Synthesis, Characterization and Antimicrobial Screening Of 3-(1H-Benzo[di]imidazol-2-ylsulfanyl) Methyl-4-[Phenoxy(Phenyl) Acetamido]-5-Mercapto-1,2,4-Triazole and Related Aryloxy Compounds

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ABSTRACT
A series of new 1H-benzo[d]imidazole derivatives of 3,4-substituted triazole. 3-(1H-Benz[d]imidazol-2-ylsulfanyl)methyl-4-[phenoxo(phenylacetamido)]-5-mercapto-1,2,4-triazole and related aryloxy compounds were synthesised, analysed and characterised by FTIR, 'H NMR 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. In addition, triazole 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 in controlling cardiovascular diseases, anticaner properties, antiinflamatory, antibacterial, antifungal, antidiabetic and anti HIV activity and some benzimidazoles are antioxidant. It has also been noticed that certain benzimidazole derivatives like ciprofloxacin and norfloxacine 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-benz[d]imidazol-2-ylsulfanyl methyl derivatives, 3-(1H-Benz[d]imidazol-2-ylsulfanyl)methyl-4-[phenoxo(phenylacetamido)]-5-mercaptop-1,3,4-triazole (BSPT) and its nine related aryloxy derivatives (B-I to 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 were ascertained from MP and estimation of nitrogen. The FTIR spectra of compounds were recorded in KBr disc on Shimadzu, IR Spectrophotometer-2500. The 'H NMR spectra of compounds were recorded on a Brucker AV-400 spectrophotometer in CDCl₃ or DMSO or DMF-d6. The CHNS analysis reports, were obtained from CDRT Lucknow or BIT Mesra, Ranchi.

RESULT AND DISCUSSION
The compound BSPT (B-I to B-IX) were synthesized using (a) Aryloxyphenylacetic acid hydrazide (A-I to A-IX) (b) Ethyl bromoacetate (c) 1H-Benzimidazole-2-thiol (d) Potassium dithiocarbazinate of (1H-benz[d]imidazol-2-ylthio)methylcarbohydrazide. adopting scheme A, B and C

(a) Phenoxy(phenylacetacetic 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-benz[d]imidazol-2-ylthio)methylcarbonohydrazed 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) [C₆H₅-CHCl-COOC₂H₅] in dioxane on steam bath for 3 hours and 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-CH(C₆H₅)-COOC₂H₅) with hydrazine takes place as shown below:

\[ \text{RO}-\text{CH(C₆H₅)}-\text{COOC₂H₅} + \text{NH₂-NH₂} \rightarrow \text{RO}-\text{CH(C₆H₅)}-\text{CO-NH-NH₂} \]

with hydrazine takes place as shown below:

- The melting point and analytical results of acecho hydrazide A-I to A-IX are given in Table 1

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 K₂CO₃ 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 ethylbromacetate (Br-CH₂COC₂H₅) 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)acetohydrazide (M.P-236-237°C Reported-236°C)

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

The triazole containing benzimidazole derivatives (B-I to B-IX) were obtained by refluxing potassium dithiocarboazinate of (1H-benzo[d]imidazol-2-thio)methylcarbonohydrazide and aryloxy(phenylacetic acid)hydrazide in pyridine as given in Scheme C

The triazole containing benzimidazole derivatives (B-I to B-IX) were obtained by refluxing potassium dithiocarboazinate of (1H-benzo[d]imidazol-2-thio)methylcarbonohydrazide and aryloxy(phenylacetic acid)hydrazide in pyridine as given in Scheme C

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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 2. The i.r
spectral data of products B-I to B-IX and A-I to A-IX are given in Table 3 and 4.

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 chloroacetate 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-benzimidazole-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-benzimidazol-2-yl) sulphonylacetoxyhydrazide. General procedure for preparation of B-I to B-IX: About 10 millimole of B was taken in 30 ml ethanol and calculated amount of 10-12 millimole CS2 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-NH2] for 3 – 4 hours. The reflux on cooling gave cream yellow crystalline precipitate of pyridinium salts of B-I to B-IX. The free mercaptotriazole was obtained by suspending the pyridinium salt in 30-40 ml water and neutralising it with dilute HCl. The free triazole was recrystallised with ethanol-THF mixture. The result of elemental analyses and mpt of B-I to B-IX are given in Table 2.

RESULT AND DISCUSSION
1HNMR Spectra: The 1HNMR spectrum of A-I shows a singlet at δ = 3.68 ppm for (–O–CH(C6H5)–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, NH2 proton signals at δ = 5.315–5.685 ppm. The 1HNMR spectrum of A-II shows a singlet at 3.726 ppm for –O–CH(C6H5)–CO and phenyl proton signals as multiplete between δ = 7.015–7.935 ppm and a broad NH, NH2 proton signals were located at 5.465–5.285 ppm. The p-nitrophenoxy derivative A-III shows a signal at 3.865 ppm with 10 millimoles of aryloxyphenyl acetic acid hydrazide [Aro-CH(Ph)CO NH-NH2] for 3 – 4 hours. The reflux on cooling gave cream yellow crystalline precipitate of pyridinium salts of B-I to B-IX. The free mercaptotriazole was obtained by suspending the pyridinium salt in 30-40 ml water and neutralising it with dilute HCl. The free triazole was recrystallised with ethanol-THF mixture. The result of elemental analyses and mpt of B-I to B-IX are given in Table 2.
= 3.745 ppm attributed from (O-CH-CO) proton and phenyl ring C—H proton signals as multiplets between δ = 7.015 and 7.895 ppm. The NH and NH₂ proton signals were observed between δ = 5.425-5.845 ppm. 

The ¹H NMR 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-CH₂) proton signals between 3.745 ppm attributed from (O-CH-CO) proton and phenyl ring C—H proton signals as multiplets between δ = 7.015 and 7.895 ppm. The NH and NH₂ proton signals were observed between δ = 5.425-5.845 ppm. 

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signal at δ = 2.281 ppm as singlet as well as acetamide – CH₂ 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 ¹H NMR 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-CH₂) proton as singlet between 2.835–2.945 ppm. The (S-CH₂) proton of B-II were observed at δ = 2.865 ppm and acetoxy (CO-CH(C₆H₅)O-) proton signal at δ = 3.685 ppm. Its phenyl proton signals were observed as multiplets 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 signals 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-
Table 5: Antibacterial and antifungal activity of compounds B-I to B-IX, Antifungal inhibition after 5 days and antibacterial inhibition after 24 hrs.

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<th>B-III</th>
<th>B-IV</th>
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<th>B-VI</th>
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Antibacterial activity

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<th>B-III</th>
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Standard for antifungal growth, Mycostatine

Standard for antibacterial activity was ciprofloxacin(a) Streptomycine(b).

methylphenoxy(phenylacetamido)-5-mercapto-1,2,4-triazole (B-VI) displays –CH₃ proton signals at δ = 1.695 ppm as singlet. The –S-CH₃ proton signal was observed as singlet at δ = 2.945 ( 2H, -S-CH₂-) and –O-CH-(C₆H₅) 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. The i.r spectra of phenoxy and related aryloxy (phenylacetic acid)hydrazide show characteristic NH₂, NH, phenyl C=H and amido CO stretches in 3µ-16µ region and NH₂, NH, C-H and CO stretches as well as NH₂ bending and phenoxy Ph-O-C stretches of compounds A-I to A-IX are recorded in Table-3. The NH₂ 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 ν(CO) of amide group. The medium band located near 1636-1628 cm⁻¹ is attributed to δ(NH₂) of hydrazide group (CO-NH-NH₂). 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 ν(NH), ring ν(NH), ν(CH₂), ν(C-H), phenyl ring, ν(C=S), ν(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-2-yl)sulfonyl]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 ν(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 –CH₂-stretches of sulfamyl methyl (-S-CH₂-) and acetamido (-CO-CH₂-) 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 NO₂ band at 1481-1483 cm⁻¹.

The ν(S-H) attached to triazole ring could not be observed indicated the predominance of thione tautomomer in the molecule. The δ(NH) of B-I to B-IX were observed between 1526-1508 cm⁻¹ and ν(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 fingerprint region are assigned to phenyl and triazole ring skeletal vibrations.

Antibacterial and antifungal activity: The antifungal activity of BSPT (Compound B-I to B-IX) were evaluated by radical growth method 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

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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.nigar. The control solution was mycostalin. The result of activity shown in Table 5. Almost all benzimidazolylsulfanylmethyl triazole derivatives have causes inhibition of fungal growth but the activity of nitrophenoxo derivatives (B-IV & B-V) were quite encouraging comparable to mycostalin. The activity of B-I to B-IX were larger with candida albicans and A.nigar.

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 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 incutated 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.

The infrared and \(^1\)HNMR spectral data of compound A-I and A-IX

A-I Phenoxy (phenylacetic acid)hydrazide or aryloxy phenylicnatic acid hydrazide show prominent I.R bands for NH; 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 i.r bands are shown in Table 3

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

ACKNOWLEDGEMENT
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REFERENCE
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