Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Electronic Supporting Information

Synthesis, Cytotoxicity and hDHFR inhibition studies of 2H-Pyrido[1,2-a]pyrimidin-2-ones

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General. (A) **Chemistry.** All Chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), or Spectrochem Pvt. Ltd. (Mumbai, India) and were used without further purification. Reactions were monitored by TLC performed on silica gel glass plates containing 60 GF-254, and visualization was achieved by UV light or iodine indicator. Column Chromatography was performed with Merk 60-120 mesh silica gel. Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR spectra were obtained on JCAMP DX-50 instrument (300 MHz for ^{1}H and 75 MHz &125 MHz for ^{13}C) and $CDCl_3$ and DMSO-d_6 used as solvents; *J* values are in Hz. Chemical shifts are reported in δ (ppm) down field from internal standard TMS. ESI spectra were recorded on thermofunigan ESI ion trap mass spectrometer. HRMS data were recorded on QSTAR XL Hybrid MS/MS system under ESI condition. IR spectra were recorded on a Thermo Nicolet NEXUS 670 spectrometer in KBr with absorption in cm-1.

(B) **Biology. (a) Cytotoxicity studies against five different cancer cell lines:**

Cellular viability in the presence of test compounds was determined by MTT-microcultured tetrazolium assay following the reported protocol[1]. Compounds were screened against cervical (HeLa), liver (HEP G-2), breast (MCF-7), neuroblastoma (SK-n-SH) and lung (A-549) cancer cells. All the five types of cancer cell lines were seeded to flat bottom 96 (10000cells/100ul) well plates and cultured in the medium containing 10% serum, incubated for 24 hours in a 5% CO_2 humid chamber so that the cells adhere to the surface. 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) was dissolved in PBS at 5 mg/ml and sterile filtered.

Five different concentrations with a ten-fold variation between 1 mM and 100 nM of the compounds were added to the adhered cells. After 48 hours, stock MTT solution (10ul) was added to the culture plate. Cells were further incubated in the CO_2 chamber for 2 hours. Following this, media was removed and 100ul of DMSO was added. Absorbance was measured at 562 nm in a multimode microplate reader (Tecan GENios). Results were represented as percentage of cytotoxity/viability. All the experiments were carried out in Triplicates. From the percentage of cytotoxicity the IC_{50} values were calculated and presented (Table 2).

(B) DHFR inhibition studies

To the 50µL reaction solution containing 20mM Tris buffer, pH 7.5, 0.5 M KCl and 0.5µg of human DHFR enzyme, different concentrations of inhibitors were added and incubated at 22 °C for 10 minutes [2]. 50 µL NADPH (25 µg) and dihydrofolate (5µg) in 20_mM Tris buffer, pH 7.5, 0.5 M KCl solution was added to the above reaction mixture. After shaking for 30 seconds at 22^0C, depletion of NADPH was monitored at 340 nm on the microplate reader (Tecan microplate spectrophotometer). All reactions were carried out in triplicates.

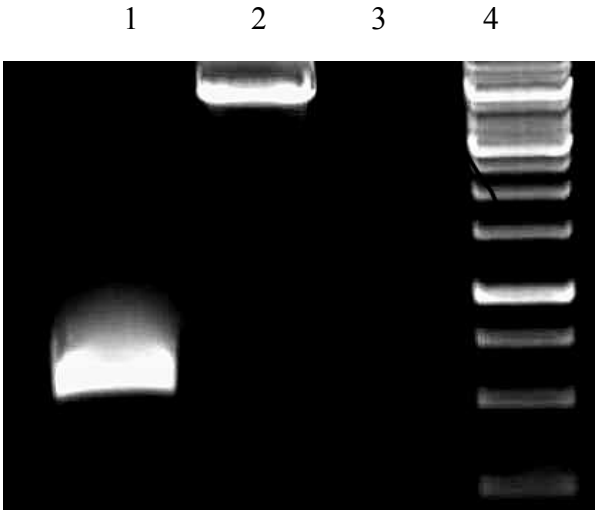
(C) Molecular Docking

Docking of the active compounds against human DHFR (PDB: 3NZD) was carried by AutoDock4 in a predefined methotrexate binding pocket [3]. Grids map was created in Autodock that define interaction of protein and ligands in binding pocket. Grid map with 60 points in each x, y, and z direction, equally spaced at 0.375 Å was used. Docking was performed using the Lamarckian genetic algorithm in AutoDock4 [4]. Each docking experiment was performed 30 times, yielding 30 docked conformations. Parameters used for the docking were as follows: population size of 150; random starting position and conformation; maximal mutation of 2 Å in

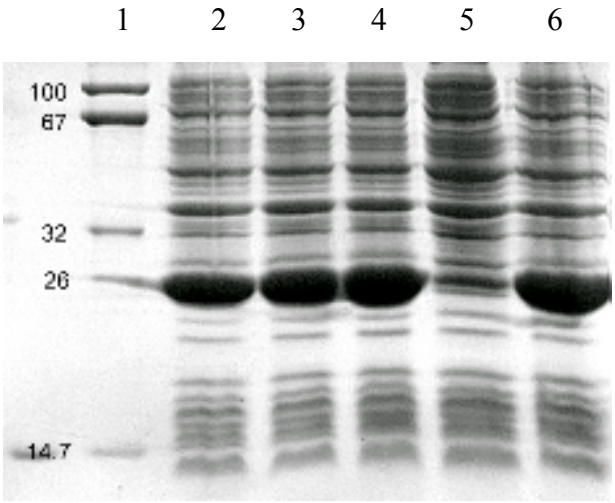
translation and 50 degrees in rotations; elitism of 1; mutation rate of 0.02 and crossover rate of 0.8; and local search rate of 0.06. Simulations were performed with a maximum of 1.5 million energy evaluations and a maximum of 50000 generations. Final docked conformations were clustered using a tolerance of 1 Å RMSD.

(D) DHFR Cloning, Expression and Purification:

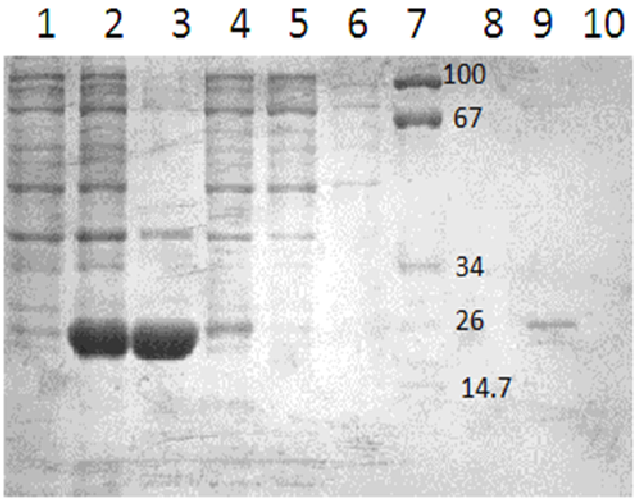
The gene encoding human DHFR was PCR-amplified from the cDNA library human acute monocytic leukemia cell line (THP-1) by using the forward (5′-CGCGGATCCATGGTTGGTTCGCTAAACTGC-3′) and reverse (5′-CCGCTCGAGTTAATCATTCTTCTCATATAC-3′) primer, followed by insertion into PET28a (Novagen, Darmstadt, Germany) vector between BamH1 and XhoI (fermentas) restriction sites introducing an N-terminal His-tag. BL21 (DE3) bacterial cells (Novagen) were used for the expression of the protein. Cells were cultured in LB media at 37^0C at 250 rpm. At OD600 of 1.2, protein expression was induced by 1 mM isopropyl β-D-thiogalactoside and then incubated at 37^0C. Cells were harvested after 3h by centrifuging at 3000 rpm for 30 min, followed by resuspension in +T/G buffer (50 mM Tris, pH 8.0/0.5 M KCl/5% glycerol/0.1% Triton X-100/5 mM imidazole) and lysed by passing through a cell disruptor. After centrifugation at 17,000 × g for 30 min, the supernatant was applied to a His select HF nickel affinity column (Nickel affinity resin, Sigma), which was pre-equilibrated with +T/G buffer. Continued application of +T/G buffer was used to wash the column until the absorption at A280 reached the baseline. The column was further washed with −T/G buffer (50 mM Tris, pH 8.0, 0.5 M KCl and 5 mM imidazole). Pure protein was eluted with 150 mM imidazole in −T/G buffer at 0.2 mg/ml and dialyzed twice in 4 liters of 50 mM Tris, pH 8.0, 200mM KCl and 5% glycerol. No further purification was done for the enzyme activity assays (Gel figure attached in supplementary).



Cloning of hDHFR: 1. huDHFR pcr product (561bp). 2. Pet28a vector (5369bp). 3. Empty lane. 4.1Kb DNA ladder.



Expression of hDHFR: 1. Protein marker. 2,3,4,6.Induced sample. 5. Uninduced sample.



Purification of hDHFR: Lane 1. Uninduced sample. 2. Induced sample. 3. Lysed pellet. 4. Lysate. 5. Flowthrough. 6. Wash. 7. Protein marker. 8,9,10. Elutions

(E) X-ray diffraction data of single crystal 3d

X-ray data for the compound **3d** was collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoK α radiation (λ=0.71073Å) with ω-scan method. Preliminary lattice parameters and orientation matrices were obtained from four sets of frames. Unit cell dimensions were determined from the setting angles of 6092 reflections for (**3d**). CCDC-760517 contains the supplementary crystallographic data for this paper. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Experimental procedure and characterization data.

Preparation of 2H-pyrido[1,2-a]pyrimidin-2-one (3): General procedure:

A mixture of Baylis Hillman acetate (2 mmol) and 2-aminopyridine (2 mmol) were taken in round bottom flaske and stirred at room temperature. The progress of the reaction is monitored

by TLC. After the completion of the reaction, the solid product obtained was filtered off by triturating with water. The white solid products collected were well characterized by physical and spectral data.

Characterization data of 2H-pyrido[1,2-a]pyrimidin-2-one (3a↕v).

3-Benzyl-*2H*-pyrido[1,2-a]pyrimidin-2-one (3a): White solid, Yield : 198 mg, 84%; m.p. 219↕222 ^{o}C (decom.); IR (KBr): ($\nu_{max,}$ cm^{-1}): 1650, 1602 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ : 3.84 (s, 2H, CH_2), 6.74-6.78 (t, 1H, *J* = 6.6 Hz, ArH), 7.21-7.30 (m, 5H, ArH), 7.48-7.53 (t, 1H, *J* = 7.3 Hz, ArH), 7.58 (s, 1H, ArH), 7.70 (s, 1H, ArH), 7.87-7.90 (d, 1H, *J* = 6.9 Hz, ArH); ^{13}C NMR (75MHz, DMSO-d_6) δ: 53.2, 129.6, 132.2, 142.1, 145.8, 147.1, 147.9 (2C), 148.4 (2C), 153.6, 155.9, 156.0, 158.3, 170.3; ESI Mass (m/z) 237 ($M+H^+$); HRMS (EI): m/z Calculated value = 237.1027, Observed value = 237.1039.

3-(4-Fluorobenzyl)-7-chloro-*2H*-pyrido[1,2-a] pyrimidin-2-one (3b): White solid, Yield : 170 mg, 59%; m.p. 240↕243^{o}C (decom.); IR (KBr): ($\nu_{max,}$ cm^{-1}): 1656, 1598 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ : 3.82 (s, 2H, CH_2), 6.73-6.78 (t, 1H, *J* = 7.1 Hz, ArH), 6.96-7.01 (t, 2H, *J* = 8.6 Hz, ArH), 7.21-7.24 (d, 1H, *J* = 8.8 Hz, ArH), 7.27-7.32 (dd, 1H, *J* = 5.6 & 8.4 Hz, ArH), 7.47-7.53 (t, 1H, *J* = 8.8 Hz, ArH), 7.74 (s, 1H, ArH), 7.90-7.92 (d, 1H, *J* = 6.7 Hz, ArH); ESI Mass (m/z) 289 (M^+); HRMS (EI): m/z Calculated value = 289.0543, Observed value = 289.0554.

3-(4-Chlorobenzyl)-7-bromo-*2H*-pyrido[1,2-a]pyrimidin-2-one (3c): White solid, Yield : 220 mg, 63%; m.p. 236↕240 ^{o}C (decom.); IR (KBr): ($\nu_{max,}$ cm^{-1}): 1653, 1614 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d_6): 3.75 (s, 2H, CH_2), 7.08-7.10 (d, 1H, *J* = 9.7 Hz, ArH), 7.23 (s, 4H, ArH), 7.51-7.53 (d, 1H, *J* = 11.7 Hz, ArH), 7.89 (s, 1H, ArH), 8.41 (s, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$-DMSO-d_6) δ: 33.7, 100.2, 112.6, 125.5, 127.9, 129.1 (2C), 130.9 (2C), 131.6,

134.2, 136.3, 138.6, 164.9, 171.3; ESI Mass (m/z) 349 (M^+); HRMS (EI): Calculated value = 348.9743, Observed value = 348.9731.

3-(4-bromobenzyl)-7-bromo-*2H*-pyrido[1,2-a]pyrimidin-2-one (3d): White solid, Yield : 256 mg, 65%; m.p.239↕240 ^oC (decom.); IR (KBr): ($\nu_{max,}$ cm^{-1}): 1652, 1615 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ : 3.78 (s, 2H, CH_2), 7.12-7.15 (d, 1H, *J* = 9.8 Hz, ArH), 7.20-7.22 (d, 2H, *J* = 8.0 Hz, ArH), 7.41-7.43 (d, 2H, *J* = 8.0 Hz, ArH), 7.54-7.57 (d, 1H, *J* = 8.9 Hz, ArH), 7.67 (s, 1H, ArH), 7.89 (s, 1H, ArH);ESI Mass (m/z) 394 (M^+); HRMS (EI): m/z Calculated value =394.9206, Observed value = 394.9216.

3-(4-Methylbenzyl)-7-chloro-*2H*-pyrido[1,2-a]pyrimidin-2-one (3e): White solid, Yield : 168 mg, 59%; m.p. 233↕236 ^oC (decom.); IR (KBr): ($\nu_{max,}$ cm^{-1}): 1655, 1616 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ : 2.31 (s, 3H, CH_2), 3.73 (s, 2H, CH_2), 7.06-7.18 (m, 5H, ArH), 7.52-7.55 (dd, 1H, *J* = 2.2 & 9.6 Hz, ArH), 7.96 (s, 1H, ArH), 8.42-8.43 (d, 1H, *J* = 1.8 Hz, ArH); ESI Mass (m/z) 285 (M^+); HRMS (EI): m/z Calculated value = 285.08 14, Observed value = 285.0822.

3-(4-Methoxybenzyl)-7-bromo-*2H*-pyrido[1,2-a] pyrimidin-2-one (3f): White solid, Yield : 203 mg, 59%; m.p. 222↕226 ^oC (decom.); IR (KBr): ($\nu_{max,}$ cm^{-1}): 1651, 1600 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ: 3.69 (s, 2H, CH_2), 3.75 (s, 3H, CH_3), 6.80 (s, 2H, ArH), 7.10 (s, 1H, ArH), 7.18 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.96 (s, 1H, ArH), 8.02 (s, 1H, ArH), 8.53 (s, 1H, ArH); ESI Mass (m/z) 345 (M^+); HRMS (EI): m/z Calculated value = 345.0238, Observed value = 345.0244.

3-(4-Nitrobenzyl)-7-chloro-2H-pyrido[1,2-a]pyrimidin-2-one (3g):

White solid, Yield: 215 mg, 68%; mp 248↕250 ^{o}C (decom.); IR (KBr): 1648, 1591 cm-^{1}; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 500 MHz): δ 3.93 (s, 2H, CH_2), 7.19-7.21 (d, 1H, *J* = 9.6 Hz, ArH), 7.50-7.53 (dd, 1H, *J* = 9.6 Hz, ArH), 7.55-7.57 (d, 1H, *J* = 8.2 Hz, ArH), 7.78-7.79 (d, 1H, *J* = 3.4 Hz, ArH), 8.13-8.14 (d, 3H, *J* = 7.5 Hz, ArH), 8.37 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75MHz, ppm): δ 33.5, 104.2, 107.7, 118.4, 123.3 (2C), 123.8, 126.3, 130.0 (2C), 131.6, 136.5, 136.8, 146.7, 179.6; ESI Mass (m/z) 316 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{15}H_{11}N_3O_3Cl$: 316.0136, found: 316.0143.

3-(4-(3,5,6-trichloropyridin-2-yloxy)benzyl)-7-chloro-2H-pyrido[1,2-a]pyrimidin-2-one (3h):

White solid, Yield: 284 mg, 61%; mp 242↕245 ^{o}C (decom.); IR (KBr): 1655, 1608 cm-^{1}; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 3.76 (s, 2H, CH_2), 7.14-7.20 (t, 3H, *J* = 8.4 Hz, ArH), 7.36-7.39 (d, 2H, *J* = 8.8 Hz, ArH), 7.72-7.76 (d, 1H, *J* = 9.4 Hz, ArH), 8.19 (s, 1H, ArH), 8.51 (s, 1H, ArH), 8.55 (s, 1H, ArH); ESI Mass (m/z) 466 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{20}H_{12}N_3O_2Cl_4$: 465.9683, found: 465.9689.

3-(3-(3,5,6-trichloropyridin-2-yloxy)benzyl)-7-bromo-2H-pyrido[1,2-a]pyrimidin-2-one (3i):

White solid, Yield: 302 mg, 59%; mp 244↕246 ^{o}C (decom.); IR (KBr): 1652, 1610 cm-^{1}; ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.80 (s, 2H, CH_2), 6.99-7.02 (d, 1H, *J* = 9.4 Hz, ArH), 7.09-7.12 (d, 2H, *J* = 9.2 Hz, ArH), 7.21-7.23 (d, 1H, *J* = 7.7 Hz, ArH), 7.34-7.39 (t, 1H, *J* = 7.7 Hz, ArH), 7.66-7.69 (d, 1H, *J* = 9.4 Hz, ArH), 8.15 (s, 1H, ArH), 8.20-8.25 (d, 1H, *J* = 9.4 Hz, ArH), 8.52 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 125MHz): δ 33.3, 105.1, 117.6, 118.9, 121.1, 122.9, 123.8, 126.2, 126.9, 129.5, 133.7, 136.1, 138.8, 140.6, 141.7, 142.4, 149.3, 152.6, 155.6, 166.9; ESI

Mass (m/z) 512 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{20}H_{12}N_3O_2BrCl_3$: 512.0238, found: 512.0244.

3-(4-Fluoro-2-trifluoromethyl-benzyl)-pyrido[1,2-a]pyrimidin-2-one (3j):

White solid, Yield: 225 mg, 70%; mp 245↕248 ^oC (decom.); IR (KBr): 1656, 1598 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 3.98 (s, 2H, CH_2), 6.78-6.83 (t, 1H, *J* = 7.1 Hz, ArH), 7.20-7.23 (d, 1H, *J* = 9.0 Hz, ArH), 7.40-7.43 (d, 1H, *J* = 9.0 Hz, ArH), 7.48-7.59 (m, 2H, ArH), 7.75 (s, 1H, ArH), 7.78-7.82 (s, 1H, ArH), 8.08 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75MHz): δ 29.8, 112.6, 113.2, 113.6, 119.3, 119.6, 122.5, 126.5, 132.5, 134.0, 136.4, 136.7, 150.7, 158.7, 161.9, 166.8; ESI Mass (m/z) 323 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{16}H_{11}N_2OF_4I$: 323.0807, found: 323.0814. 23.

3-(4-bromobenzyl)-*2H*-pyrido[1,2-a]pyrimidin-2-one (3k): White solid, Yield : 271 mg, 86%; m.p. 238↕241 ^oC (decom.); IR (KBr): (ν_{max}, cm^{-1}): 1652, 1586 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 3.79 (s, 2H, CH_2), 6.75-6.80 (t, 1H, *J* = 6.7 Hz, ArH), 7.04-7.07 (d, 1H, *J* = 8.3 Hz, ArH), 7.22- 7.28 (t, 3H, *J* = 8.3 Hz, ArH), 7.40-7.43 (d, 2H, *J* = 8.3 Hz, ArH), 7.89 (s, 1H, ArH), 8.00-8.02 (d, 1H, *J* = 6.0 Hz, ArH,); ESI Mass (m/z) 315 (M^+); HRMS (EI): m/z Calculated value = 315.0132, Observed value = 315.0143.

3-Benzyl-7-bromo-*2H*-pyrido[I,2-a]pyrimidin-2-one (3l): White solid, Yield : 214 mg, 68%; m.p. 237-240 ^oC (decom.); IR (KBr): (ν_{max}, cm^{-1}): 1653, 1616 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 3.79 (s, 2H, CH_2), 7.10-7.13 (d, 1H, *J* = 9.4 Hz, ArH), 7.27-7.29 (m, 3H, ArH), 7.56-7.60 (d, 1H, *J* = 9.4 Hz, ArH), 7.87 (s, 2H, ArH), 7.96 (s, 1H, ArH), 8.47 (s, 1H, ArH); ESI Mass (m/z) 315 (M^+); HRMS (EI): m/z Calculated value = 315.0122, Observed value = 315.0123

7-chloro-3-(4-fluoro-2-(trifluoromethyl)benzyl)-2H-pyrido[1,2-a]pyrimidin-2-one (3m): White solid, Yield: 220 mg, 62%; mp 234↕236 ^{o}C (decom.); IR (KBr): 1605, 1493 cm-1; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 3.95 (s, 2H, CH_2), 7.19-7.21 (d, 1H, *J* = 9.3 Hz, ArH), 7.27-7.31 (t, 1H, *J* = 8.3 Hz, ArH), 7.41-7.43 (d, 1H, *J* = 9.3 Hz, ArH), 7.46-7.49 (t, 1H, *J* = 8.3 Hz, ArH), 7.52-7.54 (d, 1H, *J* = 9.3 Hz, ArH), 7.74 (s, 1H, ArH), 8.43 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 29.8, 113.4, 118.5, 119.4, 119.6, 123.8, 127.0, 131.7, 132.1, 134.2, 136.1, 136.9, 149.3, 159.4, 161.4, 166.6; ESI Mass (m/z): 357 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{16}H_{10}N_2OF_4Cl$: 357.0417, found: 357.0430.

3-(4-Fluorobenzyl)-*2H*-pyrido[1,2-a]pyrimidin-2-one (3n): White solid, Yield : 189 mg, 74%; m.p. 220↕224^{o}C (decom.); IR (KBr): (ν_{max}, cm^{-1}): 1654, 1585 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ (ppm): 3.82 (s, 2H, CH_2), 6.73-6.78 (t, 1H, *J* = 6.9 Hz, ArH), 6.96-7.01 (t, 2H, *J* = 8.6 Hz, ArH), 7.21-7.24 (d, 1H, *J* = 8.8 H,z ArH), 7.27-7.32 (dd, 2H, *J* = 5.6 & 8.4 Hz, ArH), 7.47-7.53 (t, 1H, *J* = 8.1 Hz, ArH), 7.74 (s, 1H, ArH), 7.90-7.92 (d, 1H, *J* = 6.7 Hz, ArH); ^{13}C NMR (75 MHz, DMSO- d_6) δ: 52.2, 99.5, 132.3, 134.4, 134.7, 142.1, 147.0, 150.3, 150.4, 153.6, 154.3, 155.9, 156.1, 178.9, 182.1; ESI Mass (m/z) 255 $(M+H^+)$; HRMS (EI): Calculated value = 255.0933, Observed value = 255.0945.

9-amino-3-benzyl-2H-pyrido[1,2-a]pyrimidin-2-one (3o):

Black solid, yield : 195 mg, 78%; mp 196↕198 ^{o}C (decom.); IR (KBr): 1650, 1582 cm-1; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 3.80 (s, 2H, CH_2), 5.77 (s, 2H, NH_2), 6.64-6.69 (dd, 2H, *J* = 8.0 Hz, ArH), 7.17-7.20 (t, 1H, *J* = 7.0 Hz, ArH), 7.25-7.34 (m, 5H, ArH), 7.96-7.99 (d, 1H, *J* = 17.0 Hz, ArH); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 33.4, 107.9, 113.7, 120.1, 126.0, 127.6,

128.2 (2C), 128.7 (2C), 136.6, 138.6, 138.9, 143.3, 166.6; ESI Mass (m/z) 252 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{15}H_{14}N_3O$: 252.1131, found: 252.1124.

9-amino-3-benzyl-7-bromo-2H-pyrido[1,2-a]pyrimidin-2-one (3p):

Brown solid, yield: 234 mg, 71%; mp 189↕191 °C (decom.); IR (KBr): 1656, 1590 cm-1; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 3.85 (s, 2H, CH_2), 6.85 (s, 1H, ArH), 7.32 (m, 5H, ArH), 7.74 (s, 1H, ArH), 8.05 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 33.3, 108.7, 111.6, 119.3, 126.14, 128.03, 128.12 (2C), 128.6 (2C), 136.8, 137.6, 139.3, 141.3, 166.1; ESI Mass (m/z) 329 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{15}H_{13}N_3OBr$: 330.0233, found: 330.0236.

9-amino-3-(4-fluorobenzyl)-2H-pyrido[1,2-a]pyrimidin-2-one (3q):

White solid, Yield: 177 mg, 66%; mp 185↕188 °C (decom.); IR (KBr): 1653, 1584 cm-1; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz, ppm): δ 3.87 (s, 2H, CH_2), 5.61 (s, 2H, NH_2), 6.65-6.58 (t, 1H, *J* = 7.0 Hz, ArH), 6.71-6.73 (d, 1H, *J* = 8.0 Hz, ArH), 6.99-7.03 (t, 2H, *J* = 8.0 Hz, ArH), 7.19-7.23 (dd, 1H, *J* = 5.0 & 10.0 Hz, ArH), 7.29-7.32 (t, 2H, *J* = 8.0 Hz, ArH), 7.66-7.70 (t, 2H, *J* = 8.0 Hz, ArH); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 32.7, 108.0, 113.8, 114.7, 114.9, 118.1, 120.1, 127.6, 130.5, 130.6, 134.9, 136.6, 138.6, 143.3, 166.5; ESI Mass (m/z) 270 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{15}H_{13}N_3OF$: 270.1036, found: 270.1037.

9-amino-7-bromo-3-(4-fluoro-2-(trifluoromethyl)benzyl)-2H-pyrido[1,2-a]pyrimidin-2-one (3r):

White solid, Yield: 269 mg, 65%; mp 199↕201 °C (decom.); IR (KBr): 1652, 1584 cm-1; ^{1}H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 4.02 (s, 2H, CH_2), 6.03 (br s, 2H, NH_2), 6.80 (s, 1H, ArH), 7.26-7.32 58 (t, 1H, *J* = 8.1 Hz, ArH), 7.42-7.47 58 (t, 2H, *J* = 8.1 Hz, ArH), 7.52 (s, 1H,

ArH), 7.58 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 29.6, 108.1, 110.1, 112.8, 112.8, 113.2, 118.5, 118.7, 128.4, 131.1, 133.5, 133.6, 135.1, 138.8, 141.9, 158.6, 166.8; ESI Mass (m/z) 416 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{16}H_{11}N_3OF_4Br$: 416.0011, found: 416.0016.

3-((6-(benzyloxy)-2,2-dimethyl-dihydro-5H-furo[3,2-d][1,3]dioxol-5-yl)methyl)-2H-pyrido [1,2-a]pyrimidin-2-one (3s):

Yellow solid, yield: 236 mg, 58%; mp 70↕72 °C (decom.); IR (KBr): 1655, 1593 cm-1; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.08 (s, 1H, CH), 2.76-2.81 (dd, 1H, *J* = 9.1 Hz, CH), 3.11-3.14 (dd, 1H, *J* = 14.3 Hz, CH), 3.98-3.99 (d, 1H, *J* = 2.6 Hz, CH), 4.52-4.58 (m, 2H, CH_2), 4.64-4.65 (d, 1H, *J* = 3.9 Hz, CH), 4.71-4.73 (d, 1H, *J* = 11.7 Hz, CH), 5.92 (d, 1H, *J* = 3.9 Hz, CH), 6.76-6.78 (t, 1H, *J* = 6.5 Hz, ArH), 7.30-7.32 (d, 2H, *J* = 9.1 Hz, ArH), 7.34 (s, 3H, ArH), 7.47-7.50 (t, 1H, *J* = 9.1 Hz, ArH), 7.53-7.55 (d, 1H, *J* = 7.8 Hz, ArH), 7.79 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 26.2, 26.6, 27.9, 72.1, 77.7, 82.5, 82.8, 104.7, 111.7, 112.8, 124.3, 126.9, 127.7 (2C), 128.0, 128.5 (2C), 132.1, 136.0, 135.0, 137.4, 151.1, 168.4; ESI Mass (m/z) 409 $[M+H]^+$; HRMS (EI m/z) Calcd for $C_{23}H_{25}N_2O_5$: 409.1738, found: 409.1758.

3-((6-(benzyloxy)-2,2-dimethyl-dihydro-5H-furo[3,2-d][1,3]dioxol-5-yl)methyl)-7-chloro-2H-pyrido[1,2-a] pyrimidin-2-one (3t):

Yellow solid, yield : 243 mg, 55%; mp 78↕80 °C (decom.); IR (KBr): 1656, 1601 cm-1; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.30 (s, 3H, CH_3), 1.43 (s, 3H, CH_3), 2.78-2.83 (dd, 1H, *J* = 9.7Hz, CH), 3.08-3.11 (dd, 1H, *J* = 14.1 Hz, CH), 3.96-3.97 (d, 1H, *J* = 3.23 Hz, CH), 4.50-4.54 (t, 2H, *J* = 6.4 Hz, CH_2), 4.64-4.65 (d, 1H, *J* = 3.23 Hz, CH), 4.71-4.74 (d, 1H, *J* = 12.1 Hz, CH), 5.91-5.92 (d, 1H, *J* = 4.0 Hz, CH), 7.24-7.26 (s, 1H, ArH), 7.35 (m, 5H, ArH), 7.41-7.43 (d, 1H, *J* = 9.7 Hz, ArH), 7.52 (s, 1H, ArH), 7.70 (s, 1H, ArH); ^{13}C NMR ($CDCl_3$, 75M Hz): δ 26.2, 26.6,

27.8, 29.7, 72.1, 77.5, 82.4, 82.7, 104.7, 111.7, 125.3 (2C), 127.3, 127.8 (2C), 128.6, 129.6 (2C), 135.5, 136.4, 137.3, 137.3, 149.5; ESI Mass (m/z) 443 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{23}H_{25}N_2O_5Cl$: 443.1368, found: 443.1363.

3-(hydroxy(thiophen-2-yl)methyl)-2H-pyrido[1,2-a]pyrimidin-2-one (3u):

White solid, Yield: 208 mg, 80%; mp: 161↕163 ^oC (decom.); IR (KBr): 3425, 1503 cm-1; ^1H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 2.85-2.93 (q, 1H, CH), 3.90-3.99 (t, 1H, *J* = 12.4 Hz, CH), 4.17-4.24 (dd, 1H, *J* = 6.4 & 13.9 Hz, CH), 5.37-5.40 (dd, 1H, *J* = 3.3 & 5.8 Hz, CH), 6.10-6.11 (d, 1H, *J* = 3.2 Hz, ArH), 6.59-6.63 (t, 1H, *J* = 6.4 Hz, ArH), 6.72-6.75 (d, 1H, *J* = 8.6 Hz, ArH), 6.88-6.91 (t, 1H, *J* = 4.9 Hz, ArH), 6.97-6.98 (d, 1H, *J* = 3.3 Hz, ArH), 7.24-7.25 (d, 1H, *J* = 4.9 Hz, ArH), 7.53-7.58 (t, 1H, *J* = 7.36 Hz, ArH), 7.75-7.77 (d, 1H, *J* = 6.4 Hz, ArH); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz): δ 28.9, 49.4, 112.0, 121.3, 128.4, 135.3, 137.1, 138.9, 140.4, 143.4, 156.8, 174.1, 183.3; ESI Mass (m/z) 261 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{13}H_{13}N_2O_2S$: 261. 0692, found: 261. 0697.

3-(3-phenoxybenzyl)-2H-pyrido[1,2-a]pyrimidin-2-one (3v):

White solid, Yield: 288 mg, 86%; mp 194↕196 ^oC (decom.); IR (KBr): 1653, 1589 cm-1; ^1H NMR ($CDCl_3$+DMSO-d_6, 300 MHz): δ 3.86 (s, 2H, CH_2), 6.84-6.86 (d, 2H, *J* = 6.7 Hz, ArH), 6.97-7.00 (d, 3H, *J* = 7.5 Hz, ArH), 7.06-7.11 (t, 2H, *J* = 7.3 Hz, ArH), 7.23-7.35 (m, 4H, ArH) 7.54-7.59 (t, 1H, *J* = 7.5 Hz, ArH), 7.83 (s, 1H, ArH), 7.94-7.96 (d, 1H, *J* = 6.6 Hz, ArH); ^{13}C NMR ($CDCl_3$+DMSO-d_6, 75 MHz) : δ 71.0, 109.5, 150.1, 153.8, 155.8, 156.2, 156.8, 159.9, 160.7, 161.5, 164.6, 167.2, 167.4, 171.4, 173.7, 174.0, 178.4, 188.2, 193.9, 194.1, 204.6; ESI Mass (m/z) 329 $(M+H)^+$; HRMS (EI m/z) Calcd for $C_{21}H_{17}N_2O_2$: 329.1284, found: 329.1283.

Notes and references

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