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SYNTHESIS, CHARACTERIZATION AND EVALUATION OF BIOLOGICAL ACTIVITY OF SOME NOVEL BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

The reaction of benzoic acid derivatives with ammonium thiocyanate yield 4-thiocyanatobenzoicacid. The thiocyano-benzoicacid was condensed with o-phenylenediamine and carbon disulphide to get benzimidazole. These compounds were synthesized in good yield and their structures were confirmed by IR, ^{1}H-NMR and ^{13}C-NMR. Antimicrobial activity against bacteria and fungi, anti-inflammatory activity and analgesic activity were studied for the synthesized compounds.

KEYWORDS: Benzimidazole, thiocyanate, o-phenylenediamine, pharmacological activity.

INTRODUCTION

Heterocycles have often been incorporated into the organic materials to take advantage of their known chemical, thermal, thermo oxidative, and photochemical stabilities, as well as high quantum yields. Among π-conjugated molecules, those containing electron-deficient benzene-fused five-membered heteroaromatic rings with nitrogen atom(s), e.g. benzothiazoles, benzothiadiazoles, benzoxazoles, benzimidazoles, are widely employed as acceptor moieties in various optoelectronic materials because of their high electron-accepting character. More importantly, the five membered hetero aromatic rings directly bonded to a donor facilitate maximal coplanarity between the donor and the acceptor

subunits, which might be critical for the efficient charge transfer in those molecules.[1] Benzimidazole is a heterocyclic aromatic organic compound. It is an important pharmacophore and a privileged structure in medicinal chemistry. The synthesis of novel benzimidazole derivative remains a main focus of medicinal research. Benzimidazole is a group of substances have found practical applications in organic synthesis and a significant structural element in medicinal chemistry owing to its diverse biological activities.[2] Benzimidazole is isosteric with indole and purine nuclei, which are present in a number of fundamental cellular components and bioactive compounds. This heterocycle may represent a kind of privileged substructure, which may interact with different proteins and enzymes. Indeed, a number of important drugs used in different therapeutic areas contain the benzimidazole ring.[3] Benzimidazole and its derivatives have been showing hopeful activity in the treatment of several diseases, for these reasons, they achieved much attention as important pharmacophore and privileged structure in medicinal chemistry. Almost all benzimidazole derivatives with their two ring systems bear different functional substituents and this leads to essential modification of the physicochemical, metabolic and pharmacokinetic properties of these drugs.[4] Fused heterocyclic compounds, particularly, imidazole, benzimidazole and pyrazine derivatives show multifarious medicinal activities.

The benzimidazole skeleton has attracted, and still attracts, much attention from medicinal chemists because of its structural resemblance to various moieties present in the fundamental constituents of proteins and nucleic acids. Benzimidazoles are also well known for their broad spectrum of anti-parasitic properties. Synthesis of these diverse heterocyclic derivatives, ignite a great interest on the chemical community in view of their wide spectrum of medicinal activities which are therapeutically effective. Nitrogen-containing heterocycles and their derivatives are often found in natural products and in pharmaceuticals and agrochemicals.[5-6] Benzimidazole derivatives have found the application in diverse therapeutic areas including antihypertensive,[2] antiviral,[3] antifungal,[4] anticancer,[5,6] anti-histaminic,[7] antitubercular,[8] antiallergic,[9,10] antioxidant[11,12,13] anti-cancer, anti-HIV and antimicrobial activities.[14-20] The 1-*H*-benzimidazole ring, which, exhibit remarkable basic characteristics due to their nitrogen content and comprises the active substances for several drugs. In the present study, novel benzimidazoles are synthesized from benzoicacid compounds (Scheme 1).

MATERIALS AND METHODS

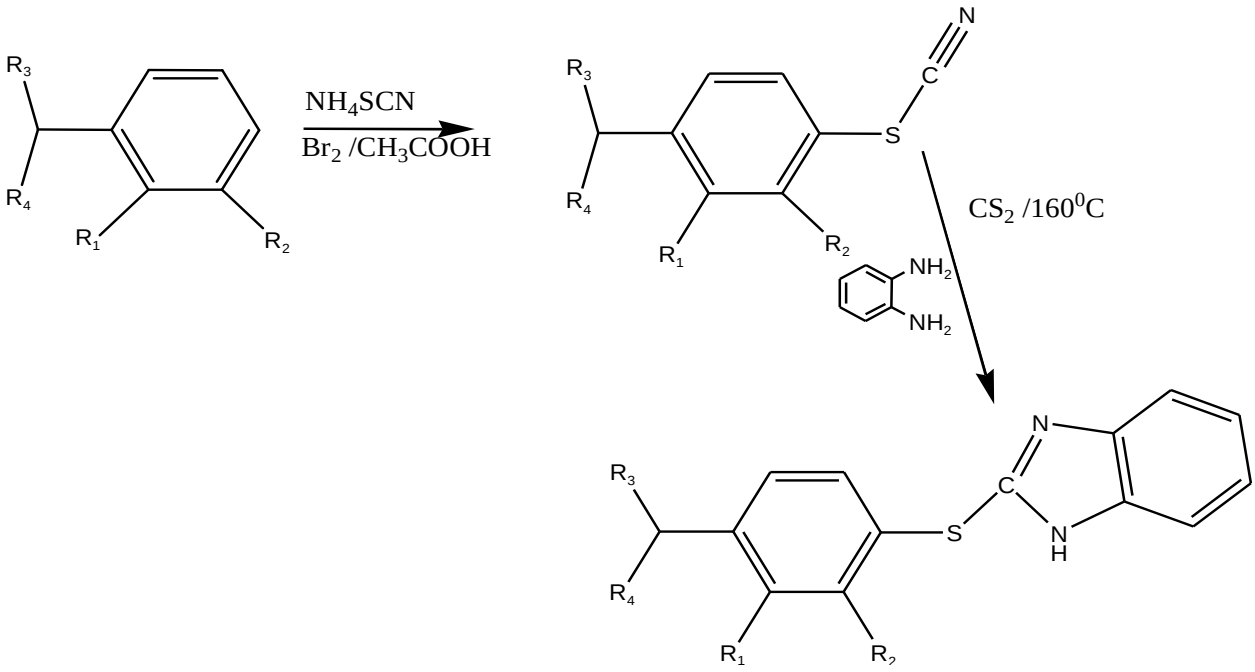
All the melting points were taken in open capillaries and are uncorrected. Elemental analysis was performed on a Perkin-Elmer analyzer. IR spectra are recorded in KBr on Shimadzu spectrometer, ^{1}H-NMR and ^{13}C-NMR in DMSO-d6 on a Bruker AC-400 spectrometer using TMS as an internal standard. The microorganisms were obtained from National Chemical Laboratory, Pune.

General procedure for the synthesis of thiocyanate (TC1-TC5)

The substituted/unsubstituted benzoic acid (0.5 mol) was dissolved in acetic acid (125 ml) and the solution was added to the solution of ammonium thiocyanate (1.05mol, 80 g) in glacial acetic acid (250 ml). This solution was cooled to 10-20º C. To this well stirred solution, a solution of bromine (0.5mol, 25.7ml) in acetic acid (250 ml) was added drop wise for thirty minutes and the temperature was maintained below 20ºC. After the addition of bromine, it was kept at room temperature for ten minutes and then it was diluted with an equal amount of water. The solid material was filtered, washed, dried and recrystallized from ethanol.

General procedure for the synthesis of benzimidazoles (Compound BI 1-BI 5)

A mixture of thiocyanate A1-A5 (0.01 mol), o-phenylenediamine (0.01mol, 1.08g) and carbon disulphide (0.1 mol, 8 ml) was heated in an oil bath at 160^{o}C for 5 hours. The resultant benzimidazole was cooled and recrystallized from ethanol.



	R1	R2	R3	R4
TC1, IM1	**H**	**H**	**OH**	**O**
TC2, IM2	**Cl_2**	**H**	**OH**	**O**
TC3, IM3	**Br**	**H**	**OH**	**O**
TC4, IM4	**H**	**NO_2**	**OH**	**O**
TC5, IM5	**OCH_3**	**H**	**OH**	**O**

Table 1: Analytical data of thiocyanate (TC1-TC5)

Thiocyanates	Yld (%)	M. Pt (° C)	Molecular Formula	Elemental Analysis (%) Reported (Calculated)							M.wt
				C	H	N	O	Cl	Br	S	
TC 1	76	205-206	$C_8H_5SN_3O_2$	53.62 (53.60)	2.81 (2.88)	7.82 (7.88)	17.86 (17.87)	-	-	17.89 (17.86)	179
TC 2	62	247-248	C_8H_4 Cl S N O_2	44.98 (45.07)	1.89 (1.94)	06.56 (06.60)	14.98 (15.05)	16.59 (16.60)		15.01 (15.04)	213
TC 3	97	277-278	C_8H_4S Br N O_2	37.23 (37.27)	1.56 (1.56)	5.43 (5.48)	12.40 (12.45)	-	30.96 (30.99)	12.42 (12.46)	258
TC 4	75	248-249	$C_8H_4N_2SO_4$	42.86 (42.90)	1.80 (1.84)	12.50 (12.56)	28.55 (28.59)	-	-	14.30 (14.34)	224
TC 5	80	251-252	$C_9H_7SNO_3S$	51.67 (51.70)	3.37 (3.41)	6.69 (6.73)	22.94 (22.99)	-	-	15.33 (15.38)	209

IR data for the thiocyanate (TC 1-TC 5)

TC-1 (4-thiocyanatobenzoicacid) - ^{v} C≡N: 2220cm-1

TC-2 (2-chloro-4-thiocyanatobenzoicacid) - ^{v} C≡N: 2168 cm-1

TC-3 (2-bromo-4-thiocyanatobenzoicacid) - ^{v} C≡N: 2160cm-1

TC-4 (3-nitro-4-thiocyanatobenzoicacid) - ^{v} C≡N: 2210 cm-1

TC-5 (2-methoxy-4-thiocyanatobenzoicacid) - ^{v} C≡N: 2192cm-1

Table 2: Analytical data of benzimidazole (BI 1-BI 5)

Benzimidazole	Yld (%)	M. Pt (° C)	Molecular Formula	Elemental Analysis (%) Reported (Calculated)							M wt
				C	H	N	O	Cl	Br	S	
BI 1	89	430-431	$C_{14}H_{10}SN_2O_2$	62.21 (62.29)	3.73 (3.81)	10.36 (10.41)	11.84 (11.90)	-	-	11.86 (11.92)	270
BI 2	76	472-473	$C_{14}H_9$ Cl S N_2 O_2	55.18 (55.25)	02.98 (03.02)	09.16 (09.21)	10.50 (10.57)	11.63 (11.69)		10.52 (10.59)	304
BI 3	67	502-203	$C_{14}H_9$S BrN_2 O_2	48.15 (48.21)	2.60 (2.67)	08.02 (08.10)	09.16 (09.22)	-	22.88 (22.96)	09.18 (09.25)	349
BI 4	75	467-468	$C_{14}H_9N_3SO_4$	53.33 (53.40)	02.88 (2.96)	13.33 (13.39)	20.30 (20.36)	-	-	10.17 (10.24)	315
BI 5	72	476-477	$C_{15}H_{12}SN_2O_2$	59.99 (60.06)	4.03 (4.14)	09.33 (11.14)	15.98 (16.07)	-	-	10.68 (10.74)	300

IR data for the Imidazole (BI 1-BI 5)

Compound IB 1: *4-(1H-benzo[d]imidazol-2-ylthio) benzoic acid)*:

IR KBr(cm ¯1):1608(C=Nstr),3425(NHstr), 2075(aromatic) ,2876(OH str), ^{1}H-NMR : δ 6.89 – 7.89 (Ar-H, multiplet),δ 10 – 13.2 (Ar-COOH , singlet).^{13}C-NMR : δ 132.7 (Ar-C), δ 146(C=N).

Compound IB 2: ***4-(1H-benzo[d]imidazol-2-ylthio)-2-chlorobenzoic acid*:**

IRKBr(cm¯1):1617(C=Nstr),3422(NHstr),3075(aromatic),2964(OHstr),746(C-Clstr)^{1}H-NMR : δ 7.0 – 7.2 (Ar-H, multiplet),δ 10 – 13.2 (Ar-COOH , singlet).^{13}C-NMR : δ 127.7 (Ar-C) , δ 149 (C=N).

Compound IB 3: *4-(1H-benzo[d]imidazol-2-ylthio)-2-bromobenzoic acid)*:

IR KBr(cm ¯1):1619(C=Nstr),3422(NH),3083(aromatic),2964(OHstr),735(C-Br str),1278(C-Ostr), ^{1}H NMR: δ7.8(aromatic proton), δ11.1(COOH singlet)proton, δ12.4(O-H)proton.^{13}C NMR: δ123-140.56(Ar-C), δ139.45(C=N).

Compound IB 4: ***4-(1H-benzo[d]imidazol-2-ylthio)-3-nitrobenzoic acid)*:**

IRKBr(cm^{-1}):1638(C=Nstr),3422(NH),3070(aromatic),2777(OHstr),1458(C-NO_{2str})1272(C-Ostr) ^{1}H NMR:δ8.6(aromatic proton)δ11.6(O-H)proton.^{13}C NMR: δ126.7(Ar-C), δ138.72(C=N).

Compound IM 5: ***4-(1H-benzo[d]imidazol-2-ylthio)-2-methoxybenzoic acid):***

IR Br(cm^{1}):1624(C=Nstr),3410(NH),3075(aromatic),2562(OHstr),2668($COCH_3$str),1258(C-Ostr)1742(C=Ostr),^{1}HNMR:δ7.3(aromaticproton),δ10.95(O-H)proton.^{13}CNMR:δ127(Ar-C), δ139.72(C=N).

RESULT AND DISCUSSION

Anti-microbial Activity

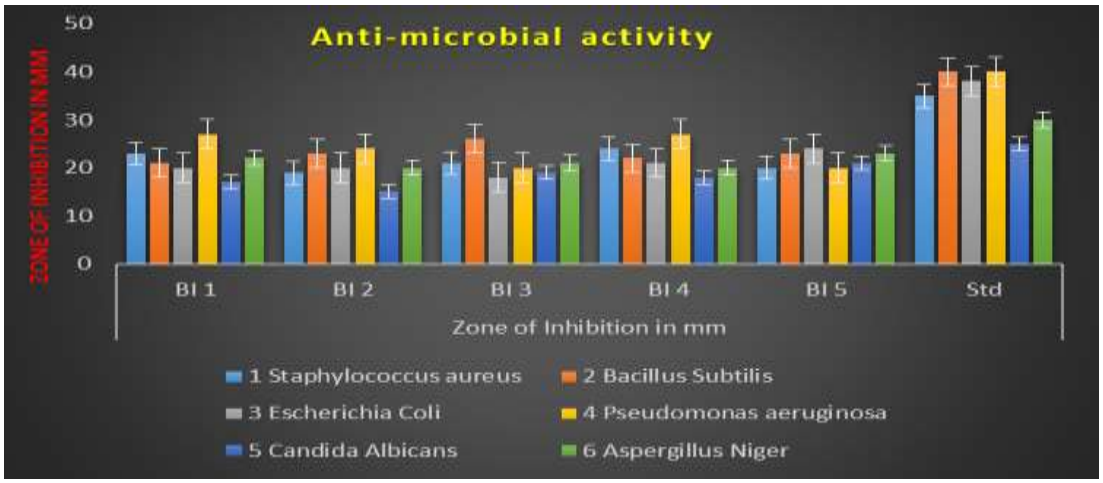
The anti-microbial activity for the sample was carried out by Disc Diffusion Technique.[21] The test microorganisms (Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus Niger) maintained by periodical subculturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The test microorganisms were obtained from National Chemical Laboratory NCL), Pune and maintained by periodical sub culturing on nutrient agar and sabouraud dextrose agar medium for bacteria and fungi respectively. The effects produced by the sample

were compared with the effect produced by the positive control (Reference standard ciprofloxacin 5 µg/disc for bacteria; Nystatin 100 units/disc for fungi).

Table 3: Anti-microbial activity of the synthesized compounds

S.No	Name of the microorganisms	Zone of Inhibition in mm					
		BI 1	BI 2	BI 3	BI 4	BI 5	Std
1	Staphylococcus aureus	23	19	21	24	20	35
2	Bacillus Subtilis	21	23	26	22	23	40
3	Escherichia Coli	20	20	21	21	24	38
4	Pseudomonas aeruginosa	27	24	20	26	20	40
5	Candida Albicans	17	15	19	18	21	25
6	Aspergillus Niger	22	19	21	20	23	30

Standard-Ciprofloxacin 5 ug/disc for bacteria; Nastatin 100 units/ disc for fungi S.C- Solvent Control (Solvent Used DMSO).



Anti inflammtory activity

Carrageenan induced hind paw edema:

Albino rats of either sex weighing 150-200gms were divided into six groups of six animals each. The dosage of the drugs administrated to the different groups were as follows: Group 1 – Control received normal saline, Group 2 to 16 received test in a dose of 50 mg/kg and Group 17-Indomethacin(10mg/Kg).All the drugs were administrated orally.

After one hour of the administration of the drugs, dose 0.1 ml of 1% w/v carrageenan solution in normal saline was injected into the subplantar tissue of the left hind paw of the rat and the right hind paw served as the control. The paw volume of the rats were measured in the digital plethysmograph(Ugo basile, Italy) at the end of 0, 60, 120 and 180 min.The increase in paw edema of the treated group was compared with that of the control and the inhibitory effect of

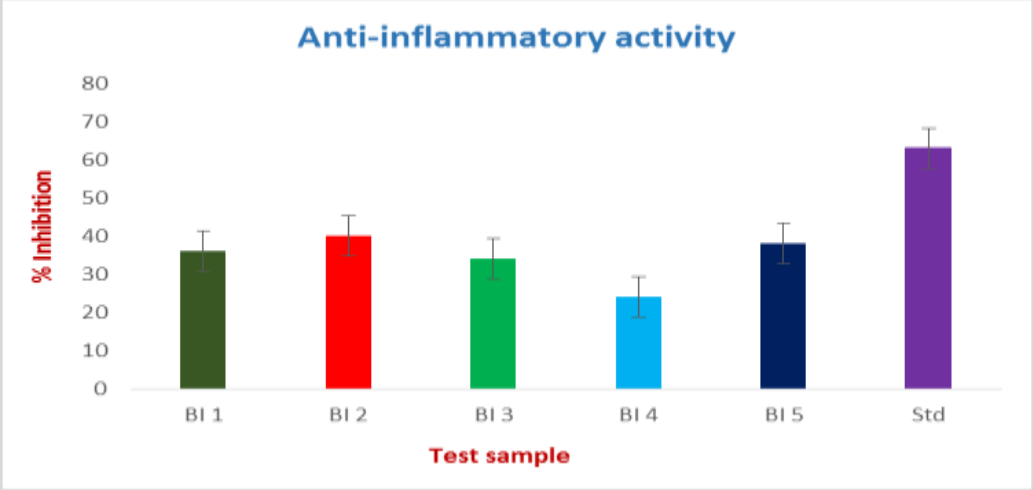
the drugs were studied. The relative potency of the drugs under investigations were calculated based upon the percentage inhibition of the inflammation.[22]

$$\text{Percentage Inhibition} = \frac{\text{Control (increase in paw volume in 3}^{rd}\text{ hour)} - \text{Test(increase in paw volume in 3}^{rd}\text{ hour)}}{\text{Control (increase in pawvolume in 3}^{rd}\text{ hour).}} \times 100$$

Table 4: Anti-inflammatory activity of the synthesized compounds

Treatment	Dose mg/kg p.o.	Paw volume Increase after 3 hr (ml)	Percentage Inhibition
Control	5 ml/kg	111.61±10.56	-
BI 1	50mg/kg	68.59±5.61	36.89
BI 2	50mg/kg	64.46±6.45	40.76
BI 3	50mg/kg	70.37±5.98	34.76
BI 4	50mg/kg	83.23±7.15	24.06
BI 5	50mg/kg	66.46±8.36	38.45
Indomethacin	10mg/kg	40.4±3.62	63.80

P< 0.001 values are expressed as ±SEM. Number of animals using are 6 in each group



Analgesic activity

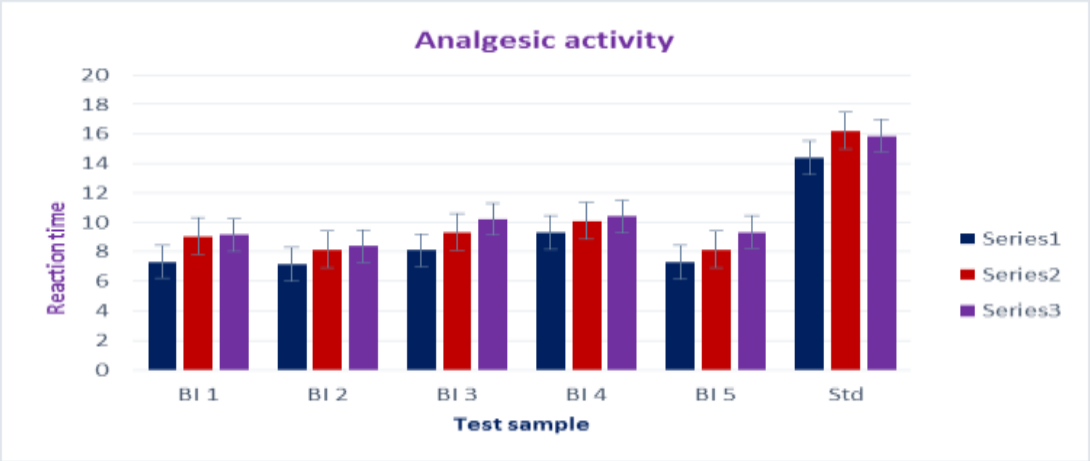
The analgesic activity of the given sample was evaluated by using Hotplate method. The albino mice of either sex were used, the animals were divided into nine groups of 5 animals each. Group 1 received normal saline (1ml/kg), group 2 received standard (pentazocine 10 mg/kg) intraperitonealy, groups 3 to 9 received the given extract (50 mg/kg) orally. Before administrating the drug, basal reaction time was studied by placing the animals in hotplate

and parameters such as paw licking, jumping response were noted. The maximum cut-off time is 15 sec. After half an hour of administration of the drug, the reaction time was noted and compared.

Table 5: Analgesic activity of the synthesized compounds

S.No	Groups	Drug	Dose (mg/kg)	Reaction time (in sec)			
				Before Administration Of drug	After Administration of drug		
					30(mins)	60(mins)	120(mins)
1.	Control	Saline	1ml/kg	4.41±0.16	4.42±0.20	4.48±0.20	4.43±0.17
2.	Test-1	BI 1	50mg/kg	4.26±0.25	7.33±0.17	9.05±0.18	09.16±0.22
3.	Test-2	BI 2	50mg/kg	4.18±0.33	7.18±0.18	8.14±0.22	8.38±0.24
4.	Test-3	BI 3	50mg/kg	4.36±0.17	8.10±0.20	9.32±0.18	10.22±0.16
5.	Test-4	BI 4	50mg/kg	4.32±0.15	9.34±0.22	10.10±0.28	10.40±0.18
6.	Test-5	BI 5	50mg/kg	4.28±0.16	7.32±0.28	8.16±0.42	9.34±0.12
7.	Standard	pentazocine	10mg/kg	5.42±0.16	14.6±0.32	16.6±0.18	15.86±0.28

Mean± S.E.M, n=5



DISCUSSION

Compound TC1-TC5 were synthesized in good yield by the reaction of benzoic acid derivatives with ammonium thiocyanate and Br_2/CH_3COOH under ice-cold condition. Compounds BI1-BI5 on reaction with o-phenylenediamine in the presence of carbon disulphide afforded compounds BI1-BI5. The purity and homogeneity of all the synthesized compounds were confirmed by their sharp melting points (uncorrected) and column chromatography. The chemical structures were confirmed by IR, ^{1}HNMR and ^{13}C_NMR techniques. The presence of OH stretching was confirmed by the peaks at 2562-3032 cm-1. Also ^{1}H-NMR spectra were useful for identifying protons. The peaks at the frequency range 6.0 – 8.6 confirm the aromatic protons and 10-13.5 confirms the COOH protons. The

compound BI 1 shows good activity and compounds BI 3, BI 4 and BI 5 show moderate activity in anti-microbial study. The compound efficiently inhibits the Diphtheria toxin. Hence the compound can be used as a cure for diphtheria but further research is needed to formulate it as a drug. Further toxicity studies have to be done to ensure the safety and efficacy of the compound to act as drug in treating inflammation.

CONCLUSION

The present investigation is focused on the synthesis, characterization and biological activities of a series of compounds from substituted benzoic acid. The findings are furnished below:

- Five compounds were prepared from substituted benzoic acid by the scheme-1.
- All the compounds synthesized by the investigator, were characterized by infrared data.
- The IR spectra of the four compounds provide the expected frequencies.
- The ^{1}H NMR and ^{13}C-NMR spectra of the four compounds provides signals.
- A study of the anti-microbial activity was carried out and the results are given.

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