ISSN 1070-4280, Russian Journal of Organic Chemistry, 2019, Vol. 55, No. 10, pp. 1569–1574. © Pleiades Publishing, Ltd., 2019.

Synthesis and Characterization of Compounds Potentially Related to the Janus Kinase Inhibitor Baricitinib

S. R. Dasari[a, b], N. Seelam[a],* S. Jayachandra[b], L. R. Vadali[b], E. R. Yerva[b], S. Tondepu[c], and M. K. Gadakar[b]

[a] *Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, India*
**e-mail: nareshvarma.klu@gmail.com*

[b] *Mylan Laboratories Ltd., Hyderabad, Telangana, India*

[c] *Department of Chemical Engineering, Vignan's Foundation for Science, Technology, and Research, Vadlamudi, Guntur, Andhra Pradesh, India*

Received April 3, 2019; revised August 15, 2019; accepted August 17, 2019

Abstract—Nine compounds potentially related to the Janus kinase inhibitor Baricitinib have been identified, synthesized by conventional methods, and characterized by IR, ^{1}H and ^{13}C NMR, and mass spectral data.

Keywords: Baricitinib, rheumatoid arthritis, synthesis, characterization, Baricitinib-related substances.

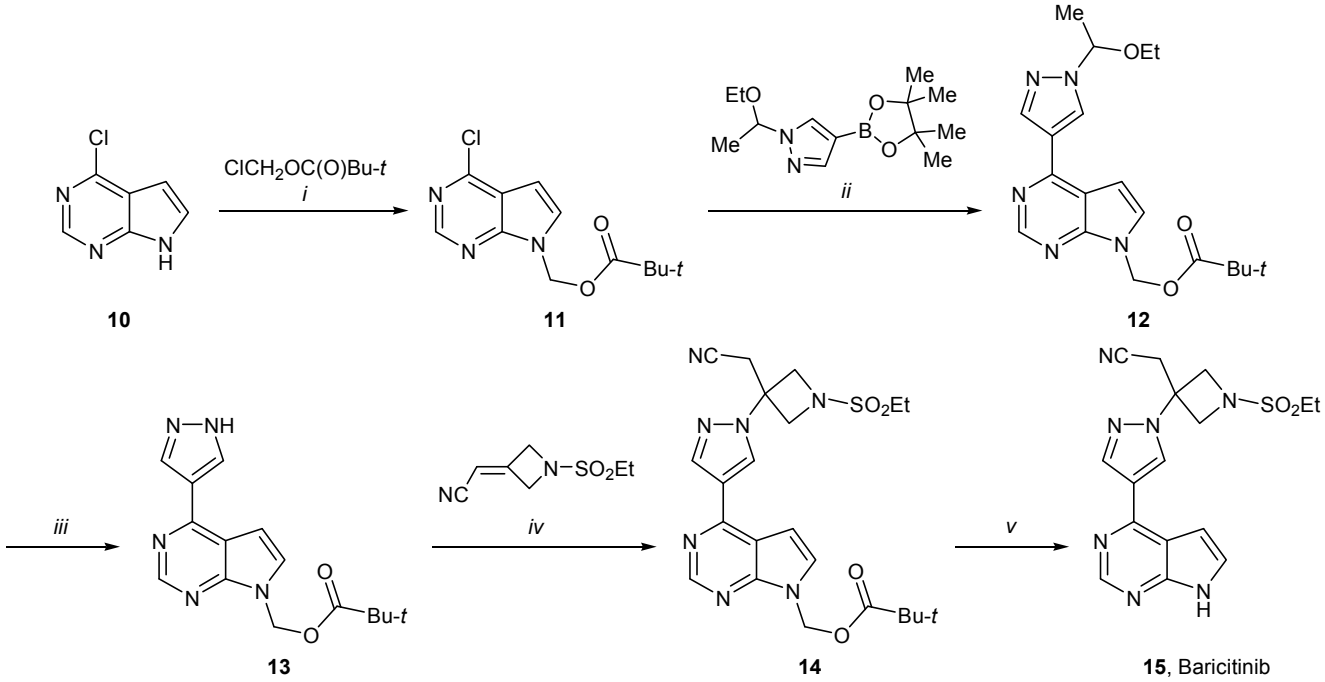
DOI: 10.1134/S1070428019100166

Baricitinib is a very recently proposed active pharma ingredient used for the treatment of all levels of rheumatoid arthritis [1]. It has been invented and is under marketing by Incyte Corporation and Eli Lilly & Co. under the trade name *Olumiant*. The drug has been approved first in Europe [2] and then in the United States of America [3] with a maximum daily dosage of 2 mg. Various methods for the synthesis of Baricitinib have been reported [4–6]. Scheme 1 outlines one of the described synthetic approaches adopted as an efficient and commercially viable method for the preparation of Baricitinib.

During the synthesis of Baricitinib according to Scheme 1, many other compounds eluting very closely to Baricitinib were detected by thin-layer chromatography. All these compounds (**1**–**9**) were isolated by column chromatography, characterized by mass, IR, and ^{1}H and ^{13}C NMR spectra, and shown to be structurally related to Baricitinib; they were called "Baricitinib related substances." It is now evident that the process of preparation of Baricitinib could be accompanied by generation of its related substances which could be mixed up with the required actual active pharma ingredient. In keeping with the latest ICH (International Conference on Harmonization) guidelines, any drug proposed for use in human medication should normally contain no more than 0.15% of any of its related substances [7] (the impurity levels may vary depending on the maximum daily dosage). To attain the required purity level of Baricitinib, an efficient purification procedure should be developed. To achieve this goal, prior knowledge of the structure and physical and chemical properties of the related substances plays a vital role. Therefore, possible Baricitinib related substances should be identified by conventional methods, isolated by various isolation techniques, characterized by different analytical methods, and synthesized easily by traditional schemes. Once the standard samples of all possible related substances have been prepared and characterized, we should make sure that the quantitative levels of each of those known related substances is below 0.15% in a given sample of Baricitinib. In view of the above stated, the present work was aimed as synthesizing Baricitinib related substances **1**–**9** and characterizing them by various spectral methods like IR spectroscopy, ^{1}H and ^{13}C NMR, and mass spectrometry.

Compounds **1**–**9** were synthesized on a multigram scale employing traditional synthetic methodologies and were characterized by various analytical techniques (Schemes 2–4). Alcohol **1** was identified in the reaction **14** → **15** (Scheme 1) and was prepared on a 10-g scale as per Scheme 2 [8]. Imidic ester **2**, ester **3**, acid **4**, and amide **5** were also identified in the

Scheme 1.

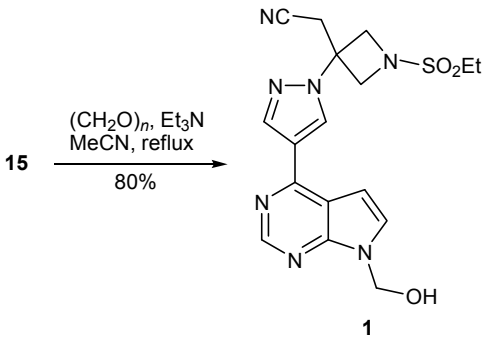


Reaction conditions: *i*: NaOH, DMF; *ii*: $Pd(PPh_3)_4$, K_2CO_3; *iii*: aq. HCl, THF, aq. NaOH; *iv*: DBU, DMF, MeCN; *v*: LiOH, H_2O, MeCN.

transformation of **14** to **15** and were prepared in multigram quantities as shown in Scheme **3** [9–11]. Compounds **6**–**9** were identified in the subsequent conversions of **10**–**14** and were prepared according to Scheme 4.

Prior awareness of highly abundant related substances and their possible formation pathways will be helpful to prepare Baricitinib with highest yield and purity. These will in turn impart robustness to the synthetic process of Baricitinib preparation on the commercial scale. Also, identification, synthesis, and characterization of Baricitinib related substances make its regulatory aspects easier to handle. So, our attempt to prepare and characterize the Baricitinib related substances may add useful knowledge for researchers.

Scheme 2.

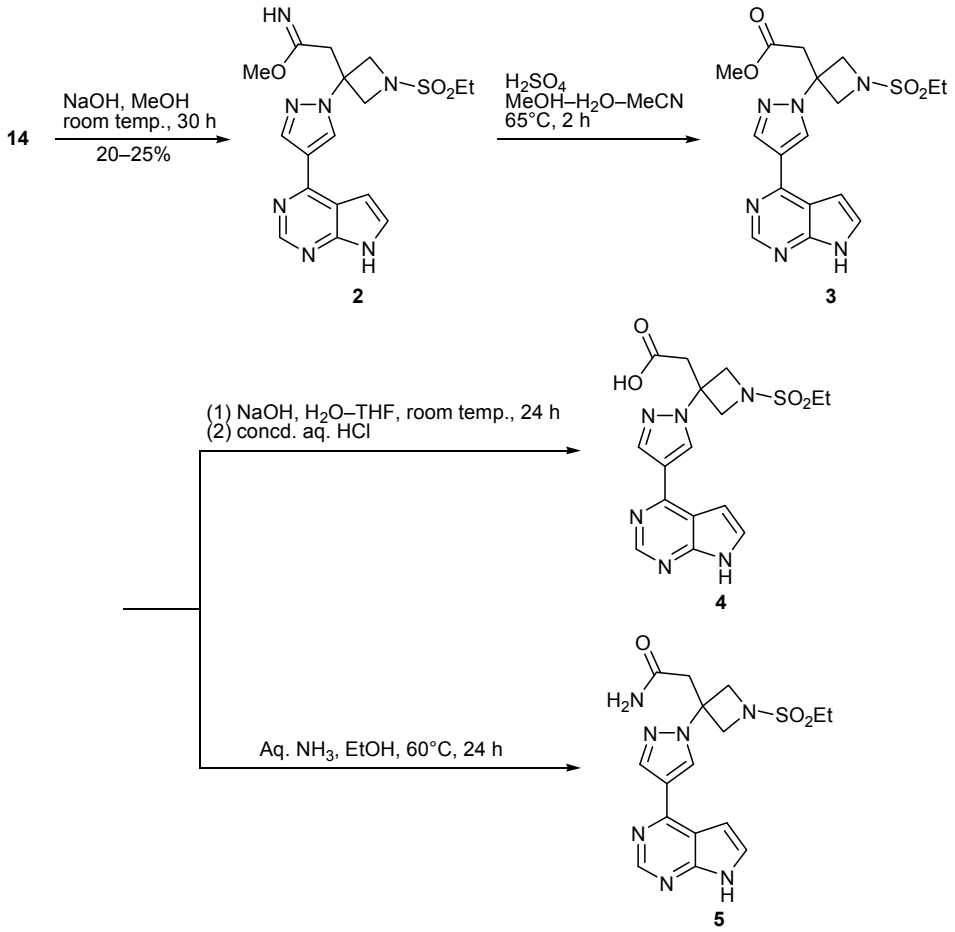


EXPERIMENTAL

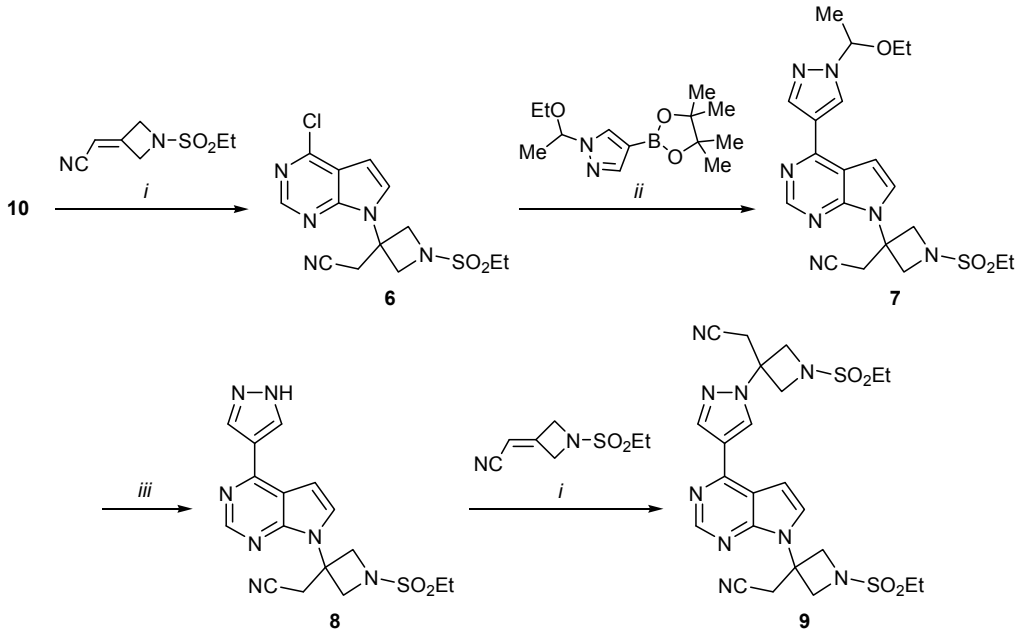
All chemicals were purchased from commercial suppliers and were used without further purification. All reactions were performed under inert nitrogen atmosphere employing dry solvents. Precoated TLC silica gel plates (Kieselgel 60 F_{254}, Merck) were used for monitoring reactions, and spots were visualized under UV lamp (λ 254 nm). Purification was performed by column chromatography using silica gel (particle size 60–120 mesh, Merck). The IR spectra were recorded in KBr on a Perkin–Elmer 400 FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance spectrometer at 300 or 500 MHz for ^{1}H with reference to TMS. The mass spectra were recorded using Waters Xevo TQS LC/MS/MS system. Elemental analysis was performed with a Thermo Finnigan Flash EA1112 elemental analyzer.

[1-(Ethanesulfonyl)-3-{4-[7-(hydroxymethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-1*H*-pyrazol-1-yl}-azetidin-3-yl]acetonitrile (1). A mixture of Baricitinib (**15**) (10.0 g, 0.027 mol), paraformaldehyde (16.2 g, 0.54 mol of CH_2O), triethylamine (54.6 g, 0.54 mol), and acetonitrile (100 mL) was heated to 80°C and stirred at that temperature for ~24 h. When the reaction was complete (TLC), excess paraformaldehyde was

Scheme 3.



Scheme 4.



Reaction conditions: *i*: (DBU), MeCN, 0°C; *ii*: $Pd(PPh_3)_4$, K_2CO_3, H_2O, 1,4-dioxane, 80°C; *iii*: 2N aq. HCl, THF, room temp., NaOH.

filtered off, the filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography using ethyl acetate–hexane (1 : 1) as eluent. Yield 5.0 g (46%), white solid. IR spectrum, ν, cm^{-1}: 3142, 2251, 1729, 1589, 1574, 1153, 1141. ^1H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm: 1.25 t (3H, J = 7.4 Hz), 3.24 q (2H, J = 7.5 Hz), 3.72 s (2H), 4.24 d (2H, J = 9.0 Hz), 4.61 d (2H, J = 9.0 Hz), 5.63 d (2H, J = 7.2 Hz), 6.67 t (OH, J = 7.4 Hz), 7.16 d (1H, J = 3.9 Hz), 7.74 d (1H, J = 3.9 Hz), 8.51 s (1H), 8.78 s (1H), 8.96 s (1H). ^{13}C NMR spectrum (300 MHz, DMSO-d_6), δ_C, ppm: 151.00, 150.75, 149.68, 139.97, 129.75, 129.54, 121.96, 116.68, 113.51, 100.13, 66.43, 59.77, 58.54, 56.10, 43.30, 26.84, 7.44. Mass spectrum: m/z 402.05 $[M + H]^+$. Found, %: C 51.25; H 5.10; N 24.12; S 7.85. $C_{17}H_{19}N_7O_3S$. Calculated, %: C 50.86; H 4.77; N 24.42; S 7.99.

Methyl 2-{1-(ethanesulfonyl)-3-[4-(7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl]azetidin-3-yl}acetimidate (2). A suspension of **14** (25.0 g, 0.051 mol) and sodium hydroxide (0.2 g, 0.005 mol) in methanol (125 mL) was stirred at room temperature for ~30 h (TLC). The crude product was isolated by filtration. In contained 20–25% of **2** (according to the LC/MS data) which was isolated by preparative HPLC. Mass spectrum: m/z 404.14 $[M + H]^+$.

Methyl 2-{1-(ethanesulfonyl)-3-[4-(7*H*-pyrrolo-[2,3-*d*]pyrimidin-4-yl)-1*H*-pyrazol-1-yl]azetidin-3-yl}acetate (3). A suspension of **2** (25.0 g, 0.062 mol) in a mixture of methanol with water and acetonitrile (8:8:2, 250 mL) containing a catalytic amount of sulfuric acid was stirred at 65°C for ~2 h (TLC). The mixture was cooled to room temperature and stirred for 2 h more complete precipitation of the product. The precipitate was filtered off and dried at 45–50°C for 5 h. Yield 25.0 g (100%). IR spectrum, ν, cm^{-1}: 3198, 3119, 2998, 1738, 1584. ^1H NMR spectrum (300MHz, DMSO-d_6), δ, ppm: 1.25 t (3H, J = 7.4 Hz), 3.25 q (2H, J = 7.5 Hz), 3.54 s (3H), 3.46 s (2H), 4.31 d (2H, J = 9.0 Hz), 4.54 d (2H, J = 9.0 Hz), 7.06 d.d (1H, J = 3.5, 1.7 Hz), 7.61 d.d (1H, J = 3.5, 2.6 Hz), 8.40 s (1H), 8.70 s (1H), 8.83 s (1H), 12.13 br.s (1H, NH). ^{13}C NMR spectrum (300 MHz, DMSO-d_6), δ_C, ppm: 169.52, 152.16, 150.91, 149.61, 139.42, 129.39, 126.82, 112.95, 99.94, 59.51, 56.83, 51.65, 42.95, 41.06, 7.43. Mass spectrum: m/z 405.04 $[M + H]^+$. Found, %: C 51.37; H 5.32; N 20.52; S 7.80. $C_{17}H_{20}N_6O_4S$. Calculated, %: C 50.48; H 4.98; N 20.78; S 7.93.

2-{1-(Ethanesulfonyl)-3-[4-(7*H*-pyrrolo[2,3-*d*]-pyrimidin-4-yl)-1*H*-pyrazol-1-yl]azetidin-3-yl}-acetic acid (4). A mixture of **3** (10.0 g, 0.025 mol) and sodium hydroxide (3.0 g, 0.075 mol) in 10% aqueous tetrahydrofuran (100 mL) was stirred for ~6 hours at room temperature (TLC). The solvent was evaporated, the residue was dissolved in 50 mL of water, the solution was acidified to pH 3–4 with concentrated aqueous HCl, and the mixture was stirred for ~2 h at room temperature. The solid product was isolated by filtration and dried. Yield 8.0 g (83%). ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.24 t (3H, J = 7.0 Hz), 3.14 s (2H), 3.21 q (2H, J = 7.5 Hz), 4.30 d (1H, J = 9.0 Hz), 4.35 d (1H, J = 9.0), 4.52 d (2H, J = 9.0 Hz), 7.02 d.d (1H, J = 3.5, 1.7 Hz), 7.55 d.d (1H, J = 3.5, 2.6 Hz), 8.41 s (1H), 8.68 s (1H), 8.76 s (1H), 12.13 br.s (1H). ^{13}C NMR spectrum (500 MHz, $CDCl_3$), δ_C, ppm: 175.4, 153.27, 150.0, 149.7, 140.3, 129.90, 128.23, 114.75, 112.62, 99.99, 59.63, 56.74, 43.57, 26.94, 7.45. Mass spectrum: m/z 391.20 $[M + H]^+$. Found, %: C 49.60; H 4.97; N 21.26; S 8.67. $C_{16}H_{18}N_6O_4S$. Calculated, %: C 49.22; H 4.65; N 21.53; S 8.21.

2-{1-(Ethanesulfonyl)-3-[4-(7*H*-pyrrolo[2,3-*d*]-pyrimidin-4-yl)-1*H*-pyrazol-1-yl]azetidin-3-yl}-acetamide (5). A suspension of **3** (10.0 g, 0.025 mol) in 20% aqueous ammonia (100 mL) was heated to 60°C and stirred for ~2 h at that temperature (TLC). The mixture was cooled to room temperature and stirred for 2 h more, and the solid product was filtered off. Yield 8.0 g (80%). ^1H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.24 t (3H, J = 7.0 Hz), 3.16 s (2H) 3.22 q (2H, J = 7.0 Hz), 4.38 d (2H, J = 9.0 Hz), 4.50 d (2H, J = 9.0 Hz), 7.0 s (1H), 7.29 s (NH), 7.55 s (1H), 7.84 s (NH), 8.63 s (1H), 8.86 m (1H), 8.98 s (1H). ^{13}C NMR spectrum (500 MHz, $CDCl_3$), δ_C, ppm: 170.4, 152.27, 151.0, 149.4, 140.02, 129.67, 126.97, 116.75, 113.16, 99.99, 58.63, 56.14, 43.47, 26.94, 7.48. Mass spectrum, m/z: 390.20 $[M + H]^+$, 412.2 $[M + Na]^+$.

2-[3-(4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)-1-(ethanesulfonyl)azetidin-3-yl]acetonitrile (6). A suspension of **10** (10.0 g, 0.065 mol) and 2-[1-(ethanesulfonyl)azetidin-3-ylidene]acetonitrile (12.12 g, 0.065 mol) in acetonitrile (50 mL) was cooled to 0°C, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.50 g, 0.00325 mol) was added, and the mixture was stirred for ~3 h (TLC). Diisopropyl ether (50 mL) was added, and the solid product was filtered off, washed with diisopropyl ether (50 mL), and dried. Yield 18.0 g (81%), pale yellow solid. ^1H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.41 t (3H, J = 7.0 Hz), 3.09 q (2H, J = 7.5 Hz), 3.42 s (2H), 4.30 d.d

(2H, J = 1.0, 8.0 Hz), 4.68 d (2H, J = 9.5 Hz), 6.74 d (1H, J = 4.0 Hz), 7.27 d (1H, J = 2.5 Hz), 8.61 s (1H). ^{13}C NMR spectrum (500 MHz, $CDCl_3$), δ_C, ppm: 152.72, 150.70, 149.90, 125.93, 117.99, 115.17, 100.91, 58.36, 52.38, 46.47, 27.20, 7.62. Mass spectrum, m/z: 340.1 $[M + H]^+$, 362.1 $[M + Na]^+$.

2-({3-[4-(1-(1-Ethoxyethyl)-1*H*-pyrazol-4-yl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl}azetidin-3-yl)acetonitrile (7). Potassium carbonate (16.1 g, 0.116 mol) and water (30 mL) were added at room temperature to a suspension of **6** (10.0 g, 0.029 mol) and 1-(1-ethoxyethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-pyrazole (7.42 g, 0.029 mol) in 1,4-dioxane (100 mL). The two-phase mixture was degassed with nitrogen for ~30 min, and tetrakis(triphenylphosphine)palladium(0) (0.60 g, 0.52 mmol) was added. The mixture was heated to 80°C and stirred at that temperature for 10–12 h (TLC). The mixture was then cooled to room temperature and filtered, the aqueous layer was separated from the filtrate, and the organic layer was mixed with ethyl acetate (100 mL), washed with water (2 × 100 ml), and concentrated under reduced pressure. The oily residue was treated with diisopropyl ether (100 mL), the mixture was stirred for 2 h at room temperature, and the precipitate was filtered off, washed with $(i\text{-Pr})_2O$ (20 mL), and dried. Yield 10.0 g (77%), yellow solid. ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.20 t (3H, J = 4.5 Hz), 1.42 t (3H, J = 7.5 Hz), 1.76 d (3H, J = 6.0 Hz), 3.08 q (2H, J = 7.5 Hz), 3.46 s (2H), 3.42–3.44 m (1H), 3.52–3.55 m (1H), 4.31 d (2H, J = 10.0 Hz), 4.72 d (2H, J = 9.0 Hz), 5.61 q (1H, J = 6.0 Hz), 6.86 d (1H, J = 3.5 Hz), 7.25 d (1H, J = 4.0 Hz), 8.25 s (1H), 8.40 s (1H), 8.76 s (1H). ^{13}C NMR spectrum (500 MHz, $CDCl_3$), δ_C, ppm: 151.87, 151.54, 150.53, 138.74, 127.08, 124.87, 121.75, 115.45, 114.49, 101.36, 87.97, 64.33, 58.60, 52.20, 46.59, 27.16, 22.1, 14.72, 7.73. Mass spectrum: m/z 420.136 $[M + H]^+$.

2-{1-(Ethanesulfonyl)-3-[4-(1*H*-pyrazol-4-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl]azetidin-3-yl} acetonitrile (8). Compound **7** (10.0 g, 0.023 mol) was dissolved in tetrahydrofuran (60 mL), 2 N aqueous hydrochloric acid (30 mL) was added at room temperature, and the mixture was stirred for ~24 h (TLC). Tetrahydrofuran was distilled off, the aqueous residue was adjusted to pH 10–12 by adding 30% aqueous sodium hydroxide at a temperature not exceeding 20°C. The mixture was stirred for 4 h at room temperature for complete precipitation, and the product was filtered off. The wet cake was taken in diisopropyl ether (100 mL), the mixture was stirred at room temperature for ~1 h, and the solid precipitate was filtered off and dried. Yield 8.0 g (100%). ^{1}H NMR spectrum (500 MHz, DMSO-d_6), δ, ppm: 1.24 t (3H, J = 7.5 Hz), 3.22 q (2H, J = 7.5 Hz), 3.62 s (2H), 4.32 d (2H, J = 9.0 Hz), 4.67 d (2H, J = 9.0 Hz), 7.20 d (1H, J = 4.0 Hz), 7.83 d (1H, J = 4.0 Hz), 8.37 s (1H), 8.71 s (1H), 8.73 s (1H), 13.5 s (1H, NH). ^{13}C NMR spectrum (500 MHz, DMSO-d_6), δ_C, ppm: 151.58, 151.05, 150.35, 138.92, 129.79, 127.12, 119.89, 116.80, 113.75, 100.85, 58.71, 58.44, 52.28, 43.32, 26.93, 7.46. Mass spectrum: m/z 372.2 $[M + H]^+$.

2-[3-(4-{1-[(3-(Cyanomethyl)-1-(ethanesulfonyl)-azetidin-3-yl]-1*H*-pyrazol-4-yl}-7*H*-pyrrolo[2,3-*d*]-pyrimidin-7-yl)-1-(ethanesulfonyl)azetidin-3-yl]-acetonitrile (9). 1,8-Diazabicyclo[5.4.0]undec-7ene (DBU, 0.01 g, 0.68 mmol) was added with stirring at 0–5°C to a suspension of **8** (10.0 g, 0.027 mol) and 2-[1-(ethanesulfonyl)azetidin-3-ylidene]acetonitrile (5.01 g, 0.027 mol) in acetonitrile (80 mL). The mixture was stirred for ~3 h (TLC) and diluted with diisopropyl ether (100 mL), the precipitate was filtered off and taken in additional diisopropyl ether (50 mL), the mixture was stirred, and the precipitate was filtered off and dried. Yield 12.0 g (80%), white solid. ^{1}H NMR spectrum (300 MHz, DMSO-d_6), δ, ppm: 1.21–1.29 m (6H), 3.18–3.28 m (4H), 3.62 s (2H), 3.70 s (2H), 4.28 d (4H, J = 9.0 Hz), 4.65 d (4H, J = 9.0 Hz), 7.29 d (1H, J = 3.6 Hz), 7.91 d (1H, J = 3.6 Hz), 8.52 s (1H), 8.78 s (1H), 8.99 s (1H). ^{13}C NMR spectrum (300 MHz, DMSO-d_6), δ_C, ppm: 150.98, 150.47, 150.41, 140.06, 130.01, 127.56, 121.64, 116.67, 116.63, 113.96, 100.84, 56.16, 52.32, 43.35, 43.31, 26.91, 26.86, 7.43. Mass spectrum: m/z 558.09 $[M + H]^+$.

ACKNOWLEDGMENTS

The authors thank their research supervisors for providing the required facilities. We also thank Department of Chemistry, Koneru Lakshmaiah Education Foundation, and Mylan Laboratories Ltd. for their help in performing this study.

CONFLICT OF INTERESTS

The authors declare the absence of conflict of interests.

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