Clean Synthesis and Antibacterial Activities of Spiro[pyrimido[4,5-*b*]-quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-pentaones

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A simple, clean and efficient method for the synthesis of spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-pentaone derivatives by condensation reaction of 6-amino-uracils and isatins in aqueous media is reported. These products were evaluated *in vitro* for their antibacterial activities.

Key words isatin; 6-amino-uracil; spiro[pyrimidoquinoline-pyrrolopyrimidine]; aqueous media

Polyfunctionalized heterocyclic compounds play important roles in the drug discovery process, and market analysis of drugs in late development shows that 68% of them are heterocycles.[1)] Therefore, it is not surprising that research in the field of synthesis of polyfunctionalized heterocyclic compounds has received special attention.

Spirocyclic systems containing one carbon atom common to two rings are structurally interesting.[2)] The asymmetric characteristic of the molecules due to the chiral spiro carbon is one of the important criteria of the biological activities. The presence of a sterically constrained spiro structure in various natural products also adds to the interest in the investigations of spiro compounds.[3)] Spiro compounds represent an important class of naturally occurring substances and their characteristic is the highly pronounced biological properties.[4,5)]

Uracil and its annelated substrates occupy a unique place in the field of medicinal chemistry as useful anticancer and antiviral drugs.[6)] The versatility of uracil derivatives for the synthesis of nitrogen containing heterocycles of biological importance has been well documented in the literature.[7)] A number of fused uracils of biological significance, such as, pyrano-, pyrido-, pyrazolo-, pyrimido-, pyridazino-pyrimidines have all been prepared by the functionalization of these important heterocyclic building blocks.[8,9)]

As part of our program which aimed to develop new selective and environmentally friendly methodologies for the preparation of heterocyclic compounds,[10—15)] we performed the synthesis of spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo-[2,3-*d*]pyrimidine]-pentaone derivatives through a cyclo-condensation reaction employing water as the reaction medium. In fact, as clearly stated by R. A. Sheldon, it is generally recognized that "the best solvent is no solvent and if a solvent (diluent) is needed it should preferably be water."[16)] The use of water as the reaction medium represent a remarkable benefit since this green solvent is highly polar and therefore immiscible with most organic compounds; moreover, the water soluble catalyst resides and operates in the aqueous phase and separation of the organic materials is thus easy.

Experimental

Apparatus Melting points were measured on an Electrothermal 9100 apparatus and are uncorrected. Mass spectra were recorded on a FINNIGAN-MAT 8430 mass spectrometer operating at an ionization potential of 70 eV. IR spectra were recorded on a Shimadzu IR-470 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a BRUKER DRX-300 AVANCE spectrometer at 300.13 and 75.47 MHz, respectively. Elemental analyses were performed using a Heracus CHN-O-Rapid analyzer.

Typical Procedure for Preparation of 1*H*-Spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3a) A mixture of 6-amino-uracil (2 mmol), isatin (1 mmol) and *p*-TSA (0.1 g) in refluxing H_2O (5 ml) was stirred for 6 h (TLC). After completion of reaction, the reaction mixture was filtered and the precipitate washed with water and then EtOH to afford the pure product **3a** as a white powder (85%). mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3270, 1747, 1681, 1653, 1628. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 6.79—7.17 (4H, m, H-Ar), 9.04 (1H, s, NH), 10.41 (1H, s, NH), 10.47 (1H, s, NH), 10.61 (1H, s, NH), 11.02 (1H, s, NH), 11.88 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 49.5, 82.6, 97.5, 116.6, 121.6, 123.7, 126.6, 128.6, 135.6, 146.4, 150.4, 151.5, 152.5, 159.0, 162.4, 181.7. MS, *m*/*z* (%): 366 (M$^+$, 25), 313 (40), 236 (44), 57 (100). *Anal.* Calcd for $C_{16}H_{10}N_6O_5$: C, 52.46; H, 2.75; N, 22.94%. Found: C, 52.50; H, 2.80; N, 22.87%.

7-Bromo-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]-pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)pentaone (3b) White powder (89%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3446, 3176, 1746, 1726, 1635. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 6.89 (1H, s, H-Ar), 6.98 (1H, d, $^3J_{HH}$=8.4 Hz, H-Ar), 7.33 (1H, d, $^3J_{HH}$=8.3 Hz, H-Ar), 9.31 (1H, s, NH), 10.51 (1H, s, NH), 10.67 (2H, s, 2NH), 11.07 (1H, br s, NH), 11.91 (1H, br s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 49.4, 82.6, 97.2, 114.8, 118.9, 123.9, 128.9, 131.5, 135.3, 146.3, 150.4, 151.6, 159.1, 162.3, 181.4. MS, *m*/*z* (%): 445 (M$^+$, 5), 430 (14), 236 (30), 149 (36), 57 (100). *Anal.* Calcd for $C_{16}H_9BrN_6O_5$: C, 43.17; H, 2.04; N, 18.88%. Found: C, 43.11; H, 2.08; N, 18.82%.

7-Nitro-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3c) Yellow powder (90%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3445, 3200, 1715, 1633, 1557. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 7.25 (1H, d, $^3J_{HH}$=8.8 Hz, H-Ar), 7.25 (1H, s, H-Ar), 8.04 (1H, d, $^3J_{HH}$=8.9 Hz, H-Ar), 9.85 (1H, s, NH), 10.55 (1H, s, NH), 10.84 (2H, s, NH), 11.28 (1H, s, NH), 12.02 (1H, s, NH), ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 49.3, 83.5, 97.8, 117.4, 122.4, 122.6, 124.7, 141.8, 142.9, 145.9, 145.9, 150.3, 151.4, 152.8, 159.1, 162.3, 181.1. MS, *m*/*z* (%): 411 (M$^+$, 12), 336 (34), 319 (80), 230 (100). *Anal.* Calcd for $C_{16}H_9N_7O_7$: C, 46.72; H, 2.21; N, 23.84%. Found: C, 46.66; H, 2.16; N, 23.78%.

1,1′,3,3′-Tetramethyl-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo-[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3d) White powder (80%); mp >380 °C; IR (KBr) (ν_{max}/cm^{-1}): 3480, 3199, 1761, 1699, 1643. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 3.01 (3H, s, CH_3), 3.09 (3H, s, CH_3), 3.40 (3H, s, CH_3), 3.49 (3H, s, CH_3), 6.94—7.30 (4H, m, H-Ar), 9.36 (1H, s, NH), 11.59 (1H, s, NH), ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 27.5, 27.9, 30.6, 31.7, 51.3, 83.2, 97.8, 117.2, 121.4, 124.1, 126.7, 128.6, 136.0, 146.4, 150.8, 151.5, 152.5, 157.3, 160.3, 181.8. MS, *m*/*z* (%): 422 (M$^+$, 5), 393 (15), 268 (100), 183 (25). *Anal.* Calcd for $C_{20}H_{18}N_6O_5$: C, 56.87; H, 4.30; N, 19.90%. Found: C, 56.92; H, 4.26; N, 19.82%.

Due to very low solubility of the products **3e** and **3f**, we can not report the ^{13}C-NMR data for these products.

1,1′,3,3′-Tetramethyl-7-nitro-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3e) Yellow powder (78%); mp >300 °C. IR (KBr) (ν_{max}/cm^{-1}): 3440,

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3106, 1769, 1710, 1635. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 2.99 (3H, s, CH_3), 3.10 (3H, s, CH_3), 3.40 (3H, s, CH_3), 3.53 (3H, s, CH_3), 7.52 (1H, d, $^3J_{HH}$=7.2 Hz, H-Ar), 7.74 (1H, s, H-Ar), 8.11 (1H, d, $^3J_{HH}$=8.1 Hz, H-Ar), 10.00 (1H, s, NH), 11.82 (1H, s, NH). MS, *m/z* (%): 466 (M^+-1, 50), 439 (40), 423 (100), 393 (30). *Anal.* Calcd for $C_{20}H_{17}N_7O_7$: C, 51.39; H, 3.67; N, 20.98%. Found: C, 51.44; H, 3.62; N, 20.90%.

1,1′,3,3′-Tetramethyl-7-bromo-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3f) White powder (80%); mp >300 °C. IR (KBr) (ν_{max}/cm^{-1}): 3487, 3283, 1758, 1696, 1649. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 3.00 (3H, s, CH_3), 3.06 (3H, s, CH_3), 3.37 (3H, s, CH_3), 3.48 (3H, s, CH_3), 7.11—7.36 (3H, m, H-Ar). 9.47 (1H, s, NH), 11.59 (1H, s, NH). MS, *m/z* (%): 500 (M^+, 20), 473 (60), 346 (100), 319 (16). *Anal.* Calcd for $C_{20}H_{17}BrN_6O_5$: C, 47.92; H, 3.42; N, 16.76%. Found: C, 47.97; H, 3.38; N, 16.69%.

1,1′-Dimethyl-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]-pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3g) White powder (86%). mp >350 °C; IR (KBr) (ν_{max}/cm^{-1}): 3463, 3188, 1747, 1689, 1616. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 3.01 (3H, s, CH_3), 3.32 (3H, s, CH_3), 6.90—7.13 (4H, m, H-Ar), 9.11 (1H, s, NH), 10.74 (1H, s, NH), 10.83 (1H, s, NH), 11.50 (1H, s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 26.8, 30.6, 50.7, 82.4, 98.1, 116.7, 121.3, 123.6, 126.9, 128.6, 135.6, 145.1, 150.5, 151.3, 154.0, 158.1, 161.5, 181.9. MS, *m/z* (%): 394 (M^+, 15), 350 (85), 227 (100). *Anal.* Calcd for $C_{18}H_{14}N_6O_5$: C, 54.82; H, 3.58; N, 21.31%. Found: C, 54.86; H, 3.53; N, 21.38%.

1,1′-Dimethyl-7-nitro-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3h) White powder (80%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3305, 3035, 1764, 1717, 1654. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 3.05 (3H, s, CH_3), 3.42 (3H, s, CH_3), 7.25—8.10 (3H, m, H-Ar), 10.00 (1H, s, NH), 10.85 (2H, s, 2NH), 11.74 (1H, s, NH), ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 26.9, 30.8, 50.4, 83.3, 98.1, 117.4, 122.2, 123.1, 124.7, 141.8, 142.0, 143.0, 144.8, 151.2, 154.4, 158.2, 161.4, 181.4. MS, *m/z* (%): 439 (M^+, 5), 368 (30), 230 (40), 43 (100). *Anal.* Calcd for $C_{18}H_{13}N_7O_7$: C, 49.21; H, 2.98; N, 22.32%. Found: C, 49.17; H, 2.94; N, 22.39%.

1,1′-Dimethyl-7-bromo-1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone (3i) White powder (90%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3200, 1761, 1721, 1671, 1642. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 3.02 (3H, s, CH_3), 3.39 (3H, s, CH_3), 6.99—7.38 (3H, m, H-Ar), 9.28 (1H, s, NH), 10.77 (1H, s, NH), 10.96 (1H, s, NH), 11.53 (1H, s, NH), ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 26.8, 30.7, 50.5, 82.4, 97.6, 115.0, 118.8, 123.7, 129.3, 131.6, 135.2, 144.9, 150.4, 151.3, 154.4, 158.1, 161.4, 181.5. MS, *m/z* (%): 472 (M^+, 4), 430 (18), 192 (43), 149 (75), 43 (100). *Anal.* Calcd for $C_{18}H_{13}BrN_6O_5$: C, 45.68; H, 2.77; N, 17.76%. Found: C, 45.72; H, 2.72; N, 17.69%.

2,2′-Bis(methylthio)-3*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo-[2,3-*d*]pyrimidine]4,4′,6′(3′*H*,7′*H*,10*H*)-trione (7a) White powder (78%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3423, 3413, 1745, 1648. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 2.51 (3H, s, SCH_3), 2.52 (3H, s, SCH_3), 6.65—7.13 (4H, m, H-Ar), 9.81 (1H, s, NH), 10.99 (1H, s, NH), 12.10 (1H, br s, NH), 12.39 (1H, br s, NH). ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 13.1, 13.4, 50.7, 89.9, 106.7, 116.1, 120.6, 122.8, 126.2, 128.6, 137.4, 149.2, 154.1, 158.1, 160.7, 161.6, 165.1, 181.4. MS, *m/z* (%): 426 (M^+, 2), 257 (10), 229 (15), 97 (43), 43 (100). *Anal.* Calcd for $C_{18}H_{14}N_6O_3S_2$: C, 50.69; H, 3.31; N, 19.71%. Found: C, 50.74; H, 3.35; N, 19.78%.

Due to very low solubility of the products **7b**—**d**, we can not report the ^{13}C-NMR data for these products.

2,2′-Bis(methylthio)-7-nitro-3*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-4,4′,6′(3′*H*,7′*H*,10*H*)-trione (7b) Yellow powder (70%); mp >300 °C. IR (KBr) (ν_{max}/cm^{-1}): 3480, 3436, 3369, 1732, 1635. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 2.41 (3H, s, SCH_3), 2.54 (3H, s, SCH_3), 7.18 (1H, d, $^3J_{HH}$=8.91 Hz, H-Ar), 7.48 (1H, s, H-Ar), 8.05 (1H, d, $^3J_{HH}$=8.88 Hz, H-Ar), 10.61 (1H, s, NH), 11.26 (1H, s, NH), 12.45 (1H, br s, NH), 12.55 (H, br s, NH). MS, *m/z* (%): 472 (M^++1, 5), 430 (38), 257 (24), 97 (62), 57 (100). *Anal.* Calcd for $C_{18}H_{13}N_7O_5S_2$: C, 45.86; H, 2.78; N, 20.80%. Found: C, 45.91; H, 2.73; N, 20.87%.

7-Bromo-2,2′-bis(methylthio)-3*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-4,4′,6′(3′*H*,7′*H*,10*H*)-trione (7c) White powder (71%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3474, 3355, 3298, 1740, 1706, 1637. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 2.40 (3H, s, SCH_3), 2.52 (3H, s, SCH_3), 6.70 (1H, d, $^3J_{HH}$=8.71 Hz, H-Ar), 7.48 (1H, s, H-Ar), 7.31 (1H, d, $^3J_{HH}$=8.73 Hz, H-Ar), 9.98 (1H, s, NH), 11.09 (1H, s, NH), 12.20 (1H, br s, NH), 12.31 (H, s, NH). MS, *m/z* (%): 503 (M^+, 10), 477 (38), 257 (34), 97 (60), 43 (100). *Anal.* Calcd for $C_{18}H_{13}BrN_6O_3S_2$: C, 42.78; H, 2.59; N, 16.63%. Found: C, 42.71; H, 2.53; N, 16.57%.

3,3′-Dimethyl-2,2′-bis(methylthio)-3*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-4,4′,6′(3′*H*,7′*H*,10*H*)-trione (7d) White powder (73%); mp >350 °C. IR (KBr) (ν_{max}/cm^{-1}): 3374, 3262, 1741, 1666, 1609. ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 2.57 (3H, s, SCH_3), 2.60 (3H, s, SCH_3), 3.26 (3H, s, NCH_3), 3.31 (3H, s, NCH_3), 6.73—7.10 (4H, m, H-Ar), 9.80 (1H, s, NH), 10.99 (1H, s, NH). MS, *m/z* (%): 454 (M^+, 20), 425 (100), 379 (25), 351 (40), 88 (40). *Anal.* Calcd for $C_{20}H_{18}N_6O_3S_2$: C, 52.85; H, 3.99; N, 18.49%. Found: C, 52.91; H, 3.95; N, 18.41%.

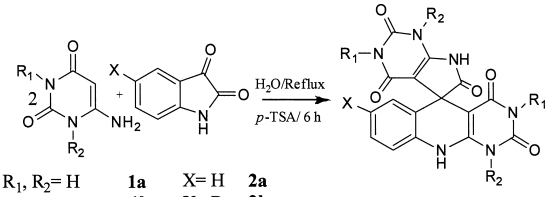
Results and Discussion

After some preliminary experiments, it was found that a mixture of 6-amino-uracil **1a** and isatin **2a** in the presence of a catalytic amount of *p*-toluene sulfuonic acid (*p*-TSA) afforded 1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaone **3a** in 85% yield in refluxing water for 6 h (Chart 1).

The ^{1}H-NMR spectrum of compound **3a** exhibited a multiplet at δ=6.79—7.17 for the four aromatic hydrogens and six singlets at δ 9.04, 10.41, 10.47, 10.61, 11.02 and 11.88 for the six NH groups. The ^{13}C-NMR spectrum of compound **3a** showed 16 signals in agreement with the structure, and the mass spectrum showed the expected molecular ion peak.

Encouraged by this success, we have extended this reaction to various 6-amino-uracils **1a**—**c** and isatines **2a**—**c** under similar conditions (using H_2O/*p*-TSA), furnishing the respective 1*H*-spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo-[2,3-*d*]pyrimidine]-2,2′,4,4′,6′(1′*H*,3*H*,3′*H*,7′*H*,10*H*)-pentaones **3a**—**i** in good yields (Chart 1).

We were not able to establish the exact mechanism for the formation of spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo-[2,3-*d*]pyrimidine]-pentaones **3** at this time, however, a reasonable suggestion is offered in Chart 2. Apparently, the reaction proceed through the intermediate **4**, formed *in situ* by reaction of the isatins **2** with 6-amino-uracils **1**, then, the intermediate **4** was converted to intermediate **5** and followed by cyclization afforded the corresponding spiro[pyrimido-[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-pentaones **3** and ammonia (Chart 2).



R_1, R_2= H **1a** X= H **2a**
R_1, R_2= Me **1b** X= Br **2b**
R_1=H, R_2= Me **1c** X= NO_2 **2c**

3a-i

Product **3**	R_1	R_2	X	*Yield (%)*[a]
a	H	H	H	85
b	H	H	Br	89
c	H	H	NO_2	90
d	Me	Me	H	80
e	Me	Me	NO_2	78
f	Me	Me	Br	80
g	H	Me	H	86
h	H	Me	NO_2	80
i	H	Me	Br	90

[a] isolated yields

Chart 1. Synthesis of Spiro[pyrimidoquinoline-5,5′-pyrrolopyrimidine]-pentaones **3**

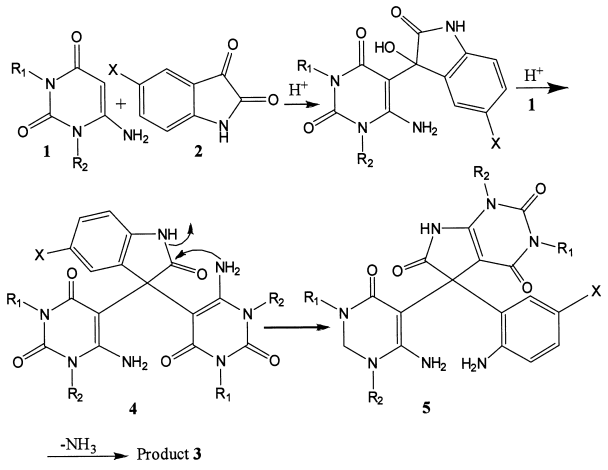
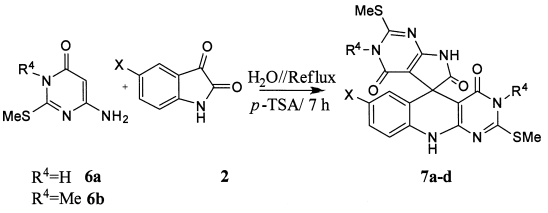


Chart 2. Mechanism for the Synthesis of Spiro[pyrimidoquinoline-5,5′-pyrrolopyrimidine]-pentaones



Product 7	R^4	X	*Yield (%)*[a]
a	H	H	78
b	H	NO_2	70
c	H	Br	71
d	Me	H	73

[a] isolated yields

Chart 3. Synthesis of Spiro[pyrimidoquinoline-5,5′-pyrrolopyrimidine]-pentaones **7**

Table 1. Antibacterial Activity of Products **3** and **7**

Product	Zone of inhibition (mm)			
	Escherichia coli	*Pseudomonas aeruginusa*	*Bacillus subtilis*	*Staphylococcus aureus*
3a	7	13	14	9
3b	9	12	16	11
3c	11	9	13	8
3d	14	11	15	13
3f	10	15	18	14
3g	10	7	17	7
3i	8	8	11	15
7a	14	13	12	9
7b	15	14	15	6
7d	17	10	9	8

To further explore the potential of this protocol for the spiro-heterocyclic synthesis, we investigated reaction involving 6-amino-thiouracils **6a**, **b** and isatins **2** and obtained 2,2′-bis(methylthio)-2′,3′-dihydro-3*H*-spiro[pyrimido-[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine]-4,4′,6′-(1′*H*,7′*H*,10*H*)-trione derivatives **7a**—**d** in 70—78% yields (Chart 3).

Finally, compounds **4a**, **b**, **c**, **d**, **f**, **g**, **i** and **7a**, **b**, **d**, **f** were screened for antimicrobial activity using disc diffusion method.[17] The microorganisms used in this study were

Table 2. MIC (μg/ml) Values of Products **3** and **7**

Product	*Escherichia coli*	*Pseudomonas aeruginusa*	*Bacillus subtilis*	*Staphylococcus aureus*
3a	12	17	11	12
3b	10	15	8	7
3c	15	12	10	9
3d	9	10	7	12
3f	8	9	8	16
3g	11	18	8	14
3i	14	14	9	11
7a	15	11	7	7
7b	11	9	8	10
7d	10	13	6	9
Norfloxacin	<2	20	2	16

Escherichia coli ATCC 25922, *Pseudomonas aeruginusa* ATCC 85327 (Gram-negative bacteria), *Bacillus subtilis* ATCC 465, *Staphylococcus aureus* ATCC 25923 (Gram-positive bacteria). All of the compounds were dissolved in DMSO (100 μg/ml) and 25 μl of them were loaded to 6 mm paper discs. 100 μl of 10^9 cell/ml suspension of the microorganisms were spread on sterile Muller Hilton Agar plates and the discs were placed on the surface of culture plates. Table 1 shows the inhibition zones of compounds around the discs. The minimum inhibitory concentration (MIC) of the selected compounds which showed antibiotic activity in disc diffusion tests, were also determined by microdillution method[18] and compared to a commercial antibiotic (Table 2).

As can be seen from Table 2, good to improved antibacterial activity was observed for most of the compounds against all species of Gram-positive and Gram-negative bacteria used in the study.

Conclusions

In summary, we have described an efficient and green synthesis for the preparation of spiro[pyrimido[4,5-*b*]quinoline-5,5′-pyrrolo[2,3-*d*]pyrimidine] *via* a condensation reaction of 6-amino-uracils and isatins in aqueous media. These products were evaluated *in vitro* for their antibacterial activities. Almost most of the compounds exhibited good to excellent antibacterial activity against all the tested strains.

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