A Convenient Synthesis and Pharmacological Activity of Novel Annelated Pyrimidine Derivatives

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Simple and convenient synthesis for a series of 2,3-diglycosylpyrimidine **4**, pyrazolo[3,4-d]pyrimidine **8**, ditetrazolo[1,5-a;1′,5′-c]pyrimidine **9**, 2,9a,10-triazaanthracene **12**, thieno[2,3-d]pyrimidine **14**, 1,3,5,7-tetraazafluorene-8-one **15**, 1,3,5-triazafluorene-8-one **16**, 1,3-diazafluorene **21a,b** derivatives have been synthesized *via* a sequence of heterocyclization reactions of suitably functionalized 6-[5-(4-bromophenyl)oxazol-4-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**2**) with different electrophiles and nucleophiles. The new compounds were prepared with the objective to study their pharmacological properties.

Keywords: 2-Methylsulfanylpyrimidine; Pyrazolo[3,4-d]pyrimidine; Thieno[2,3-d]pyrimidine; Azafluorenone.

INTRODUCTION

Pyrimidines and fused pyrimidines, being an integral part of DNA and RNA, play an essential role in several biological processes and have considerable chemical and pharmacological importance. Particularly, the pyrimidine ring can be found in the nucleoside antibiotics, antibacterial, antitumor, cardiovascular as well as agrochemical and veterinary products.[1-9] In view of these observations and in continuation of our interest in developing efficient syntheses of polyfunctionally substituted heterocycles utilizing the readily obtainable pyrimidine as starting material,[10-12] it is worthwhile to explore their potential utility for synthesis of polyfunctionally substituted pyrimidine derivatives useful for optimization of biological activity.

RESULTS AND DISCUSSION

In the present work, 6-[5-(4-bromophenyl)oxazol-4-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**2**), required as a starting material, was conveniently prepared from the reaction of 5-(4-bromophenyl)oxazole-4-carbaldehyde (**1**)[13] with ethyl cyanoacetate and thiourea.[14,15] Reaction of **2** with 2,3,4,6-tetra-*O*-acetyl-α-*D*-glucopyranosyl bromide (**3**)[16] in the presence of aqueous potassium hydroxide gave 6-[5-(4-bromophenyl)oxazol-4-yl]-4-oxo-2-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosylsulfanyl)-3-(2,3,4,6-tetra-*O*-acetyl-β-*D*-glucopyranosyl)-3,4-dihydropyrimidine-5-carbonitrile (**4**). The structure of compound **4** was confirmed on the bases of its elemental analysis and spectral data. Its ir spectrum showed absence of NH group and presence of absorption band at 1685 cm^{-1} due to the carbonyl of pyrimidinone in addition to the acetoxy carbonyl groups at 1765-1750 cm^{-1}. Also, the ^1H NMR spectrum showed the presence of eight OAc., and a doublet at δ 5.60 with spin-spin coupling constant 10.55 Hz which corresponds to diaxial orientation of the H-1′ and H-2′ protons, indicating the presence of only the β-configuration.[17]

We then invested **2** in synthesis of S-alkylsulfanylpyrimidine derivatives which have been recently identified as highly specific reverse transcriptase inhibitors of human immunodeficiency virus.[18] Thus, the reaction of **2** with methyl iodide in the presence of ethanolic sodium ethoxide solution afforded 4-[5-(4-bromophenyl)oxazol-4-yl]-6-hydroxy-2-methylsulfanylpyrimidine-5-carbonitrile (**5**) in good yield.

The electron deficient nature of the pyrimidine ring and the high reactivity of the methylthio group towards nucleophilic reagents facilitate the synthesis of a large number of condensed pyrimidine *via* nucleophilic aromatic substitution.[19,20] Thus, reaction of **5** with $POCl_3/PCl_5$ on a water bath yields 4-[5-(4-bromophenyl)oxazol-4-yl]-6-chloro-2-methylsulfanylpyrimidine-5-carbonitrile (**6**) which was used as a precursor employed for synthesis of annelated pyrimidine of

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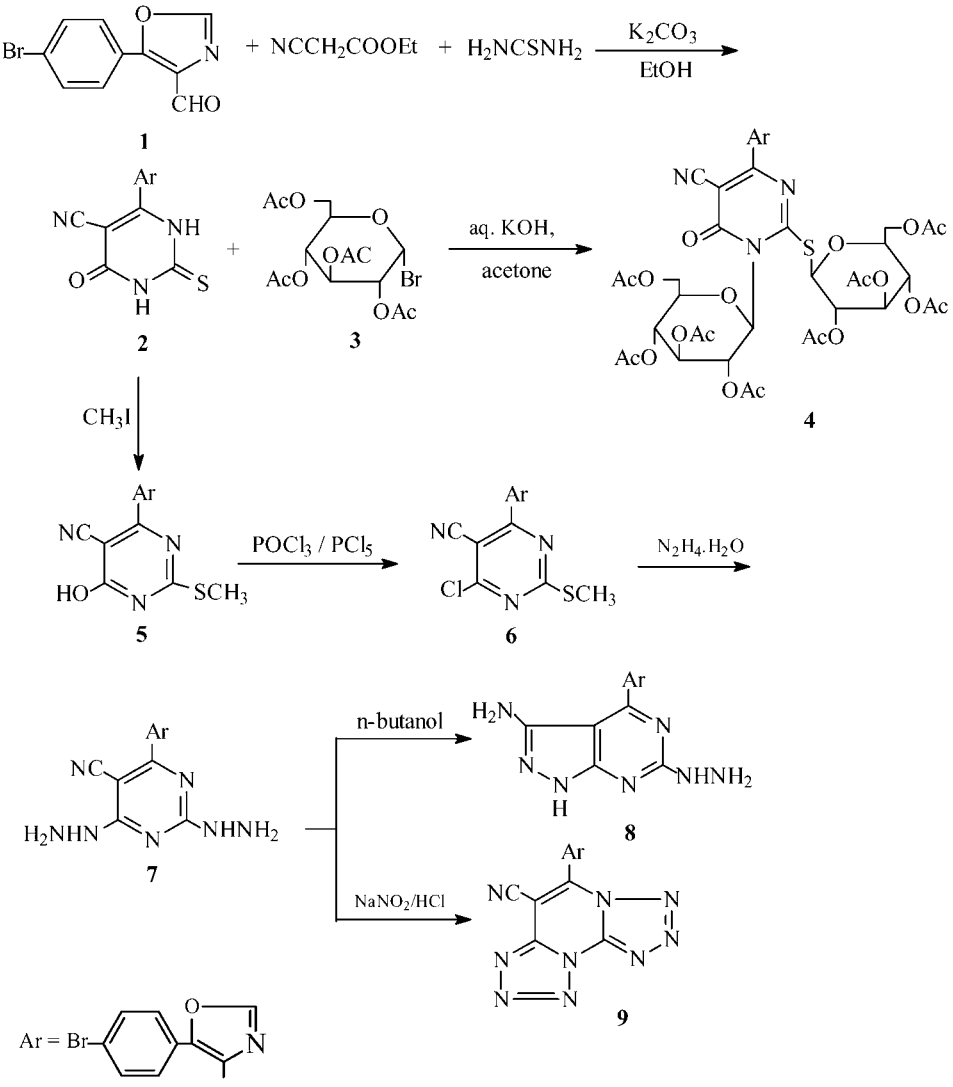
medicinal application. Hence, treatment of **6** with hydrazine hydrate in refluxing 1-butanol for 2 hrs afforded 4-[5-(4-bromophenyl)oxazol-4-yl]-2,6-dihydrazinopyrimidine-5-carbonitrile (**7**) which was further cyclized to pyrazolopyrimidine **8** by refluxing in 1-butanol for 5 hrs. The formation of **8** proceeds *via* nucleophilic attack of the hydrazino moiety to the cyano group in *ortho* position leading to fused pyrimidine **8**. The ir spectrum of **8** displays lack of absorption of the cyano group and presence of different bands in the region between 3340-3195 cm^{-1} (NH_2 and $NHNH_2$). Moreover, the presence of latter groups was confirmed by the ^1H NMR spectrum which consisted of a series of D_2O exchangeable signals that present different chemical shifts. Also, when an acidic solution of **7** was allowed to react with an aqueous solution of sodium nitrite at 0-5 °C ditetrazolo[1,5-a;1′,5′-c]pyrimidine **9** was obtained (Scheme I).

Treatment of **6** with thiourea[21] in ethanolic solution produced 4-[5-(4-(bromophenyl)oxazol-4-yl]-6-mercapto-2-methylsulfanylpyrimidine-5-carbonitrile (**10**). Treatment of **10** with anthranilic acid in 1-butanol afforded pyrimidine derivative **11** which cyclized to 3-[5-(4-bromophenyl)oxazol-4-yl]-1-methylsulfanyl-9-oxo-9H-2,9a,10-triazaanthracene-4-carbonitrile (**12**) by heating in acetic anhydride. Also, the alkylation of **10** with ethyl chloroacetate in ethanolic solution containing sodium acetate afforded S-alkylated derivative **13** which cyclized to thienopyrimidine derivative **14** in ethanol containing sodium ethoxide. Of particular interest, a cyclocondensation reactions of thienopyrimidine **14** with

Scheme I



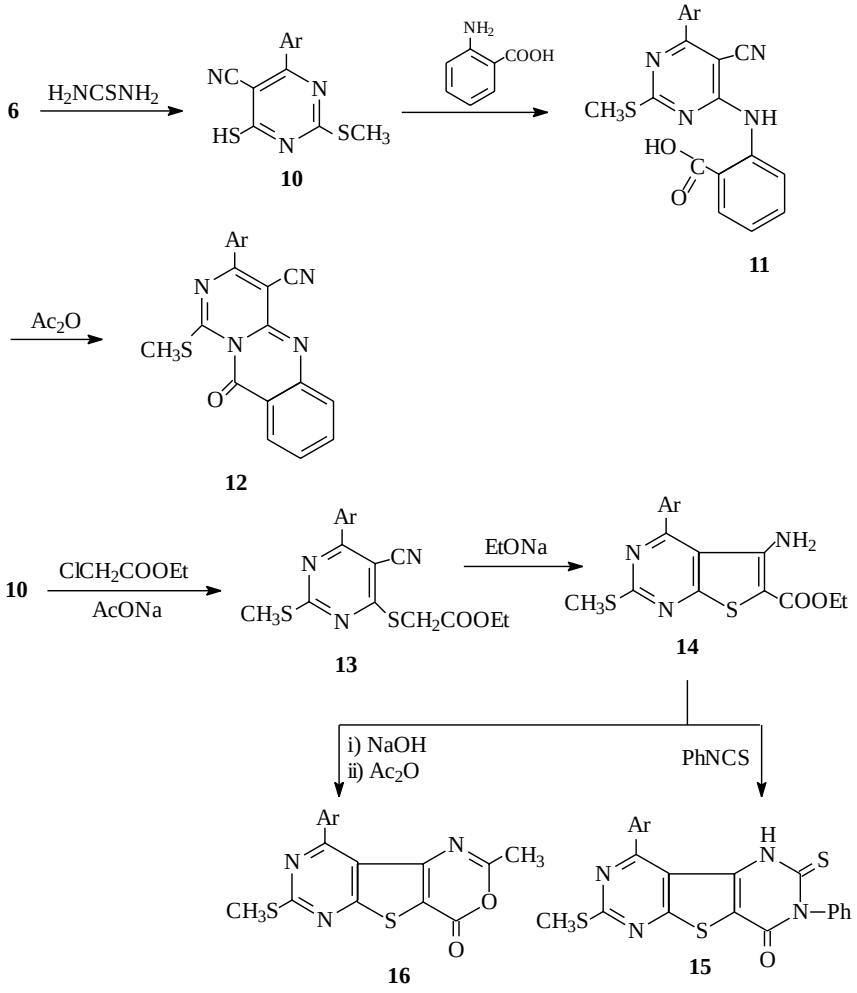
phenyl isothiocyanate or by heating in acetic anhydride resulted in the formation of tricyclic heterocycles **15** and **16**, respectively (Scheme II).

Saponification of the o-aminoester **14** resulted in the formation of the corresponding carboxylic acid **17** which upon treatment with orthophosphoric acid at room temperature underwent decarboxylation[22] to give amino compound **18**. On the other hand, treating of **17** with orthophosphoric acid at 100 °C, the product was identified as thienopyrimidine **19** which was also prepared *via* treatment of **18** with orthophosphoric acid at 100 °C. The condensation of **19** with aromatic aldehydes (*viz*. benzaldehyde and *p*-anisaldehyde) in refluxing ethanol containing catalytic amounts of piperidine furnished the chalcones **20a** and **20b**, while the reaction of **19** with arylmethylene malononitrile provided 6-amino-4-[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanyl-8-(phenyl/4-methoxyphenyl)-8H-5-oxa-9-thia-1,3-diazafluorene-7-carbonitrile (**21a** and **21b**) (Scheme III). The structure of the synthesized compounds was assigned on the basis of elemental analysis and spectral data (cf. experimental).

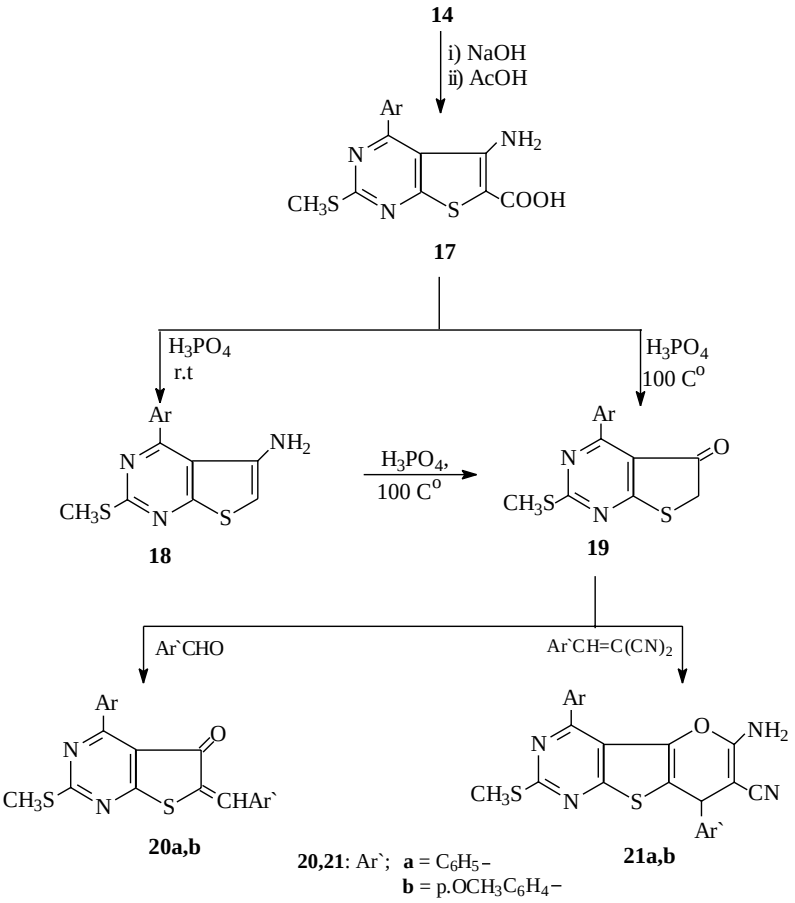
ANTIMICROBIAL ACTIVITY

The antimicrobial activities of some synthesized compounds were determined in *vitro* using hole plate and filter paper disc methods.[23] A variety species of Gram positive and Gram negative bacteria in addition to some fungal plant pathogens were used. Also, a comparison between the activity of our synthesized compounds and sulphadiazine as standard drug was discussed. The tested compounds were dissolved in 10% acetone (v/v); different concentrations have been chosen (125, 250, 500 μg/mL). A qualitative screen was performed on all compounds while quantitative assays were

Scheme II



Scheme III



done on active compounds only. The results are summarized in Table 1.

The data indicated that most of the tested compounds **4**, **5**, **10**, **15**, **16**, **21b** were highly active towards the selected

Table 1. The antimicrobial activity of the tested compounds

Compd. No.	*Bacillus subtilis*		*Bacillus cereus*		*Escherichia coli*		*Aspergillus niger*	
	A	MIC	A	MIC	A	MIC	A	MIC
4	+++	500	+	250	++	125	++	500
5	++	250	+++	250	++	500	+	250
9	+	500	+	250	+	250	+	500
10	+++	250	++	250	++	125	++	125
12	+	250	+	125	+	125	+	250
14	++	250	+	125	+	250	+	250
15	+++	500	++	250	++	125	+	250
16	+++	250	+++	125	++	250	+	500
18	++	125	+	250	+	125	+	250
20a	++	125	+	250	++	500	+	250
21b	++	250	++	250	+++	250	+	500

A = antimicrobial activity of tested compounds.
MIC = minimum inhibitory concentration.
+ > 5 mm slightly active.
++ > 7 mm moderately active.
+++ > 9 mm highly active.

pathogens, while the compounds **9**, **12**, **14**, **18**, **20a** are moderately active towards the different strains of bacteria and fungi as compared with standards.

In summary, we have demonstrated the ability of pyrimidine derivative **2** to undergo annelation reactions under rather mild conditions providing an efficient synthetic method for the preparation of various pyrimidine derivatives which enhanced antibacterial and antifungal activity.

EXPERIMENTAL

Melting points are uncorrected. IR spectra in KBr were recorded on a Perkin Elmer 298 spectrophotometer. ^{1}H NMR spectra were obtained on a Varian Gemini 200 MHz instrument using TMS as internal reference (Chemical shifts are expressed as δ, ppm). Mass spectra were recorded on a Shimidzu GCMS-QP 1000 EX instrument (70 ev EI mode). All reactions were monitored by thin layer chromatography, carried out on 0.2 mm silica gel 60 F254 (Merck) plates.

6-[5-(4-bromophenyl)oxazol-4-yl]-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (2)

A mixture of 5-(4-bromophenyl)oxazole-4-carbaldehyde (**1**)[13] (0.01 mol), ethyl cyanoacetate (0.01 mol), thiourea (0.01 mol), and potassium carbonate (0.01 mol) in absolute ethanol (40 mL) was refluxed for 24 h. The precipitate which formed after cooling and acidification was filtered off and crystallized from DMF-water mixture to give **2**.[14,15] Yield, 52%, mp. 236-8 °C, IR: ν = 3200-3190 (NH), 2221 (C≡N), 1679 (CO), 1610 (C=N), 1265 cm^{-1} (CS); ^{1}H NMR (DMSO): δ = 7.10-8.02 (m, 5H, ArH and oxazole H-2) and 11.7 (br s, 2H, 2NH exchangeable); MS: *m/z* (%) 375 (M^+, 86.4), 376 (M^++1, 1.08); Anal. Calcd. for $C_{14}H_7BrN_4O_2S$: C, 44.82; H, 1.88; N, 14.93%. Found: C, 44.70; H, 1.73; N, 14.81%.

6-[5-(4-Bromophenyl)oxazol-4-yl]-4-oxo-2-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylsulfanyl)-3-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-3,4-dihydropyrimidine-5-carbonitrile (4)

To a solution of **2** (0.001 mol) in aqueous potassium hydroxide (0.002 mol) in distilled water (10 mL) was added a solution of **3** (0.0021 mol) in acetone (40 mL). The reaction mixture was stirred for 4 h at room temperature until the starting material was consumed (TLC). The mixture was evaporated under reduced pressure and the residue was washed with distilled water to remove the potassium bromide formed. The solid product was dried and crystallized from ethanol to give **4**. Yield, 58%, mp. 182-4 °C, IR: ν = 2226 (C≡N), 1765-1750 ($COCH_3$), 1685 (CO), 1618 (C=N), 1559 cm^{-1} (C=C); ^{1}H NMR (DMSO): δ = 1.77, 1.98, 2.06, 2.11, 2.15, 2.19, 2.21, 2.30 (8s, 24H, 8CH_3CO), 3.70-4.28 (m, 6H, H-5′, H-5″, 2 × H-6′, 2 × H-6″), 4.89-5.32 (m, 2H, H-4′, H-4″), 5.40-5.48 (m, 4H, H-3′, H-3″, H-2″, H-1″), 5.59 (t, 1H, *J* = 9.1 Hz, H-2′), 5.60 (d, 1H, *J* = 10.55 Hz, H-1′), 7.11-7.98 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{42}H_{43}BrN_4O_{20}S$: C, 48.70; H, 4.18; N, 5.41%. Found: C, 48.83; H, 4.30; N, 5.30%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-6-hydroxy-2-methylsulfanylpyrimidine-5-carbonitrile (5)

A solution of **2** (0.01 mol) and methyl iodide (0.01 mol) in ethanolic sodium ethoxide (prepared by dissolving 1.0 gm of sodium metal in 50 mL of ethanol). The reaction mixture was cooled and poured onto ice-cold water. The solid product obtained after acidification with hydrochloric acid was filtered off, washed with water and crystallized from ethanol to give **5**. Yield, 85%, mp. 201-3 °C, IR: ν = 3480 (OH), 2218 (C≡N), 1615 (C=N), 1601 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ = 2.53 (s, 3H, CH_3), 7.1-8.10 (m, 5H, ArH and oxazole H-2) and 10.61 (s, 1H, OH, exchangeable); MS: *m/z* (%) 389 (M^+, 71.2), 390 (M^++1, 2.01); Anal. Calcd. for $C_{15}H_9BrN_4O_2S$: C, 46.29; H, 2.33; N, 14.39%. Found: C, 46.40; H, 2.46; N, 14.51%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-6-chloro-2-methylsulfanylpyrimidine-5-carbonitrile (6)

A mixture of **5** (0.0095 mol), $POCl_3$ (0.29 mol) and PCl_5 (0.015 mol) was refluxed on a water bath for 4 h. The reaction mixture was poured gradually on crushed ice and the solid that separated was filtered off and crystallized from benzene to give **6**. Yield, 70%, mp. 195-7 °C, IR: ν = 2223 (C≡N), 1620 (C=N), 1603 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ = 2.56 (s, 3H, CH_3), 7.13-8.12 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{15}H_8BrClN_4OS$: C, 44.19; H, 1.98; N, 13.74%. Found: C, 44.30; H, 1.85; N, 13.86%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-2,6-dihydrazinopyrimidine-5-carbonitrile (7)

A mixture of chloropyrimidine **6** (0.06 mol) and hydrazine hydrate (2.3 mL) in 1-butanol was refluxed for 2 h. The resulting solid was collected by filtration and crystallized from 1-butanol to give **7**. Yield, 78%, mp. 211-3 °C, IR: ν = 3330-3180 ($NHNH_2$), 2219 (C≡N), 1625 (C=N), 1601 cm^{-1} (C=C); ^{1}H NMR ($CDCl_3$): δ = 5.91, 5.96 (br s, 4H, 2NH_2), 7.03-8.11 (m, 5H, ArH and oxazole H-2) and 9.11, 9.13 (2br s, 2H, 2NH, exchangeable); Anal. Calcd. for

$C_{14}H_{11}BrN_8O$: C, 43.43; H, 2.86; N, 28.94%. Found: C, 43.55; H, 2.74; N, 28.82%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-6-hydrazino-1H-pyrazolo[3,4-d]pyrimidin-3-ylamine (8)

A solution of dihydrazinopyrimidine **7** (0.0014 mol) in 1-butanol (20 mL) was refluxed for 5 h. The solvent was removed at reduced pressure and the residue crystallized from 1-butanol to give **8**. Yield, 88%, mp. 283-5 °C, IR: ν = 3340-3195 (NH_2 and $NHNH_2$), 1612 (C=N), 1605 cm^{-1} (C=C); ^1H NMR (DMSO): δ = 5.68 (br s, 2H, NH_2, exchangeable), 6.38 (br s, 2H, NH_2, exchangeable), 6.99-8.12 (m, 5H, ArH and oxazole H-2) and 9.12, 9.35 (2s, 2H, 2NH, exchangeable); Anal. Calcd. for $C_{14}H_{11}BrN_8O$: C, 43.43; H, 2.86; N, 28.94%. Found: C, 43.55; H, 2.74; N, 28.83%.

5-[5-(4-Bromophenyl)oxazol-4-yl]ditetrazolo[1,5-a;1′,5′-c]-pyrimidine-6-carbonitrile (9)

A solution of sodium nitrite (0.0021 mol) in water (10 mL) was added to ice cooled water and a stirred solution of compound **7** (0.001 mol) in 20% aqueous hydrochloric acid (10 mL). The mixture was allowed to react for 2 h at the same temperature. Then the formed precipitate was collected by filtration and crystallized from methanol to furnish **9**. Yield, 69%, mp. 176-8 °C, IR: ν = 2218 (C≡N), 1614 (C=N), 1095-1055 cm^{-1} (tetrazole ring); ^1H NMR (DMSO): δ = 6.99-8.13 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{14}H_5BrN_{10}O$: C, 41.10; H, 1.23; N, 34.23%. Found: C, 41.21; H, 1.34; N, 34.12%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-6-mercapto-2-methylsulfanylpyrimidine-5-carbonitrile (10)

To a solution of chloropyrimidine **6** (0.0012 mol) in ethanol (25 mL), thiourea (0.0012 mol) was added and the reaction mixture was heated under reflux for 10 h. The solid obtained after cooling was crystallized from ethanol to give **10**. Yield, 81%, mp. 190-2 °C, IR: ν = 2370 (SH), 2216 (C≡N), 1618 (C=N), 1601 cm^{-1} (C=C); MS: *m/z* (%) 405 (M^+, 70.1), 406 (M^++1, 1.98); Anal. Calcd. for $C_{15}H_9BrN_4OS_2$: C, 44.45; H, 2.24; N, 13.82%. Found: C, 44.56; H, 2.35; N, 13.71%.

2-{6-[5-(4-Bromophenyl)oxazol-4-yl)]-5-cyano-2-methylsulfanylpyrimidin-4-ylamino}benzoic acid (11)

A mixture of **10** (0.011 mol) and anthranilic acid (0.011 mol) in 1-butanol was heated under reflux for 8 h. The reaction mixture was then cooled and the solid obtained was filtered off and crystallized from 1-butanol to yield **11**. Yield, 73%, mp. 231-3 °C, IR: ν = 3470-3205 (OH and NH), 2215 (C≡N), 1705 (CO), 1614 (C=N), 1598 cm^{-1} (C=C); MS: *m/z* (%) 508 (M^+, 32.5), 509 (M^++1, 2.6); Anal. Calcd. for $C_{22}H_{14}BrN_5O_3S$: C, 51.98; H, 2.78; N, 13.78%. Found: C, 51.87; H, 2.65; N, 13.89%.

3-[5-(4-Bromophenyl)oxazol-4-yl]-1-methylsulfanyl-9-oxo-9H-2,9a,10-triazaanthracene-4-carbonitrile (12)

A mixture of **11** (0.002 mol) and acetic anhydride (20 mL) was refluxed for 4 h. The solid separated on cooling was filtered and crystallized from DMF to give **12**. Yield, 67%, mp. 201-3 °C, IR: ν = 2223 (C≡N), 1680 (CO), 1615 cm^{-1} (C=N); ^1H NMR (DMSO): δ = 2.53 (s, 3H, CH_3), 7.02-7.99 (m, 9H, ArH and oxazole H-2); Anal. Calcd. for $C_{22}H_{12}BrN_5O_2S$: C, 53.89; H, 2.47; N, 14.28%. Found: C, 53.58; H, 2.58; N, 14.38%.

Ethyl{6-[5-(4-bromophenyl)oxazol-4-yl]-5-cyano-2-methylsulfanylpyrimidin-4-yl]sulfanyl}acetate (13)

A mixture of **10** (0.0019 mol), sodium acetate (0.0035 mol) and ethyl chloroacetate (0.0019 mol) in ethanol (40 mL) was heated under reflux for 3 h. The precipitate that formed on cooling was filtered and crystallized from benzene to yield **13**. Yield, 68%, mp. 211-3 °C, IR: ν = 2218 (C≡N), 1725 (CO), 1617 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ = 1.2 (t, *J* = 7.41 Hz, 3H, CH_3CH_2), 2.59 (s, 3H, SCH_3), 3.62 (s, 2H, SCH_2), 3.98 (q, *J* = 7.41 Hz, 2H, CH_2CH_3), 7.01-8.01 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{19}H_{15}BrN_4O_3S_2$: C, 46.44; H, 3.08; N, 11.40%. Found: C, 46.31; H, 3.19; N, 11.53%.

Ethyl 5-amino-4-[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanylthieno[2,3-d]pyrimidine-6-carboxylate (14)

To a solution of **13** (0.002 mol) in absolute ethanol (20 mL), sodium ethoxide solution (50 mg sodium in 25 mL absolute ethanol) was added dropwise and the reaction mixture was heated under reflux for 30 min. The solid that formed while hot was collected and crystallized from ethanol to furnish **14**. Yield, 83%, mp. 187-9 °C, IR: ν = 3320, 3300 (NH_2), 1720 (CO), 1620 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ = 1.3 (t, *J* = 7.2 Hz, 3H, CH_3CH_2), 2.56 (s, 3H, SCH_3), 4.11 (q, *J* = 7.2 Hz, CH_2CH_3), 5.81 (br s, 2H, NH_2), 6.96-7.98 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{19}H_{15}BrN_4O_3S_2$: C, 46.44; H, 3.08; N, 11.40%. Found: C, 46.55; H, 3.19; N, 11.51%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-2-methylsulfanyl-7-phenyl-6-thioxo-6,7-dihydro-5H-9-thia-1,3,5,7-tetraazafluorene-8-one (15)

To a solution of **14** (0.0011 mol) in pyridine (25 mL)

was added phenyl isothiocyanate (0.001 mol) and the reaction mixture was refluxed in an oil bath for 20 h. The reaction mixture after cooling was poured into ice/HCl and the solid separated was filtered, washed with cold aqueous ethanol, dried and crystallized from ethanol-DMF (2:1) to give **15**. Yield, 59%, mp. 214-6 °C, IR: ν = 3280 (NH), 1680 (CO), 1621 (C=N), 1260 cm^{-1} (CS); ^1H NMR ($CDCl_3$): δ = 2.58 (s, 3H, CH_3), 6.97-8.01 (m, 10H, ArH and oxazole H-2) and 9.01 (s, 1H, NH, exchangeable); Anal. Calcd. for $C_{24}H_{14}BrN_5O_2S_3$: C, 49.66; H, 2.43; N, 12.06%. Found: C, 49.79; H, 2.55; N, 12.17%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-6-methyl-2-methylsulfanyl-7-oxa-9-thia-1,3,5-triazafluorene-8-one (16)

A sodium salt of the acid derivative **14** (0.0011 mol) (which resulted from boiling of **14** in ethanolic sodium hydroxide solution for 2 h) in acetic anhydride (20 mL) was heated under reflux for 3 h, then concentrated and allowed to cool. The solid product was collected, washed with water and crystallized from xylene to yield **16**. Yield, 61%, mp. 226-8 °C, IR: ν = 1695 (CO), 1625 (C=N), 1602 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ = 2.01 (s, 3H, CH_3), 2.56 (s, 3H, SCH_3), 6.99-8.11 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{19}H_{11}BrN_4O_3S_2$: C, 46.83; H, 2.28; N, 11.50%. Found: C, 46.94; H, 2.39; N, 11.61%.

5-Amino-4-[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanylthieno[2,3-d]pyrimidine-6-carboxylic acid (17)

A suspension of **14** (0.001 mol) in ethanolic sodium hydroxide solution 10% (50 mL) was heated under reflux for 4 h. The alkaline solution was acidified with diluted acetic acid and extracted with ether. The solid separated after evaporation of the dried ethereal layer was crystallized from ethanol to furnish **17**. Yield, 79%, mp. 241-3 °C, IR: ν = 3475-3160 (OH and NH_2), 1707 (CO), 1615 (C=N), 1602 cm^{-1} (C=C); ^1H NMR (DMSO): δ = 2.54 (s, 3H, CH_3), 5.75 (br s, 2H, NH_2), 6.96-7.89 (m, 5H, ArH and oxazole H-2) and 10.95 (br s, 1H, OH, exchangeable); Anal. Calcd. for $C_{17}H_{11}BrN_4O_3S_2$: C, 44.07; H, 2.39; N, 12.09%. Found: C, 44.18; H, 2.50; N, 12.20%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-2-methylsulfanylthieno-[2,3-d]pyrimidin-5-ylamine (18)

A solution of **17** (0.0012 mol) in orthophosphoric acid 85% (30 mL) was stirred for 20 min (until the evolution of CO_2 gas ceased). The reaction mixture was poured on 80 mL ice-water and then neutralized with 8% aqueous sodium carbonate. The solid product so formed was filtered and crystallized from 1-butanol to afford **18**. Yield, 72%, mp. 216-8 °C, IR: ν = 3360-3330 (broad NH_2), 1621 (C=N), 1604 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ = 2.55 (s, 3H, CH_3), 5.51 (br s, 2H, NH_2), 6.01 (s, 1H, thiophene ring), 7.01-8.11 (m, 5H, ArH and oxazole H-2); Anal. Calcd. for $C_{16}H_{11}BrN_4OS_2$: C, 45.83; H, 2.64; N, 13.36%. Found: C, 45.72; H, 2.75; N, 13.47%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-2-methylsulfanylthieno-[2,3-d]pyrimidine-5-one (19)

Method a: A solution of **17** (0.0011 mol) in orthophosphoric acid 85% (30 mL) was heated on a water bath for 8 h. The reaction mixture was poured on ice-water (50 mL) where upon the solid formed; it was collected by filtration and crystallized from toluene to give **19**, yield 70%.

Method b: A solution of **18** (0.0011 mol) in 85% orthophosphoric acid (30 mL) was heated on a water bath for 7 h. The reaction mixture was poured on ice-water (50 mL) and the solid that separated was filtered and crystallized to yield a product identified to be **19** by mp and mmp determination. Yield, 73%, mp. 207-9 °C, IR: ν = 1698 (CO), 1622 (C=N), 1605 cm^{-1} (C=C); ^1H NMR ($CDCl_3$): δ = 2.56 (s, 3H, CH_3), 3.71 (s, 2H, CH_2), 7.13-8.12 (m, 5H, ArH and oxazole H-2); MS: *m/z* (%) 420 (M^+, 25.1), 421 (M^++1, 1.65); Anal. Calcd. for $C_{16}H_{10}BrN_3O_2S_2$: C, 45.72; H, 2.40; N, 10.00%. Found: C, 45.61; H, 2.52; N, 10.12%.

General procedure of the synthesis of 6-arylidene-4-[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanylthieno[2,3-d]pyrimidine-5-one (20a,b)

A mixture of **19** (0.002 mol) and aromatic aldehydes (0.0013 mol), namely benzaldehyde and/or *p*-anisaldehyde in ethanol (25 mL) containing a few drops of piperidine, was refluxed for 2 h. The precipitate that separated while hot was collected by filtration and crystallized from dioxane to furnish **20a,b**.

6-Benzylidene-4-[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanylthieno[2,3-d]pyrimidine-5-one (20a)

Yield, 89%, mp. 261-3 °C, IR: ν = 1675 (CO), 1625-1620 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ = 2.54 (s, 3H, CH_3), 6.98-7.95 (m, 10H, ArH and oxazole H-2), 7.85 (s, 1H, CH=C); Anal. Calcd. for $C_{23}H_{14}BrN_3O_2S_2$: C, 54.34; H, 2.78; N, 8.26%. Found: C, 54.22; H, 2.89; N, 8.38%.

4-[5-(4-Bromophenyl)oxazol-4-yl]-6-(4-methoxybenzylidene)-2-methylsulfanylthieno[2,3-d]pyrimidine-5-one (20b)

Yield, 83%, mp. 283-4 °C, IR: ν = 1680 (CO), 1624-

1620 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ = 2.53 (s, 3H, SCH_3), 3.81 (s, 3H, OCH_3), 6.91-7.31 (m, 9H, ArH and oxazole H-2) and 7.81 (s, 1H, CH=C); Anal. Calcd. for $C_{24}H_{16}BrN_3O_3S_2$: C, 53.54; H, 3.00; N, 7.80%. Found: C, 53.63; H, 3.11; N, 7.69%.

General procedure for the synthesis of 8-aryl-6-amino-4-[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanyl-8H-5-oxa-9-thia-1,3-diazafluorene-7-carbonitrile (21a,b)

A mixture of **19** (0.0012 mol) and arylmethylene malononitrile (0.0012 mol) in ethanol (25 mL) containing a few drops of piperidine was heated under reflux for 3 h and the precipitate that formed while hot was collected by filtration and crystallized from dioxane to afford **21a,b**.

6-Amino-4[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanyl-8-phenyl-8H-5-oxa-9-thia-1,3-diazafluorene-7-carbonitrile (21a)

Yield, 81%, mp. 273-5 °C, IR: ν = 3350-3330 (broad NH_2), 2230 (C≡N), 1620 cm^{-1} (C=N); ^1H NMR (DMSO): δ = 2.53 (s, 3H, CH_3), 5.10 (s, 1H, pyran-CH), 5.89 (br s, 2H, NH_2), 6.85-7.48 (m, 10H, ArH and oxazole H-2); Anal. Calcd. for $C_{26}H_{16}BrN_5O_2S_2$: C, 54.36; H, 2.81; N, 12.19%. Found: C, 54.48; H, 2.70; N, 12.30%.

6-Amino-4[5-(4-bromophenyl)oxazol-4-yl]-2-methylsulfanyl-8-(4-methoxyphenyl)-8H-5-oxa-9-thia-1,3-diazafluorene-7-carbonitrile (21b)

Yield, 80%, mp. 237-9 °C, IR: ν = 3360-3340 (broad NH_2), 2235 (C≡N), 1623 cm^{-1} (C=N); ^1H NMR ($CDCl_3$): δ = 2.51 (s, 3H, SCH_3), 3.89 (s, 3H, OCH_3), 5.13 (s, 1H, pyran CH), 5.97 (br s, 2H, NH_2), 6.91-7.96 (m, 9H, ArH and oxazole H-2); Anal. Calcd. for $C_{27}H_{18}BrN_5O_3S_2$: C, 53.65; H, 3.00; N, 11.59%. Found: C, 53.76; H, 3.12; N, 11.47%.

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