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Synthesis and biological evaluation of benzo[*d*]imidazolyl chromeno[2,3-*d*]pyrimidinones

Anisetti Ravindernath · Malladi Srinivas Reddy · Vodela Sunil

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Abstract A series of benzo[*d*]imidazolyl chromeno[2,3-*d*]pyrimidnones were described. The key intermediate 2-cyano-*N*-(2-mercapto-1*H*-benzo[*d*]imidazol-5-yl)acetamide (**3**) is obtained by reacting 5-amino-2-mercaptobenzimidazole (**1**) with ethyl cyanoacetate (**2**). Compound **3** on reaction with substituted salicylaldehydes afforded 2-imino-*N*-(2-mercapto-1*H*-benzo[*d*]imidazol-5-yl)-2*H*-chromene-3-carboxamides (**5**) in good yields. Compounds **5** on condensation with formalin furnished the title compounds viz., 3-(2-mercapto-1*H*-benzo[*d*]imidazol-5-yl)-2*H*-chromeno[2,3-*d*]pyrimidin-4(3*H*)-ones (**7**). All the synthesized compounds were screened for their anti-microbial and anti-oxidant activities.

Keywords
Benzo[*d*]imidazolyl chromeno[2,3-*d*]pyrimidinones · Condensation · Anti-bacterial activity · Anti-fungal activity · Anti-oxidant activity

Introduction

Chromeno derivatives are an important class of compounds, widely present in plants, including edible vegetables and fruits (Curini *et al*., 2006). Numerous bioactive natural products have been identified, and the presence of the chromeno-based structure has been associated with the capacity to prevent diseases (O'Kennedy and Thornes, 1997). Synthetic analogs have been developed over the years, some of them displaying remarkable effects as pharmaceuticals (Borges *et al*., 2005), including anti-fungal (Tangmouo *et al*., 2006), anti-microbial (Kitamura *et al*., 2006), molluscidial (Abdelrazek *et al*., 2007), anti-coagulant, spasmolytic, diuretic, anti-cancer, and anti-anaphylactic characteristics (Singh *et al*., 1996). Pyrimidines and its fused derivatives play an essential role in several biological processes and chemical and pharmacological importance. In particular, pyrimidine nucleus can be found in a broad variety of anti-bacterial and anti-tumor agents as well as in agrochemical and veterinary products (Ismali *et al*., 2008; El-Gaby *et al*., 2006; Prikazchikova *et al*., 1975; Brown *et al*., 1984).

Benzimidazole derivatives are also reported to possess analgesic (Sondhi *et al*., 2002), anti-helminthic (Hazelton *et al*., 1995; Labaw and Webb, 1981), anti-inflammatory (Ito *et al*., 1982), anti-microbial, anti-arthritic, anti-bacterial, anti-tumor, and anti-HIV activities (Rao *et al*., 2002). This in view of diverse therapeutic activity of chromenes, pyrimidines, and benzimidazoles and in the course of our search for an effective biomimetic model compounds (Ravindernath and Srinivas Reddy, 2012), chromeno[2,3-*d*]pyrimidines, which can be regarded as a 5-deazaflavin analog, was selected as a target molecule (Fig. 1; Scheme 1).

Experimental

Materials and methods

All the melting points were determined on Cintex melting point apparatus and are uncorrected. Analytical TLC was

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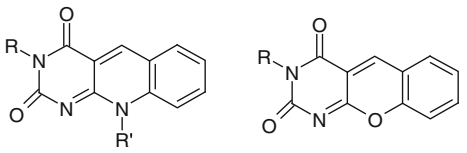


5-deazaflavin **5-deaza-10-oxaflavin**

Fig. 1 Biologically active chromeno[2,3-*d*]pyrimidines as design templates

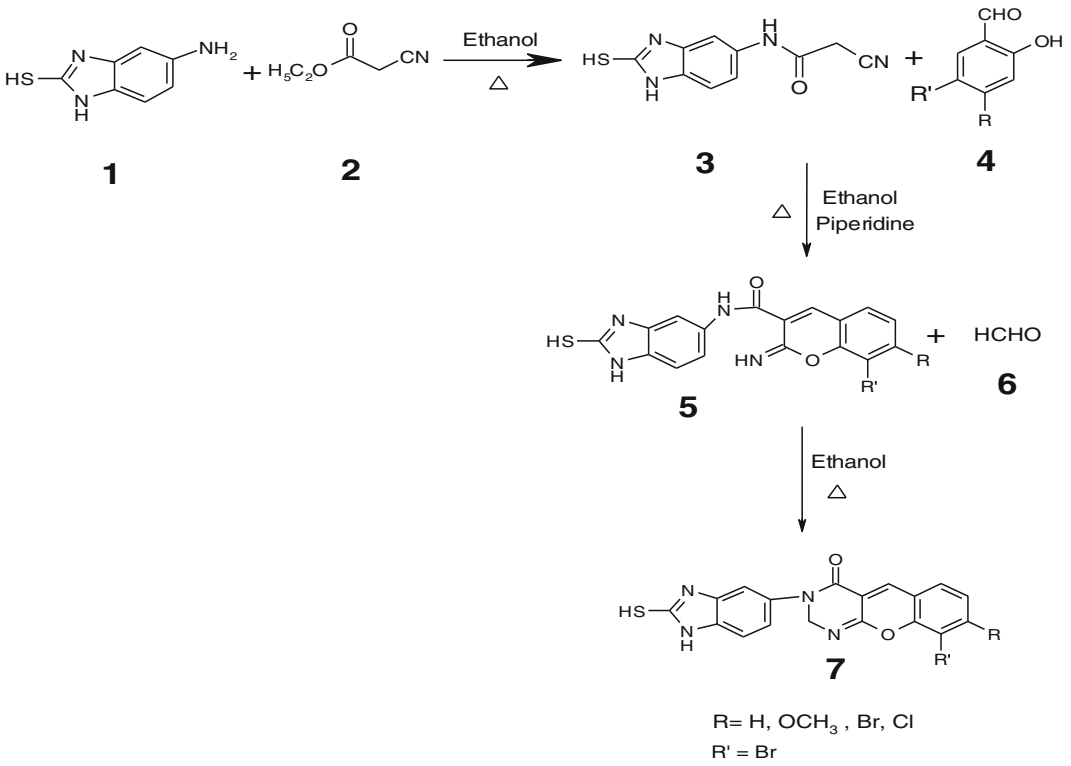
performed on Merck precoated 60 F_{254} silica gel plates. Visualization was done by exposure to iodine vapor. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in ppm (δ) with tetramethyl silane as an internal standard. Mass spectral measurements were carried out by EI method on a Joel JMC-300 spectrometer at 70 eV. Elemental analyses were performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

Synthesis

*General procedure for the synthesis of 2-cyano-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)acetamide (**3**)* A mixture of 5-amino-2-mercapto benzimidazole **1** (1 mmol) and ethyl cyanoacetate **2** (1 mmol) in ethanol (15 mL) were refluxed for 5 h. After the completion of the reaction (monitored by TLC), reaction mixture was cooled and the solid obtained was filtered off and recrystallized from ethyl acetate to give benzimidazolyl cyanoacetamide **3**.

*2-Cyano-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)acetamide (**3**)* m.p. 116–118 °C, Yield, 78 %; IR (KBr) cm^{-1}: 3363, 3176, 2471, 1618. ^{1}H NMR ($CDCl_3$) δ (ppm): 3.58 (s, 1H, SH) 3.80 (s, 2H, CH_2CN), 4.98 (s, 1H, NH, D_2O exchangeable), 6.93 (s, 1H), 7.14 (d, 1H), 7.18 (d, 1H), 8.58 (s, 1H, CONH, D_2O exchangeable). ESI–MS *m/z* 233 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$): 23.90, 108.22, 115.48, 123.74, 125.71, 134.57, 135.29, 139.20, 168.24, 168.45. Anal. Calcd. for $C_{10}H_8N_4OS$: C, 51.71; H, 3.47; N, 24.12. Found: C, 51.65; H, 3.50; N, 24.07 %.

*General procedure for the synthesis of benzimidazolyl chromene-3-carboxamides (**5a–e**)* To a solution of compound **2** (1 mmol) in absolute ethanol (20 mL) containing piperidine (0.5 mL), substituted salicylaldehydes **4** (1 mmol) was added. The reaction mixture was heated under reflux for 3 h and then allowed to cool. The precipitate obtained on pouring the reaction mixture into crushed ice was filtered off, washed with ethanol, dried, and recrystallized from ethyl acetate to afford **5**. Spectral data of each compound are given below.



Scheme 1 Synthesis of benzimidazolyl chromeno[2,3-*d*]pyrimidin-4(3*H*)-ones (**7a–e**)

*2-Imino-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromene-3-carboxamide (**5a**)* m.p. 168–170 °C Yield, 79 %; IR (KBr) cm^{-1}: 3310, 3250, 3200, 1770. ^1H NMR ($CDCl_3$) δ (ppm): 4.42 (s, 1H, SH), 6.24 (s, 1H, =CH), 6.98–7.40 (m, 7H, Ar–H), 8.62 (s, 1H, NH, D_2O exchangeable), 9.46 (s, 1H, =NH), 10.20 (s, 1H, CONH, D_2O exchangeable). ESI–MS *m/z* 337 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 107.37, 113.81, 115.20, 115.57, 119.01, 121.67, 123.72, 127.98, 130.16, 132.65, 134.54, 138.90, 140.45, 141.54, 155.23, 162.67, 164.10. Anal. calcd for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66; Found C, 60.76; H, 3.58; N, 16.68 %.

*2-Imino-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)-7-methoxy-2H-chromene-3-carboxamide (**5b**)* m.p. 151–153 °C. Yield, 75 %; IR (KBr) cm^{-1}: 3210, 3125, 3053, 1677. ^1H NMR ($CDCl_3$) δ (ppm): 3.63 (s, 3H, OCH_3), 4.38 (s, 1H, SH), 6.18 (s, 1H, =CH), 6.87–7.61 (m, 6H, Ar–H), 8.32 (s, 1H, NH, D_2O exchangeable), 9.48 (s, 1H, =NH), 10.12 (s, 1H, CONH, D_2O exchangeable). ESI–MS *m/z* 367 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 63.14, 106.96, 114.15, 114.50, 115.07, 119.01, 120.67, 122.42, 126.98, 129.96, 131.85, 133.94, 137.99, 141.05, 142.04, 154.85, 163.32, 164.19. Anal. calcd for $C_{18}H_{14}N_4O_3S$: C, 59.01; H, 3.85; N, 15.29; Found C, 59.05; H, 3.81; N, 15.31 %.

*7-Bromo-2-imino-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromene-3-carboxamide (**5c**)* m.p. 163–165 °C Yield, 81 %; IR (KBr) cm^{-1}: 3330, 3231, 3225, 1770. ^1H NMR ($CDCl_3$) δ (ppm): 4.36 (s, 1H, SH), 6.23 (S, 1H, =CH), 6.97–7.20 (m, 6H, Ar–H), 8.22 (s, 1H, NH, D_2O exchangeable), 9.37 (s, 1H, =NH), 10.01 (s, 1H, CONH, D_2O exchangeable). ESI–MS *m/z* 415 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm):108.01, 114.21, 114.90, 115.01, 120.21, 122.07, 122.92, 126.98, 131.16, 133.05, 133.94, 139.01, 139.95, 140.84, 154.93, 163.67, 163.99. Anal. calcd for $C_{17}H_{11}BrN_4O_2S$: C, 49.17; H, 2.67; N, 13.49; Found C, 49.21; H, 2.61; N, 13.54 %.

*7-Chloro-2-imino-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromene-3-carboxamide (**5d**)* m.p. 170–172 °C. Yield, 84 %; IR (KBr) cm^{-1}: 3353, 3240, 3215, 1737. ^1H NMR ($CDCl_3$) δ (ppm): 4.20 (s, 1H, SH), 6.11 (s, 1H, =CH), 6.82–7.71 (m, 6H, Ar–H), 8.43 (s, 1H, NH, D_2O exchangeable), 9.40 (s, 1H, =NH),10.18 (s, 1H, CONH, D_2O exchangeable). ESI–MS *m/z* 371 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 106.96, 112.90, 114.80, 115.01, 120.01, 122.45, 122.85, 127.02, 131.08, 133.32, 134.98, 137.89, 141.55, 142.54, 154.83, 162.94, 163.90. Anal. calcd for $C_{17}H_{11}ClN_4O_2S$: C, 55.06; H, 2.99; N, 15.11; Found C, 55.10; H, 2.94; N, 15.15 %.

*7,8-Dibromo-2-imino-N-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromene-3-carboxamide (**5e**)* m.p. 177–179 °C. Yield, 75 %; IR (KBr) cm^{-1}: 3311, 3232, 3201, 1770. ^1H NMR ($CDCl_3$) δ (ppm): 4.20 (s, 1H, SH), 6.10 (s, 1H, =CH), 6.91–7.81 (m, 5H, Ar–H), 8.30 (s, 1H, NH, D_2O exchangeable), 9.21 (s, 1H, =NH), 10.00 (s, 1H, CONH, D_2O exchangeable). ESI–MS *m/z* 495 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 107.96, 114.21, 116.20, 116.71, 119.88, 120.99, 122.72, 126.98, 131.16, 133.15, 134.86, 137.90, 141.45, 142.24, 154.99, 163.47, 164.89. Anal. calcd for $C_{17}H_{10}Br_2N_4O_2S$: C, 41.32; H, 2.04; N, 11.34; Found C, 41.37; H, 2.10; N, 11.38 %.

*General procedure for the synthesis of benzimidazolyl chromeno[2,3-d]pyrimidin-4(3H)-ones (**7a–e**)* A mixture of compound **5** (1 mmol) and formalin **6** (37 % 1 mmol) was refluxed in ethanol (15 mL) for 4 h. The reaction mixture after cooling to room temperature was poured into ice-cold water. The separated solid was filtered and recrystallized from benzene to get pure compounds **7a–e**. Spectral data of each compound are given below.

*3-(2-Mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromeno[2,3-d]pyrimidin-4(3H)-one (**7a**)* m.p. 203–205 °C. Yield, 77 %; IR (KBr) cm^{-1}: 3375 (NH), 1631 (C=O). ^1H NMR ($CDCl_3$) δ (ppm): 4.50 (s, 2H, CH_2), 4.56 (s, 1H, SH), 6.51 (s, 1H, =CH), 6.81–7.66 (m, 7H, Ar–H), 8.13 (s, 1H, NH, D_2O exchangeable). ESI–MS *m/z* 349 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 61.91, 106.45, 115.37, 115.92, 116.53, 121.75, 122.17, 122.54, 127.42, 129.93, 131.53, 133.78, 138.30, 142.16, 142.72, 154.63, 162.67, 164.53. Anal. calcd for $C_{18}H_{12}N_4O_2S$: C, 62.06; H, 3.47; N, 16.08; Found C, 62.01; H, 3.40; N, 16.14 %.

*3-(2-Mercapto-1H-benzo[d]imidazol-5-yl)-8-methoxy-2H-chromeno[2,3-d]pyrimidin-4(3H)-one (**7b**)* m.p. 210–214 °C. Yield, 79 %; IR (KBr) cm^{-1}: 3250, 1609.^1H NMR ($CDCl_3$) δ (ppm): 3.63 (s, 3H, OCH_3), 4.22 (s, 1H, SH), 4.55 (s, 2H, CH_2), 6.11 (s, 1H, =CH), 6.92–7.30 (m, 6H, Ar–H), 8.18 (s, 1H, NH, D_2O exchangeable). ESI–MS *m/z* 379 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 62.11, 63.56, 107.15, 114.98, 116.22, 117.43, 120.98, 121.77, 123.24, 127.98, 128.33, 132.83, 133.08, 137.87, 142.93, 143.55, 154.88, 163.07, 164.96. Anal. calcd for $C_{19}H_{14}N_4O_3S$: C, 60.31; H, 3.73; N, 14.81; Found C, 60.22; H, 3.78; N, 14.86 %.

*8-Bromo-3-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromeno[2,3-d]pyrimidin-4(3H)-one (**7c**)* m.p. 218–222 °C. Yield, 74 %; IR (KBr) cm^{-1}: 3215, 1730. ^1H NMR ($CDCl_3$) δ (ppm): 4.33 (s, 2H, CH_2), 4.62 (s, 1H, SH), 6.51 (s, 1H, =CH), 6.34–7.22 (m, 6H, Ar–H), 8.21 (s, 1H, NH, D_2O exchangeable). ESI–MS *m/z* 427 $[M+H]^+$.

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 62.11, 107.35, 116.07, 116.92, 117.23, 122.35, 123.57, 123.98, 127.42, 130.63, 132.65, 134.48, 138.99, 143.11, 143.82, 153.58, 163.27, 164.85. Anal. calcd for $C_{18}H_{11}BrN_4O_2S$: C, 50.60; H, 2.59; N, 13.11; Found C, 50.63; H, 2.55; N, 13.15 %.

8-Chloro-3-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromeno[2,3-d]pyrimidin-4(3H)-one (***7d***) m.p. 220–224 °C. Yield, 79 %; IR (KBr) cm^{-1}: 3225, 1740. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.31 (s, 2H, CH_2), 4.68 (s, 1H, SH), 6.44 (s, 1H, =CH), 6.45–7.28 (m, 6H, Ar–H), 8.24 (s, 1H, NH, D_2O exchangeable).ESI–MS *m/z* 383 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 62.56, 105.99, 116.37, 116.97, 117.32, 120.99, 121.47, 123.24, 128.02, 129.01, 132.33, 134.48, 138.86, 141.93, 142.01, 153.63, 161.85, 164.53. Anal. calcd for $C_{18}H_{11}ClN_4O_2S$: C, 56.47; H, 2.90; N, 14.64; Found C, 56.52; H, 2.94; N, 14.58 %.

8,9-Dibromo-3-(2-mercapto-1H-benzo[d]imidazol-5-yl)-2H-chromeno[2,3-d]pyrimidin-4(3H)-one (***7e***) m.p. 250–254 °C. Yield, 82 %; IR (KBr) cm^{-1}: 3245, 1760. ^{1}H NMR ($CDCl_3$) δ (ppm): 4.25 (s, 2H, CH_2), 4.62 (s, 1H, SH), 6.38 (s, 1H, =CH), 6.51–7.66 (m, 5H, Ar–H), 8.30 (s, 1H, NH, D_2O exchangeable). ESI–MS *m/z* 507 $[M+H]^+$. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 60.99, 107.35, 114.89, 116.22, 116.89, 120.75, 122.78, 123.44, 126.12, 128.63, 132.23, 133.87, 137.75, 141.76, 143.27, 153.73, 161.34, 162.99. Anal. calcd for $C_{18}H_{10}Br_2N_4O_2S$: C, 42.71; H, 1.99; N, 11.07; Found C, 42.75; H, 1.95; N, 11.11 %.

The structures of all newly synthesized compounds were confirmed by analytical and spectral data (IR, ^{1}H NMR, and MS).

Pharmacological screening

Anti-bacterial assay

The newly synthesized compounds **7a–e** were evaluated for their in vitro anti-bacterial activity against Gram-positive bacteria viz., *Bacillus subtilis, Bacillus cereus* and Gram-negative bacteria viz. *Micrococcus luteus, Entamoeba Coli* at 100 μg mL^{-1} concentration. The in vitro anti-bacterial activity of the tested compounds was assessed by minimum inhibitory concentration (MIC) using broth dilution method (NCCLS, 1982). Ciprofloxacin was used as standard comparison. The readymade nutrient broth media (Himedia, 24 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb inc^{-2} for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound was dissolved in DMSO and a concentration of 250 μg mL^{-1} of the test compounds was added in the first test tube, which was serially diluted. A fixed volume of 0.5 mL of overnight culture was added in all the test tube which was incubated at 37 °C for 24 h. After 24 h, these tubes were measured for turbidity.

Anti-fungal assay

The target compounds **7a–e** were also evaluated for their anti-fungal activity against *Candida albicans* and *Aspergillus niger* in acetone by agar cup bioassay method (Margery Linday, 1962), using fluconazole as the standard drug. The MIC was recorded in μg mL^{-1}. The readymade potato dextrose agar media (Himedia, 39 g) was suspended in distilled water (100 mL) and heated until it dissolved completely. The medium and Petri dishes were autoclaved at a pressure of 15 lb inc^{-2} for 20 min. The medium poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of the (week-old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone (100 μg mL^{-1}). Agar inoculation cups were scooped out with 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, 100 μg mL^{-1} of test solution was added. Controls were maintained with acetone and clotrimazole (100 μg mL^{-1}). The treated and the control were kept at room temperature for 72–96 h. Inhibition zones were measured and the diameter was calculated in millimeters. Three to four replicates were maintained for each treatment.

Anti-oxidant activity

For the evaluation of anti-oxidant activity, we have used a stable free radical α,α-diphenyl-β-picryl hydrazyl (DPPH), at the concentration of 0.2 mM in methanol (Ranjit *et al.*, 2010). To 0.1 mL of test compound (at different concentrations), 1.5 mL of methanol and 0.5 mL of DPPH solution were added, mixed thoroughly and absorbance (OD) was read at 517 nm against the blank. The % reduction of free radical concentration (OD) with different concentration of test compounds was calculated and was compared with standard, ascorbic acid. The results were expressed as IC_{50} values (the concentration of test required to scavenge 50 % free radicals).

Results and discussion

Synthesis

The key intermediate, 2-cyano-*N*-(2-mercapto-1*H*-benzo[*d*]imidazol-5-yl)acetamide **3** required for synthesis of target compounds was obtained by reacting 5-amino-2-mercapto benzimidazole **1** with ethyl cyanoacetate **2** in refluxing ethanol. IR spectrum of benzimidazolyl cyanoacetamide **3**

showed absorption bands 3363, 3176, 2471, and 1618 cm^{-1} benzimidazole-NH, NH, CN, and C=O functional group stretching vibrations, respectively. ^1H NMR spectra of **3** showed singlet at δ 3.80 due to CH_2 proton, broad singlet at δ 8.58 CONH proton. The mass spectrum of **3** is in conformity with the structure by displaying the molecular ion peak at $[M+H]^+$ *m/z* 233.

Cyclocondensation of benzimidazolyl cyanoacetamide **3** with substituted salicylaldehydes **4** in boiling absolute ethanol containing a catalytic amount of piperidine afforded the chromeno derivatives viz., 2-imino-*N*-(2-mercapto-1*H*-benzo[*d*]imidazol-5-yl)-2*H*-chromene-3-carboxamides **5** in good yields (75–85 %). The scope and generality of this reaction is illustrated by reacting with benzimidazolyl cyanoacetamide and different substituted salicylaldehydes afforded their corresponding derivatives **5a–e** without any difficulty. In the IR spectra of compounds **5** exhibited three NH stretching absorptions at 3310, 3250, 3200, and amide carbonyl at 1770 cm^{-1} confirm the cyclization. Further support was obtained from ^1H NMR spectra of **5**, in which benzimidazole-NH, imine-NH, and amide-NH proton signals appeared as singlets at δ 8.62, 9.46, and 10.20, respectively, which are D_2O exchangeable. The mass spectrum of **5a** is in conformity with the assigned structure, which displayed the molecular ion peak at $[M+H]^+$ *m/z* 337 corresponding to its molecular formula. Elemental analyses satisfactorily confirmed elemental composition and purity of the synthesized compounds.

Compounds **5** on heating with formalin in ethanol afforded the corresponding 3-(2-mercapto-1*H*-benzo [*d*]imidazol-5-yl)-2*H*-chromeno[2,3-*d*]pyrimidin-4(3*H*)-ones **7** in good yields. The IR spectra of compounds **5** showed the disappearance of amide-NH and imine-NH functional group stretching absorption bands, present in its precursor **5** at 3310 and 3250 cm^{-1}, indicating the evidence for cyclization and formation of title compound **5**. In ^1H NMR spectra of **5,** the absence of imine-NH and amide-NH proton signals (present in its precursor **5** at δ 9.46 and 10.20) and appearance of a new signal as a singlet at δ 4.50 is due to –N–CH_2–N– protons clearly confirms the product formation **7**. Moreover, the mass spectrum of **7a** showed the molecular ion peak at *m/z* 349 $[M+H]^+$, which well agrees with the molecular weight of the proposed structure. C, H, N analyses are also in full conformity with the assigned structures **7a–e**.

Biological screening

Anti-microbial activity

The newly synthesized compounds **7a–e** were evaluated for their in vitro anti-bacterial activity against Gram-positive bacteria viz., *B. subtilis*, *B. cereus* and Gram-negative bacteria viz., *M. luteus, E. Coli* at 100 μg mL^{-1} concentration. The results of anti-bacterial screening (Table 1) reveal that the compounds **7a–e** displayed a better activity and more active than the standard Ciprofloxacin. Compound **7b** possessing methoxy group as substituent on the benzene ring showed a better activity. Compound **7a** exhibited least activity because it has no substituent on the benzene ring. However, the degree of inhibition varied both with the test compound and with the bacteria used.

The title compounds **7a–e** were also evaluated for their anti-fungal activity against *C. albicans* and *A. niger* in acetone by agar cup bioassay method (**19**), using clotrimazole as standard drug. The anti-fungal activity results (Table 2) indicate that compounds **7a–e** are significantly toxic toward all the fungi under investigation. Compound **7c** possessing bromo substituent on benzene ring are highly toxic toward all the fungi. However, the degree or spore germination inhibition varied with the test compound as well as with the fungi under investigation.

Anti-oxidant activity

The anti-oxidant DPPH free radical scavenging activity of all the synthesized compounds **7a–e** were performed using DPPH method is shown in Table 3. The IC_{50} values of all the compounds **7a–e** were found between 12.62 and 56.45 μM, with anti-oxidant activity. In the series,

Table 1 Anti-bacterial activity of benzimidazolyl chromeno[2,3-*d*]pyrimidin-4(3*H*)-ones (**7a–e**) (minimum inhibitory concentration)

Compound	*B. subtilis*	*B. cereus*	*M. luteus*	*E. coli*
7a	21	23	18	22
7b	18	20	17	19
7c	23	20	19	26
7d	20	26	24	20
7e	26	21	18	23
Ciprofloxacin	24	24	25	25

Negative control (acetone)—no activity; concentration 100 μg mL^{-1}

Table 2 Anti-fungal activity of benzimidazolyl chromeno[2,3-*d*]pyrimidin-4(3*H*)-ones (**7a–e**) (zone of inhibition in mm)

Compound	*C. albicans*	*A. niger*
7a	22	24
7b	22	23
7c	36	27
7d	20	28
7e	24	20
Fluconazole	24	18

Negative control (acetone)—no activity; concentration 100 μg mL^{-1}

Table 3 Anti-oxidant activity of benzimidazolyl chromeno[2,3-*d*]pyrimidin-4(3*H*)-ones (**7a–e**)

Compound	R	R′	IC_{50} (μM)
7a	H	H	56.45
7b	OCH_3	H	45.20
7c	Br	H	21.36
7d	Cl	H	29.46
7e	Br	Br	12.62
Ascorbic acid	–	–	8.64

compounds **7c** and **7e** possessing bromo and dibromo substituent's on the benzene ring showed better activity against DPPH free radicals.

Conclusion

In conclusion, we have demonstrated a simple, facile convenient, and efficient synthesis of benzo[*d*]imidazolyl chromeno[2,3-*d*]pyrimidones from commercially available materials and screened for their anti-bacterial, anti-fungal, and anti-oxidant activities. All the synthesized compounds **7a–e** exhibited good anti-bacterial, anti-fungal, and anti-oxidant activities.

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