antimicrobial activity on the basis of their clinical consequence of causing diseases in humans. Two Gram-positive bacteria (*S.Aureus* MTCC 96 and *S.Pyogenus* MTCC 443); two Gram-negative bacteria (*E.Coli* MTCC 442 and *P.Aeruginosa* MTCC 441) and three funguses (*C.Albicans* MTCC 227, *A.Niger* MTCC 282 and *A.Clavatus* MTCC 1323) were used for the evaluation of antimicrobial activities of the newly synthesized chemical compounds. The bacterial and fungal cultures were procured from Institute of Microbial Technology, Chandigarh. The bacteria were sub-cultured on Nutrient agar and fungi were sub-cultured on Sabouraud's dextrose agar (SDA) and incubated aerobically at 37 °C. All compounds and standards were dissolved in DMSO initially at 2000 μg/mL and then were serially diluted in to the following two series: 1000, 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 μg/mL, 800, 400, 200, 100, 50, 25, 12.5 and 6.25 μg/mL concentrations.

3.1. *In vitro* antibacterial and antifungal activity

Antibacterial and antifungal activity of compounds (BTa-d) was carried out by using cup plate method. The culture of bacterial and fungal strains was prepared in 4 mL of Muller Hinton broth at 37 °C for 24 hours in incubator. The turbidity of culture suspension was adjusted with sterile Muller Hinton broth in order to obtain turbidity comparable to a No. 1 McFarland turbidity standard. One mL of this suspension was pipetted into the Muller Hinton agar plate and distributed evenly over the surface of the medium by gently stirring the plate. The surface of the

medium was allowed to dry for 15 minutes at room temperature. Compound (220 μg) impregnated discs were applied to the surface of inoculated plates. The Petri plates were placed in an incubator at 37 °C. After 24 hours of incubation the Petri plates was examined [9].

3.2. Determination of MIC

The minimum inhibitory concentration (MIC) of the compounds was determined by the micro broth dilution technique using Muller Hinton broth. Serial twofold dilutions ranged from 1000 to 6.5 μg mL^{-1} for compounds. The inoculums was prepared in broth which had been kept overnight at 37 °C and which had been diluted with Muller Hinton broth to give a final concentration of 10^8 cfu mL^{-1} (where cfu = Colony forming unit) in the test tray. The trays were covered and placed in plastic bags to prevent drying. After incubation at 37 °C for 24 hours, the MIC value was defined as the lowest concentration of the compound giving complete inhibition of visible growth [10].

4. RESULT AND DISCUSSION

4.2. Antimicrobial activity

All the benzothiazole (BTa-d) compounds were evaluated for *in vitro* antibacterial activity against *E.Coli* MTCC 442, *P.Aeruginosa* MTCC 441, *S.Aureus* MTCC 96 and *S.Pyogenus* MTCC 443. All the compounds were also evaluated for antifungal activity against *C.Albicans* MTCC 227, *A.Niger* MTCC 282 and *A.Clavatus* MTCC 1323. Compounds (BTa-d) showed zones of inhibition ranging between 10 to 20 mm.

Table -1: Physical and analytical data of 2-(substituted)-benzo[*d*]thiazoles (BTa-d)

Comp.	Mole. For.	m.p. °C	Elemental analysis Calcd. (Found)		
			C	H	N
BTa	$C_{22}H_{15}N_3S$	180	74.76(74.60)	4.28(4.06)	11.89(11.74)
BTb	$C_{23}H_{17}N_3OS$	155	72.04(71.88)	4.47(4.29)	10.96(10.83)
BTc	$C_{22}H_{15}N_3OS$	130	71.52(71.39)	4.09(3.88)	11.37(11.22)
BTd	$C_{22}H_{14}N_4O_2S$	170	66.32(66.16)	3.54(3.39)	14.06(13.89)

Table -2: Anti bacterial activity of compounds (BTa-d) (μg mL^{-1})

Compound	*E.Coli* MTCC 442	*P.Aeruginosa* MTCC 441	*S.Aureus* MTCC 96	*S.Pyogenus* MTCC 443
BTa	125	200	100	125
BTb	100	12.5	6.25	100
BTc	125	125	25	200
BTd	200	250	250	250
Standard drugs				
Ampicillin	100	100	250	100
Ciprofloxacin	25	25	50	50

Table -3: Anti fungal activity of compounds (BTa-d) (μg mL^{-1})

Compound	*C.Albicans* MTCC 227	*A.Niger* MTCC 282	*A.Clavatus* MTCC 1323
BTa	500	125	500
BTb	250	500	500
BTc	1000	>1000	>1000
BTd	250	500	1000
Standard drugs			
Nystatin	100	100	100
Griseofulvin	500	100	100

On the basis of the zones of inhibition produced against the test bacteria, all the compounds (BTa-d) were found to be most effectual against *S.Aureus*, showing the maximum zones of inhibition at 15 to 20 mm, only one compound (BTb) was found much active against *S.Pyogenus* as similar to Ampicillin standard drug (100 μg/mL) and against *P.Aeruginosa* and *S.Aureus* more active than Ciprofloxacin (12.5 and 6.25 μg/mL). All the compounds showed comparatively fair activity against gram-negative bacteria (100 and 250 μg/mL) (Table II). The MIC (minimum inhibitory concentration) values of all tested chemical compounds ranged between 25 and 250 μg/mL against gram-positive bacteria. Compound (BTc) displayed good antibacterial activity with the lowest MIC value at 25 μg/ml against *S.Aureus*. Compound, (BTb) possessed antibacterial activity with MIC value of 100, 12.5, 6.25 and 100 μg/mL against *E.Coli*, *P.Aeruginosa*, *S.Aureus* and *S.Pyogenus* respectively (Table II). Amongst the synthesized compounds, three compounds (BTa), (BTb) and (BTd) showed more mycelia growth inhibition against *C.Albicans*, the (BTa) is as similar to standard drug Griseofulvin and rest of two (BTb) and (BTd) more active than Greseofulvin. The compounds, (BTa), (BTb) and (BTd) were found to be moderately active against *A.Niger* and *A.Clavatus* (Table III). The compound (BTc) is less active against all fungal strains showing the mycelia growth inhibition at concentration of 1000 μg/mL and > 1000 μg/mL.

From the overall antibacterial and antifungal result it is conspicuous that (BTa-d) compounds could be recognized as biologically active members with good antimicrobial profile.

5. CONCLUSION

The 2-(1,3-substituted-1*H*-pyrazol-4-yl)-1*H*-benzo[*d*]thiazoles (BTa-d) has been synthesized for the discovering of effortful new structure escorts. Compounds (BTa-d) were found to be most effectual against *S.Aureus* showing the maximum zones of inhibition of 15 and 20 mm, and compound (BTb) was found to be most effectual against all bacterial strains. Furthermore, the compounds (BTa), (BTb) and (BTd) showed more mycelia growth inhibition only against *C.Albicans* whereas, compound (BTc) was found to be less active against *C.Albicans*, *A.Niger* and *A.Clavatus*; compounds (BTb) and (BTc) found more energetic than the reference drugs Ampicillin and Ciprofloxacin against *S.Aureus* and (BTa), (BTb) and (BTd) compounds were found energetic similar to the reference drug Griseofulvin against *C.Albicans* strain.

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