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Synthesis of some nitrogen heterocycles and in vitro evaluation of their antimicrobial and antitumor activity

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Abstract Treatment of ethyl β-aryl-α-cyanoacrylate (**2a**, **b**) with thiourea, guanidine hydrochloride, and thiosemicarbazide in presence of anhydrous potassium carbonate in methanol led to formation of pyrimidine derivatives **3** and **5** and thiosemicarbazone derivative **9**. Thiazole derivative **10** was prepared via cyclization of thiosemicarbazone derivative **9** with 4-methoxy phenacyl bromide. Acetylation of **3a**, **5**, and **10** with acetic anhydride yielded the acetoxy and *N*-acetyl derivatives **4**, **6**, and **11**. The mass-spectral fragmentation patterns of nitrogen heterocycles were investigated to elucidate the structure of the prepared compounds. Biological studies of nitrogen heterocycles were carried out to investigate their antimicrobial and anticancer activities; it was found that compounds **5**, **10**, and **11** were highly active against bacteria and fungi, and compounds **3a** and **3b** were also active against bacteria and fungi.

Keywords Synthesis · Biological activity · Pyrimidine · Thiazole

Introduction

Nitrogen heterocycles in general, and pyrimidines in particular, are found in several biologically active natural products and exhibit considerable therapeutic potential [1]. The wide-spectrum biological activities such as antiallergic [2], antitumor [3], antipyretic [4], anti-inflammatory [4], and antiparasitic [5] activities exhibited by synthetic pyrimidine-based scaffolds and a number of analogs have attracted considerable attention. During a screening effort for antiviral agents, we found that

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multifunctional tetrahydropyrimidine derivatives bearing bulky C-2 alkyl substituents exhibit cytostatic activity and inhibit proliferation of murine leukemia and murine mammary carcinoma cells [6]. 3,4-Dihydropyrimidine-2-(1*H*)-ones (DHLMS) and their appropriately functionalized derivatives have interesting pharmacological profiles [7, 8].

In view of this, 6-aryl-5-cyano-4-hydroxy-2-mercaptopyrimidines (**3**), 6-(3-nitrophenyl)-5-cyano-4-hydroxy-2-aminopyridine (**5**), and 5-bromo-2-hydroxybenzaldehyde thiosemicarbazone (**9**) were prepared by condensation of ethyl β-aryl-α-cyanoacrylate (**2**) with thiourea, guanidine hydrochloride, and thiosemicarbazide in presence of anhydrous potassium carbonate in methanol. These pyrimidine and thiazole derivatives were investigated for antimicrobial and antitumor activity.

Materials and methods

Melting points were determined in capillaries with a MEL-TEMP II apparatus and are reported uncorrected. Infrared spectra were taken on a PerkinElmer 337 spectrophotometer using KBr wafers. Proton nuclear magnetic resonance (NMR) spectra were obtained on a Varian EM 360 spectrometer using a solution in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Mass spectra were recorded on a VG Autspec GEI FAB and a Hewlett Packard MS-Engine thermospray with ionization by electron impact at 70 eV. The accelerating voltage was 6 kV. The temperature of the source was ~200 °C, and the emission current was ~100 mA. Microanalyses were conducted using a PerkinElmer 2408 CHN analyzer.

Ethyl β-aryl-α-cyanoacrylate (**2a**, **b**)

A mixture of ethyl cyanoacetate (0.01 mol) and aromatic aldehydes (such as 5-bromo-2-hydroxybenzaldehyde and 3-nitrobenzaldehyde) (0.01 mol) in methanol in the presence of anhydrous potassium carbonate (0.03 mol) was heated under reflux for 1.5 h. The reaction mixture was cooled and acidified with diluted hydrochloric acid. The solid formed was filtered off, washed with water, dried, and purified by recrystallization from ethanol to give **2**.

Ethyl β-(5-bromo-2-hydroxyphenyl)-α-cyanoacrylate as pale yellow crystals, m.p. 122 °C. IR (KBr): 3,350–2,850 (br.OH), 2,250 (CN), 1,756 (C=O), 1,625 (C=C), 1,215, 1,095 (C–O) cm^{-1}. ^1H-NMR (DMSO-d_6): δ 1.3 (t, 3H, CH_3), 4.35 (q, 2H, OCH_2), 7.53–8.01 (m, 4H, Ar–H and H-olefinic), 11.33 (s, 1H, OH) ppm. MS (*m*/*z*, %) = 297 (M^++2, 53.3), 295 (M^+, 56.30), 251 (56.40), 226 (87.50), 224 (100), 89 (81.30), 88 (62.50), 62.20 (93.80), 57 (68.80), 54 (75.00). Anal. Found: C, 48.61; H; 3.35; N, 4.71. $C_{12}H_{10}NBrO_3$ requires: C, 8.81; H, 3.39; N, 4.74.

*Ethyl β-(3-nitrophenyl)-α-cyanoacrylate (**2b**)* as pale yellow, yield 76 %, m.p. 124 °C. IR (KBr): 2,223 (CN), 1,751 (C=O), 1,621 (C=C), 1,210, 1,095 (C–O) cm^{-1}. ^1H-NMR ($CDCl_3$): δ 1.4 (t, 3H, CH_3), 4.45 (q, 2H, OCH_2), 7.12–7.89 (m, 5H, Ar–H and H-olefinic) ppm. MS: *m*/*z* (%) = 247 (M^++1, 3.20), 246 (M^+, 13.1), 218 (41.20), 150 (41.20), 148 (58.80), 136 (64.70), 121 (41.20), 117 (41.20), 105

(41.20), 101 (52.90), 91 (41.20), 85 (41.20), 83 (41.20), 76 (52.90), 69 (41.20), 67 (58.80), 66 (47.10), 65 (70.60), 60 (100), 58 (76.50), 57 (52.90), 55 (76.50), 50 (5.90). Anal. Found: C, 58.51; H, 4.06; N, 11.35. $C_{12}H_{10}N_2O_4$ requires: C, 58.54; H, 4.06; N, 11.38.

6-Aryl-5-cyano-4-hydroxy-2-substituted pyrimidines (**3a** and **5**)

*5-Bromo-2-hydroxybenzaldehyde thiosemicarbazone (**9**)*

A mixture of **2a**, **b** and thiourea, guanidine hydrochloride, and thiosemicarbazide (0.01) in presence of anhydrous potassium carbonate (0.03 mol) in methanol (50 ml) was heated under reflux for 2–3 h, then cooled and poured into dilute hydrochloric acid (1 %). The solid formed was filtered off, washed with water, dried, and purified by recrystallization from suitable solvent to give **3a**, **5**, and **9**.

6-(5-Bromo-2-hydroxyphenyl)-5-cyano-4-hydroxy-2-mercaptopyrimidine (**3a**) as pale yellow crystals, yield 68 %, m.p. 286 °C. IR (KBr): 3,390–2,850 (br-OH), 2,250 (CN), 1,635 (C=N), 1,618, 1,585 (C=C), 1,385 (C=S), 1,210, 1,083 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 7.31–8.01 (m, 3H, Ar–H), 10.4 (s, 1H, NH), 11.30 (s, 1H, OH), 11.51 (s, 1H, OH) ppm. MS: *m*/*z* (%) = 325 (M^++2, 83.60), 324 (M^++1, 100), 323 (M^+, 84.40), 310 (15.60), 309 (13.30), 308 (10.40), 307 (16.50), 297 (31.10), 296 (13.30), 293 (11.10), 292 (17.60), 291 (14.40), 290 (13.30), 269 (0.7), 268 (48.90), 267 (34.10), 266 (51.1), 265 (65.8), 264 (13.3), 239 (9.80), 238 (7.40), 237 (13.30), 94 (13.30), 93 (10.40), 90 (11.90), 89 (27.30), 86 (33.30), 85 (23.80), 82 (25.90), 81 (18.50), 80 (21.10), 79 (14.80), 71 (18.50), 69 (23.00), 68 (25.20), 64 (23.00), 63 (20.70), 61 (15.80), 60 (23.00), 59 (26.30), 58 (10.40), 56 (17.00), 53 (12.60), 52 (20.70), 50 (17.00). Anal. Found: C, 40.84; H, 1.83; N, 12.89; S, 9.89. $C_{11}H_6N_3BrO_2S$ requires: C, 40.87; H, 1,86; N, 13.00; S, 9.91.

*6-(3-nitrophenyl)-5-cyano-4-hydroxy-2-mercaptopyrimidine (**3b**)* as yellow crystals, yield 72 %, m.p. 278 °C. IR (KBr): 3,233 (NH), 3,390–2,850 (br.OH), 2,251 (CN), 1,631 (C=N), 1,617, 1,585 (C=C), 1,123 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 7.31–8.15 (m, 4H, Ar–H), 10.21 (br-s, 1H, NH), 11.21 (s, 1H, OH) ppm. MS: *m*/*z* (%) = 275 (M^{+1}+1, 20.00), 274 (M^+,23.00), 217 (12.50), 216 (30.00), 176 (50.00), 175 (95.00), 174 (52.50), 167 (17.50), 166 (47.50), 154 (17.50), 153 (12.30), 152 (15.00), 151 (22.50), 146 (30.00), 145 (32.50), 144 (25.00), 136 (100), 135 (42.50), 131 (22.50), 130 (20.00), 129 (60.00), 128 (77.50), 127 (37.50), 120 (20.00), 119 (90.00), 118 (52.50), 116 (57.50), 113 (37.50), 112 (15.00), 105 (30.00), 104 (22.50), 103 (70.00), 102 (55.00), 101 (47.50), 98 (40.00), 97 (22.50), 92 (12.50), 91 (67.50), 90 (62.50), 89 (52.50), 87 (25.00), 84 (32.50), 82 (22.50), 77 (82.50), 75 (42.50), 74 (32.50), 73 (35.00), 71 (40.00), 70 (40.00), 69 (65.00), 65 (40.00), 64 (52.50), 63 (80.00), 62 (27.50), 51 (47.50), 50 (65.00). Anal. Found: C, 48.03; H, 2.17; N, 20.35; S, 11.63. $C_{11}H_6N_4O_3S$ requires: C, 48.17; H, 2.19; N, 20.44; S, 11.68.

*6-(3-nitrophenyl)-5-cyano-4-hydroxy-2-aminopyrimidine (**5**)* as pale yellow, yield 63 %, m.p. 260 °C. IR (KBr): 3,385–2,875 (br.OH), 3,289, 3,171 (NH_2), 2,255 (CN), 1,635 (C=N), 1,613, 1,589 (C=C), 1,172 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 6.21 (s, 2H, NH2), 7.31–8.10 (m, 4H, Ar–H), 11.23 (br-S, IH, OH) ppm. MS: *m/z* (%) = 258 (M^++1, 27.30), 257 (M^+, 100), 256 (M^+-1, 48.50), 255 (27.30), 239 (12.10), 238 (18.20), 216 (21.20), 215 (48.50), 214 (36.40), 210 (30.30), 205 (18.20), 185 (21.20), 169 (45.50), 168 (33.30), 167 (18.20), 165 (15.20), 159 (21.20), 157 (33.30), 156 (21.20), 151 (24.20), 150 (27.30), 149 (21.20), 143 (18.20), 142 (30.30), 141 (45.50), 131 (15.20), 129 (24.20), 128 (30.30), 127 (39.40), 118 (24.20), 116 (21.20), 115 (30.30), 114 (84.80), 113 (24.20), 106 (27.30), 105 (24.20), 103 (33.30), 102 (45.50), 101 (39.40), 99 (30.30), 98 (33.30), 97 (21.20), 91 (30.30), 89 (36.30), 87 (30.30), 84 (30.30), 82 (21.20), 81 (21.20), 80 (33.30), 77 (33.30), 76 (54.50), 75 (51.50), 73 (36.40), 71 (30.30), 70 (33.30), 69 (48.50), 68 (42.40), 67 (33.30), 66 (36.40), 65 (39.40), 64 (45.50), 63 (51.50), 62 (30,30), 57 (63.60), 56 (36.40), 55 (45.50), 54 (27.30), 51 (57.60), 50 (63.60). Anal. Found: C; 51.32; H; 2.69; N, 27.13. $C_{11}H_7N_5O_3$ requires: C, 51.36; H, 2.72; N, 27.24.

*5-Bromo-2-hydroxybenzaldehyde thiosemicarbazone (**9**)* as pale yellow crystals, yield 78 %, m.p. 225 °C. IR (KBr): 3,410–2,980 (br-OH), 3,320, 3,170 (NH_2), 3,221 (NH), 1,631 (C=N), 1,382 (C=S), 1,185 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.83 (s, 2H, NH_2), 7.12–7.91 (m, 3H, Ar–H), 8.31 (s, 1H, CH=N), 9.31 (s, IH, NH) ppm. MS: *m/z* (%) = 275 (M^++2, 47.60), 274 (M^++1, 26.70), 273 (M^+, 56.20), 258 (10.60), 257 (6.30), 256 (10.80), 200 (35.00), 199 (35.30), 198 (26.20), 197 (21.70), 187 (10.80), 186 (4.80), 171 (19.10), 170 (12.30), 164 (14.40), 157 (5.30), 155 (4.50), 143 (10.60), 135 (5.30), 134 (10.80), 119 (13.40), 118 (8.30), (27.0), 89 (12.10), 88 (7.10), 79 (13.40), 78 (21.40), 77 (69.50), 76 (64.00), 75 (29.50), 74 (25.90), 65 (21.00), 64 (25.90), 63 (73.30), 62 (35.30), 61 (25.70), 60 (100), 59 (31.00), 58 (13.10), 53 (39.00), 52 (17.90), 51 (47.40), 50 (34.50). Anal. Found: C, 35.01; H, 2.79; N, 15.23; S, 11.55; $C_8H_8N_3BrOS$ requires: C, 35.16; H, 2.93; N, 15.38; S, 11.72.

Acetylation of **3a** and **5**: formation of acetyl derivatives **4** and **6**

A solution of **3a** and/or **5** (0.01 mol) in acetic anhydride (15 ml) was heated under reflux for 2 h, then cooled and poured into ice-water. The solid formed was filtered off, washed with water, dried, and purified by recrystallization from benzene to give **4** and **6**.

*6-(5-Bromo-2-acetoxyphenyl)-5-cyano-4-hydroxy-2-mercaptopyrimidine (**4**)* as yellow crystals, yield 56 %, m.p. 160 °C. IR (KBr): 3,390–2,950 (br.OH), 2,253 (CN), 1,765 (CO), 1,633 (C=N), 1,615, 1,598 (C=C), 1,225, 1,097 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 2.41 (s, 3H, $COCH_3$), 7.21–7.98 (m, 3H, Ar–H), 9.21 (s, 1H, SH), 11.02 (s, 1H, OH) ppm. MS: *m/z* (%) = 367 (M^++2, 6.50), 366 (M^++1, 41.40), 365 (M^+, 28.50), 364 (43.00), 363 (25.30), 349 (26.30), 348 (46.80), 347 (45.70), 346 (47.30), 345 (28.00), 344 (15.60) 98 (10.20), 97 (7.50), 90 (11.50), 89 (12.40), 88 (4.00), 87 (29.00), 86 (15.10), 85 (10.20), 80 (10.20), 79 (12.40), 77 (17.70), 76 (16.70), 75 (18.80),74 (10.80), 73 (8.60), 69 (25.30), 68 (12.40),65 (11.30), 64 (14.00), 63 (23.10), 62 (22.70), 61 (59.70), 60 (26.90), 53 (10.80), 51 (12.90), 50 (18.80). Anal. Found: C, 42.57; H, 2.05; N, 11.33; S, 8.61. $C_{13}H_8N_3BrO_3S$ requires C, 42.74; H, 2.19; N, 11.51; S, 8.77.

*6-(3-Nitrophenyl)-5-cyano-4-hydroxy-2-(acetyl)aminopyrimidine (**6**)* as pale yellow crystals, yield 63 %, m.p. 124 °C. IR (KBr): 3,385–2,960 (br.OH), 3,221 (NH), 2,256 (CN), 1,695 (CO), 1,629 (C=N), 1,607, 1,593 (C=C), 1,121 (C–O), cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.31 (s, 3H, $COCH_3$), 7.41–8.01 (m, 4H, Ar–H), 10.10 (s, 1H, NH), 11.21 (s, 1H, OH) ppm. MS: *m/z* (%) = 300 (M^{+}+1, 10.20), 299 (M*, 28.60), 292 (21.40), 259 (10.70), 258 (28.60), 257 (100), 229 (17.90), 218 (39.30), 217 (28.60), 216 (14.30), 215 (25.00), 212 (17.90), 211 (21.40), 210 (25.00), 201 (53.60), 200 (35.70), 195 (25.00), 186 (17.90), 185 (17.90), 183 (17.90), 182 (17.90), 176 (17.80), 175 (21.40), 174 (53.60), 173 (42.90), 172 (28.60), 155 (32.10), 152 (25.00), 150 (25.00), 143 (28.60), 142 (17.90), 141 (21.40), 140 (14.30), 137 (17.90), 136 (14.30), 129 (39.30), 128 (57.10), 127 (46.40), 126 (25.00), 117 (28.60), 116 (50.20), 103 (32.10), 102 (25.00), 101 (46.40), 100 (42.90), 99 (21.40), 91(14.30), 90 (25.00), 89 (39.30), 88 (35.70), 84 (32.10), 77 (32.10), 76 (50.10), 75 (57.10), 74 (42.90), 73 (39.30), 72 (25.00), 66 (25.00), 65 (25.00), 64 (35.70), 63 (64.30), 61 (39.30), 60 (71.40), 57 (39.30), 56 (28.60), 52 (32.10), 51 (60.70), 50 (67.90). Anal. Found: C, 52.01; H, 2.97; N, 23.22. $C_{13}H_9N_5O_4$ requires: C, 52.17; H, 3.01; N, 23.41.

7-(3-Nitrophenyl)-6-cyano-5-hydroxy-imidazolo[2,1-*b*]pyrimidin-1-one (**7**)

A mixture of **5** (0.01 mol), ethyl chloroacetate (0.01 mol), and fused sodium acetate (0.03 mol) in glacial acetic acid (30 ml) was heated under reflux for 4 h, then cooled and poured into water. The resulting solid was filtered off, washed with water, dried, and purified by recrystallization from dimethylformamide to give **7** as pale yellow crystals, yield 62 %, m.p 293 °C. IR (KBr): 3,781–2,453 (br.OH), 3,026 (NH), 2,453 (CN), 1,718 (CO), 1,606 (C=N), 1,527 (C=C), 1,091 (C–O), cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.25 (s, 2H, $COCH_2N$), 7.21–8.01 (m, 4H, Ar–H), 11.31 (s, 1H, OH) ppm. MS: *m/z* (%) = 297 (M^{+}, 5.80), 271 (7.10), 258 (14.90), 257 (100), 256 (49.40), 99 (20.10), 90 (11.00), 89 (13.60), 88 (16.90), 87 (13.00), 86 (10.40), 77 (18.20), 76 (31.80), 75 (33.80), 74 (20.80), 69 (20.80), 68 (14.90), 65 (14.40), 64 (18.80), 63 (18.80), 62 (16.20), 57 (20.10), 56 (13.60), 55 (10.40), 51 (40.30), 50 (48.70). Anal. Found: C, 52.33; H, 2.25; N, 23.47. $C_{13}H_7N_5O_4$ requires: C, 52.23; H, 2.53; N, 23.57.

5-(4-Methoxyphenyl)-2-[(5-bromo-2-hydroxybenzylidene)-hydrazino]-thiazole (**10**)

A mixture of **9** (0.01 mol), 4-methoxy phenacyl bromide (0.01 mol), and fused sodium acetate (0.03 mol) in methanol (50 ml) was heated under reflux for 6 h, then cooled and poured into water. The solid formed was filtered off, washed with water, dried, and purified by recrystallization from ethanol to give **10** as orange crystals, yield 67 %, m.p. 245 °C. IR (KBr): 3,950–2,890 (br.OH), 3,227 (NH), 1,635 (C=N), 1,605–1,585 (C=C), 1,211, 1,095 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.95 (s, 3H, OCH_3), 6.89–7.95 (m, 9H, Ar–H and H-thiazole), 8.31 (s, IH, CH=N), 10.33 (s, IH, NH), 11.31 (s, 1H, OH) ppm. MS: *m/z* (%) = 405 (M^{+}+2, 12.30), 404 (M^{+}+1, 6.20), 403 (M^{+}, 15.30), 388 (15.20), 387 (17.40), 386 (14.50), 385 (5.80), 370

(5.80), 369 (9.40), 368 (5.80), 358 (5.80), 355 (5.80), 338 (4.30), 336 (4.30), 288 (10.90), 287 (10.10), 286 (42.00), 285 (16.70), 284 (40.60), 283 (32.60), 279 (18.10), 278 (5.80), 277 (17.40), 276 (10.10), 271 (27.50), 270 (18.80), 269 (27.80), 242 (9.40), 241 (9.40), 239 (10.10), 237 (5.10), 229 (14.50), 228 (6.50), 227 (13.80), 212 (10.10), 211 (10.10), 210 (10.10), 207 (13.80), 206 (59.40), 205 (32.60), 204 (53.60), 203 91 (37.70), 90 (37.00), 89 (37.00), 82 (28.30), 81 (31.90), 80 (33.30), 79 (21.70), 77 (35.50), 76 (30.40), 75 (34.10), 69 (22.50), 68 (12.30), 66 (17.40), 65 (47.10), 64 (38.40), 63 (100), 62 (71.70), 61 (31.90), 53 (31.90), 51 (44.90), 50 (46.40). Anal. Found: C, 50.46; H, 3.27; N, 10.21; S, 7.79.

5-(4-Methoxyphenyl)-2-[(5-bromo-2-acetoxybenzylidene)-acetaldehyde-azino]-thiazole (**11**)

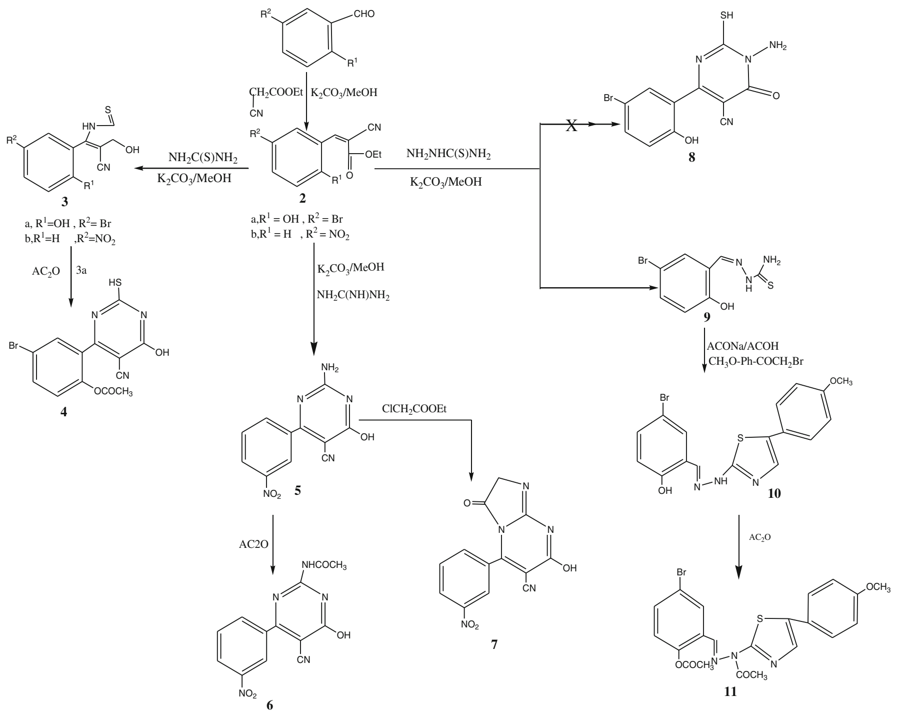
A solution of **10** (0.01 mol) in acetic anhydride (25 ml) was heated under reflux for 2 h, then cooled and poured into ice-water. The resulting solid was filtered off, washed with water, dried, and purified by recrystallization from benzene to give **11** as brown crystals, yield 53 %, m.p. 145 °C. IR (KBr): 1,705–1,689 (br.CO), 1,632 (C=N), 1,605, 1,585 (C=C), 1,225, 1,095 (C–O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.31 (s, 3H, $COCH_3$), 2.61 (s, 3H, $OCOCH_3$), 3,89 (s, 3H, OCH_3), 6.91–7.81 (m, 9H, Ar–H and H-thiazole), 8.21 (s, 1H, CH=N) ppm. MS: m/z (%) = 489 (M^++2, 9.10), 488 (M^++1, 10.80), 487 (M^+, 12.50), 486 (10.20), 485 (11.40), 484 (13.60), 483 (25.00), 482 (26.10), 481 (13.60), 480 (14.80), 468 (13.10), 467 (11.40), 466 (21.00), 465 (14.30), 464 (13.10), 447 (13.60), 446 (23.30), 445 (35.80), 444 (26.10), 405 (24.40), 404 (10.80), 403 (27.30), 402 (7.40), 388 (34.70), 387 (24.50), 386 (42.60), 385 (30.10), 286 (27.80), 285 (22.70), 284 (36.90), 271 (14.20), 269 (14.20), 268 (11.90), 242 (4.50), 241 (10.80), 233 (8.00), 232 (13.60), 103 (11.40), 102 (13.60), 97 (11.40), 94 (10.80), 92 (19.90), 91 (27.80), 90 (19.90), 89 (14.30), 77 (33.00), 76 (31.30), 75 (32.40), 74 (13.10), 65 (15.20), 64 (22.70), 63 (47.70), 62 (24.40), 60 (29.50), 59 (13.10), 57 (15.90), 53 (16.50), 51 (20.50), 50 (28.40). Anal. Found: C, 51.57; H, 3.48; N, 8.45; S, 6.37. $C_{21}H_{18}N_3BrO_4S$ requires: C, 51, 75; H, 3.69, N, 8.62; S, 6.57.

Results and discussion

Chemistry

The synthetic pathways leading to the new pyrimidine and thiazole derivatives are illustrated in Scheme 1. Condensation of 5-bromo-2-hydroxybenzaldehyde and 3-nitrobenzaldehyde with ethyl cyanoacetate in presence of anhydrous potassium carbonate yielded the corresponding ethyl β-aryl-α-cyanoacrylate (**2a**, **b**). Treatment [9] of compound **2** with thiourea in the presence of anhydrous potassium carbonate in methanol under reflux resulted in the formation of 6-aryl-5-cyano-4-hydroxy-2-mercaptopyrimidine (**3a**, **b**).

Acetylation of 6-(5-bromo-2-hydroxyphenyl)-5-cyano-4-hydroxy-2-mercapto-pyrimidine (**3a**) with acetic anhydride under reflux led to the formation of



Scheme 1 Representations of new reactions performed in the laboratory

6-(5-bromo-2-acetoxyphenyl)-5-cyano-4-hydroxy-2-mercaptopyrimidine (**4**). Cyclocondensation [10] of ethyl-β-(3-nitrophenyl)-α-cyanoacrylate (**2b**) with guanidine hydrochloride in the presence of anhydrous potassium carbonate in methanol under reflux afforded the corresponding 6-(3-nitrophenyl)-5-cyano-4-hydroxy-2-aminopyrimidine (**5**). 6-(3-Nitrophenyl)-5-cyano-4-hydroxy-2-(acetylamino)-pyrimidine (**6**) was prepared via acetylation of **5** with acetic anhydride under reflux.

The reaction of 2-aminopyrimidine (**5**) with ethyl chloroacetate in presence of fused sodium acetate in acetic acid under reflux gave the corresponding 7-(3-nitrophenyl)-6-cyano-5-hydroxy-imidazolo[2,1-*b*]-pyrimidine-1-one (**7**).

Treatment [11] of ethyl-β-(5-bromo-2-hydroxyphenyl)-α-cyanoacrylate (**2a**) with thiosemicarbazide in presence of anhydrous potassium carbonate in methanol under reflux gave the corresponding 5-bromo-hydroxybenzaldehyde thiosemicarbazone (**9**), which does not give the expected product **8** (Scheme 1). 5-(*p*-Methoxyphenyl)-2-[(5-bromo-2-hydroxybenzylidene)-hydrazino]-thiazole (**10**) was prepared from the reaction of (5-bromo-2-hydroxy)benzaldehyde thiosemicarbazone (**9**) with 4-methoxy phenacyl bromide in presence of fused sodium acetate. Acetylation of thiazole derivative **10** with acetic anhydride under reflux led to the formation of 5(*p*-methoxyphenyl)-2-[(5-bromo-2-acetoxybenzylidene)-acetylhydrazino]-thiazole (**11**) (Scheme 1).

Mass spectrometry

The mass-spectral decomposition modes [12, 13] of the prepared heterocyclic compounds containing pyrimidine and thiazole ring were investigated. The mass spectra of the pyrimidine derivatives (**3a**, **b**, **4**, **5**, and **6**) showed intense molecular ion peaks at *m/z* 323, 274, 365, 257, and 299, consistent with the molecular formulae $C_{11}H_6N_3BrO_2S$, $C_{11}H_6N_4O_3S$ and $C_{13}H_8N_3BrO_3S$, $C_{11}H_7N_5O_3$, and $C_{13}H_9N_5O_4$, respectively.

The molecular ion of compound **5** (Fig. 1) underwent fragmentation to produce a peak at *m/z* 215 by losing a NH_2CN group. The loss of the formyl group (CHO) from the ion with *m/z* 215 resulted in an ion at *m/z* 186. The ion of *m/z* 186 broke to give an ion of *m/z* 148. The ion of *m/z* 148 fragmented to give an ion at *m/z* 102, which lost a nitro group (NO_2). The ion of *m/z* 102 underwent loss of HCN and C_2H_2 molecules to give peaks at *m/z* 76 and 50, respectively. Furthermore, the molecular ion of compound **5** (*m/z* 257) underwent the loss of NH=C=N to give a peak at *m/z* 216. Loss of the isocyanate group (NCO) from the ion at *m/z* 216 gave a peak at *m/z* 174. It further lost NO_2, C_2HCN, and acetylene molecules to give peaks at *m/z* 128, 77, and 51, respectively.

From the mass spectrum of compound **6** (Fig. 2), it was concluded that the molecular ion was at *m/z* 299. The ion of *m/z* 299 underwent fragmentation to produce a peak at *m/z* 257 by losing a ketene molecule (CH_2=C=O) corresponding to the base peak and the molecular ion of compound **5**. The stable fragment of *m/z* 257 further broke down via a pathway similar to that of compound **5** (Scheme 2).

The mass spectra of thiazole derivatives **10** and **11** (Figs. 3, 4) are fully consistent with the assigned structures. In most cases, intense molecular ion peaks were observed. Thus, compounds **10** and **11** showed intense molecular ion peaks at *m/z* 403/405 and 487/489, consistent with the molecular formulae $C_{17}H_{14}N_3BrO_2S$ and $C_{21}H_{18}N_3BrO_4S$, respectively. The $M + 2$ peak was also observed along with the molecular ion peak due to the presence of isotopes of a bromine atom present in these compounds.

The molecular ion of compound **10** (Scheme 3) underwent fragmentation to produce a peak at *m/z* 206, corresponding to the molecular ion of 5-(4-methoxyphenyl)-2-aminothiazole. The loss of an amino cyanide (NH_2CN) group from the ion with *m/z* 206 resulted in an ion at *m/z* 164. It further underwent the loss of sulfur atom (S), formaldehyde (CH_2O), C_2H, and C_2H_2 to give peaks at *m/z* 132, 102, 77, and 51, respectively.

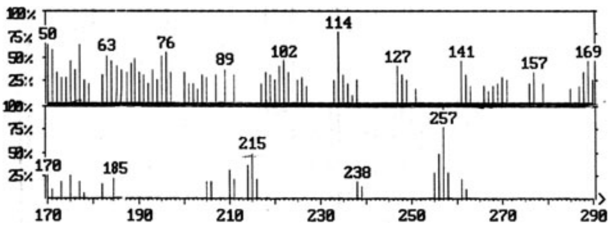


Fig. 1 70 eV mass spectrum of compound **5**

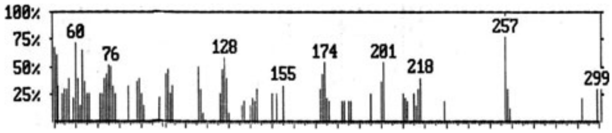
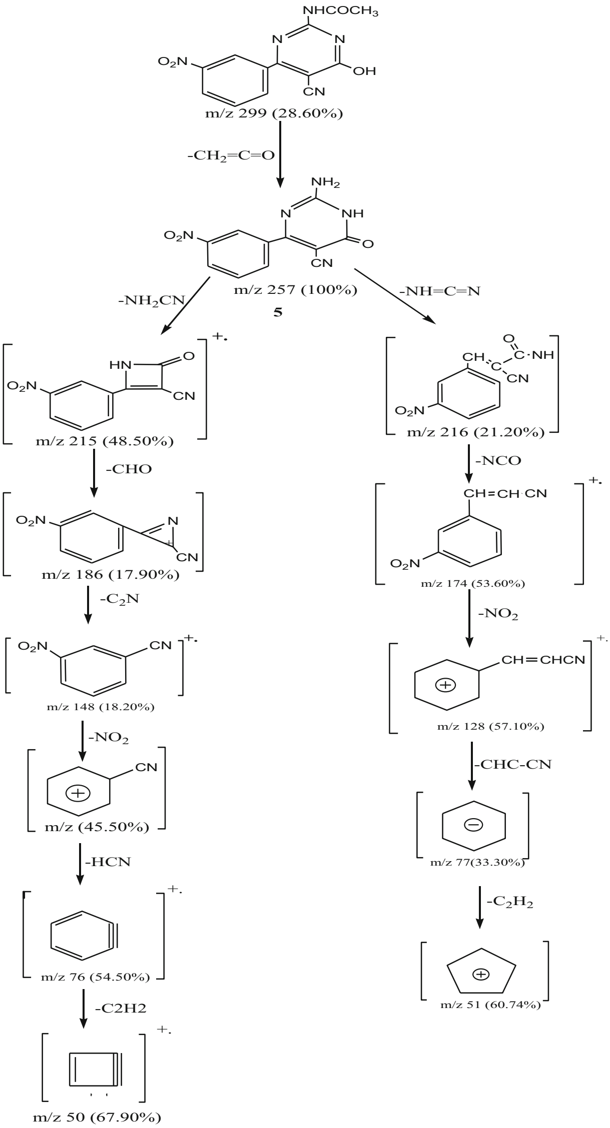


Fig. 2 70 eV mass spectrum of compound **6**



Scheme 2 Main fragmentation pathway of compounds **5** and **6**

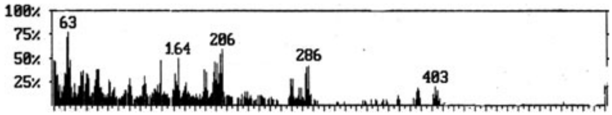


Fig. 3 70 eV mass spectrum of compound **10**

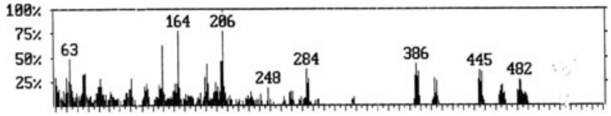


Fig. 4 70 eV mass spectrum of compound **11**

The molecular ion of compound **10** was also found to undergo fragmentation to produce the ion of 2-cyano-4-bromophenol at *m/z* 197. The ion at *m/z* 197 underwent loss of bromine atom (Br), formyl group (CHO), and acetylene molecule (C_2H_2) to give peaks at *m/z* 118, 89, and 63, respectively. The loss of the ketene molecules (CH_2CO) from the molecular ion of compound **11** resulted in an ion at *m/z* 445. The ion at *m/z* 445 underwent loss of the ketene molecules (CH_2=C=O) to give the ion at *m/z* 403, corresponding to the molecular ion of compound **10**. The fragment ion of *m/z* 403 further broke down via a pathway similar to that of compound **10**. Base peaks at *m/z* 63 and 206 were found in the mass spectra of compounds **10** and **11**.

Biological assays

Antimicrobial activity

The antimicrobial activities of the synthesized compounds **3**, **5**, **10**, and **11** were determined by agar well diffusion method [13, 14]. The compounds were evaluated for antibacterial activity against *Bacillus subtilis* (RCMBOO107) and *Streptococcus pneumonia* (RCMBOO105) as Gram-positive bacteria, and *Escherichia coli* (RCMBO-O103) and *Pseudomonas* sp. (ATCC9027) as Gram-negative bacteria.

Antifungal [15] activity was evaluated against *Aspergillus niger* and *Penicillium* sp. as fungi. The antibiotic streptomycin and clotrimazole were used as reference drug for antibacterial and antifungal activity, respectively. Dimethylsulfoxide (1 %, DMSO) was used as control without compound.

All compounds were tested at 10, 50, and 100 mg concentration. The zone of inhibition was measured in mm and compared with standard drug. The data are summarized in Table 1, showing that all compounds display certain antimicrobial activity.

In comparison with standard antibacterial streptomycin and antifungal clotrimazole, compounds **5**, **10**, and **11** were found to be highly active against bacteria and fungi. Compounds **3a** and **3b** were also found to be active against bacteria and fungi.

Scheme 3 Main fragmentation pathway of compounds **10** and **11**

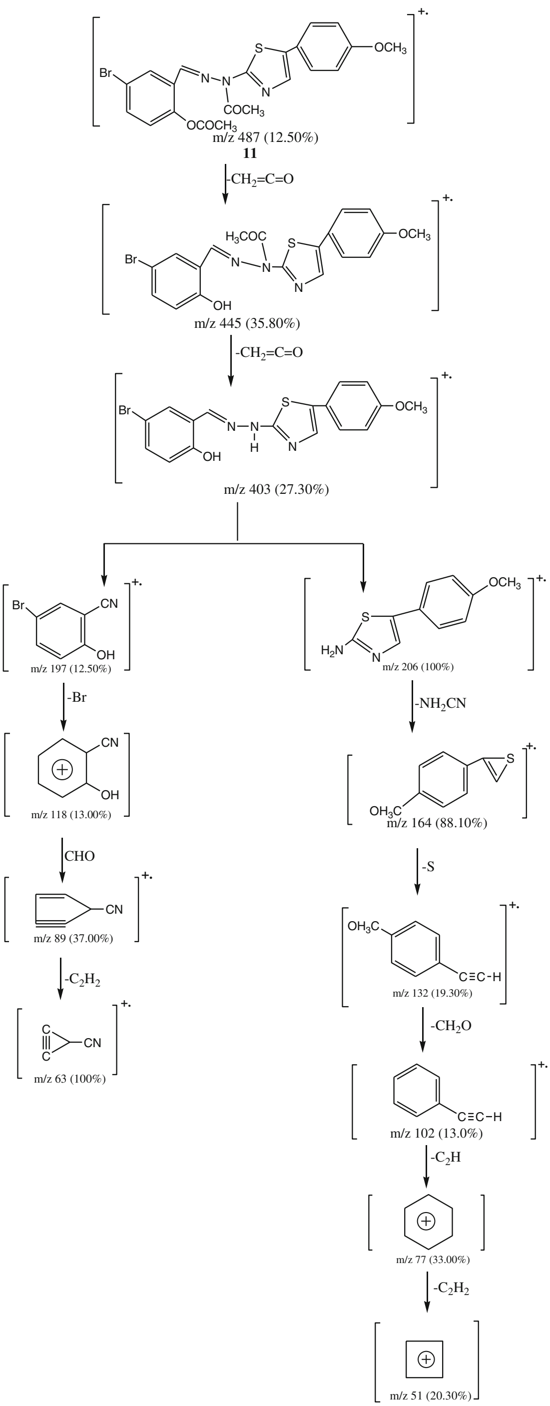


Table 1 Antimicrobial activity of prepared compounds **3**, **5**, **10**, and **11**

Compound	Gram-positive bacteria						Gram-negative bacteria						Fungi					
	Bacillus subtilis			*Streptococcus pneumonia*			*Escherichia coli*			*Pseudomonas* sp.			*Aspergillus niger*			*Penicillium* sp.		
	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg	10 mg	50 mg	100 mg
3a	−	+	++	−	++	++	−	+	+	−	+	++	−	+	+	−	++	++
3b	+	+	++	−	++	+++	+	++	++	+	++	++	+	++	++	−	++	+
5	++	+++	+++	++	+++	+++	++	++	+++	+	++	+++	++	+++	+++	++	++	+++
10	+	+	+++	+	++	+++	++	++	+++	+	++	+++	++	++	++	+	++	+++
11	++	+++	+++	++	+++	+++	+	++	+++	++	++	+++	++	+++	+++	++	+++	+++
Streptomycin	++	++	+++	+	++	+++	+	+	++	+	++	+++	−	−	−	−	−	−
Clotrimazole	−	−	−	−	−	−	−	−	−	−	−	−	++	++	+++	+	++	+++

− Inactive, + slightly active, ++ moderately active, +++ highly active

Anticancer activity

Cytotoxic and antitumor activity of prepared compounds **3–11** were evaluated against cell lines MCF-7, HePG-2, and HCT according to the method of Mosmann and Vijayan et al. [16]. The drug vinblastine was used as standard.

Inhibitory activity against breast carcinoma cells (MCF-7 cell line), hepatocellular carcinoma cells (HePG-2 cell line), and colon carcinoma cells (HCT cell line) was tested by using different concentrations of the samples (50, 25, 12.50, 6.25, 3.125, and 1.56 μgm), and cell viability (%) was determined by colorimetric method.

The 50 % inhibitory concentration (IC_{50}) of the MCF-7 cell line was calculated from Table 2 and Figs. 5 and 6.

The 50 % inhibitory concentration (IC_{50}) of the HePG-2 cell line was calculated from Table 3 and Figs. 7 and 8.

Table 2 Evaluation of cytotoxicity of prepared compounds against cell line MCF-7

Sample conc. (μg)	Viability (%)							
	3a	**3b**	**4**	**5**	**6**	**10**	**11**	Vinblastine standard
50	67.82	40.65	93.14	41.94	63.62	74.94	69.12	7.82
25	82.94	59.21	100	65.29	94.15	93.15	80.41	15.18
12.5	93.56	73.03	100	81.76	97.82	98.46	98.50	29.60
6.25	100	89.26	100	94.09	100	100	100	48.75
3.125	100	97.42	100	99.65	100	100	100	60.35
1.56	100	100	100	100	100	100	100	76.24
0	100	100	100	100	100	100	100	100

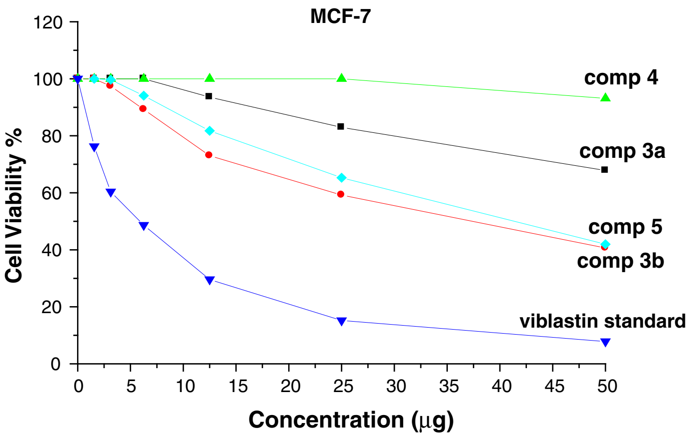


Fig. 5 Evaluation of cytotoxicity of prepared compounds **4**, **3a**, **5**, **3b** against MCF-7 cell line

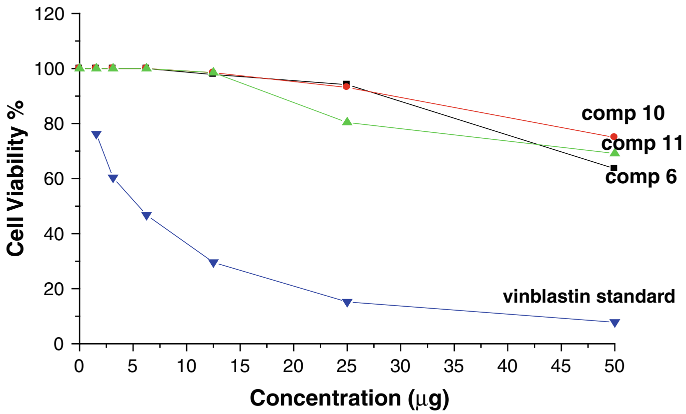


Fig. 6 Evaluation of cytotoxicity of prepared compounds **10**, **11**, **6** against MCF-7 cell line

Table 3 Evaluation of cytotoxicity of prepared compounds against cell line HePG-2

Sample conc. (μg)	Viability (%)							
	3a	**3b**	**4**	**5**	**6**	**10**	**11**	Vinblastine standard
50	59.68	29.64	95.86	31.34	25.67	82.14	62.54	14.38
25	74.66	73.93	100	62.90	69.15	93.52	86.44	16.13
12.50	91.42	84.55	100	81.65	87.05	98.66	97.18	24.25
6.25	97.74	92.85	100	92.19	93.75	100	100	45.13
3.125	100	95.54	100	98.88	99.29	100	100	55.00
1.56	100	99.11	100	100	100	100	100	72.13
0	100	100	100	100	100	100	100	100

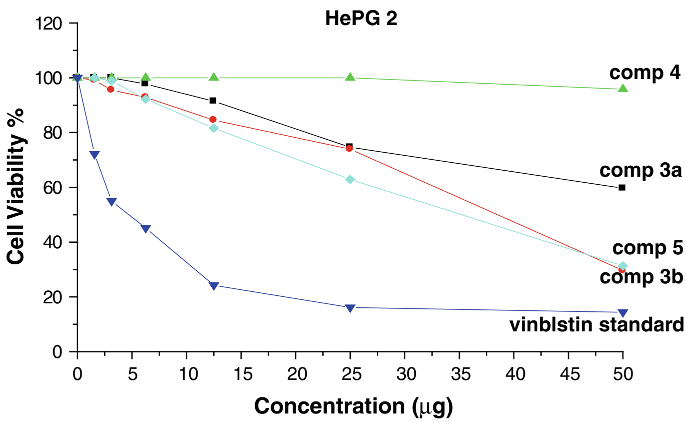


Fig. 7 Evaluation of cytotoxicity of prepared compounds **3a**, **3b**, **4**, **5** against HePG-2 cell line

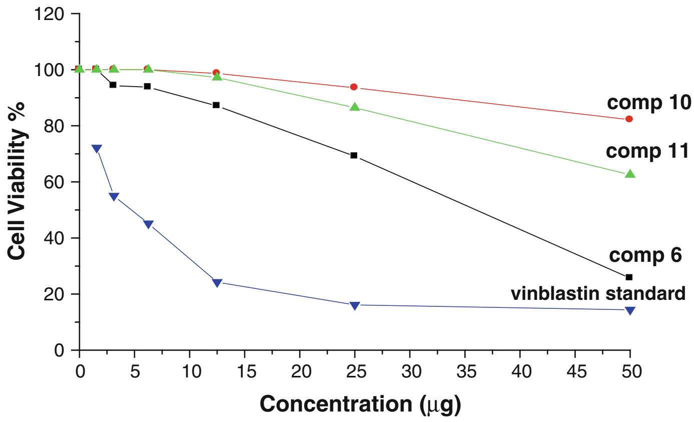


Fig. 8 Evaluation of cytotoxicity of prepared compounds **6**, **10**, **11** against HePG-2 cell line

Table 4 Evaluation of cytotoxicity of compounds **3** and **5** against HCT cell line

Sample conc. (μgm)	Viability (%)			
	3a	**3b**	**5**	Vinblastine standard
50	41.58	36.94	74.48	15.38
25	78.42	69.62	91.12	23.08
12.5	93.65	82.14	98.66	27.35
6.25	97.96	93.88	100	43.59
3.125	100	98.04	100	53.85
1.56	100	100	100	69.23
0	100	100	100	100

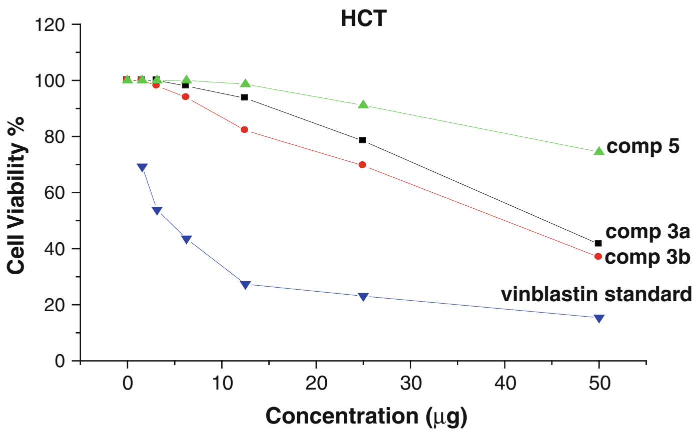


Fig. 9 Evaluation of cytotoxicity of compounds **3** and **5** against HCT cell line

Table 5 IC_{50} (μgm) values of prepared compounds after 72 h continuous exposure of tumor cell lines

Compound	Tumor type/cell line		
	MCF-7	HePG-2	HCT
3a	>50	>50	41.58
3b	37.4	38.5	40.98
4	Not inhibitory	Not inhibitory	>50
5	41.4	35.20	–
6	>50	36.00	–
10	>50	>50	–
11	>50	>50	–
Vinblastine standard	6.10	4.60	9.80

The IC_{50} value is the concentration that induces 50 % growth inhibition compared with untreated control cells

MCF-7 human breast carcinoma cell line

HePG-2 human hepatocellular carcinoma cell line

HCT human colon carcinoma cell line

Also, the 50 % inhibitory concentration (IC_{50}) of the HCT cell line was calculated from Table 4 and Fig. 9.

The results of 50 % inhibitory concentration (IC_{50}) data are summarized in Table 5.

In comparison with standard antitumor drug vinblastine, compound **3a** was found to be active against the HCT cell line. Compound **3b** was also found to be active against MCF-7, HePG-2, and HCT cell lines. Compounds **5** and **6** were observed to be active against MCF-7 and HePG-2 cell lines. As compared with standard antitumor drug, compounds **10** and **11** were observed to be weakly active against MCF-7 and HePG-2 cell lines. Compound **4** exhibited no inhibitory active against MCF-7 and HePG-2 cell lines.

Conclusions

A series of nitrogen heterocycles were synthesized, and their antimicrobial and anticancer activity was compared with that of a standard drug. Compounds **5**, **10**, and **11** were found to exhibit the highest antimicrobial activity. These compounds showed in vitro growth inhibition activity against MCF-7, HePG-2, and HCT cell lines that was comparable to or less than that of vinblastine.

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