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Synthesis and biological studies of novel 2-(4-substitutedbenzyl-thio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4- chlorophenyl)pyrido--[2,3-*d*]-pyrimidin-4(3*H*)-one derivatives

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Abstract: A series of novel 2-(4-substitutedbenzylthio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-*d*]pyrimidin-4(3*H*)-one derivatives were synthesized and evaluated for their antibacterial and antifungal activity**.** All the derivatives were efficiently synthesized in four steps. The structure of the newly synthesized compounds was elucidated by their IR, ^{1}H-NMR, ^{13}C NMR, LCMS mass spectra and elemental analysis.

Keywords: 2-(4-substitutedbenzylthio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-d]-pyrimidin-4(3*H*)-one; antibacterial; antifungal.

1. Introduction

Developing new antimicrobial agents (antibacterial and antifungal) continues to attract attention and is an area of rigorous research. Although a large number of antibiotics and chemotherapeutics are available for medical use, the antimicrobial resistance created a substantial need of new class of antimicrobial agents in the last decades.[1–3] Pyrido[2,3-d]pyrimidines and its derivatives have distinct status as pharmaceutical agents. They have been found to have high therapeutic value as herbicide antidotes[4], diuretic[5], anti-inflammatory[6] and insecticidal agents[7], anticancer and antiviral[8-12], anticonvulsive[13], growth regulator[14], antileishmaniasis[15], and anticancer agents[16,17] Recently, based upon

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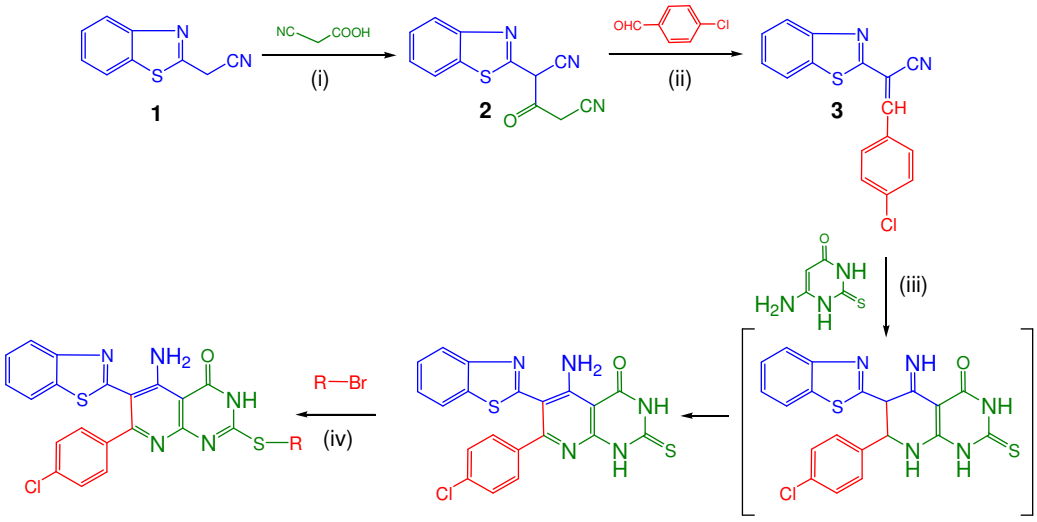
a diverse range of biological properties and the potential for folate antagonists[18,19] to elicit highly species-specific responses as antitumour[20,21], in addition pyrido[2,3-d]pyrimidines derivatives revealing promising antimicrobial activities.[22–27] In continued quest of new antimicrobial agents, we designed and synthesized novel pyrido[2,3-d]pyrimidines having benzothiazole group. Structures of the products were characterized by IR, ^{1}H-NMR, ^{13}C NMR, LCMS mass spectrometry, and elemental analysis. Results of biological activities indicate that some compounds possess potential antimicrobial activity.

2. Results and Discussion

Our synthesis was started from 2-(benzo[d]thiazol-2-yl)acetonitrile (**1**). The condensation of **1** with 2-cyanoacetic acid in Ac_2O gave compound **2**. Knoevenagel condensation of **2** with 4-chlorobenzaldehyde gave compound **3.** 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one was reacted with compound **3** in the air atmosphere gave pyrido[2,3-*d*]pyrimidin-4(1*H*)-one **4**. Compound 4 was alkylated with benzyl halides to give **5a-j**.



Compound	R	Compound	R
5a	CH_2	**5f**	Me_2N–CH_2
5b	Br–CH_2	**5g**	Cl–CH_2
5c	Et–CH_2	**5h**	Pr^i–CH_2
5d	Me–CH_2	**5i**	Bu^t–CH_2
5e	MeO–CH_2	**5j**	CH_2

Reagents and conditions: (*i*) Acetic anhydride, reflux 20 min; (ii) EtOH, piperidine, reflux 4 h; (iii) EtOH, piperidine, reflux 6 h; (iv) DMF, K_2CO_3, 1h.

Scheme 1. Synthetic pathway for compound **5 a-j.**

3. Pharmacological assay

3.1. Antibacterial assay

A standard inoculum (1-2 X 10^7 c.f.u/cm^3 0.5 McFarland standards) was introduced on to the surface of sterile agar plates, and a sterile glass spreader was used for even distribution of the inoculum. The discs measuring 6.25 mm in diameter were prepared from Whatman no.1 filter paper and sterilized by dry heat at 140 ^oC for 1 h. The sterile disc previously soaked in a known concentration of the test compounds were placed in nutrient agar medium. Solvent and growth controls were kept. The plates were inverted and incubated for 24 h at 37 ^oC. The inhibition zones were measured and compared with the controls. Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compound and controls were inoculated with approximately
5 X 10^5 c.f.u of actively dividing bacteria cells. The cultures were incubated for 24 h at 37 ^oC and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentrations (*MIC*). Ciprofloxacin was used as a standard drug. The diameter of the zone of inhibition and minimum inhibitory concentration values are given in **Table 1**.

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes* and *Klebsiella pneumoniae* (recultured) bacterial strains by disc diffusion method.[28,29] The investigation of antibacterial screening data revealed that all the tested compounds showed moderate to good bacterial inhibition. The compounds **5a, 5b, 5e, 5f, 5i** and **5j** showed very good activity against all the bacterial strains.

3.2. Antifungal assay

Sabouraud's agar media was prepared by dissolving 1 g peptone, 4 g D-glucose, and 2 g agar in 100 cm^3 distilled water, and adjusting pH to 5.7 using buffer. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loop full of particular fungal strains was transferred to 3 cm^3 saline to get a suspension of corresponding species. 20 cm^3 of agar media was poured in to each Petri dish. Excess of suspension was decanted and the plates were dried by placing in a incubator at 37 ^oC for 1 h. Using an agar punch, wells were made and each well was labeled. A control was also prepared in triplicate and maintained at 37 ^oC for 3-4 d. The inhibition zones in diameter were measured and compared with the controls. The Nutrient Broth, which contained logarithmic serially two fold diluted amount of test compound and controls was inoculated with approximately 1.6 X 10^4-6 X 10^4 c.f.u cm^{-3}. The cultures were incubated for 48 h at 35 ^oC and the growth was monitored. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as minimum inhibitory concentrations (*MIC*). Amphotericin B was used as the standard drug. The diameter of zone of inhibition and minimum inhibitory concentration values are given in **Table 2**.

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Penicillium marneffei* and *Trichophyton mentagrophytes* (recultured) in DMSO by serial plate dilution method[30,31]. The antifungal screening data showed moderate to good activity. Compounds **5a, 5c, 5f, 5i** and **5j** emerged as very good active against all the fungal strains.

Table 1: Antibacterial activity of thiazolotriazinoes (**5a-j**).

Compound no	*Staphylococcus aureus*	*Eschrichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Streptococcus pyogenes*
5a	20 (6.25)	25 (6.25)	29 (6.25)	18 (6.25)	23 (6.25)
5b	23 (6.25)	28 (6.25)	30 (6.25)	18 (6.25)	21 (6.25)
5c	10 (12.5)	--	--	17 (6.25)	11 (6.25)
5d	8 (25)	23 (6.25)	9 (25)	--	8 (12.5)
5e	22 (6.25)	27 (6.25)	32 (6.25)	20 (6.25)	24 (6.25)
5f	21 (6.25)	29 (6.25)	32 (6.25)	20 (6.25)	23 (6.25)
5g	10 (12.5)	15 (25)	--	--	17 (12.5)
5h	12 (12.5)	--	21 (6.25)	--	8 (25)
5i	21 (6.25)	24 (6.25)	29 (6.25)	19 (6.25)	23 (6.25)
5j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard[a]	24 (6.25)	30 (6.25)	33 (6.25)	23 (6.25)	25 (6.25)

-- Indicates bacteria is resistant to the compounds at > 100 µg/ml, MIC values are given in brackets. MIC (µg/ml) = minimum inhibitory concentration, ie. Lowest concentration to completely inhibit bacterial growth. Zone of inhibition in mm.

[a] Ciprofloxacin was used as standard.

Table 2: Antifungal activity of thiazolotriazinoes (**5a-j**).

Compound no	*Aspergillus fumigatus*	*Aspergillus flavus*	*Trichophyton mentagrophytes*	*Penicillium marneffei*	*Candida albicans*
5a	22 (6.25)	22 (6.25)	25 (6.25)	22 (6.25)	20 (6.25)
5b	8 (25)	--	12 (12.5)	--	17 (6.25)
5c	22 (6.25)	20 (6.25)	22 (6.25)	25 (6.25)	17 (6.25)
5d	15 (6.25)	--	7 (25)	21 (6.25)	18 (6.25)
5e	5 (25)	18 (6.25)	--	12 (12.5)	17 (6.25)
5f	24 (6.25)	21 (6.25)	21 (6.25)	23 (6.25)	18 (6.25)
5g	11 (12.5)	12 (25)	--	--	14 (12.5)
5h	9 (25)	--	12 (12.5)	9 (25)	10 (12.5)
5i	22 (6.25)	19 (6.25)	20 (6.25)	23 (6.25)	19 (6.25)
5j	21 (6.25)	26 (6.25)	32 (6.25)	21 (6.25)	24 (6.25)
Standard[b]	25 (6.25)	21 (6.25)	23 (6.25)	25 (6.25)	19 (6.25)

-- Indicates fungus is resistant to the compounds at >100 mg/ml, MIC values are given in brackets. MIC (mg/ml) = minimum inhibitory concentration, ie. Lowest concentration to completely inhibit fungal growth. Zone of Inhibition in mm.

[b] Amphotericin was used as standard.

4. Conclusion

The investigation of antibacterial screening data reveals that among the 10 compounds screened, five compounds showed good bacterial and six compounds showed fungal inhibition almost equivalent to that of standard.

5. Experimental

Novel 2-(4-substitutedbenzyl-thio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4- chlorophenyl)pyrido- [2,3-*d*]-pyrimidin-4(3*H*)-one derivatives

All reagents and solvents were purchased and used without further purification. Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Crude products were purified by column chromatography on silica gel of 60–120 mesh. IR spectra were obtained on a Perkin Elmer BX serried FT-IR 5000 spectrometer using KBr pellet. NMR spectra were recorded on a Varian 300 MHz spectrometer for ^{1}H NMR. The ^{13}C NMR spectra were recorded on JEOL. The chemical shifts were reported as ppm down field using TMS as an internal standard. Mass spectra were recorded on a MASPEC low resolution mass spectrometer operating at 70 eV.

5.1. 2-(Benzo[d]thiazol-2-yl)-3-oxopentanedinitrile (2)

To a solution of cyanoacetic acid (0.01 mol) and acetic anhydride (15 mL) that was heated on water bath for 5 min, 2-(benzo[*d*]thiazol-2-yl)acetonitrile (**1**) (0.01 mol) was added and the reaction mixture was refluxed for 20 min at 85-95 ^{o}C. After the mixture was cooled the formed solid was filtered off, dried and recrystallized from ethanol to afford 1.69 g (70%) of **2**; mp 275 ^{o}C; brown crystals; IR (KBr) (υ, cm^{-1}): 2185, 2199 (2CN), 1700 (CO). ^{1}H NMR (DMSO-*d*6): δ, 4.52 (s, 2H, CH_2), 5.10 (s, 1H, CH), 6.67-7.50 (m, 4H, Ar-H). LCMS: (m/z, %): 241 $(M)^{+}$. *Anal*. Calcd. for $C_{12}H_7N_3OS$: C, 59.74; H, 2.92; N, 17.42. Found: C, 59.92; H, 2.97; N, 17.53.

5.2. 2-(Benzo[d]thiazol-2-yl)-3-(4-chlorophenyl)-acrylonitrile (3):

To a solution of **2** (0.01 mol) and *p*-chlorobenzaldehyde (0.01 mol) in ethanol (20 mL), was added a few drops of piperidine and the reaction mixture was refluxed for 4 h, then left to cool (Scheme 1). The precipitate that formed was filtered off, washed with ethanol and purified by recrystallized from ethanol to afford 2.11 g (71%) of **3**; mp > 300 ^{o}C; yellow powder; IR (KBr) (υ, cm^{-1}): 3119 (NH), 2188 (CN). ^{1}H NMR (DMSO-*d*6): δ, 7.32-8.12 (m, arom. + vinylic H). LCMS: (m/z, %): 298 $(M+1)^{+}$. *Anal*. Calcd. for $C_{16}H_9ClN_2S$: C, 64.75; H, 3.06; N, 9.44. Found: C, 64.84; H, 3.17; N, 9.51.

5.3. 5-Amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)-2-thioxo-2,3-dihydropyrido-[2,3-d]pyrimidin-4(1H)-one (4):

To a mixture of **3** (0.01 mol), 6-aminothiouracil (0.01 mol) in ethanol (15 mL), a catalytic amount of piperidine was added. The reaction mixture was refluxed for 6 h, allowed to cool and poured into ice cold water. The precipitated solid obtained was filtered off, dried and recrystallized from ethanol to furnish 2.72 g (62%) of 4; mp 230 ^{o}C; pale yellow powder; IR (KBr) (υ, cm^{-1}): 3402, 3389 (NH_2), 3141 (NH), 1698 (CO), 1221 (C=S). ^{1}H NMR (DMSO-*d*6): δ, 6.22 (s, 2H, NH_2), 7.32-8.49 (m, 8H, Ar-H), 13.51 (s, 1H, NH), 13.59 (s, 1H, NH). LCMS: (m/z, %): 438 $(M)^{+}$. *Anal*. Calcd. for $C_{20}H_{12}ClN_5OS_2$: C, 54.84; H, 2.76; N, 15.99. Found: C, 54.94; H, 2.82; N, 16.04.

5.4. General Procedure of 2-(4-substituted benzylthio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-*d*]-pyrimidin-4(3*H*)-ones (5a-j):

An ice cold solution of cyclic compound of 5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)-2-thioxo-2,3-dihydropyrido-[2,3-d]pyrimidin-4(*1H*)-one **(4)** (1 mol) in DMF (4 vol), potassium carbonate (1.5 mol) and substituted benzyl halides (1.3 mol) was taken in a 1 liter round bottomed flask equipped with magnetic stirrer and stirred for 1 hour. The residual portion was poured on to crushed ice, neutralized with dilute acid and the product obtained 2-(4-substituted benzylthio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-*d*]-pyrimidin-4(3*H*)-one derivatives **(5a-j)** was collected by filtration.

5-Amino-6-(benzo[d]thiazol-2-yl)-2-(benzylthio)-7-(4-chlorophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one (5a): Pale yellow solid; Yield 69%; m.p. 196-198 ^{o}C; IR (KBr): υ 3402-3389 (NH_2), 3141 (NH), 1698 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.10 (s, 2H, CH_2), 6.23 (s, 2H, NH_2), 7.32-8.49 (m, 13H, Ar-H), 13.58 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 168.23, 164.52, 161.72, 158.36, 157.44, 155.86, 154.21, 141.29, 138.67, 137.12, 136.84, 133.46, 133.10, 132.53, 131.64, 130.92, 128.29, 127.83, 124.52, 124.19, 120.89, 110.92, 40.10. LCMS: (*m*/*z*) 528 (M+1).$^{+}$ *Anal*. Calcd. for $C_{27}H_{18}ClN_5OS_2$: C, 61.41; H, 3.44; N, 13.26. Found: C, 61.55; H, 3.53; N, 13.39.

2-(4-Bromobenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one *(5b):* Yellow solid; Yield 63%; m.p. 214-216 ^{o}C; IR (KBr): υ 3410-3383 (NH_2), 3155 (NH), 1680 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 4.15 (s, 2H, CH_2), 6.25 (s, 2H, NH_2), 7.10-8.49 (m, 12H, Ar-H), 13.55 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 168.45, 164.59, 161.70, 158.43, 157.48, 155.81, 153.97, 140.87, 138.77, 137.25, 136.93, 135.41, 134.13, 133.55, 133.12, 128.32, 127.88, 125.24, 124.50, 124.23, 121.10, 110.98, 40.57. LCMS (*m*/*z*) 608 (M+2)$^{+}$. *Anal*. Calcd. for $C_{27}H_{17}BrClN_5OS_2$: C, 53.43; H, 2.82; N, 11.54. Found: C, 54.14; H, 2.95; N, 11.65.

2-(4-Ethylbenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one (5c): Yellow solid; Yield 75%; m.p. 175-176 ^{o}C; IR (KBr): υ 3410-3370 (NH_2), 3155 (NH), 1698 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.4 (t, 3H, CH_3, J = 6.2), 2.35 (q, 2H, CH_2 J = 7.4), 4.15 (s, 2H, SCH_2), 6.30 (s, 2H, NH_2), 7.20-8.49 (m, 12H, Ar-H), 13.58 (s, 1H, NH); LCMS (*m*/*z*) 556 (M+1)$^{+}$. *Anal*. Calcd. for $C_{29}H_{22}ClN_5OS_2$: C, 62.64; H, 3.99; N, 12.59. Found: C, 63.12; H, 4.21; N, 12.73.

2-(4-Methylbenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one (5d): Pale yellow solid; Yield 65%; m.p. 224-225 ^{o}C; IR (KBr): υ 3410-3365 (NH_2), 3165 (NH), 1710 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.15 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 6.30 (s, 2H, NH_2), 7.10-8.45 (m, 12H, Ar-H), 13.51 (s, 1H, NH); LCMS (*m*/*z*) 542 (M+1)$^{+}$. *Anal*. Calcd. for $C_{28}H_{20}ClN_5OS_2$: C, 62.04; H, 3.72; N, 12.92. Found: C, 62.43; H, 3.96; N, 13.04.

2-(4-Methoxybenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-lorophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one *(*5e): Yellow solid; Yield 76%; m.p. 171-173 ^{o}C; IR (KBr): υ 3400-3380 (NH_2), 3165 (NH), 1710 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.06 (s, 3H, OCH_3), 4.21 (s, 2H, CH_2), 6.30 (s, 2H, NH_2), 7.20-8.45 (m, 12H, Ar-H), 13.51 (s, 1H, NH); LCMS (*m*/*z*) 558 (M+1)$^{+}$. *Anal*. Calcd. for $C_{28}H_{20}ClN_5O_2S_2$: C, 60.26; H, 3.61; N, 12.55. Found: C, 60.62; H, 3.88; N, 12.83.

2-(4-(Dimethylamino)benzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl) -pyrido[2,3-d]-pyrimidin-4(3H)-one (5f): Yellow solid; Yield 82%; m.p. 210-212 ^{o}C; IR (KBr): υ 3400-3365 (NH_2), 3160 (NH), 1710 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.02 (s, 6H, $N(CH_3)_2$), 4.20 (s, 2H, CH_2), 6.25 (s, 2H, NH_2), 7.10-8.49 (m, 12H, Ar-H), 13.51 (s, 1H, NH); LCMS (*m*/*z*) 571 (M+1)$^{+}$. *Anal*. Calcd. for $C_{29}H_{23}ClN_6OS_2$: C, 60.99; H, 4.06; N, 14.71. Found: C, 61.49; H, 4..61; N, 14.93.

2-(4-Chlorobenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido[2,3-d]-pyrimidin-4(3H)-one (5g): Yellow solid; Yield 80%; m.p. 252-254 ^{o}C; IR (KBr): υ 3410-3345 (NH_2), 3160 (NH), 1720 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): 4.20 (s, 2H, CH_2), 6.30 (s, 2H, NH_2), 7.0-8.50 (m, 12H, Ar-H), 13.51 (s, 1H, NH); LCMS (*m*/*z*) 562 (M+1)$^{+}$. *Anal*. Calcd. for $C_{27}H_{17}Cl_2N_5OS_2$: C, 57.65; H, 3.05; N, 12.45. Found: C, 57.94; H, 3.54; N, 12.82.

2-(4-Isopropylbenzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido

Novel 2-(4-substitutedbenzyl-thio)-5-amino-6-(benzo[*d*]thiazol-2-yl)-7-(4- chlorophenyl)pyrido- [2,3-*d*]-pyrimidin-4(3*H*)-one derivatives

-[2,3-d]-pyrimidin-4(3H)-one (5h): Yellow solid; Yield 78%; m.p. 185-187 ^{o}C; IR (KBr): υ 3410-3330 (NH_2), 3190 (NH), 1720 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20 (d, 6H, $(CH_3)_2$), 2.70-2.87 (m, 1H, CH), 4.10 (s, 2H, CH_2), 6.20 (s, 2H, NH_2), 7.20-8.50 (m, 12H, Ar-H), 13.58 (s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 168.17, 164.48, 161.69, 158.27, 157.40, 155.81, 154.18, 148.32, 139.34, 138.62, 137.08, 136.79, 133.50, 132.96, 132.12, 130.71, 128.32, 127.81, 124.47, 124.17, 120.88, 110.88, 40.12, 38.74, 28.63. LCMS (*m/z*) 570 $(M+1)^{+}$. *Anal*. Calcd. for $C_{30}H_{24}ClN_5OS_2$: C, 63.20; H, 4.24; N, 12.28. Found: C, 63.69; H, 4.63; N, 12.55.

2-(4-Tert-butyl-benzylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido [2,3-d]-pyrimidin-4(3H)-one (5i): Pale yellow solid; Yield 71%; m.p. 202-204 ^{o}C; IR (KBr): υ 3410-3320 (NH_2), 3190 (NH), 1720 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.35 (s, 9H, $(CH_3)_3$), 4.10 (s, 2H, CH_2), 6.25 (s, 2H, NH_2), 7.20-8.50 (m, 12H, Ar-H), 13.51 (s, 1H, NH); LCMS (*m/z*) 584 $(M+1)^{+}$. *Anal*. Calcd. for $C_{31}H_{26}ClN_5OS_2$: C, 63.74; H, 4.49; N, 11.99. Found: C, 64.06; H, 4.87; N, 12.21.

2-((1,2,3,4-Tetrahydronaphthalen-5-yl)methylthio)-5-amino-6-(benzo[d]thiazol-2-yl)-7-(4-chlorophenyl)pyrido-[2,3-d]-pyrimidin-4(3H)-one (5j): Pale yellow solid; Yield 69%; m.p. 221-223 ^{o}C; IR (KBr): υ 3400-3310 (NH_2), 3155 (NH), 1710 (CO) cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.45-2.65 (m, 7H), 4.10 (s, 2H, CH_2), 6.20 (s, 2H, NH_2), 7.20-8.50 (m, 11H, Ar-H), 13.51 (s, 1H, NH); LCMS (*m/z*) 582 $(M+1)^{+}$. *Anal*. Calcd. for $C_{31}H_{24}ClN_5OS_2$: C, 63.96; H, 4.16; N, 12.09. Found: C, 64.23; H, 4.42; N, 12.38.

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