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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF 3-(1H-BENZO[d]IMIDAZOL-2-YLSULFANYL) METHYL-4-[PHENOXY(PHENYL) ACETAMIDO)-5-MERCAPTO-1,2,4-TRIAZOLE AND RELATED ARYLOXY COMPOUNDS

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Keywords:

Synthesis, Microbial activity, benzo[d]imidazol-2-ylsulfanyl methyl triazole derivatives

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ABSTRACT: A series of new 1*H*-benzo[d]imidazole derivatives of 3,4-substituted triazole. 3-(1H-Benzo[d]imidazol-2-ylsulfanyl)methyl-4-[phenyloxy(phenylacetamido)]-5-mercapto-1,2,4-triazole and related aryloxy compounds were synthesised, analysed and characterised by FTIR, ^{1}HNMR and elemental analysis. These compounds were screened for antibacterial and antifungal activity. The antibacterial activities were compared against chlorophenicol and antifungal activity with mycostalin. Some triazole derivatives showed a little antibacterial but appreciable antifungal activity.

INTRODUCTION: A perusal of literature revealed that benzimidazole and triazole ring containing heterocyclic molecules possess wide range of antibacterial activity and medicinal properties [1-10].

In addition, triazolo benzimidazoles possess broad range of antimicrobial spectrum and have privileged nuclei to display medicinal activity. Benzimidazole derivatives possess great importance in medicinal chemistry due to wide variety of pharmacological activity [11, 12] in controlling cardiovascular diseases [13], anticancer properties [14], anti-inflammatory [15], antibacterial [16], antifungal [17], antidiabetic [18] and anti HIV [19] activity and some benzimidazoles are antioxidant [20-24].

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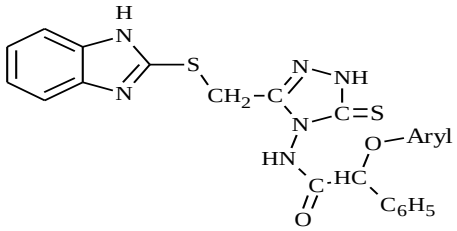
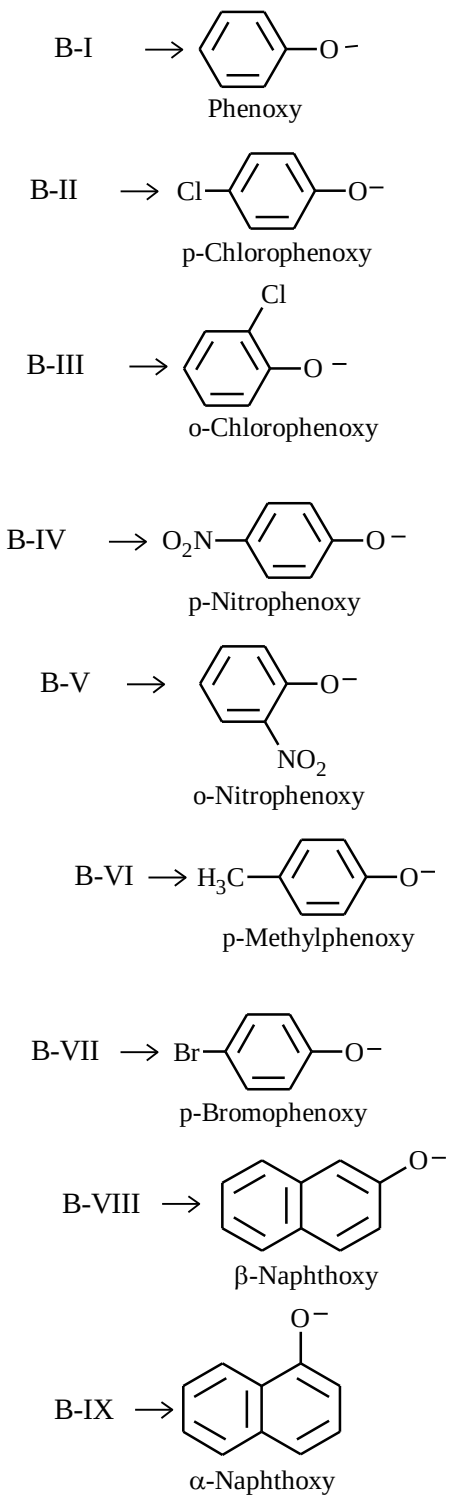
It has also been noticed that certain benzimidazole derivatives like ciprofloxacin and norfloxacin are most essential and popular antibiotics. In view of substantial pharmacological importance and multidimensional applications of benzimidazole-triazole mixed heterocyclic compounds we were motivated to study their chemistry and here we report synthesis, characterisation and antibacterial activity of new substituted triazole ring containing 1H-benzo[d]imidazole-2ylsulfanylmethyl derivatives, 3-(1H-Benzo[d]imidazole-2-ylsulfanyl) methyl-4-[phenoxy(phenyl)acetamido)-5-mercapto-1,3,4-triazole (BSPT) and its nine related aryloxy derivatives (B-I to B-IX).



BSPT
(B-I to B-IX)

Aryloxy-group (-O-Aryl) for BI – B-IX



METHODS AND MATERIALS: All the reagents and chemicals were obtained from E Merck, Loba chem, Chem pure, Sigma Aldrich and Fluka (Germany). Solvents used for synthesis were analytical grade reagent. The purity of the products was checked by TLC. The purity of known and reported chemicals was ascertained from MP and estimation of nitrogen.

The FTIR spectra of compounds were recorded in KBr disc on Shimadzu, IR Spectrophotometer-2500.

The ^{1}HNMR spectra of compounds were recorded on a Brucker AV-400 spectrophotometer in $CDCl_3$ or DMSO or DMF-d6.

The CHNS analysis reports were obtained from CDRT Lucknow or BIT Mesra, Ranchi.

Result and Discussion

The compound BSPT (BI-B-IX) were synthesized using

(a) Aryloxyphenylacetic acid hydrazide (A-I to A-IX)

(b) Ethyl bromoacetate

(c) 1H-Benzoimidazole-2-thiol

(d) Potassium dithiocarbazinate of (1H-benzo[d]imidazole-2-ylthio)methylcarbohydrazide. adopting scheme A, B and C

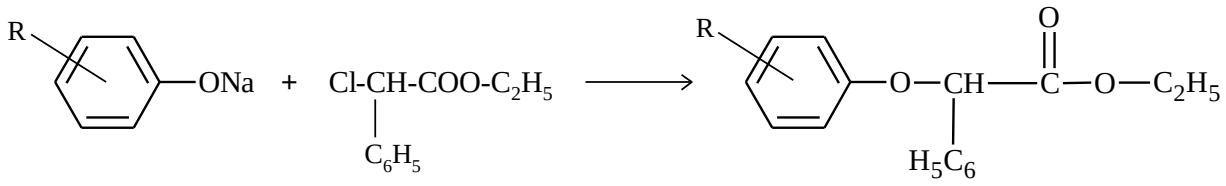
(a) Phenoxy(phenylacetic acid) hydrazide and its derivatives were synthesised using **Scheme A**.

(b) The compound ‘b’ and ‘c’ were obtained from market and they were Fluka Product. Both b and c were used without further purification.

(c) Potassium dithiocarbazinate of (1H-benzo[d]imidazole-2-ylthio)methylcarbono hydrazide was prepared adopting Scheme B.

Scheme A:

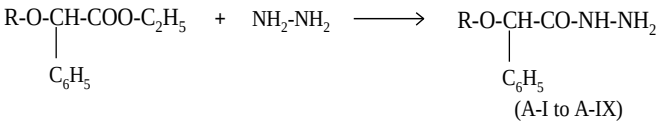
Preparation of aryloxy phenyl acetic acid hydrazide: Sodium salt of phenol and substituted phenols were refluxed with ethyl (phenyl chloroacetate) [C_6H_5-CHCl-$COOC_2H_5$] in dioxane on steam bath for 3 hours and



ethyl (substitutedphenoxy)(phenyl)acetate

The crude aryloxy compound aryloxy ethyl (phenylacetate) obtained above was refluxed with 98% hydrazine hydrate on a steam bath for 3-4 hours. The product formed was triturated with ether to remove unreacted phenol and ester. The white mass left was recrystallised with aqueous ethanol. The related substituted aryloxyphenylacetic acid hydrazides (A-I to A-IX) were also prepared following the above procedure.

The reaction of aryloxy ester ($RO\text{-}CH(C_6H_5)\text{-}COOC_2H_5$) with hydrazine takes place as shown below:-

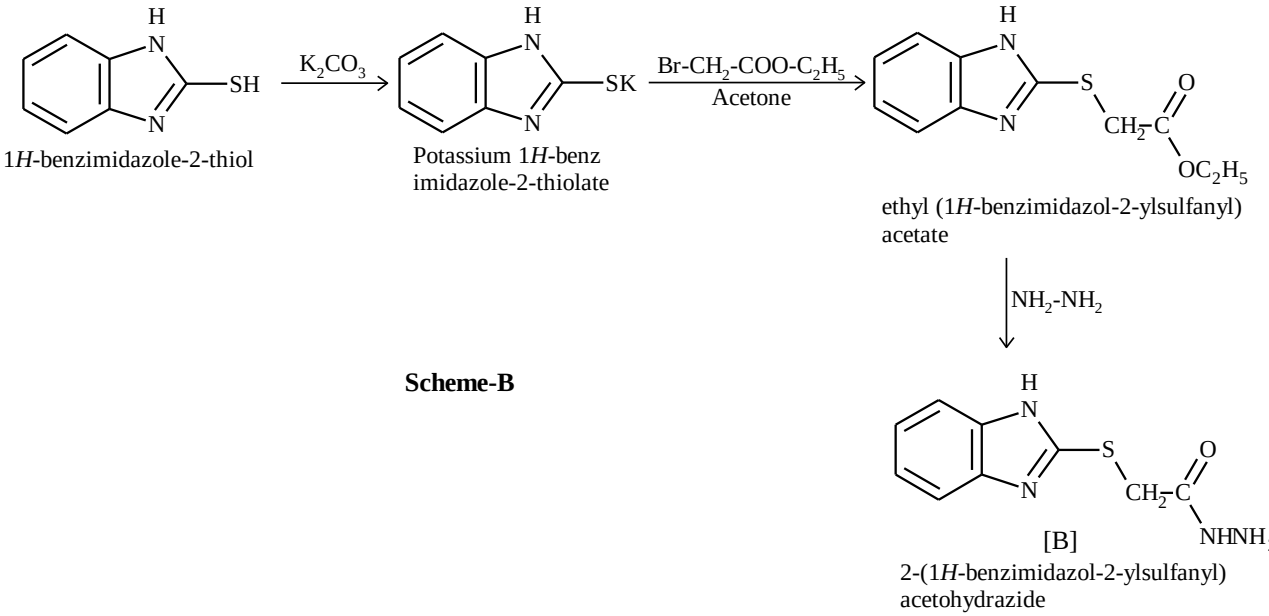


(A-I to A-IX)

The melting point and analytical results of aceto hydrazide A-I to A-IX are given in **Table-A**

Scheme B:

Preparation of (1H-benzo[d]imidazol-2-ylsulfanyl)acetic acid hydrazide, from 1H-benzo[d]imidazole-2-thiol: Potassium salt of 1H-Benzo[d]imidazole-2-thiol (BtH) was prepared by heating aqueous ethanol solution of thiol (BtH) with calculated amount of K_2CO_3 and the potassium 1H-benzo-[d]-imidazol-2-thiolate (KBt) was obtained by evaporating the solution to dryness. The dried product was suspended in dry acetone and refluxed with ethylbromoacetate ($Br\text{-}CH_2COOC_2H_5$) with stirring. The resulting solution was filtered and solvent evaporated to get solid product. The crude ester obtained was refluxed with 98% hydrazine hydrate in 30 ml THF to yeild 2-[1H-benzo[d]imidazol-2-ylsulfanyl)acetohydra zide (M.P- 236-237°C Reported-236°C)



1*H*-benzimidazole-2-thiol

Potassium 1*H*-benz imidazole-2-thiolate

ethyl (1*H*-benzimidazol-2-ylsulfanyl) acetate

2-(1*H*-benzimidazol-2-ylsulfanyl) acetohydrazide

Scheme-B

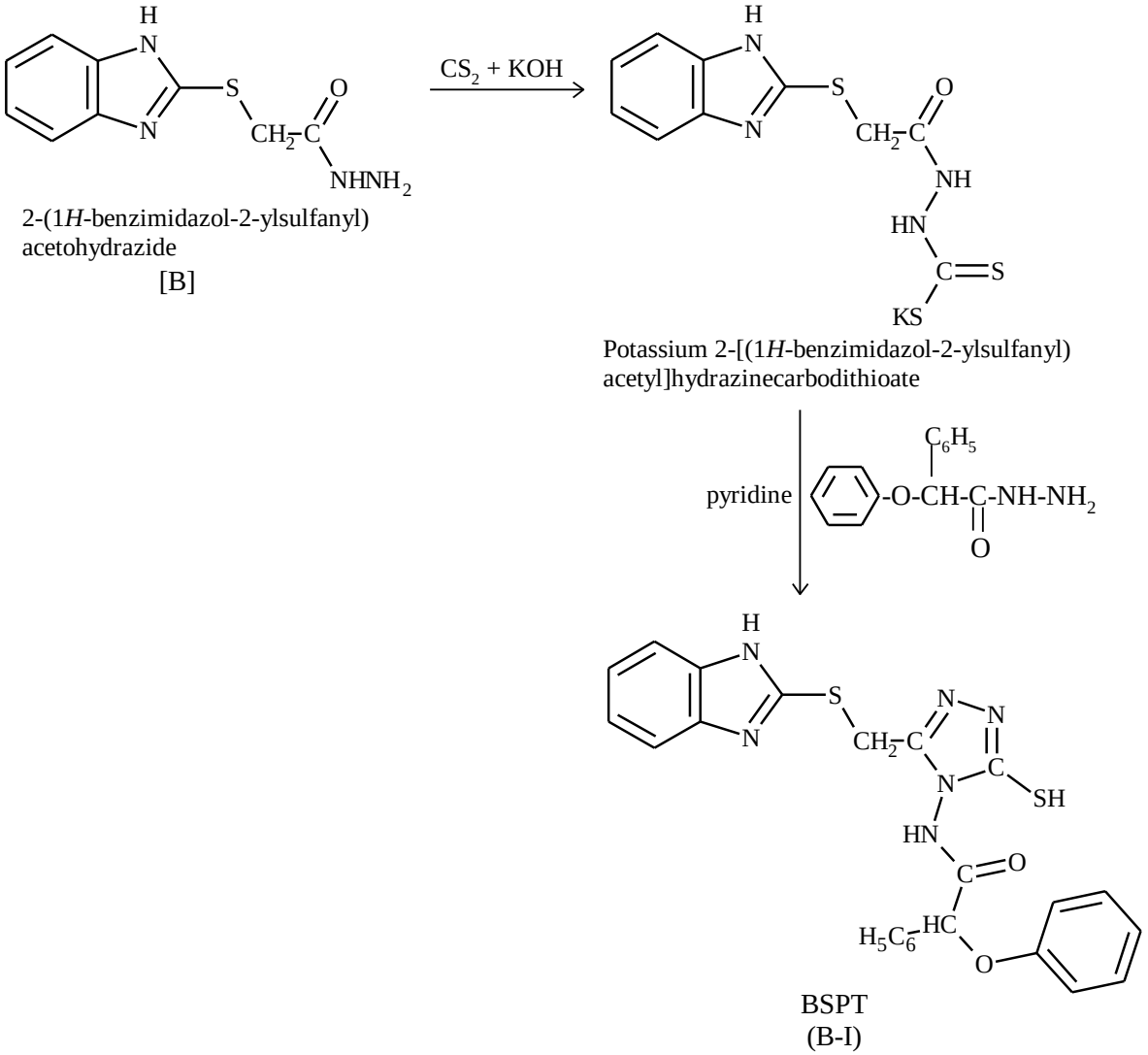
The purity of products was ascertained from TLC, melting point and C, H, N analysis of recrystallized product (B).

The triazole containing benzimidazole derivatives (B-I to B-IX) were obtained by refluxing potassium dithiocarboazinate of (1H-benzo[d]imidazol-2-ylthio)methylcarbonohydrazide and aryloxy-

(phenylacetohydrazide in pyridine as given in **Scheme C.**

2-(1H-Benzo[d]imidazole-2-ylsulfanyl)acetohydra zide was suspended in ethanol-dioxane mixture and treated with calculated amount of CS_2 and KOH and stirred for two hour to get potasssium salt of dithiocarbazinate of B. Potassium salt of dithiocarbazinate of 'B' was refluxed with aryloxy(phenylacetic acid)hydrazide in pyridine when pyridinium salt of 3-(1H-benzo[d]imidazole-2-ylsulfanyl)methyl-4-[aryloxyphenylacetamido]-5-mercapto-1,3,4-triazole [BSPT] was obtained. The free mercapto triazole derivatives (B-I to B-IX) were obtained by neutralising the product with dilute hydrochloric acid.

Scheme C:



2-(1*H*-benzimidazol-2-ylsulfanyl) acetohydrazide

[B]

Potassium 2-[(1*H*-benzimidazol-2-ylsulfanyl) acetyl]hydrazinecarbodithioate

BSPT
(B-I)

The compounds B-II to B-IX were obtained by the same procedure using appropriate aryloxy group. The analytical results of product B-I to B-IX are given in **Table-B**. The i.r spectral data of products B-I to B-IX and A-I to A-IX are given in **Table C and D**

EXPERIMENTAL:

General method for preparation of A-I to A-IX: 10 millimoles of phenol or substituted phenol or naphthols was taken in 50 ml dioxan or THF and refluxed with calculated amount of KOH to get potassium salt of phenol. The potassium phenolate was treated with 10 millimole of ethylphenyl chloroacetrate and refluxed gently for three hours and solid separated (KCl) was removed by filtration. The filtrate containing ethyl (aryloxy phenyl acetate) was treated with hydrazine hydrate (98%) and refluxed gently for three to four hours on steam bath and solvent evaporated to get syrupy mass.

The product on cooling gave solid product which was recrystallised with hot aqueous ethanol or tetrahydrofuran (yield 80-85%).

The method of preparation of B: About 0.1 mole of potassium salt of (1H-benzo[d]midazol-2-thiol) was taken in dry acetone and refluxed on steam bath with 0.1 mole of ethylbromoacetate with stirring for 3 hours. The sulfanylacetate was obtained on evaporation of acetone. The product was collected and recrystallised with ethanol tetrahydrofuran mixture. The acetate was refluxed with 98% hydrazide-hydrate to get 2-(1H-benzo[d]imidazol-2-yl) sulphanylacetohydrazide.

General procedure for preparation of B-1 to B-IX: About 10 millimole of B was taken in 30 ml ethanol and calculated amount of 10-12 millimole CS_2 and 10 millimole KOH were mixed and refluxed with stirring for 2-hour when potassium salt of dithocarbazinate separated. The product was dissolved in 20 ml pyridine and refluxed with 10 millimoles of aryloxyphenyl acetic acid hydrazide [Aro-CH(Ph)CO NH-NH_2] for 3 – 4 hours. The refluxate on cooling gave cream yellow crystalline precipitate of pyridinium salts of B-I to B-IX. The free mercaptotriazole was obtained by suspending the pyridinum salt in 30-40 ml water and neutralising it with dilute HCl. The free triazole was recrystallized with ethanol-THF mixture. The result of elemental analyses and mpt of B-1 to B-IX are given in **Table B**.

RESULT AND DISCUSSION:

^{1}HNMR Spectra: The ^{1}HNMR spectrum of A-I shows a singlet at δ = 3.68 ppm for (-O-CH(C_6H_5)-CO) for aceto (C-H) proton. The phenyl ring CH proton signals were observed between δ = 6.965 and 7.835 ppm as multiplete and broad NH, NH_2 proton signals at δ = 5.315 -5.685 ppm.

The ^{1}HNMR spectrum of A-II shows a singlet at 3.726 ppm for –O-CH(C_6H_5)CO and phenyl proton signals as multiplete between δ = 7.015–7.935 ppm and a broad NH, NH_2 proton signals were located at 5.465–5.285 ppm.

The p-nitrophenoxy derivative A-III shows a signal at 3.865 ppm and phenyl ring proton signals between δ = 7.115–8.215 ppm as multiplete. The NH and NH_2 proton signals was broad at δ = 5.415–5.845 ppm.

The β-naphtholoxy compound A-VIII shows a singlet at δ = 3.745 ppm attributed from (-O-CH-CO) proton and phenyl ring C—H proton signals as multiplete between δ = 7.015 and 7.895 ppm. The NH and NH_2 proton signals were observed between δ = 5.425-5.845 ppm.

The ^{1}HNMR spectra of hydrazides A-I to A-IX are consistent with proposed structure and these are supported by FTIR and elemental analysis.

The proton NMR spectrum of B-I shows (-S-CH_2) proton signal at δ = 2.281 ppm as singlet as well as acetamide –CH- proton at δ = 3.965 ppm as singlet. The ring NH proton signal was observed at 8.652 ppm, 8.925 ppm. The phenyl proton signals were observed at δ = 7.055-7.985 ppm as multiplet.

The ^{1}HNMR spectrum of chloro, bromo and nitro-aryloxy derivatives are almost identical. The phenyl proton signals were located between 6.943–7.855 ppm and (S-CH_2) proton as singlet between 2.835–2.945 ppm.

The (S-CH_2) proton of B-II were observed at δ = 2.865 ppm and acetoxy (CO-CH(C_6H_5)-O-) proton signal at δ = 3.685 ppm. Its phenyl proton signals were observed as multiplete between δ = 7.154-7.925 ppm. The rings NH of triazole and benzimidazole proton signals were located at 8.765 and 9.254 ppm. The acetamido (NH) proton signal were located at 5.45 ppm as singlet as broad band.

The proton NMR of 3-[2-(1H-benzo[d]imidazole-2-ylsulfanyl)methyl]-4-[(p-methylphenoxy(phenyl acetatemido)]-5-mercapto-1,2,4-triazole (B-VI) displays –CH_3 proton signals at δ = 1.695 ppm as singlet. The –S-CH_2- proton signal was observed as singlet at δ = 2.945 (2H, -S-CH_2-) and –O-CH-(C_6H_5)CO proton signal at δ = 3.875 ppm. The broad singlet at 5.45 ppm was assigned to acetamide (-HN-CO-CH-) proton signal. The phenyl ring (C-H) proton signals were located between δ = 7.025-7.845 ppm. The ring NH proton signals were located as singlet at 8.735 and 8.952 ppm. Based on spectral data of the compound, the structures suggested for the synthesized product

(BSPT) were also supported by the analytical compositions of benzimidazole derivatives.

The I.R. spectral band positions of A-I to A-IX and triazolo product B-I to B-IX are recorded in **Table 3 & 4.**

The I.R. spectra of phenoxy and related aryloxy (phenylacetic acid)hydrazide show characteristic NH_2, NH, phenyl C-H and amido CO stretches in 3μ-16μ region and NH_2, NH, C-H and CO stretches as well as NH_2 bending and phenoxy Ph-O-C stretches of compounds A-I to A-IX are recorded in **Table 3**. The NH_2 and NH stretches were located between 3348-3105 cm^{-1} and phenyl ring (C—H) stretches between 3085-3050 cm^{-1}. The strong band located at 1685-1698 cm^{-1} is assigned to υ(CO) of amide group. The medium band located near 1636-1628 cm^{-1} is attributed to δ(NH_2) of hydrazide group (CO-NH-NH_2). A medium band located at 1063 to 1050 cm^{-1} (**Table 3**) is attributed to aryloxy (C—O—C) stretching vibration. These i.r bands of compound A-I to A-IX are consistent with proposed structure of aryloxy(phenylacetic acid)hydrazide.

The prominent IR band due to υ(NH), ring υ(NH), υ(CH_2), υ(C-H), phenyl ring, υ(C=S), υ(CO), δ(NH) etc were consistent with proposed structure of triazolo derivatives and are recorded in **Table 4**. The i.r spectrum of B-I, 3-[2-(1H-benzo[d]imidazol-2ylsulfanyl)methyl]-4-[phenoxy (phenylacetamido)]-5-mercapto-1,2,4-triazole shows NH and (C-H) stretching vibrations at 3265, 3105, 2940 and 2865 cm^{-1} (**Table 4**)

The i.r spectra of all benzimidazole derivatives B-I to B-IX show strong υ(CO) vibration between 1685-1705 cm^{-1} confirming the presence of acetamide (-CONH) group. The ring NH and amide NH stretches were observed as medium band between 3265–3105 cm^{-1}. The –CH_2- stretches of sulfanyl methyl (-S-CH_2-) and acetamido (-CO-CH-) group were located at 2860-2940 cm^{-1}. The I.R bands at 1590-1610 cm^{-1} observed in B-I to B-IX are assigned to ring (C=N) stretching vibrations. The nitro aryloxy compound B-IV and B-V show NO_2 band at 1481-1483 cm^{-1}.

The υ(S-H) attached to triazole ring could not be observed indicated the predominance of thione tautomer in the molecule. The δ(NH) of B-I to B-IX were observed between 1526-1508 cm^{-1} and υ(C=S) band could be assigned to a strong i.r band observed between 1342-1305 cm^{-1}. The phenoxy (-C-O-C-) stretch can be assigned to a medium i.r band near 1020 ± 10 cm^{-1}. A large number of IR band located in finger print region are assigned to phenyl and triazole ring skeletal vibrations.

Antibacterial and antifungal activity: The antifungal activities of BSPT (Compound B-I to B-IX) were evaluated by radical growth method[26] using Czepek agar medium prepared by dissolving 20 g starch. 20 g agar 20 g glucose in one litre distilled water. The resulting solution was added requisite amount of test compound to get 100 and 200 ppm solution. The medium was then poured into petri plate and spore of fungi were placed on medium with the help of inoculum needle. These petri plate were wrapped in polythene bags by mixing 2 drops of ethanol and placed in an incubator at 30 ± 0.5°C. The linear growth of fungi was calculated by measuring the fungal colony diameter after five days. The percentage inhibition was calculated using the relation;

$$\frac{C - T}{C} \times 100$$

Where C & T are the diameter of the fungus colony and control test plate respectively. The fungi used in present microbial screening are *Candida albicans, F oxysporum, Aspergillus flavus, R. phaseoli* and *A. niger*. The control solution was mycostalin. The result of activity is shown in **Table 5**. Almost all benzimidazolylsulfanylmethyl triazole derivatives have causes inhibition of fungal growth but the activity of nitrophenoxy derivatives (B-IV & B-V) were quite encouraging comparable to mycostalin. The activities of B-I to B-IX were larger with *Candida albicans* and *A. niger*.

The antibacterial activity against *E. coli, S. aureues* and *Bacillus subtilis* were studied for compounds B-I to B-IX and zone of inhibition was observed in all the derivatives. The activity was studied by zone inhibition technique [27].

The nutrient agar medium was prepared by dissolving 5 g peptone 5 g beef extract, 5 g NaCl and 20 g agar agar in one litre distilled water. The medium solution was pipetted into petri plate and dried; the dried plate was seeded with bacteria and test compound dissolved in DMF (250 ppm 500 ppm strength). The disc of whatman filterpaper soaked with these solutions to 5 mm diameter discs were dried and placed on medium previously soaked with organism in petriplate at suitable distance and incubated at 30 ± 1°C for 24 hours. The zone of inhibition was measured accurately in mm. The results of inhibition are recorded in **Table 5**. It was encouraging to note that compounds were highly active on Escherichia coli. The standard used was chlorophenicol.

A-I Phenoxy (phenylacetic acid)hydrazide or aryloxy phenylacetic acid hydrazide show prominent I.R bands for NH_2, NH, C-H, aromatic C-H, amido (CO), phenoxy (C—O—C) stretches and phenyl ring skeletal vibration in 3 μ-16μ region. The diagnostic IR bands are shown in **Table 3.**

TABLE 1: ELEMENTAL ANALYSIS OF COMPOUND A-I TO A-IX

S. No.	Molecular formula	M.P ^{0}C	% analysis, found (Calculated)		
			C	H	N
A-I 242	$C_{14}H_{14}N_2O_2$	243-244	69.65 (69.42)	6.03 (5.78)	11.71 (11.57)
A-II 276.54	$C_{14}H_{13}N_2O_2Cl$	257-258	60.06 (60.75)	4.66 (4.70)	10.01 (10.12)
A-III 276.54	$C_{14}H_{13}N_2O_2Cl$	249-250	60.83 (60.75)	4.83 (4.70)	10.23 (10.12)
A-IV 287	$C_{14}H_{13}N_3O_4$	262-263	58.17 (58.53)	4.36 (4.52)	14.43 (14.63)
A-V 287	$C_{14}H_{13}N_3O_4$	260-261	58.41 (58.53)	4.63 (4.52)	14.63 ?
A-VI 256	$C_{15}H_{16}N_2O_2$	241-242	70.13 (70.31)	6.11 (6.25)	10.83 (10.93)
A-VII 321	$C_{14}H_{13}N_2O_2Br$	248-249	52.16 (52.33)	3.98 (4.05)	8.69 (8.72)
A-VIII 292	$C_{18}H_{16}N_2O_2$	258-259	73.73 (73.79)	5.61 (5.48)	9.39 (9.59)
A-IX 292	$C_{18}H_{16}N_2O_2$	263-264	73.68 (73.19)	5.53 (5.48)	9.41 (9.59)

TABLE 2: ELEMENTAL ANALYSIS OF COMPOUND B-I TO B-IX

S. No.	Molecular formula	M.P ^{0}C	% analysis, found (Calculated)			
			C	H	N	S
B-I 488.3	$C_{24}H_{20}N_6O_2S_2$	276-77	58.78 (58.99)	4.12 (4.09)	17.32 (17.21)	13.26 (13.10)
B-II 522.84	$C_{24}H_{19}N_6O_2S_2Cl$	281-82	55.12 (55.08)	3.76 (3.63)	15.94 (16.06)	12.13 (12.24)
B-III 522.84	$C_{24}H_{19}N_6O_2S_2Cl$	280-81	54.93 (55.08)	3.72 (3.63)	15.89 (16.06)	12.16 (12.24)
B-IV 533.4	$C_{24}H_{19}N_7O_4S_2$	294-95	54.13 (54.03)	3.61 (3.56)	18.26 (18.37)	11.86 (12.00)
B-V 533.4	$C_{24}H_{19}N_7O_4S_2$	296-97	54.01 (54.03)	3.51 (3.56)	18.15 (18.37)	11.92 (12.00)
B-VI 502.3	$C_{25}H_{22}N_6O_2S_2$	284-85	59.58 (59.73)	4.46 (4.38)	16.58 (16.72)	12.64 (12.74)
B-VII 568.2	$C_{24}H_{19}N_6O_2S_2Br$	291-92	50.63 (50.68)	3.28 (3.34)	15.21 (15.13)	11.16 (11.26)
B-VIII 538.2	$C_{28}H_{22}N_6O_2S_2$	288-84	64.32 (64.46)	4.12 (4.08)	15.51 (15.60)	11.68 (11.89)
B-IX 538.2	$C_{28}H_{22}N_6O_2S_2$	290-91	64.14 (64.46)	4.21 (4.08)	15.71 (15.60)	11.81 (11.89)

TABLE 3: THE INFRARED AND ^{1}HNMR SPECTRAL DATA OF COMPOUND A-I AND A-IX

Prominent I.R bands in cm^{-1} of compounds A-I to A-IX

Compound	υ NH_2, υNH, υC–H	υ CO	δ NH_2	υ (C-O-C)
A-I	3335, 3220, 3105, 3070	1698	1634	1065
A-II	3325, 3218, 3140, 3082	1692	1628	1055
A-III	3340, 3241, 3165, 3075	1685	1632	1054
A-IV	3316 , 3205, 3148, 3070	1690	1636 υ(NO_2^-) 1483	1056
A-V	3345, 3233, 3140, 3060	1688	1630 υ(NO_2) 1481	1050
A-VI	3340,3231, 3150, 3073, 2982, 2860	1695	1633	1062
A-VII	3348, 3268, 3140, 3065	1690	1638	1061
A-VIII	3340, 3240, 3165, 3069	1687	1636	1063
A-IX	3335, 3245, 3160, 3085	1698	1630	1058

TABLE 4: DIAGNOSTICS IR BANDS OF COMPOUNDS (B-I TO B-IX) IN CM^{-1}

S. No.	υ(NH)	υ(CH_2) + υ(C-H)	υ(CO)	υ(C=N)	δ(NH)	υ(C=S)	υ(C-O-C)
B-I	3265, 3105	2865, 2940	1695	1601	1512	1320	1024
B-II	3260, 3132	2950, 2840	1690	1608	1508	1312	1015
B-III	3246, 3135	2940, 2830	1685	1595	1521	1342	1018
B-IV	3211, 3130	2955, 2845	1692	1598	1526	1338	1022
B-V	3245, 3136	2962, 2842	1690	1605	1522	1320	1030
B-VI	3256, 3151	2960, 2841	1696	1601	1513	1324	1015
B-VII	3220, 3140	2965, 2845	1700	1605	1508	1305	1028
B-VIII	3245, 3160	2932, 2890	1685	1602	1518	1321	1021

TABLE 5: ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COMPOUNDS B-I TO B-IX

ANTIFUNGAL INHIBITION AFTER 5 DAYS AND ANTIBACTERIAL INHIBITION AFTER 24 HRS

Fungi or bacteria	Conc. in ppm	B-I	B-II	B-III	B-IV	B-V	B-VI	B-VII	B-VIII
A. flavus	100 ppm	30	25	30	32	45	46	16	25
	200 ppm	42	38	45	46	55	56	32	30
Candida albicans	100 ppm	55	50	52	48	53	36	30	35
	200 ppm	70	68	68	50	68	50	35	40
F. oxysporum	100 ppm	45	38	39	40	40	38	30	41
	200 ppm	56	49	52	48	52	52	36	48
R. Phaseoli	100 ppm	48	45	40	42	50	33	25	22
	200 ppm	60	56	53	50	56	48	32	43
A. Niger	100 ppm	42	45	50	52	54	50	35	40
	200 ppm	55	60	62	64	68	62	48	52

ANTIBACTERIAL ACTIVITY

	Conc. in ppm	B-I	B-II	B-III	B-IV	B-V	B-VI	B-VII	B-VIII	Reference
E. Coli	250	6	5	6	8	8	5	3	3	22a
	500	8	7	8	14	15	7	5	5	24a
S. aureus	250	4	4	5	10	9	6	4	4	22a
	500	7	6	7	14	13	8	6	7	24 a
B. Subtilis	250	5	5	6	11	10	5	5	5	23 b
	500	7	8	8	14	14	9	7	8	26 b

Standard for antifungal growth, Mycostalin, Standard for antibacterial activity was ciprofloxacin (a) Streptomycin (b).

CONCLUSION: The mixed triazole, benzimidazole derivative show positive antibacterial properties as well as antifungal effect. The antifungal properties of retrosubstituted products are larger than other.

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