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SYNTHESIS, SPECTRAL CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL PYRIMIDOBENZIMIDAZOLE DERIVATIVES

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ABSTRACT
Pyrimidobenzimidazole derivatives were synthesized, by reaction of 2/-amino-5/-cyano-4/-hydroxy pyrimido[1,2-a]benzimidazole **1(a-b)** with paraformaldehyde in formic acid to give **2(a-b)** which upon further reaction with methyl chloroacetate in presence of anhydrous potassium carbonate in acetone gave **3(a-b)** which on condensation with hydrazine hydrazide gave compounds **4(a-b)** which on further treatment with carbon disulfide in absolute ethanol in presence of potassium hydroxide formed potassium dithiocarbazate salt which when refluxed with hydrazine hydrate gave **5(a-b).** These compounds were evaluated for their antimicrobial and antifungal activities which showed promising results. The structure of all synthesized compounds was confirmed by IR, ^{1}HNMR, mass spectral data and elemental analysis.

KEYWORDS: Pyrimidobenzimidazole, triazole, antimicrobial activity, fungicidal activity.

INTRODUCTION
In the last few years fused benzimidazole and their substituted derivatives have been one of the mostly studied modules of heterocyclic compounds. They have received much interest from synthetic organic chemists due to their applications in several areas such as optical laser [i], fluorescent tags in DNA sequencing[ii]. Fused benzimidazoles show antimicrobial activities[iii], antiviral[iv] and antifungal[v] activities. During the last few decades, a great interest has been dedicated towards synthesis of 1,2,4-triazole-derivatives which possesses wide range of bioactivities.[vi-viii] 5-mercapto-(1,2,4)-trizole derivatives have found applications as antibacterial, herbicides, pesticides and anti-inflammatory agents.[ix-xii] In extension of our work on benzimidazole analogs, we have synthesized novel substituted pyrimidobenzimidazole derivatives with enhanced antimicrobial and antifungal activities.

EXPERIMENTAL SECTION

The reactions were monitored by TLC using 0.25 mm E-Merck silica gel plates, which were visualized in Iodine Chamber. Melting points were taken in open capillaries and are uncorrected. ^{1}H NMR spectra was performed in DMSO-d6 on 300 MHz using TMS as an internal standard.

SYNTHESIS OF COMPOUNDS

5′-Cyano-2′-(dimethylamino)-4′-hydroxy-3′-pyrimido [1,2-*a*]benzimidazole 2(a-b)

The powder of 2′-Amino-5′-cyano-4′-hydroxy-3′-pyrimido[1,2-a]benzimidazole **1(a-b)** (0.61g, 1.32 mole) was charged in formic acid (2.25g, 0.01 mole) and stirred for 30 min, till the clear solution was obtained. Paraformaldehyde (0.3g, 0.30 mole) was charged in the above reaction mass and refluxed for 12 h. The progress and completion of reaction was monitored by TLC using chloroform: methanol (9.5:0.5) as a solvent system. After completion of reaction, the reaction mass was cooled to 10-15^o C then neutralized by sodium hydroxide solution. A solvent was evaporated under reduced pressure, which resulted into the yellow colored solid which was recrystallized from aqueous ethanol gave 5′-Cyano-2′-(dimethylamino)-4′-hydroxy-3′-pyrimido [1,2-*a*]benzimidazole **2(a-b)**. Compounds were synthesized as per Ref [xiii-xiv] (Eschweiler-Clarke methylation *or* reductive alkylation).

5′-Cyano-2′-(dimethylamino)-4′-ethylacetoxy-3′-pyrimido[1,7-a]benzimidazole 3(a-b)

5′-Cyano-2′-(dimethylamino)-4′-hydroxy-3′-pyrimido[1,2-*a*]benzimidazole **2(a-b)** (2.5g, 0.01 mole) and anhydrous potassium carbonate (1g) mixture was stirred in 30 ml dry acetone and methyl chloroacetate (1.3g, 0.012 mole) was added drop wise at room temperature within 1h. The reaction mixture was refluxed for 5 h. The inorganic solid was filtered off and filtrate was poured onto crushed ice and water. Brown colored amorphous solid obtained was filtered and recrystallized from aqueous ethanol to give 5′-Cyano-2′-(dimethylamino)-4′-hydroxy-3′-pyrimido [1,2-*a*]benzimidazole **2(a-b)**.

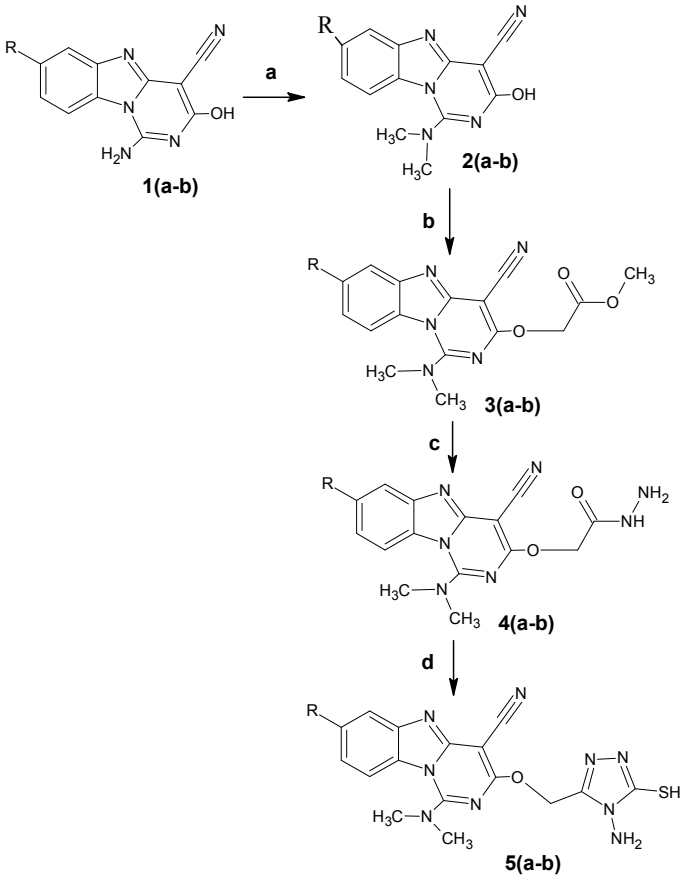
5′-Cyano-2′-(dimethylamino)-4′-acetoxyhydrazino-3′-pyrimido[1,7-a]benzimidazole 4(a-b)

5′-Cyano-2′-(dimethylamino)-4′-hydroxy-3′-pyrimido [1,2-*a*]benzimidazole **2(a-b)** (3.25g, 0.01mole) was dissolved in dry methanol and 98% hydrazine hydrate (0.35g, 0.011 mole) was added and refluxed for 5 h. The progress and completion of reaction was monitored by TLC using chloroform: methanol (9.5:0.5) as a solvent system. After completion of reaction, mixture was cooled to 25-30°C and filtered then washed with small quantity of cold methanol. The resulting yellow solid of 5′-Cyano-2′-(dimethylamino)-4′-acetoxyhydrazino-3′-pyrimido[1,7-a]benzimidazole **4(a-b)** was recrystallized from methanol. Compounds synthesized as per Ref. [xv] (Reactions of hydrazines with esters and carboxylic acids).

5′-cyano-2′-(dimethylamino)-4′-(4″-amino-1″,2″,4″-triazole-3″-mercapto-5-yl)methoxy -3′-pyrimido[1,7-a]benzimidazole 5(a-b)

4′-Acetoxyhydrazino-5′-Cyano-2′-(dimethylamino)-3′-pyrimido[1,7-a]benzimidazole **4(a-b)** (4.29g, 0.01mole) and potassium hydroxide (0.84g, 0.015 mol) stirred in absolute ethanol. To this carbon disulfide (1.15g, 0.015mole) was added gradually at low temperature. The reaction mixture was stirred at room temperature for 6 h where a light yellow precipitate of potassium dithiocarbazate salt was obtained. The potassium dithiocarbazate salt was stirred in dry ether at room temperature for 1 h then filtered off, washed with dry ether and dried. The salt was then

suspended in alcohol containing 98% hydrazine hydride (0.48g, 0.015mol), stirred and refluxed for 4 h. The reaction mixture was cooled at room temperature, diluted with ice cold water and neutralized with 10% hydrochloric acid. The precipitate obtained was filtered, washed thoroughly with cold water and recrystallized from ethanol to afford 5′-cyano-2′-(dimethylamino)-4′-(4″-amino-1″,2″,4″-triazole-3″-mercapto-5-yl)methoxy-3′-pyrimido [1,7-a] benzimidazole **5(a-b)**. Compounds synthesized as per Ref.[xvi]



CHARACTERIZATION OF SYNTHESIZED DERIVATIVES

5′-Cyano-2′-(dimethylamino)-4′-hydroxy-3′-pyrimido[1,7-*a*]benzimidazole (2a)

Yellow solid, Yield: 63 %, Melting Point: 190-194ºC. IR (KBr): 3561 (OH), 3001 (CH), 2245 (CN), 1560, 1310, 1188 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6/TMS): δ 3.17 (s, 6H, N-CH_3), δ 7.45.-7.85 (m, 4H, Ar-H), δ 12.55 (s, 1H, OH, D_2O exchangeable). M^{+1}: 253.25. Elemental Analysis % Calculated for $C_{13}H_{11}N_5O$: C, 61.65; H, 4.38; N, 27.65. Found: C, 62.58; H, 4.49; N, 27.31.

5′-Cyano-2′-(dimethylamino)-6-fluoro-4′-hydroxy-3′-pyrimido[1,7-*a*]benzimidazole (2b)
Dark yellow solid, Yield: 59 %, Melting Point: 210-215ºC. IR (KBr): 3610 (OH), 3196 (CH), 2255 (CN), 1556, 1340, 1315, 1184cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d6/TMS): δ 3.11 (s, 6H, N-CH_3), δ 7.55.-7.78 (m, 3H, Ar-H), δ 12.35(s, 1H, OH, D_2O exchangeable). M^{+1}: 271.25. Elemental Analysis % Calculated for $C_{13}H_{10}N_5FO$: C, 57.56; H, 3.72; F, 7.00; N, 25.82. Found: C, 58.01; H, 3.79; F, 6.91; N, 25.91.

5′-Cyano-2′-(dimethylamino)-4′-ethylacetoxy-3′-pyrimido[1,7-a] benzimidazole (3a)
Brown solid, Yield: 61%, Melting Point: 220-224ºC. IR (KBr): 2291 (CH), 2240 (CN), 1660 (>C=O), 1566, 1318, 1181cm-^{1}.^{1}H NMR (300 MHz, DMSO-d6/TMS): δ 3.20 (s, 6H, N-CH_3), δ 3.70 (s, 3H, OCH_3), δ 4.60 (s, 2H, OCH2), δ 7.65-7.83 (m, 4H, Ar-H). M^{+1}: 325.32. Elemental Analysis % Calculated for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53. Found: C, 58.96; H, 4.49; N, 21.41.

5′-Cyano-2′-(dimethylamino)-4′-ethylacetoxy-6-fluoro-3′-pyrimido-[1,7-a] benzimidazole (3b)
Brown solid, Yield: 72%, Melting Point: 185-190ºC. IR (KBr): 2280 (CH), 2259 (CN), 1640 (>C=O), 1555, 1325, 1328, 1161cm^{-1}.^{1}H NMR (300 MHz, DMSO-d6/TMS): δ 3.07 (s, 6H, N-CH_3), δ 3.65 (s, 3H, OCH_3), δ 4.50 (s, 2H, OCH_2), δ 7.40-7.63 (m, 3H, Ar-H). M^{+1}: 343.31. Elemental Analysis % Calculated for $C_{16}H_{14}N_5FO_3$: C, 55.98; H, 4.11; F, 5.53; N, 20.40. Found: C, 56.01; H, 3.79; F, 5.71; N, 20.91.

4′-Acetoxyhydrazino-5′-cyano-2′-(dimethylamino)-3′-pyrimido(1,7-a)benzimidazole (4a)
Yellow solid, Yield: 72%, Melting Point : 190-194ºC, IR (KBr): 3440 (NH_2), 3256 (NH), 2980 (CH), 2225 (CN), 1690 (HN-C=O), 1569, 1310, 1188cm^{-1}.^{1}H NMR (300 MHz, DMSO-d6/TMS): δ 3.18 (s, 6H, N-CH_3), δ 4.55 (s, 2H, OCH_2), δ 7.45-7.75 (m, 4H, Ar-H), δ 5.11(s, 1H, NH, D_2O exchangeable), δ 5.95 (s, 2H, NH_2, D_2O exchangeable). M^{+1}: 325.32. Elemental Analysis % Calculated for $C_{15}H_{15}N_7O_2$: C, 55.38; H, 4.65; N, 30.14. Found: C, 55.58; H, 4.59; N, 30.30.

4′-Acetoxyhydrazino-5′-cyano-2′-(dimethylamino)-6-fluoro-3′-pyrimido[1,7-a] benzimidazole (4b)
Yellow solid, Yield: 72%, Melting Point: 241-244ºC. IR (KBr): 3430 (NH_2), 3250 (NH), 2990 (CH), 2230 (CN), 1680 (HN-C=O), 1579, 1318, 1185cm^{-1}.^{1}H NMR (300 MHz, DMSO-d6/TMS): δ 3.09 (s, 6H, N-CH_3), δ 4.69 (s, 2H, OCH_2), δ 7.45.-7.83 (m, 3H, Ar-H), δ 4.91 (s, 1H, NH, D_2O exchangeable), δ 6.05 (s, 2H, NH_2, D_2O exchangeable). M^{+1}: 343.31. Elemental Analysis % Calculated for $C_{15}H_{14}N_7FO_2$: C, 52.48; H, 4.11; F, 5.53; N, 28.56. Found: C, 56.01; H, 3.99; F, 5.62; N, 28.71.

5′-Cyano-2′-(dimethylamino)-4′-(4″-amino-1″,2″,4″-triazole-3″-mercapto-5-yl) methoxy-3′-pyrimido[1,7-a]benzimidazole (5a)
Brown solid, Yield: 70 %, Melting Point : 187-191ºC, IR (KBr): 3450 (NH_2), 2970 (CH), 2389 (SH), 2220 (CN), 1650 (C=N), 1596, 1527, 1120 and 1082 cm^{-1}.^{1}H NMR (300 MHz, DMSO-d6/TMS): at δ 3.15 (s, 6H, N-CH_3), δ 4.55 (s, 2H, OCH_2), δ 7.35-7.72 (m, 4H, Ar-H), δ 5.54 (s, 2H, NH_2) and δ 2.14 (s, 1H, SH), which were D_2O exchangeable. M^{+1}: 381.41. Elemental

Analysis % calculated for $C_{16}H_{15}N_9OS$: C, 50.38; H, 3.96; N, 33.05; S, 8.41. Found: C, 50.49; H, 4.19; N, 33.25; S, 8.39.

5/-Cyano-2/-(dimethylamino)-6-fluro-4/-(4//-amino-1//,2//,4//-triazole-3//-mercapto-5-yl) methoxy-3/- pyrimido[1,7-a]benzimidazole (5b)

Brown solid, Yield: 52.2%, Melting Point : 214-219ºC.IR (KBr): 3455 (NH_2), 2980 (CH), 2379 (SH), 2230 (C≡N), 1655 (C=N), 1586, 1537, 1130 and 1081 cm^{-1}. ^1H NMR (300 MHz, DMSO-d6/TMS): at δ 3.21 (s, 6H, N-CH_3), δ 4.88 (s, 2H, CH_2, OCH_2), δ 7.38-7.61 (m, 3H, Ar-H), δ 6.04 (s, 2H, NH_2) and δ 2.11 (s, 1H, SH), which were D_2O exchangeable. M^{+1}: 399.41. Elemental Analysis % calculated for $C_{16}H_{14}N_9FOS$: C, 48.11; H, 3.53; F, 4.76; N, 31.56; S, 8.03. Found: C, 48.21; H, 3.69; F, 4.63; N, 31.99; S, 7.89.

ANTIMICROBIAL SCREENING

Antibacterial activity

All the synthesized derivatives 1(a-b), 2(a-b), 3(a-b), 4(a-b) and 5(a-b) were screened for their antibacterial activity by drug diffusion method by preparing the paper discs of the drug. The Muller-Hinton agar and 5 mm diameter paper discs of Whatman paper no. 1 were used. The activity was tested against three bacterial strains *Escherichia coli, Salmonella typhi* and *Staphylococcus aureus*. The compounds were dissolved in DMSO. The filter paper discs were soaked in different concentrations (50 µg/ml and 100 µg/ml) of the compounds, dried and then placed in the petriplates previously seeded with the test organisms *S. typhi, E. coli* and *S. aureus*. The plates were incubated for 24–30 h at 28±2°C and the inhibition zone around each disc was measured. The extent of inhibition was observed by measuring zone of inhibition in mm. As DMSO also has antimicrobial activity, DMSO also was used as a blank and its zone of inhibition was measured. The results are shown in Table: 1. Antibacterial activity exhibited by the derivatives was compared with Streptomycin as antibacterial standard.

Antifungal screening

The antifungal activity of the compounds was evaluated against *Aspergillus niger* by the paper disc method. The Sabouraud dextrose agar and 5 mm diameter paper discs of Whatman paper no.1 were used. The compounds were dissolved in DMSO. The filter paper discs were soaked in different solutions of the compounds, dried and then placed in the petriplates previously seeded with the test organisms *A. niger.* The plates were incubated for 48 h at 25±2°C and the inhibition zone around each disc was measured. The results are shown in Table: 1.

RESULTS AND DISCUSSION

In order to synthesize various pyrimidobenzimidazole derivatives, 2/-amino-5/-cyano -4/-hydroxy-3/-pyrimido[1,2-a]benzimidazole **1(a-b)** was used as a building block. Treatment of **1(a-b)** with paraformaldehde and formic acid provided compounds 5/-cyano-2/-(dimethylamino)-4/-hydroxy-3/-pyrimido[1,2-*a*]benzimidazole **2(a-b)**. IR spectra of **2(a-b)** shows significant characteristic absorption band of (OH) at 3561cm^{-1} and absence of significant characteristic absorption band of (NH_2) at 3450 cm^{-1}. ^1H NMR: at δ 3.17 (s, 6H, N-CH_3), δ 7.45-7.85 (m, 4H, aromatic), δ 12.55 (s, 1H, OH, D_2O exchangeable). MS (m/z): 253.25 (M^+). (Found: C, 62.58; H, 4.49; N, 27.31 required for $C_{13}H_{11}N_5O$: C, 61.65; H, 4.38; N, 27.65). Thus the data obtained confirmed the conversion of NH_2 to N-$(CH_3)_2$. **2(a-b)** condensed with methyl chloroacetate gave compounds 5/-cyano-2/-(dimethylamino)-4/-ethylacetoxy-3/pyrimido[1,7-a]benzimidazole **3(a-b)**. IR spectra show significant characteristic absorption

band at 1740 cm^{-1} (>C=O) due to carbonyl of ester and do not show significant characteristic absorption band of (OH) at 3561 cm^{-1}. ^1H NMR spectra of compounds shows singlet's at δ 3.65 (s, 3H, OCH_3), δ 4.60 (s, 2H, OCH_2) and absence of singlet at δ 12.40 (s, 1H, OH). MS (m/z): 253.25 (M^+). (Found C, 58.96; H, 4.49; N, 21.41 require for $C_{16}H_{15}N_5O_3$: C, 59.07; H, 4.65; N, 21.53). Thus the data obtained confirmed the conversion of hydroxyl to ester. **3(a-b)** which were refluxed with hydrazine hydrate in methanol gave compounds 4/-acetoxyhydrazino-5/-cyano-2/-(dimethylamino)-3/-pyrimido[1,7-a]benzimidazole **4(a-b)**. IR spectra showed strong bands at 3440 cm^{-1} (NH_2), 3256 cm^{-1} (NH), 1690 cm^{-1} (C=O) of amide. ^1H NMR displayed singlet at δ 4.55 (s, 2H, OCH_2), δ 5.12 (s, 1H, NH, D_2O exchangeable), δ 5.95 (s, 2H, NH_2, D_2O exchangeable). MS (m/z): 325.32 (M^+). (Found: C, 55.58; H, 4.59; N, 30.30 require for $C_{15}H_{15}N_7O_2$: C, 55.38; H, 4.65; N, 30.14). Thus the data obtained, confirmed formation of acetoxyhydrazino compounds. **4(a-b)** treated with carbon disulfide in presence of potassium hydroxide in absolute ethanol formed potassium dithiocarbazate salt. The salt was treated with 98% hydrazine hydrate and neutralized with hydrochloric acid gave 5/-cyano-2/-(dimethylamino)-4/-(4//-amino-1//,2//,4//-triazole-3//-mercapto-5-yl)methoxy-3/- pyrimido[1,7-a]benzimidazole **5(a-b)**. IR spectra showed strong band at 3450 cm^{-1} (NH_2), 2389 cm^{-1} (SH) and absence of band at 1690 cm^{-1} (C=O) of amide. ^1H NMR showed presence of peaks at δ 5.54 (s, 2H, NH_2), δ 2.14 (s, 1H, SH) which were D_2O exchangeable and absence of δ 5.12 (s, 1H, NH, D_2O exchangeable). MS (m/z): 381.41 (M^+). (Found: C, 50.49; H, 4.19; N, 33.25; S, 8.39 require for $C_{16}H_{15}N_9OS$: C, 50.38; H, 3.96; N, 33.05; S, 8.41). Thus the data obtained, confirmed formation of 4-amino-3-mercapto -1,2,4-triazole derivatives of pyrimido [1,2-*a*] benzimidazole.

The Inhibition zone against the bacteria *S. aureus, E. coli, S. typhi* and fungus *A. niger* due to the different substituted pyrimidobenzimidazole derivatives are shown in Table 1. Highest antimicrobial potential was observed with compounds 2b, 3b, 4b, 5a, and 5b against *S. aureus.* Compounds 3b, 4b, 5a, and 5b were found to be potent against *S.typhi* whereas compounds 3b, 4b, 5a, 5b exhibited remarkable activity against *E. coli*. On the other hand compounds 2b, 3b, 4b and 5b showed highest antifungal potential against *A. niger.*

Table: 1 Antibacterial and antifungal activity

Compounds	Zone of Inhibition (mm)							
	S.aureus		*S.typhi*		*E. coli*		*A. niger*	
	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
1a	10	14	10	16	09	12	10	13
1b	12	15	13	15	12	15	13	16
2a	12	16	12	16	13	15	11	15
2b	16	18	11	15	16	19	17	20
3a	10	15	12	16	12	15	15	21
3b	17	19	15	18	16	18	16	18
4a	15	20	13	10	14	17	12	17
4b	18	21	16	20	16	20	15	19
5a	15	18	15	19	15	20	14	16
5b	16	19	16	19	15	18	16	20

CONCLUSION

Spectral techniques used in the scheme confirmed synthetic route and the formation of novel pyrimidobenzimidazole derivatives. From the result of antibacterial and antifungal activity, it is seen that synthesized derivatives exhibited significant to moderate activity. This confirms that all the newly synthesized ethylacetoxy, acetoxyhydrazino, 1,2,4- triazole derivatives of pyridobenzimidazole are biologically active towards the tested microbial strains.

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