Research Article

Synthesis and Antimicrobial Activity of 3-Cyano-4-imino-9-methoxy-4*H*-pyrimido [2, 1-*b*] pyrimido [4, 5-*b*] quinoline and 2-Substituted Derivatives

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The 2-amino-7-methoxypyrimido[4,5-*b*]quinoline (**1**) on treatment with bis(methylthio)methylene malononitrile (**2**) in ethyl alcohol and catalytic amount of TEA gives 3-cyano-4-imino-9-methoxy-2-methylthio-4*H*-pyrimido[2,1-*b*]pyrimido[4,5-*b*]quinoline (**3**). The latter were further reacted with selected N-, O-, and C-nucleophiles such as aryl amines, hetryl amines, substituted phenols, and compounds containing an active methylene group.

1. Introduction

Quinoline derivatives represent the major class of heterocycles and a number of preparations have been known since the late 1800s. The quinoline ring system occurs in various natural products, especially in alkaloids. The quinoline skeleton is often used for the design of many synthetic compounds with diverse pharmacological properties. In 1820, quinine was isolated as the active ingredient from the bark of cinchona tree and successively replaced the crude bark for the treatment of malaria. Despite its relatively low efficacy and tolerability, quinine still plays an important role in the treatment of multiresistant malaria. This molecule has also played a historical role in organic chemistry as a target for structural determination and total synthesis [1] and recently both stereoselective [2] and enantioselective [3] total syntheses. Quinoline and its derivatives are receiving important due to their wide range of biological activities as a drug analgesics [4], antiamebic [5–8], trypanocidal [9], antiseptic [10], and antiserotonin [11]. In addition to these, derivatives also exhibit good antimalarial [12, 13], antitubercular [14], antibacterial [15], antihistaminic [16], anti-neurodegenerative [17], anticonvulsant [18], antitumor [19], anticancers [20, 21], and antiallergics [22] activities.

In the light of these valid observations, such fused quinoline with pyrimidine ring would exhibit some interesting pharmacological activities; further, the ring annelation to amino groups containing nitrogen heterocycles with ketene dithioacetals as reagent has been reported [23–25]. Recently, we report one pot synthesis of 3-cyano-9-methyl-2-methylthio-4-oxo-4*H*-pyrimido [2,1-*b*] pyrimido [4,5-*b*] quinoline and its reaction with selected nucleophiles [26]. In continuation, this remains an opportunity for further development of milder condition with better yields. The compound **3** was prepared by the reaction of 2-amino-7-methoxy pyrimido [4,5-*b*] quinolone **1** with bis(methylthio)methylene malononitrile **2** in presence of ethyl alcohol and catalytic amount of TEA, Scheme 1. A plausible mechanism for the formation of parent compound **3** can be adduced as shown in Scheme 2. Compound **3** possesses an active methylthio group at the 2 position that is activated by the ring 1-nitrogen atom and the electron withdrawing 3-cyano group. Compound **3** was reacted with selected N-, O-, and C-nucleophiles like aryl amines, substituted phenols, heteryl amines, and compound containing active methylene group. Hence, compound **3** independently reaction with different substituted anilines, substituted phenols, active methylene compounds, and hetryl amines in presence of

ii) EtOH/TEA

SCHEME 1

SCHEME 2: Mechanism: plausible mechanism 3-cyano-4-imino-9-methoxy-2-methylthio-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinolone.

ethyl alcohol and catalytic amount of TEA afforded new compounds 4a–e, 5a–f, 6a–6d, 7a–7d, and Scheme 3.

2. Experimental Section

Melting points were determined by an open capillary method and are uncorrected. The chemicals and solvents were used for laboratory grade and were purified. IR spectra were recorded (in KBr pellets) on Shimadzu spectrophotometer.

^1H NMR spectra were recorded (in $\text{DMSO}-d_6$) on Avance-300 MHz spectrometer using TMS as an internal standard. The mass were recorded on EI-Shimadzu GC-MS spectrometer. Elemental analyses were performed on a Heraeus CHN-O rapid analyzer.

3-Cyano-4-imino-9-methoxy-2-methylthio-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(3). A mixture of 2-amino-7-methoxy pyrimido [4,5-b] quinoline 1 (2.26 g, 0.01 mmol) and bis(methylthio)methylene malononitrile 2 (1.70 g, 0.01 mmol) was refluxed in the presence of ethyl alcohol

SCHEME 3

and TEA for 4 hr. The reaction mixture was cooled to room temperature and poured into ice cold water. The separated solid was filtered, washed with water, and recrystallized from N,N'-dimethyl formamide-ethanol mixture to afford compound 3.

Brown powder, (76% yield), Mp: 238–240°C; EI-MS (m/z-RA%): 372 (M⁺ Na), IR (cm⁻¹, KBr): 3224 (=NH), 2213 (CN), ¹HNMR (DMSO-d₆, ppm): 2.6 (s, 3H, SCH₃), 3.7 (s, 3H, Ar-OCH₃), 6.8–7.8 (m, 4H, Ar-H), 8.4 (s, 1H-C=CH quin.), 8.6 (s, 1H, =NH), ¹³CNMR (300 MHz, DMSO-d₆, ppm) 15, 55, 80, 104, 115, 116, 121, 123, 125, 127, 135, 145, 152, 163, 163.8, 164, 165. Anal. Calcd. For: C₁₇H₁₂N₆OS; C, 58.61; H, 3.47; N, 24.12; Found: C, 58.50; H, 3.31; N, 24.02.

3-Cyano-4-imino-9-methoxy-2-(4'-methyl anilino)-4H-pyrimido [2,1-b]pyrimido [4,5-b] quinoline (4a-e). A mixture of compound 3 (3.48 g, 0.01 mmol), ethyl alcohol

catalytic amount TEA, and appropriate substituted anilines (0.01 mmol) was refluxed for 4 hr. The reaction mixture was cooled to room temperature and poured into ice cold water. The products 4a-e thus obtained were recrystallized from N,N'-dimethyl formamide-ethanol solvent.

3-Cyano-4-imino-9-methoxy-2-(4'-methyl anilino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (4a). Brown powder, (85% yield), Mp: 214°C; IR (cm⁻¹, KBr): 3315 (NH), 3222 (=NH), 2210 (CN); ¹HNMR (DMSO-d₆, ppm): 2.6 (s, 3H, Ar-CH₃), 3.8 (s, 3H, Ar-OCH₃), 4.3 (s, 1H, -NH), 6.5–8.0 (m, 7H, Ar-H), 8.3 (s, 1H, CH=N-), 8.5 (s, 1H, -C=CH), 8.8 (s, 1H, =NH) EI-MS (m/z-RA%): 407 (M⁺) Anal. Calcd. For: C₂₃H₁₇N₇O; C, 67.80; H, 4.21; N, 24.06; Found: C, 67.59; H, 4.10; N, 23.85.

3-Cyano-4-imino-9-methoxy-2-(4'-methoxy anilino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (4b). Brown powder, (89% yield), Mp: 208°C; IR (cm⁻¹, KBr): 3321 (NH), 3222

(=NH), 2211 (CN), ^1H NMR (DMSO- d_6 , ppm): 3.7(s, 6H, Ar-OCH $_3$), 4.2 (s, 1H, -NH), 6.5–8.0 (m, 7H, Ar-H), 8.2 (s, 1H, CH=N), 8.5 (s, 1H, -C=CH), 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 423 (M^+) Anal. Calcd. For: C $_{23}$ H $_{17}$ N $_7$ O $_2$; C, 65.24; H, 4.05; N, 23.16; Found: C, 65.02; H, 3.82; N, 23.00.

3-Cyano-4-imino-9-methoxy-2-(4'-chloro anilino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(4c). Brown powder, (80% yield), Mp: 219°C; IR (cm $^{-1}$, KBr): 3330 (NH), 3224 (=NH); 2210 (CN), ^1H NMR (DMSO- d_6 , ppm): 3.7 (s, 3H, Ar-OCH $_3$), 4.0 (s, 1H, -NH), 6.4–8.0 (m, 7H, Ar-H), 8.1 (s, 1H, CH=N-), 8.5 (s, 1H, -C=CH), 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 428 (M^+) Anal. Calcd. For: C $_{22}$ H $_{14}$ Cl N $_7$ O; C, 61.76; H, 3.30; N, 22.92; Found: C, 61.05; H, 2.56; N, 22.28.

3-Cyano-4-imino-9-methoxy-2-(3'-methoxy anilino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(4d). Brown powder, (66% yield), Mp: 217°C; IR (cm $^{-1}$, KBr): 3331 (NH), 3220 (=NH), 2208 (CN); ^1H NMR (DMSO- d_6 , ppm): 3.8 (s, 6H, -OCH $_3$), 4.0 (s, 1H, -NH), 6.4–8.0 (m, 7H, Ar-H), 8.1 (s, 1H, CH=N-), 8.5 (s, 1H, -C=CH), 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 423 (M^+) Anal. Calcd. For: C $_{23}$ H $_{17}$ N $_7$ O $_2$; C, 65.24; H, 4.05; N, 23.16; Found: C, 64.70; H, 3.64; N, 22.62.

3-Cyano-4-imino-9-methoxy-2-(4'-nitro anilino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(4e). Brown powder, (62% yield), Mp: 213°C; IR (cm $^{-1}$, KBr): 3318 (NH), 3219 (=NH), 2210 (CN); ^1H NMR (DMSO- d_6 , ppm): 2.6 (s, 3H, Ar-CH $_3$), 3.8 (s, 3H, -OCH $_3$), 4.0 (s, 1H, -NH), 6.4–8.0 (m, 7H, Ar-H), 8.0 (s, 1H, CH=N-), 8.5 (s, 1H, -C=CH), 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 438 (M^+) Anal. Calcd. For: C $_{22}$ H $_{14}$ N $_8$ O $_3$; C, 60.27; H, 3.22; N, 25.56; Found: C, 59.67; H, 2.72; N, 24.84.

3-Cyano-4-imino-9-methoxy-2-(phenoxy/4'-methyl phenoxy/4'-methoxy phenoxy/4'-chloro phenoxy/4'-nitro phenoxy/2'-hydroxy biphenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5a-f). A mixture of compound 3 (3.48 g, 0.01 mmol), ethyl alcohol catalytic amount of triethyl amine, and appropriate substituted phenols (0.01 mmol) was refluxed for 4 hrs. The reaction mixture was cooled to room temperature and poured into ice cold water. The products 5a-f thus obtained were recrystallized from N,N'-dimethyl formamide-ethanol solvent.

3-Cyano-4-imino-9-methoxy-2-(phenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5a). Brown powder, (82% yield), Mp: 204–205°C; IR (cm $^{-1}$, KBr): 3222 (=NH), 2209 (CN); ^1H NMR (DMSO- d_6 , ppm): 3.5 (s, 3H, Ar-OCH $_3$), 6.6–8.5 (m, 9H, Ar-H), 8.4 (s, 1H, =NH), 8.5 (s, 1H, CH=C quin.); EI-MS (m/z-RA%): 395 (M^+) Anal. Calcd. For: C $_{22}$ H $_{14}$ N $_6$ O $_2$; C, 67.00; H, 3.58; N, 21.31; Found: C, 66.49; H, 3.01; N, 21.01.

3-Cyano-4-imino-9-methoxy-2-(4'-methyl phenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5b). Brown powder, (78% yield), Mp: 202–204°C; IR (cm $^{-1}$, KBr): 3218 (=NH), 2213(CN); ^1H NMR (DMSO- d_6 , ppm): 3.8 (s, 3H, Ar-OCH $_3$), 6.6–7.9 (m, 8H, Ar-H), 8.5 (s, 1H, CH=C quin.); 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 408 (M^+) Anal. Calcd. For: C $_{23}$ H $_{16}$ N $_6$ O $_2$; C, 67.64; H, 3.95; N, 20.58 Found: C, 67.02; H, 3.14; N, 19.85.

3-Cyano-4-imino-9-methoxy-2-(4'-methoxy phenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5c). Brown powder, (74% yield). Mp: 207°C; IR (cm $^{-1}$, KBr): 3220 (=NH), 2209 (CN); ^1H NMR (DMSO- d_6 , ppm): 3.6 (s, 3H, Ar-OCH $_3$), 6.6–7.8 (m, 8H, Ar-H), 8.3 (s, 1H, CH=C quin.), 8.5 (s, 1H, =NH). EI-MS (m/z-RA%): 424 (M^+) Anal. Calcd. For: C $_{23}$ H $_{16}$ N $_6$ O $_3$, Calcd: C, 65.09; H, 3.80; N, 19.80, Found: C, 64.48; H, 2.60; N, 19.18.

3-Cyano-4-imino-9-methoxy-2-(4'-chloro phenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5d). Brown powder, (70% yield), Mp: 214–216°C; IR (cm $^{-1}$, KBr): 3219 (=NH), 2213 (CN); ^1H NMR (DMSO- d_6 , ppm): 3.7 (s, 3H, Ar-OCH $_3$), 6.6–7.9 (m, 8H, Ar-H), 8.3 (s, 1H, CH=C quin.), 8.5 (s, 1H, =NH). EI-MS (m/z-RA%): 429 (M^+) Anal. Calcd. For: C $_{22}$ H $_{13}$ ClN $_6$ O $_2$; C, 61.62; H, 3.06; N, 19.60. Found: C, 61.02; H, 2.70; N, 19.13.

3-Cyano-4-imino-9-methoxy-2-(4'-nitro phenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5e). Brown powder, (73% yield), Mp: 211–213°C; IR (cm $^{-1}$, KBr): 3222 (=NH), 2209 (CN); ^1H NMR (DMSO- d_6 , ppm): 3.5 (s, 3H, Ar-OCH $_3$), 6.7–8.0 (m, 8H, Ar-H), 8.5 (s, 1H, CH=C quin.) 8.7 (s, 1H, =NH). EI-MS (m/z-RA%): 439 (M^+) Anal. Calcd. For: C $_{22}$ H $_{13}$ N $_7$ O $_4$; C, 60.14; H, 2.98; N, 22.31, Found: C, 60.00; H, 2.60; N, 22.02.

3-Cyano-4-imino-9-methoxy-2-(2'-hydroxy biphenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline(5f). Brown powder, (82% yield), Mp: 224–226°C; IR (cm $^{-1}$, KBr): 3220 (=NH); 2209 (CN), ^1H NMR (DMSO- d_6 , ppm): 3.7 (s, 3H, Ar-OCH $_3$), 6.7–7.9 (m, 13H, Ar-H), 8.5 (s, 1H, CH=C quin.), 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 470 (M^+) Anal. Calcd. For: C $_{28}$ H $_{18}$ N $_6$ O $_2$; Calcd: C, 71.48; H, 3.86; N, 17.66; Found: C, 71.00; H, 3.41; N, 17.43.

3-Cyano-4-imino-9-methoxy-2-(pyrrolidino/piperidino/morpholino/piperazino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinolone (6a-d). A mixture of compound 3 (3.48 g, 0.01 mmol), ethyl alcohol catalytic amount of triethyl amine, and appropriate cyclic secondary amines (0.01 mmol) was refluxed for 4 hrs and the mixture was poured into ice cold water. The products 6a–d thus obtained were recrystallized from N,N'-dimethyl formamide-ethanol solvent.

3-Cyano-4-imino-9-methoxy-2-(pyrrolidino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (6a). Brown powder, (82% yield), Mp: 218°C; IR (cm $^{-1}$, KBr): 3216 (=NH); 2211 (CN), ^1H NMR (DMSO- d_6 , ppm): 1.6 (t, 4H, CH $_2$), 2.7 (t, 4H, -NCH $_2$ -), 3.7 (s, 3H, Ar-OCH $_3$), 6.7–7.8 (m, 4H, Ar-H), 8.5 (s, 1H, CH=C quin.), 8.8 (s, 1H, =NH). EI-MS (m/z-RA%): 371 (M^+) Anal. Calcd. For: C $_{20}$ H $_{17}$ N $_7$ O; C, 64.68; H, 4.61; N, 26.40; Found: C, 64.12; H, 4.10; N, 25.92.

3-Cyano-4-imino-9-methoxy-2-(piperidino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (6b). Brown powder, (80% yield), Mp: 222–224°C; IR (cm $^{-1}$, KBr): 3216 (=NH); 2208 (CN), ^1H NMR (DMSO- d_6 , ppm): 1.5 (t, 6H, three -CH $_2$ -), 2.8 (t, 4H, two-NCH $_2$ -), 3.7 (s, 3H, Ar-OCH $_3$), 6.7–7.8 (m, 4H, Ar-H), 8.5 (s, 1H, CH=C quin.); 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 385 (M^+); Anal. Calcd. For: C $_{21}$ H $_{19}$ N $_7$ O; C, 65.44; H, 4.97; N, 25.44, Found: C, 65.15, H, 4.20; N, 25.02.

3-Cyano-4-imino-9-methoxy-2-(morpholino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (6c). Brown powder, (88% yield), Mp: 228–230°C; IR (cm^{-1} , KBr): 3222 (=NH); 2211 (CN), ^1H NMR (DMSO- d_6 , ppm): 2.7 (t, 4H, $-\text{NCH}_2$), 3.6 (s, 3H, Ar- OCH_3), 3.8 (t, 4H, OCH_2), 6.9–7.9 (m, 4H, Ar-H), 8.5 (s, 1H, $\text{CH}=\text{C}$ quin.), 8.7 (s, 1H, =NH). EI-MS (m/z-RA%): 388 (M+1) Anal. Calcd. For: $\text{C}_{20}\text{H}_{17}\text{N}_7\text{O}_2$. Calcd: C, 62.01; H, 4.42; N, 25.31; Found: C, 61.60; H, 4.00; N, 24.82.

3-Cyano-4-imino-9-methoxy-2-(piperazino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (6d). Brown powder, (85% yield), Mp: 212–214°C; IR (cm^{-1} , KBr): 3226 (=NH); 2210 (CN), ^1H NMR (DMSO- d_6 , ppm): 2.1 (s, 1H, NH), 2.7 (m, 8H, four CH_2 , $J = 7.5$ Hz), 3.4 (s, 3H, Ar- OCH_3), 6.7–7.9 (m, 4H, Ar-H), 8.5 (s, 1H, $\text{CH}=\text{C}$ quin.), 8.6 (s, 1H, =NH). EI-MS (m/z-RA%): 387 (M+1) Anal. Calcd. For: $\text{C}_{20}\text{H}_{18}\text{N}_8\text{O}$; C, 62.17; H, 4.70; N, 29.00; Found: C, 61.81; H, 4.34; N, 28.52.

3-Cyano-4-imino-9-methoxy-2-(α -acetyl acetonil/ α -ethylacetoacetyl/ α -ethylcyanoacetyl/ α -malononitriyl)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (7a-d). A mixture of compound 3 (3.48 g, 0.01 mmol), ethyl alcohol catalytic amount of triethyl amine, and appropriate compounds containing an active methylene group (0.01 mmol) was refluxed for 4 hrs. The reaction mixture was cooled at room temperature and poured into ice cold water. The products 7a–d thus obtained were recrystallized from N,N'-dimethyl formamide-ethanol.

3-Cyano-4-imino-9-methoxy-2-(α -acetyl acetonil)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (7a). Brown powder, (75% yield), Mp: 205–207°C; IR (cm^{-1} , KBr): 3322 (=NH); 2210 (CN), 1685 (CO), ^1H NMR (DMSO- d_6 , ppm): 2.2 (s, 6H, two COCH_3), 3.6 (s, 3H, Ar- OCH_3), 3.9 (s, 1H, $-\text{CH}$), 6.8–7.7 (m, 4H, Ar-H), 8.5 (s, 1H, $\text{CH}=\text{C}$ quin.); 8.8 (s, 1H, =NH); EI-MS (m/z-RA%): 401 (M+1) Anal. Calcd. For: $\text{C}_{21}\text{H}_{16}\text{N}_6\text{O}_3$; C, 62.99; H, 4.03; N, 20.99; Found: C, 62.35; H, 2.64; N, 20.42.

3-Cyano-4-imino-9-methoxy-2-(α -ethylacetoacetyl)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (7b). Brown powder, (77% yield), Mp: 212°C; IR (cm^{-1} , KBr): 3400 (=NH); 2209 (CN), 1733 (CO of ester), 1684 (CO), 1245 (C–O); ^1H NMR (DMSO- d_6 , ppm): 1.4 (s, 3H, $-\text{CH}_3$), 2.2 (s, 3H, COCH_3), 3.7 (s, 3H, Ar- OCH_3), 3.9 (s, 1H, $-\text{CH}$), 4.2 (d, 2H, $-\text{OCH}_2-$), 6.8–7.9 (m, 4H, Ar-H), 8.6 (s, 1H, $\text{CH}=\text{C}$ quin.); 8.8 (s, 1H, =NH); Anal. Calcd. For: $\text{C}_{22}\text{H}_{18}\text{N}_6\text{O}_4$; C, 61.39; H, 4.22; N, 19.53; Found: C, 60.84; H, 3.92; N, 19.02.

3-Cyano-4-imino-9-methoxy-2-(α -ethylcyanoacetyl)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (7c). Brown powder, (84% yield), m.p. 218–220°C; IR (cm^{-1} , KBr): 3338 (=NH); 2224 (CN), 1740 (CO of ester), 1258 (C–O); ^1H NMR (DMSO- d_6 , ppm): 1.4 (s, 3H, $-\text{CH}_3$), 3.7 (s, 3H, Ar- OCH_3), 3.9 (s, 1H, $-\text{CH}$), 4.2 (s, 2H, $-\text{OCH}_2-$), 6.9–7.8 (m, 4H, Ar-H), 8.7 (s, 1H, $\text{CH}=\text{C}$ quin.); 8.9 (s, 1H, =NH); EI-MS (m/z-RA%): 413 (M+) Anal. Calcd. For: $\text{C}_{21}\text{H}_{15}\text{N}_7\text{O}_3$; C, 61.01; H, 3.66; N, 23.72. Found: C, 60.50; H, 3.05; N, 23.28.

3-Cyano-4-imino-9-methoxy-2-(α -malononitriyl)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (7d). Brown powder, (79% yield), Mp: 226–228°C; IR (cm^{-1} , KBr): 3418 (=NH); 2211 (CN), ^1H NMR (DMSO- d_6 , ppm): 3.7 (s, 3H,

Ar- OCH_3), 4.3 (s, 1H, $-\text{CH}$), 6.6–7.8 (m, 4H, Ar-H), 8.5 (s, 1H, $\text{CH}=\text{C}$ quin.); 8.8 (s, 1H, =NH); EI-MS (m/z-RA%): 367 (M+1); Anal. Calcd. For: $\text{C}_{19}\text{H}_{10}\text{N}_8\text{O}$; C, 62.29; H, 2.75; N, 30.59; Found: C, 61.56; H, 2.18; N, 30.02.

3. Result and Discussion

In the present investigation, we have developed new methodology towards the synthesis of 3-cyano-4-imino-9-methoxy-2-methylthio-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline and their substituted derivatives. 3 Our method gives single product with high yield. The reaction started with 2-amino-7-methoxypyrimido [4,5-b] quinoline 1 and bis (methylthio)methylene malononitrile 2 were refluxed in ethyl alcohol in presence of catalytic amount of triethyl amine (TEA) to afford 3, Scheme 1.

Compound 3 posses a replaceable active methylthio group at 2-position which is activated by ring 1-nitrogen atom and electron withdrawing group 3-cyano group. Compound 3 reacted with selected N-, O-, C-nucleophiles like aryl amines hetryl amines, substituted phenols, and compounds containing an active methylene group. The compound (3) on independent reaction with p-methyl aniline, and p-methoxy aniline, p-chloro aniline, m-methoxy aniline, p-nitro aniline, in ethyl alcohol and catalytic amount of triethyl amine, afforded 3-cyano-4-imino-9-methoxy-2-(4'-methyl anilino/4'-methoxy anilino/4'-chloro anilino/3'-methoxy anilino/4'-nitro anilino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (4a–e), respectively, Scheme 3.

Under similar experimental condition compound 3 reacted independently with hetryl amines like pyrrolidine, piperidine, morpholine, and piperazine to yield 3-cyano-4-imino-9-methoxy-2-(pyrrolidino/piperidino/morpholino/piperazino)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline. (6a–d), respectively, Scheme 3.

3-Cyano-4-imino-9-methoxy-2-(phenoxy/4'-methyl phenoxy/4'-methoxy phenoxy/4'-chloro phenoxy/4'-nitro phenoxy/2'-hydroxy biphenoxy)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (5a–f), respectively, Scheme 3 were obtained by condensation of 3 with phenol, p-methyl phenol, p-methoxy phenol, p-chloro phenol, p-nitro phenol, o-hydroxy biphenol, in ethyl alcohol and catalytic amount of TEA.

Compounds on reaction with acetyl acetone, ethyl acetoacetate, ethyl cyano acetate, malononitrile in presence of ethyl alcohol and catalytic amount of TEA yielded compounds 3-cyano-4-imino-9-methoxy-2-(α -acetyl acetonil/ α -ethylacetoacetyl/ α -ethylcyanoacetyl/ α -malononitriyl)-4H-pyrimido [2,1-b] pyrimido [4,5-b] quinoline (7a–d), respectively, Scheme 3.

Compounds 4a–e, 5a–f, 6a–6d, and 7a–7d show absorption bands in their IR spectra in the range of 3200 cm^{-1} to 3460 cm^{-1} and 2190 cm^{-1} to 2230 cm^{-1} due to =NH and CN stretching, respectively, ^1H NMR and Mass spectral data are also in agreement with structures of newly synthesized compounds 4a–e, 5a–f, 6a–6d, and 7a–7d.

The structures of these newly synthesized compounds were established on the basis of elemental analysis, IR, PMR,

TABLE 1: Antimicrobial activity of compounds (4a–e, 5a–f, 6a–6d, and 7a–7d).

Serial number	Compound code	Zone of inhibition in mm			
		Fungal species		Bacterial species	
		<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>E. coli</i>	<i>B. subtilis</i>
1	3	—	—	16	22
2	4a	12	10	10	26
3	4b	14	13	12	13
4	4c	16	17	20	18
5	4d	10	13	10	15
6	4e	12	16	22	21
7	5a	15	15	13	23
8	5b	08	06	10	21
9	5c	11	16	13	06
10	5d	14	13	22	18
11	5e	12	15	18	13
12	5f	06	14	10	23
13	6a	09	16	13	10
14	6b	17	15	26	13
15	6c	11	12	12	22
16	6d	15	10	09	21
17	7a	13	13	04	16
18	7b	05	15	11	13
19	7c	09	12	10	17
20	7d	13	18	26	23
Positive control		26	25	32	30
		Fluconazole		Streptomycin	

and MASS spectral data, spectral studies of all compounds show that compounds are stable and do not exhibit any tautomerism.

4. Antimicrobial Activity

The synthesized compounds were evaluated for their antifungal and antibacterial activity against species *Aspergillus flavus*, *Aspergillus niger*, and *E. coli* and *B. subtilis* by paper disc diffusion method [27]. All the synthesized compounds were dissolved in Dimethyl sulphoxide. The synthesized compounds exhibited zone of inhibition of 09–23 mm in diameter where as standard Fluconazole exhibited zone of inhibition of 26 and 25 mm Streptomycin exhibited zone of inhibition of 32 and 30 mm in diameter against *E. coli* and *B. subtilis*, respectively. Amongst the synthesized compounds 3, compound 4c (16, 17 mm), 4e (12, 16 mm), 5d (14, 13 mm), 5e (12, 15 mm), 6b (17, 15 mm), and 7d (13, 18 mm) showed higher zone of inhibition against *Aspergillus flavus*, *Aspergillus niger*, respectively. And 4c (20, 18 mm), 4e (22, 21 mm), 5d (22, 18 mm), 5e (18, 13 mm), 6b (26, 13 mm), and 7d (26, 23 mm) showed higher zone of inhibition against *E. coli* and *B. subtilis*. It seems that the presence of nitro and chloro group increases antifungal activity (Table 1).

5. Conclusion

In this communication all synthesized compounds reported first time and describe the simple route of their synthesis in mild condition with good yield. The present study showed that all the title compounds were exhibiting significant antibacterial and antifungal activities. However, further studies are required to establish the mechanism of action of the title compounds. From the screening data, it was found that 4c, 4e, 5d, 5e, 6b, and 7d derivative have encouraging antibacterial and antifungal activity, which needs to be further investigated to get better antibacterial and antifungal agents.

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