REVIEW   **Open Access**

Pyrimidine: a review on anticancer activity with key emphasis on SAR

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Abstract

Background: Cancer is a global health challenge, it impacts the quality of life and its treatment is associated with several side effects. Resistance of the cancer cells to the existing drugs has led to search for novel anticancer agents. Pyrimidine, a privileged scaffold, is part of living organisms and plays vital role in various biological procedures as well as in cancer pathogenesis. Due to resemblance in structure with the nucleotide base pair of DNA and RNA, it is recognized as valuable compound in the treatment of cancer.

Main text: Many novel pyrimidine derivatives have been designed and developed for their anticancer activity in the last few years. The present review aims to focus on the structure activity relationship (SAR) of pyrimidine derivatives as anticancer agent from the last decade.

Conclusion: This review intends to assist in the development of more potent and efficacious anticancer drugs with pyrimidine scaffold.

Keywords: Pyrimidine, Anticancer activity, Structure activity relationship, Heterocyclic compounds

Background

Cancer is a life-threatening ailment worldwide [1]. Present treatment modalities like chemotherapy and radiotherapy suffers serious setbacks with multidrug resistance (MDR) being the major challenge. Search for diverse and novel structural framework may pave way to develop new effective anticancer drugs. Pyrimidine (1) is considered a vital heterocyclic moiety on account of its large spectrum of biological and pharmacological activities. These six-membered 1,3-diazine ring containing nitrogen at 1 and 3 position are part of naturally occurring substances such as nucleotides, nucleic acids, vitamins, coenzymes, purines, pterins, and uric acids. The widespread therapeutic applications of pyrimidine may be accounted for its presence in the structure of DNA and RNA. 5-halogenated derivatives of pyrimidine were among the first analogs tested for biological activity. This heterocyclic moiety is part of several drugs like

zidovudine, stavudine, 5-flurouracil, methotrexate, imatinib, dasatinib, pazopanib, nilotinib, uramustine, tegafur, cytarabine, trimethoprim, sulfamethazine, minoxidil, phenobarbital, primidone, and risperidone [2, 3]. This review emphasizes advances over the last decades in pyrimidine containing hybrids with in vitro anticancer potential and its correlation with the SAR.

Main Text

Pyrimidine derivatives as anticancer agent

Pyrimidine belongs to an electron rich nitrogen containing heterocycle. Synthetic versatility of pyrimidine allows generation of structurally diverse derivatives which includes analogs derived from substitution of the aryl ring, dervatization of pyrimidine nitrogen and substitutions at of carbon at 2, 4, 5, and 6 positions [4].

Disubstituted pyrimidine derivative
2,4-Disubstituted pyrimidine derivative
Some novel 2,4-disubstituted pyrimidines were developed and tested for antiproliferative activity using MTT assay with VX-680 as positive control. Compound **2** was

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moderate to highly active against the A549 (IC$_{50}$ = 12.05 ± 0.45 μM), HTC-116 (IC$_{50}$ = 1.31 ± 0.41 μM), and MCF-7 (IC$_{50}$ = 20.53 ± 6.13 μM) cell lines and exhibited potent aurora kinase inhibition against both aurora A and B kinase. Apoptosis was induced due to upregulation of Bax and downregulation in Bcl-xl. The SAR studies suggests that the benzene ring when replaced with cyclohexyl group gave better activity and replacement of NH in urea with CH$_2$ lead to decrease in activity [5]. It was established further that the blockade of G2-M phase of cell cycle occurred by accumulation of the contents at S phase due to decrease in mitochondrial membrane potential making 2,4-diaminopyrimidine derivatives potential anticancer agents. **3a** (IC$_{50}$ = 2.14 to 5.52 μM) and **3b** (IC$_{50}$ = 1.98 to 4.27 μM) were most potent against the PC-3, A549, MCF-7, and HCT-116 cancer cell lines due to the variation of substitution on aromatic ring and terminal aniline on the pyrimidine moiety [6]. The induction of apoptosis was observed for cancer cell line K562 by novel anilino substituted pyrimidine sulfonamides. Cell viability was tested through MTT and tunnel assay. Compounds **4(a–c)** demonstrated a promising activity with IC$_{50}$ range = 5.6 to 12.3 μM [7]. Figure 1 depicts the chemical structure of 2,4-disubstituted pyrimidine derivatives.

2,5-Disubstituted pyrimidine derivatives
In 2015, Reddy and co-workers synthesized 2,5-disubstituted pyrimidines by Suzuki coupling and reported for moderate anticancer activity against the HeLa cell lines using MTT cell proliferation assay. Compound **5** (IC$_{50}$ = 82.7 μM) showed the best activity (Fig. 2) [8].

4,6-Disubstituted pyrimidine derivatives
A series of novel 4, 6-disubstituted pyrimidine derivatives were evaluated for in vitro activities on cancerous cell lines SIHA, IMR-32, A549, PANC-1, DU145, and MDA-MB-231. **6a** was found to be an efficacious inhibitor of IMR32, **6b** in MDA-MB-231, and **6c** in SIHA and DU145, whereas **6d** in case of PANC-1 and A549 respectively. The SAR is depicted in Fig. 3 [9].

Trisubstituted pyrimidines
2, 5, 6-Trisubstituted pyrimidines
A new series of 2,4-diaminopyrimidines were reported as potent and selective aurora A kinase inhibitors. The best potency of the compounds was elucidated against HeLa, A-549, HCT-8, and Hep-G2 cells compared with the VX-680 as positive control by MTT assay. Compound **7** exhibited highest cytotoxicity with an IC$_{50}$ = 0.5–4.0 μm as well as led to cell cycle arrest in HeLa cells at G2/M phase [10].
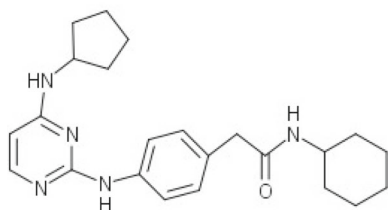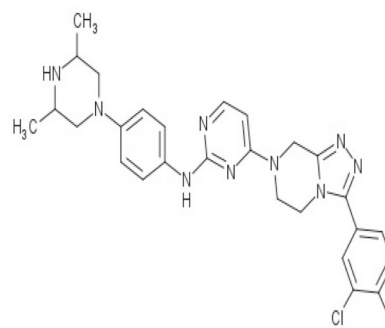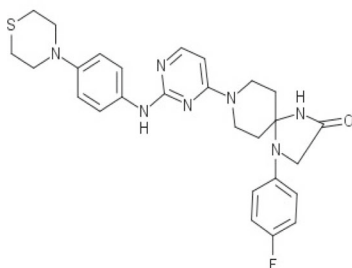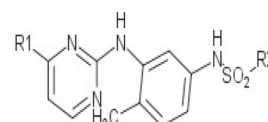
2, 4, 5-Trisubstituted pyrimidines
New 5-alkyl pyrimidine derivatives, alkyl N-methoxymethyl pyrimidine derivatives, and 5,6-dihydro-furo[2,3-d]pyrimidines were reported with cytostatic activities using 5-FU as the positive control. 5-chloroethyl-2,6 dichloro pyrimidine **8** (IC$_{50}$ = 0.8 ± 0.2 μM) exerted cytostatic effect on HCT-116 cancer cell line which led to cell cycle arrest at G2/M phase due to DNA damage. The SAR suggests that the presence of two aromatic and an aliphatic chlorine atom linked to the pyrimidine ring gave the compound with maximum potential [11]. Furthermore, 2-arylaminopyrimidine derivative bearing a 2-amino-N-methylbenzamide at C4 and chlorine at C5 positions were designed as a potent inhibitor of c-Met in cellular and enzymatic assays. C2 benzazepinone were found to be the most potent c-Met inhibitors, **9** (IC$_{50}$ = 10 nM) being the best analog. Incorporation of fluorine at C3 position of aminobenzamide moiety led to selectivity for c-Met kinase [12].

2, 4, 6-Trisubstituted pyrimidines
Recently, in a study of synthesis and anticancer activity of trisubstituted pyrimidines and their N-alkyl derivatives was studied via ELISA, BRU, and MTT assay and **10** posed exceptional activity. They were tested against A549, Hep3B, HT29 FL, MCF-7, and HeLa cell lines with the IC$_{50}$ range from 2 to10 μm/ml [13]. Moreover, anthranillic acid ester moiety-linked 2,4,6-trisubstituted pyrimidines were tested for cytotoxic activty. The compounds were screened against U-937, CEM-13, MDA-MB-231, DU-145, and BT-474 cancer cell lines by conventional MTT assays. **11a** and **11b** were known to be the most potent in the series and also as CDK9 inhibitors. The SAR studies reveal that the major activity is due to the (E)-styryl moiety at C-6 position, methyl group at R$_2$ position, and the presence of methylanthranilate moiety with an EDG at C-4 lead to better activity (Fig. 4) [14].

Pyrimidines and triazolopyrimidines as antiproliferative agents exhibited COX-1/2 inhibitory potential. Compound **12** (IC$_{50}$ range = 8.68 ± 0.2 to 36.56 ± 0.9 μg/ml) displayed in vitro activity against cancer cell lines HepG-2, MCF-7, CaCo-2, and A549 alongside COX-2 inhibition using 5-FU as the reference drug [15]. Previously in a study, combretastatin bridged pyrimidine derivatives were tested for antitumor activity against the MCF-7 and A549 using MTT assay. **13a** (IC$_{50}$ = 4.67 μM; 3.38 μM) and **13b** (IC$_{50}$ = 0.63 μM; 3.71 μM) were concluded to have the best potential. **13a** induced apoptosis by ROS-regulated intrinsic apoptotic pathway; they were non-toxic to harmful cells and were more potent inhibitors than cholchicne in the tunnel assay. The SAR demonstrated that the R$_2$ and R$_3$ substituted rings affected the activity, EWG such as 2,4-dichlorosubstitution

Fig. 1 Chemical structure of 2, 4-disubstituted pyrimidine derivatives

4a;R₁=3-pyridyl , R2=3quinonyl

4b; R₁= 3,4,5-Trimethoxyphenyl, R₂= 4-Biphenyl

**4c**;R₁=3,4,5-Trimethoxyphenyl, R₂=3,4-Dimethoxyphenyl

on the rings manifested good activity, and interchange of amine, methyl with hydrogen in R_1 position of the pyrimidine ring displayed no activity. Replacement of rings with napthyl gave less activity and no substitution in any of the three rings also depicted activity [16].

In a library of N-trisubstituted pyrimidine scaffold, compound **14** (IC_{50} = 12.2 nM) elucidated the best activity in the inhibition of U937 cell line. It caused the inhibition by inducing polyploidy (4N, 8N, and 16N) in the cancer cells by inducing defects in both chromosome formation and spindle formation. The SAR studies are depicted in Fig. 5 [17].

In a continued study, a series of pyrimidine-benzimidazole compound **15** with an IC_{50} = 1.06 to 12.89 μM, was tested against the MGC-803, SMMC-7721, EC-9706, and MCF-7 cell lines antiproliferative

activity. The cell cycle came to rest at G2/M phase by the active compound accompanied by an increase in apoptotic cell death of MGC-803 [18]. Additionally, in the previous year, the novel thiazolopyrimidine derivatives were studied against the human cancer cell lines and primary CLL cells. **16** displayed excellent anticancer activity against the cell lines and led to cell death by apoptosis as it inhibited the CDK enzyme [19].

Evaluation for pharmacological activity was conducted for some novel 1, 2, 4-triazole containing pyrimidine derivatives. **17(a–b)** were found to have effective activity on HOP-92 as evaluated using the MTT assay [20]. In an earlier work, MTT assay was conducted to determine cytotoxic study of the series novel pyrimidine derivatives **18a** (% inhibition at 50 μg/mL = 43.62) containing 4-chlorophenyl substitution on 6th position of pyrimidine

Fig. 2 Chemical structures of 2,5-disubstituted pyrimidine derivatives
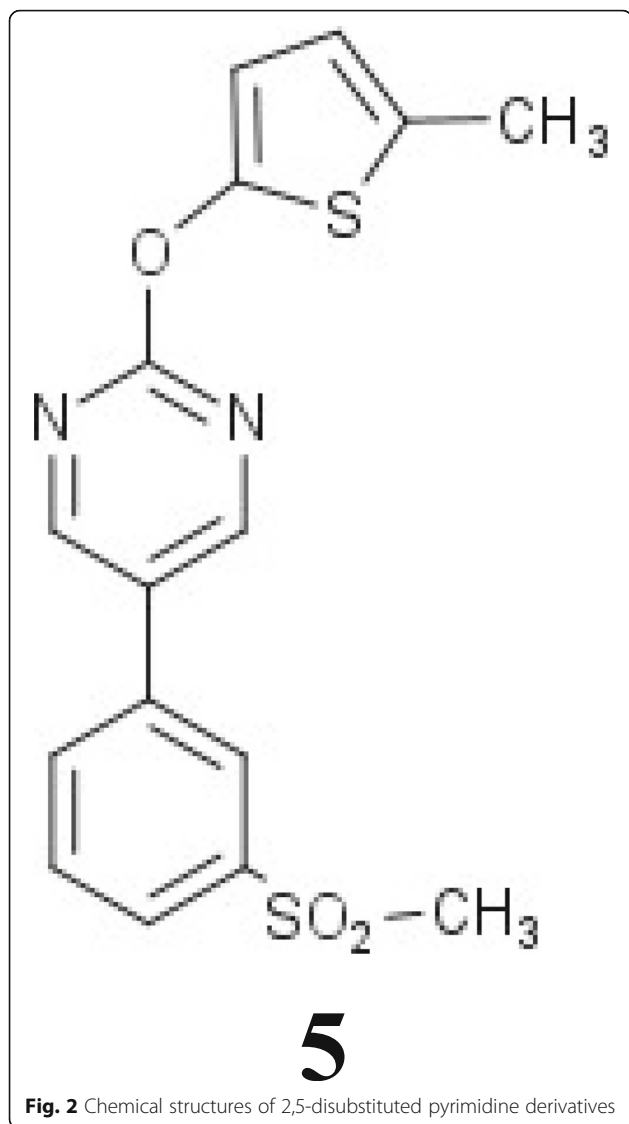
nucleus and **18b** (% inhibition at 50 μg/mL = 39.52) containing thiophene ring, showed the most potency. The SAR revealed that the most of the activity was due to the substitution of mono or di chlorine at the R1 position of the phenyl ring [21].
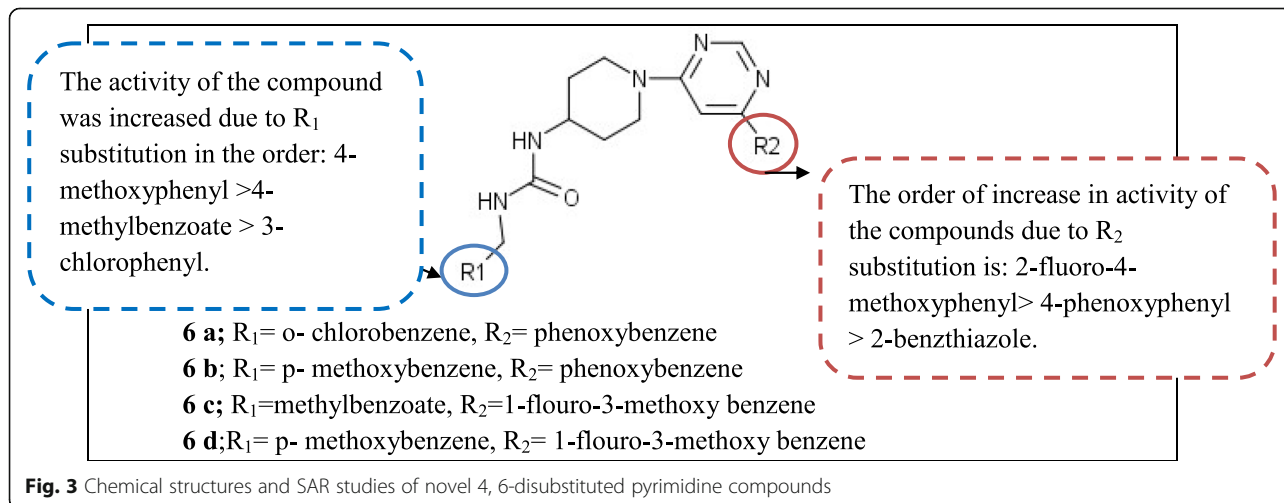
The in vitro anticancer activity of novel pyrimidine derivatives was reported in which it was concluded that **19** showed the best activity against the whole panel of 60 cancer cell lines (especially lung cancer cell lines). The cell lines were tested at different concentration of the compounds, and the SAR studies revealed that the EDG groups like NH_2 and EWG like Cl and C=O at ortho- and paraposition of the ring is highly influential for the activity (Fig. 6) [22].

Tetrasubstituted pyrimidine derivatives
2,4,5,6-Tetrasubstituted pyrimidines

A study reported ERα and VEGFR-2 ligands in the form of 2, 4-disubstituted pyrimidine derivatives and tested them against the MCF-7 cancer cell lines. **20** had ERα binding affinity (IC_{50} = 1.64 μM) and inhibition activity against VEGFR-2 (IC_{50} = 0.085 μM). It acted by suppressing the progesterone inhibition mRNA and in vivo angiogenesis inhibition in CAM assay. Suppression of cell migration, apoptosis, and transduction reticence of Raf-1/MAPK/ERK in MCF-7 cells were also reported. SAR studies showed that the hydrogen bonding interaction at the head is significant for increase in ERα binding affinity (Fig. 7) [23].

A series of pyrimidines including thioxopyrimidine, iminopyrimidine, bicyclic thiazolopyrimidine, and arylidine derivatives of thiazolopyrimidine were reported for anticancer activity and studied against HCT-116, PC-3, and Hep-2 cancer cell lines. Compound **21a**, **21b**, and **21d** exhibited higher activity against PC-3 with IC_{50} = 66.6 ± 3.6 μg/ml, 69.6 ± 2.1 μg/ml, and 65.8 ± 2.8 μg/ml

The activity of the compound was increased due to R$_1$ substitution in the order: 4-methoxyphenyl >4-methylbenzoate > 3-chlorophenyl.

The order of increase in activity of the compounds due to R$_2$ substitution is: 2-fluoro-4-methoxyphenyl> 4-phenoxyphenyl > 2-benzthiazole.

6 a; R$_1$= o- chlorobenzene, R$_2$= phenoxybenzene
6 b; R$_1$= p- methoxybenzene, R$_2$= phenoxybenzene
6 c; R$_1$=methylbenzoate, R$_2$=1-flouro-3-methoxy benzene
6 d;R$_1$= p- methoxybenzene, R$_2$= 1-flouro-3-methoxy benzene

Fig. 3 Chemical structures and SAR studies of novel 4, 6-disubstituted pyrimidine compounds

7   **8**

9   **10**

11a; R₁= 2,3-OMe ;
11b R₁= 2,5-OMe ;
a-b; R₂= Me

12

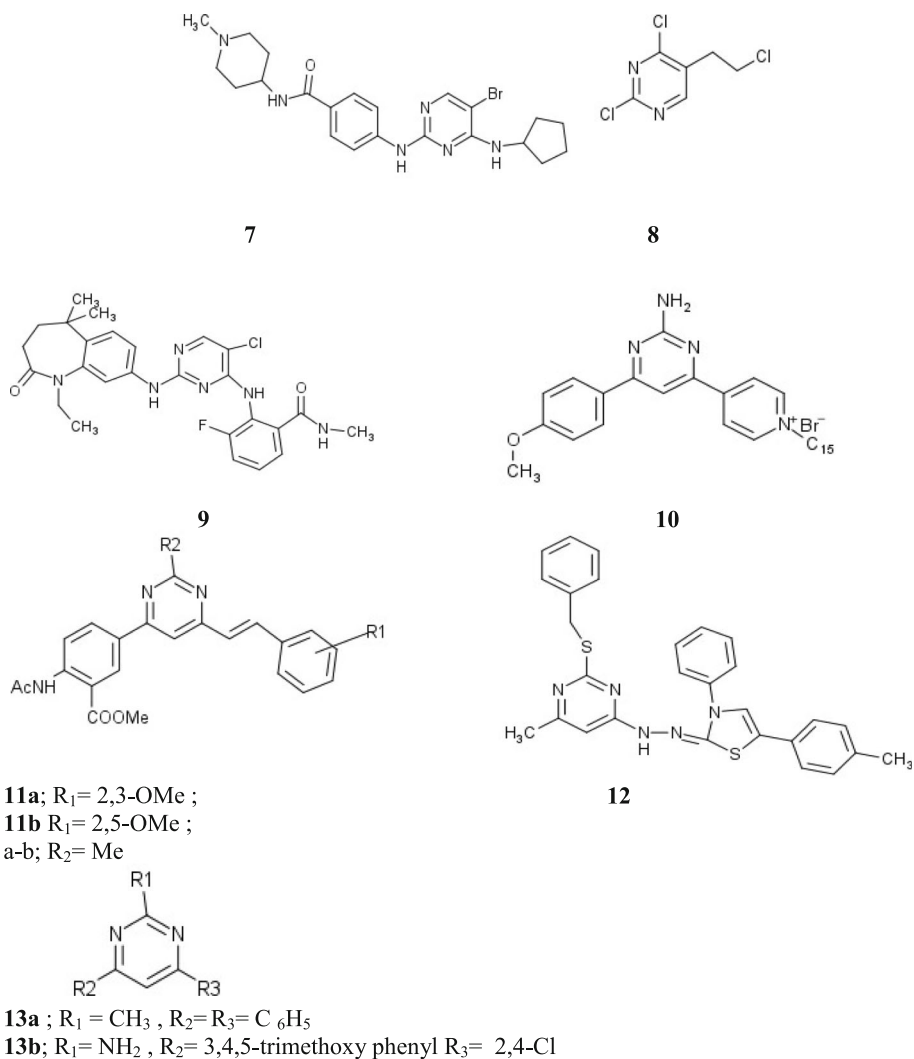**13a** ; R₁ = CH₃ , R₂=R₃= C₆H₅
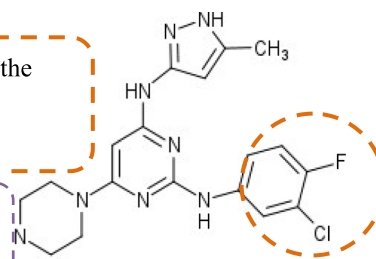**13b**; R₁= NH₂ , R₂= 3,4,5-trimethoxy phenyl R₃= 2,4-Cl

**Fig. 4** Chemical structures of trisubstituted pyrimidine derivatives

Substitutions like 3, 4- Cl, F and trimethoxy on the aniline moiety at C-2 position highly effects the activity.

Fluorine in phenyl ring is significant for activity

14

**Fig. 5** Chemical structure and SAR studies of trisubstituted pyrimidine

Fig. 6 Chemical structures of some 2,4,6-trisubstituted pyrimidine derivatives
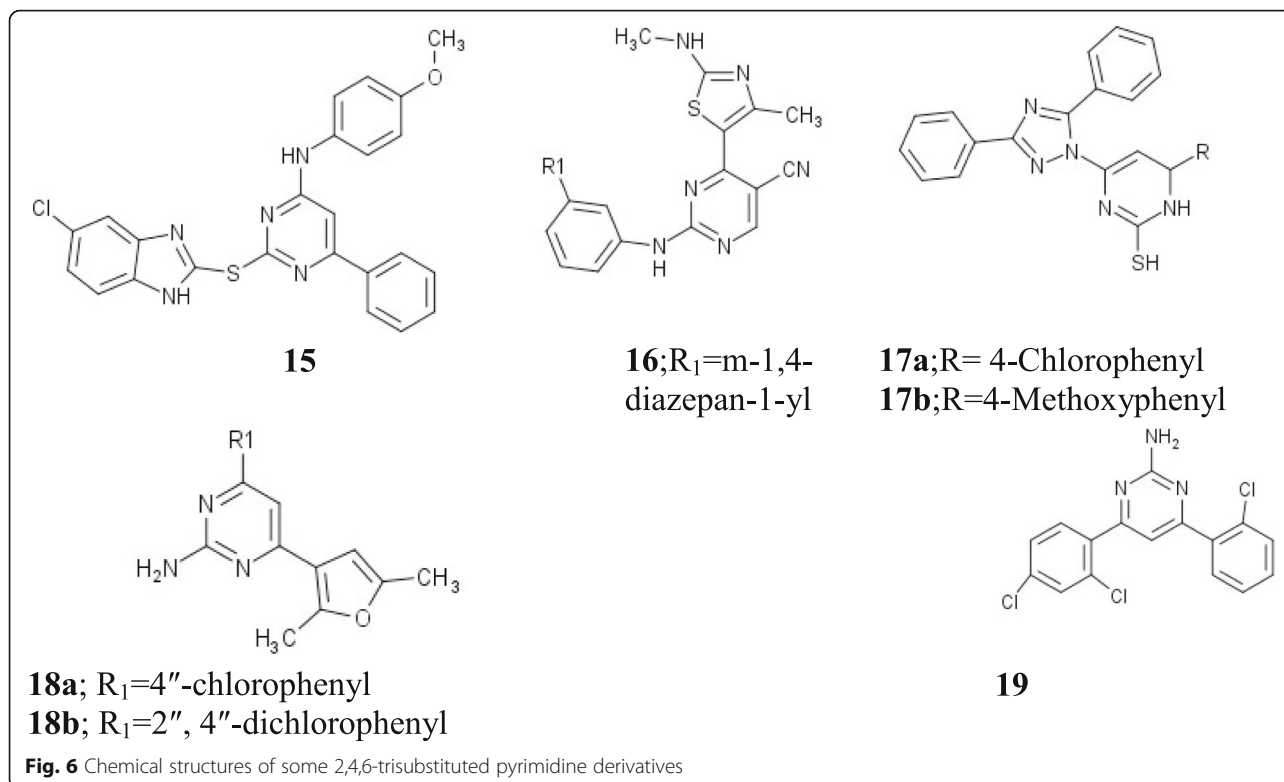
respectively whereas **21c** and **21d** showed higher activity against HCT-116 (IC$_{50}$ values 60.9 ± 1.8 μg/ml and 58.2 ± 5.1 μg/ml). The SAR study is illustrated in Fig. 8 [24].

A novel series of 2,4-disubstituted-2-thiopyrimidine derivatives was reported as VEGFR-2 inhibitor and tested against the HepG2 and UO-31 cancer cell lines. **22a** (IC$_{50}$ = 1.23 μM) and **22b** (IC$_{50}$ = 3.78 μM) were found to be active inhibitor of VEGFR-2 as well as

Fig. 7 Chemical structure of tetrasubstituted pyrimidines derivatives

displayed activity against HePG2 with the IC$_{50}$ = 13.06 μM and IC$_{50}$ = 8.35 μM. The SAR studies reveal that the potency was due to the hydrophobic interaction of the substitutions at position-2 and phenyl group at position-4 of thiouracil moiety [25].
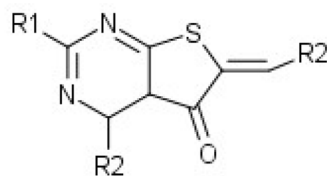
Pyrimidine substituted with 1, 2, 3-triazole-urea was screened for oncogenic activity against MGC-803, B16-F10, EC-109, and MCF-7 cancer cell lines. Compounds **23a**, **23b**, and **23c** were reported to exhibit potent activity against B16-F10 with an IC$_{50}$ = 32 nM, 35 nM, and 42 nM respectively. The SAR revealed that the electronic effect on the phenyl ring affects the activity, an EDG group, gives more activity and at R$_1$ the presence of 4-methyl and 4-methoxyl attributes better activity than 2-methyl, 3-methyl, and 2-methoxyl substitutions [26]. Additionally, anticancer activity of pyrimidine-thiourea derivatives was studied. The derivatives inhibited histone LSD1 which was over expressed in many tumor cells. Compound **24** (IC$_{50}$ = 0.65 ± 0.12 μM) was most potent against gastric cancer cell line and it inhibited cell migration and invasion with tumor suppressing and anti-metastasis functions. Amino, thio, and urea groups are essential for the LSD-1 inhibition. Thio urea instead of urea offered better activity and subsequently the proprgyl and trimethoxy phenyl group increases anticancer activity (Fig. 9) [27].

1, 2, 3-Triazole substituted pyrimidines were studied for anticancer activity against the MGC-803, EC-109,
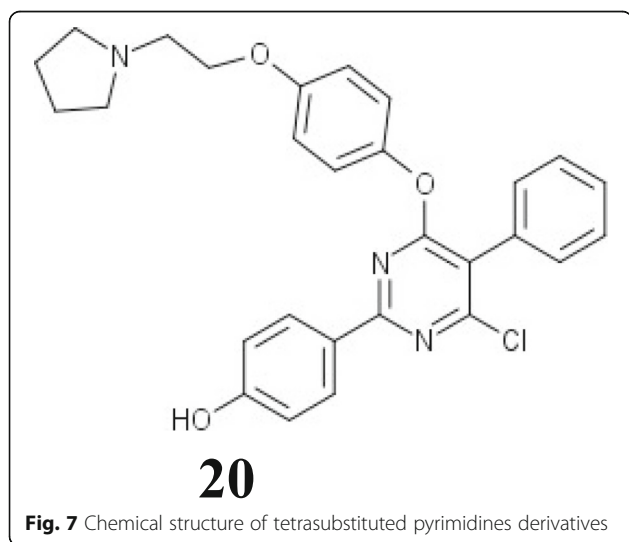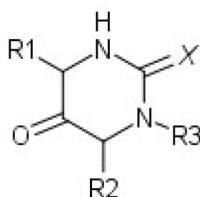
Anti- cancer activity is lost when C-6 is functionalized with oxo-group.

Substitution of thiopyrimidine nucleus at N3 with amino group lead to increased anticancer activity.

21 a R$_1$= Thiophen-2-yl   R$_2$= 3,4,5- (OCH$_3$) $_3$ C $_6$H $_2$

Phenyl/ dimethoxyphenyl moiety at 6th position of the pyrimidine ring gave higher activity against selective cell lines.

1, 3-unsubstituted pyrimidinedione derivatives had increased activity in contrast to 2- thioxo analogues.

21b; R$_1$= Thiophen-2-yl, R$_2$= -C $_6$H $_5$, R$_3$= H X=S
**21c**; R$_1$= Thiophen-2yl, R$_2$= 3,4-(OCH$_3$) $_3$ C $_6$H $_3$, R$_3$= NH$_2$, X=S
21d; R$_1$= Thiophen-2-yl, R$_2$= - C $_6$H $_5$, R$_3$=H, X= O

Fig. 8 Chemical structure and SAR studies of arylidine derivatives of thiazolopyrimidine

MCF-7, and B16-F10 cancer cell lines with 5-FU as positive control. Compound **25** (IC$_{50}$ = 1.42 to 6.52 μM) was the most potent and induced apoptosis as well as arrested cell cycle at G2/M phase in EC-109 cells. The main activity was due to presence of triazo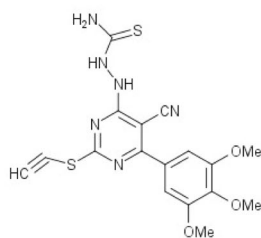le moiety, substitution at 2 position of benzyl group, and substitution at 4 position of arylamine group with an EDG than at 3 positions [28]. 4-Substituted thiopyrimidine analogs were reported and tested against 60 cancer cell line panel. **26a** and **26b** depicted high potential against the leukemia cell lines. SAR is given in Fig. 10 [29].
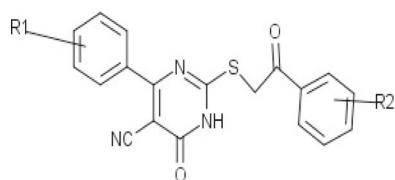
22a; R$_1$ = 4-OMe , R$_2$= 4-OMe
22b; R$_1$ = 4-OMe   R$_2$= 2-OMe
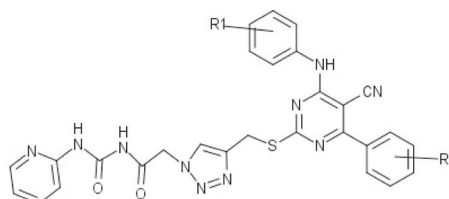
23a; R$_1$= *p*-CH$_3$;R$_2$= *m,p,m*-(OCH$_3$)$_3$
23b; R$_1$= *p*-CH(OCH$_3$)$_2$; R$_2$= *p*-CH$_3$
23c ; R$_1$ = *p*-CH(CH$_3$)$_2$; R$_2$= *p*-Br

24
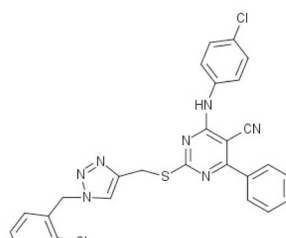
25

Fig. 9 Chemical structures of tetrasubstituted pyrimidine derivatives

An active chlorine atom at R$_2$ position gives more activity compared to the piperidine moiety. Sulfamethoxazole moiety increased activity.

Morpholino derivative at R$_2$ is more preferred than piperidino congener.

Fusion of thiazolone ring with the pyrimidinone nucleus results in low activity.

26a; R$_1$= 4-Fluorobenzene , R$_2$= Chlorine , R$_3$= dimethyl **26b** ; R$_1$= 4- Chlorobenzene , R$_2$= aminosulfamethoxazole , R$_3$= methyl

Fig. 10 Chemical structure and SAR studies of thiopyrimidine analogs 26(a–b)

A library of pyrimidine substituted with polymethoxy chalcones and thiazolopyrimidine for anticancer potential was reported. The activity was tested against 60 cell cancer line panel. **27** exhibited significant inhibition of tumor growth being highly efficient with cytotoxic and cytostatic attribute. SAR illustrated in Fig. 11 [30].

2,3,4,6–Tetra substituted pyrimidine

In a report, novel substituted pyrimidines and triazolo-pyrimidines were evaluated for antiproliferative activity against PC3, HCT116, MCF-7, and RPE1 cancer cell lines. It was discovered that **28** (IC$_{50}$ = 66 ± 6 μm) had the best potential against the RPE1 cell line. From the SAR studies, it was concluded that triazolpyrimidine glucosides/xylosides were found to be less active than substituted pyrimidine glycosides (Fig. 12) [31].
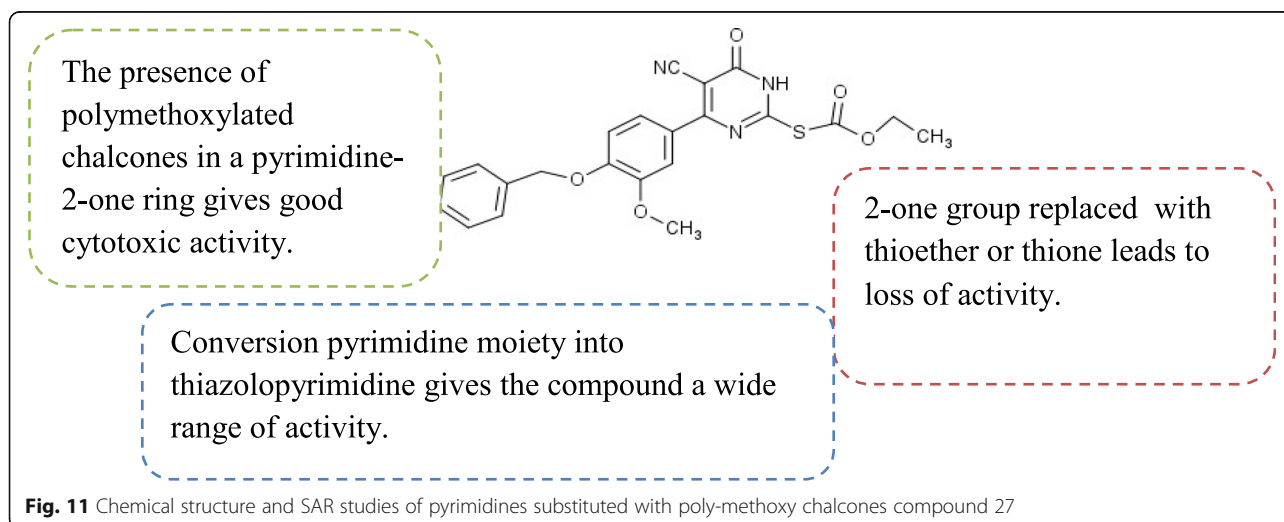
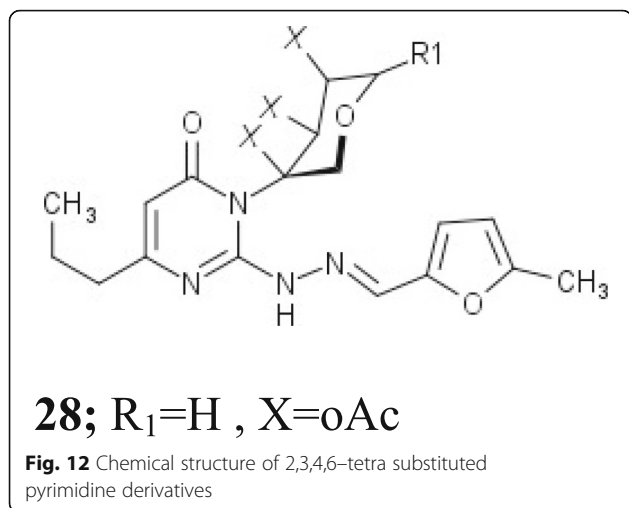### Pyrimidine fused with heterocyclic rings
Pyrazole-pyrimidine derivatives

The heterocyclic moieties pyrazole and pyrimidines constitute two pharmacophores, which have potent antitumor activity. Various isomeric forms of pyrazolo-pyrimidine are known to possess excellent anticancer activity depending on the position of both the rings namely pyrazolo[1,5-a]pyrimidines, pyrazolo[3,4-*d*]pyrimidines, and pyrazolo[4,3-*d*]pyrimidines.

Pyrazolo [1, 5-a]pyrimidines

In a recent report, some novel fused pyrazolo-pyrimidine derivatives were studied for anticancer activity as well as COX-2 inhibition against a 60 cancer cell line panel. Compound **29** was potent in case of both the studies. It was selective towards COX due to the presence of 5-amino-1-oxo-substituted-pyrazole-4-

The presence of polymethoxylated chalcones in a pyrimidine-2-one ring gives good cytotoxic activity.

2-one group replaced with thioether or thione leads to loss of activity.

Conversion pyrimidine moiety into thiazolopyrimidine gives the compound a wide range of activity.

Fig. 11 Chemical structure and SAR studies of pyrimidines substituted with poly-methoxy chalcones compound 27

28; R$_1$=H , X=oAc

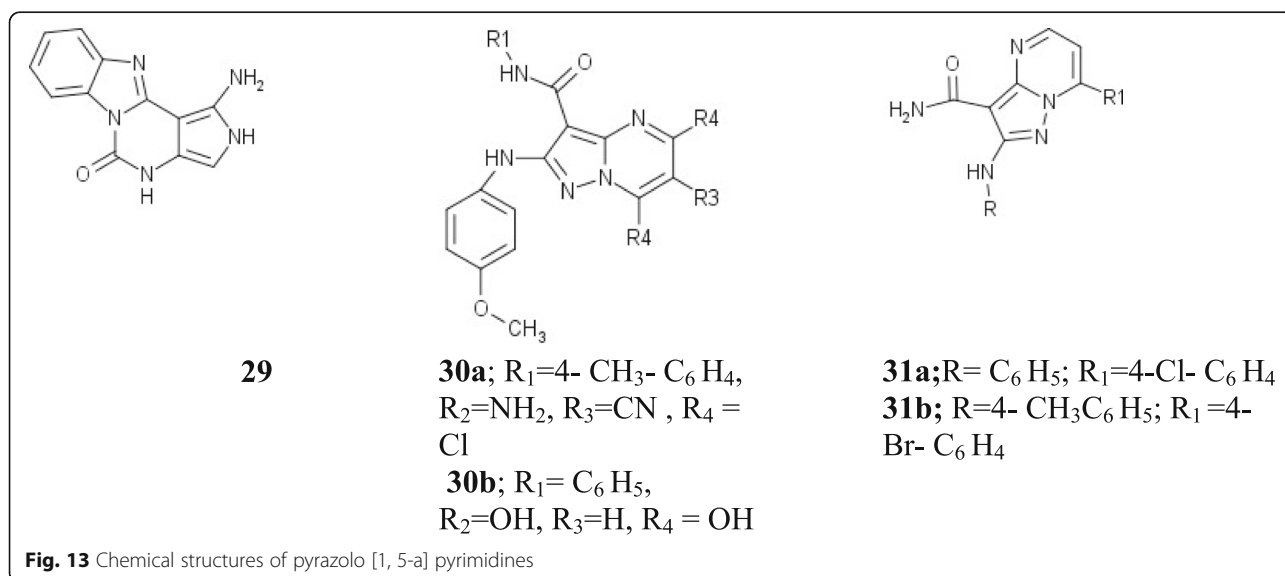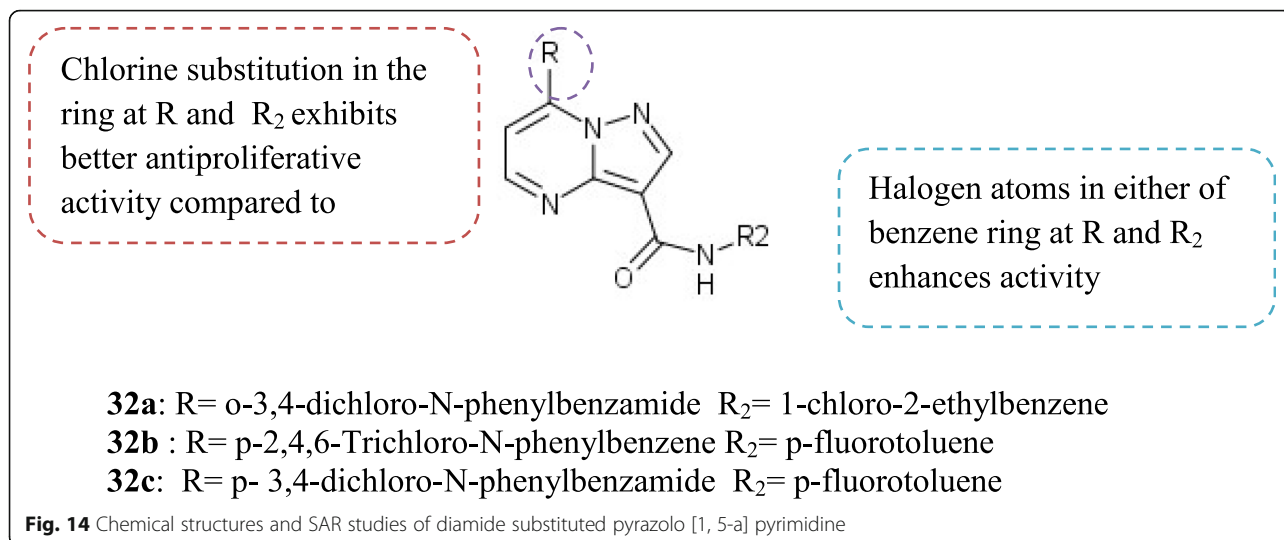**Fig. 12** Chemical structure of 2,3,4,6–tetra substituted pyrimidine derivatives

carbonitrile moiety [32]. Pyrazolo [1,5-a] pyrimidine derivatives which were tested for cytotoxicity by the MTT assay against cancer cell lines PC-3, HCT116, and HepG-2. **30a** (IC$_{50}$ = 67.27 ± 3.8 μM/mL) and **30b** (IC$_{50}$ = 58.44 ± 3.8 μM/mL) demonstrated the best activity against HCT116 and PC-3 cell lines. SAR studies revealed that the order of antitumor activity was 4 methyl phenyl> 4 chloro phenyl> phenyl derivative against the cell lines and chlorine atom at 2 position was more active than 3 and 4 positions [33]. In another study by the same group, antitumor activities of pyrazolo [1, 5-a] pyrimidines was screened against HepG-2 and MCF-7 using MTT assay. **31a** (IC$_{50}$ =63.2 ± 5.9 μg/mL) was reported have the best potential against MCF-7 carcinoma cells and **31b** (IC$_{50}$=70.3 ±4.1 μg/mL) against HepG2 carcinoma cells. SAR suggested that substitutions with bulky groups like methoxy and bromo gave significant antitumor activity (Fig. 13) [34]. A new series of diamide

substituted pyrazolo [1, 5-a] pyrimidine derivatives were reported. **32(a–c)** were active against HeLa cell lines where IC$_{50}$ value of each was less than 10 μM better than the marketed drug cisplatin. MTT assay was conducted to evaluate the cytotoxicity of the compounds. SAR discussed in Fig. 14 [35].

Chalcone-linked pyrazolo [1, 5-a] pyrimidines were reported as potential anticancer agents. **33** (IC$_{50}$ = 2.6 μM) was most potent against the MDA-MB231 cancer cell line as screened by MTT assay. The compound caused increase in the expression of apoptosis-inducing proteins like p53, p21, and Bax, and decreased proapoptotic proteins like Bcl-2 and procaspase-9, while stopping the cell cycle at sub G1 phase. The activity was mainly due to C-5 substitution phenylprop-2-en-1-one and C-7 phenyl ring [36].

The antiproliferative activity of the pyrazolo [1, 5-a] pyrimidine derivatives bearing nitrogen mustard moiety were tested for antitumor activity against cell lines A549, SH-SY5Y, HepG2, MCF-7, and DU145 via MTT assay. **34** inhibited the cell growth at the G1 phase of the cell cycle by inducing apoptosis in all the five cancer cell lines with the IC$_{50}$ range = 0.2 to 8.3 μM. The compound was tested against human HepG2, HCC tumor xenograft in nude mice, and gave better potency than positive control drug sorafenib and cyclophosphamide also being less toxic to normal human cells. N-mustard pharmacophore at C-7 and other substituent at C-5 of the pyrrazolo-pyrimidine derivatives exhibited potent in vitro cytotoxicity but when N-mustard pharmacophore is attached at C-5 and various aniline moieties at C-7 the compound formed is found to be ineffective [37]. In a consecutive study a regioselective synthesis of pyrazolo [1, 5-a] pyrimidine derivatives was conducted in presence of KHSO4(aq) assisted by ultrasound. The

29

30a; R$_1$=4- CH$_3$- C$_6$ H$_4$, R$_2$=NH$_2$, R$_3$=CN , R$_4$ = Cl
30b; R$_1$= C$_6$ H$_5$, R$_2$=OH, R$_3$=H, R$_4$ = OH

31a;R= C$_6$ H$_5$; R$_1$=4-Cl- C$_6$ H$_4$
31b; R=4- CH$_3$C$_6$ H$_5$; R$_1$ =4-Br- C$_6$ H$_4$

Fig. 13 Chemical structures of pyrazolo [1, 5-a] pyrimidines

Chlorine substitution in the ring at R and R_2 exhibits better antiproliferative activity compared to

Halogen atoms in either of benzene ring at R and R_2 enhances activity

32a: R= o-3,4-dichloro-N-phenylbenzamide R_2= 1-chloro-2-ethylbenzene
32b : R= p-2,4,6-Trichloro-N-phenylbenzene R_2= p-fluorotoluene
32c: R= p- 3,4-dichloro-N-phenylbenzamide R_2= p-fluorotoluene

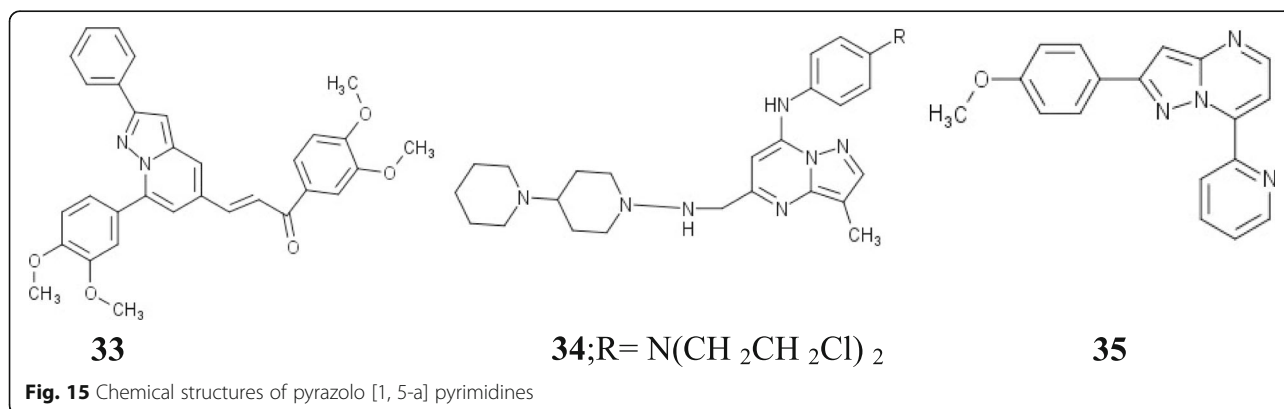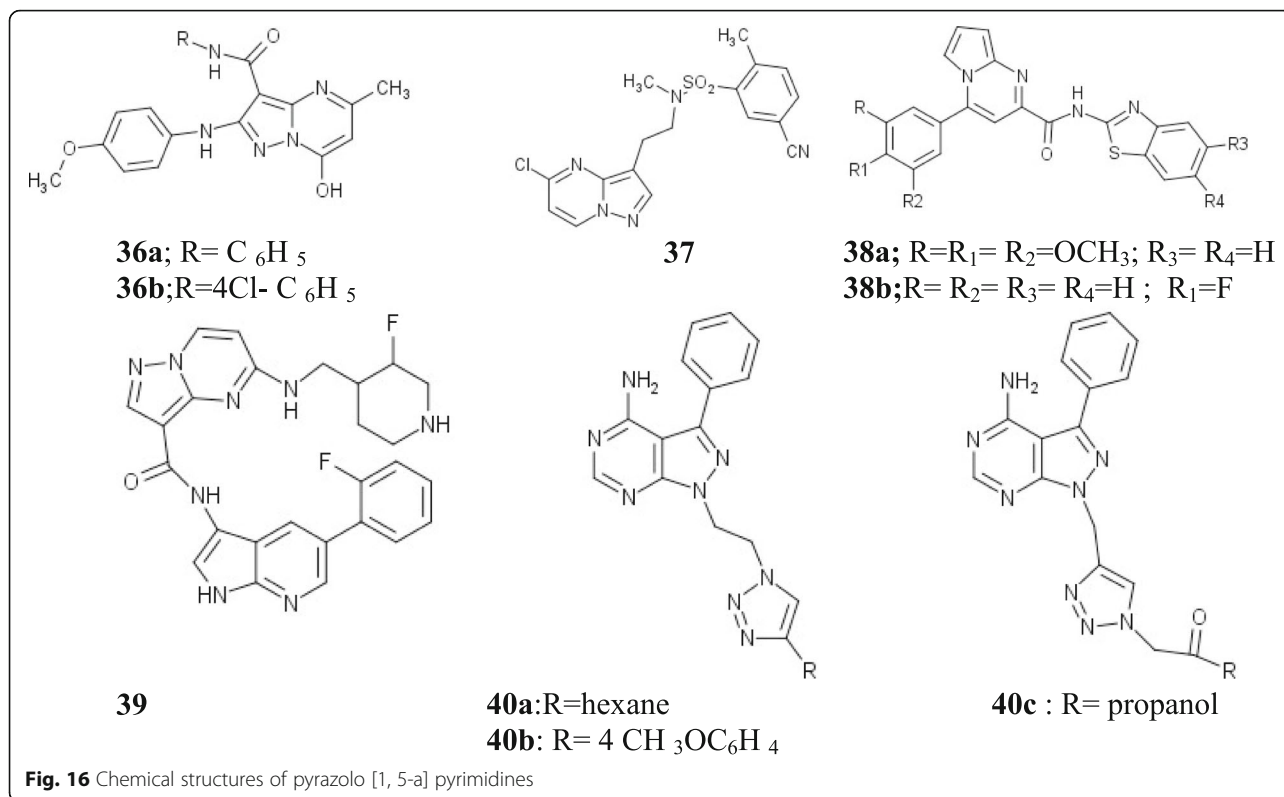**Fig. 14** Chemical structures and SAR studies of diamide substituted pyrazolo [1, 5-a] pyrimidine

metabolically viable cells cleave the MTT to purple forzman at 570 nM and results showed that compound **35** highly reduced the metabolism of MTT (Fig. 15) [38].

In vitro cytotoxicity of pyrazolo [1,5-a] pyrimidine against cell lines HCT-116, A549, HepG2, and MCF-7 was reported. **36a** and **36b** have the best cytotoxicity and cancer cell growth inhibitory properties compared to the standard drug DOX. Order of activity in substituted pyrazolo [1,5-a] pyrimidines was phenyl groups> 4-chlorophenyl >4-methylphenyl group [39]. Moreover, some novel pyrazolo [1,5-a]pyrimidines were reported as the inhibitors of CDK9 which is often linked to cancer. The study used PIK-75 as a positive control and found out that the synthesized compound **37** (IC_{50} = 203–> 1000 nM) is a better lead compound compared to PIK-75 due to lack of structural liabilities. The compound was also effective against FLT3 and MV4; 11 (IC_{50} = 0.177 and 219 µM respectively) cancer cell lines (Fig. 16) [40].

The synthesis of 2-aminobenzothizole conjugate linked with pyrazole [1,5-a] pyrimidines were tested for antitumor activity against cell lines namely A549, DU-145, MCF-7, ACHN, and HeLa via MTT assay. **38a** (IC_{50} range =1.94–3.46 µM) and **38b** (IC_{50} range = 2.01–7.07 µM) showed the best activity. Apoptosis was induced in caspase-3-dependent manner along with the arrest of cell cycle at G2/M and lowering of CDK1expression. SAR analysis suggests that substitution on the phenyl ring of aminobenzothiazole group does not show any promising effect on the activity whereas substitution on the 7-phenyl ring of pyrazolo [1,5-a]pyrimidine group is significant for the activity [41]. Interestingly, pim kinase inhibitors in the form of novel pyrazolo [1,5-a]pyrimidines were reported and it was concluded that compound **39** is a potential lead with low picomolar potency on the three isoforms of pim kinase. This particular enzyme is a significant target for cancer therapeutics but is hard to inhibit due to high affinity for ATP [42]. Novel 3-phenylpyrazolopyrimidine-1,2,3-triazole conjugates were studied for Src kinase inhibition and anticancer activity against SK-Ov-3, MDA-MB231, and HT-29 cell lines. **40a** and **40c** inhibited the enzyme with the

33   **34;**R= N(CH$_2$CH$_2$Cl)$_2$ **35**

Fig. 15 Chemical structures of pyrazolo [1, 5-a] pyrimidines

Fig. 16 Chemical structures of pyrazolo [1, 5-a] pyrimidines

IC_{50} range = 5.6 to 9.1 μM and **40b** also inhibited the HT-29 cell line growth by 73%. SAR suggests the presence of bulky group at N1 position is not tolerated (Fig. 16) [43].

Pyrazolo [3,4-d] pyrimidine derivatives

Novel pyrazolo [3,4 d]pyrimidine derivatives were antagonists to human cancer cell malignancy and ROS induced apoptosis. A panel comprising of 60 cancer cell lines were used to test the compounds and it was found that **41** effected the cell line growth in dose dependent manner at IC_{50} = 2 μM and also generate ROS species [44]. Previously, a series of 4,6-disubstituted pyrazolo[3,4-*d*]pyrimidine analogs inhibited the activity against the enzyme CDK2/cyclin E and Abl kinases as well as had anticancer attributes against MCF-7 and K-562 cell lines. **42** with IC_{50} = 19.8 μM (K-562) and 18.9 μM (MCF-7) was the most efficacious and as per reports on CHO cell line it is non-toxic to normal human cells [45].

In a subsequent study, compound **43** showed the best anticancer activity against enzymes CDK2/cyclin E Abl kinases with significant activity against the K-562 and MCF-7 cell lines. Better CDK2 inhibition was observed with compounds with thiophenethyl group at C-6 and monosubstituted aniline at C-4 positions compared to thiopentane at C-6 and disubstituted aniline at C-4 position. Significant enzyme inhibitory activity was reported

for compounds with 2-chloro, 3-nitro, and 4-methythio aniline at C-4 [46].

Synthesis as well as SAR studies of 6´-fluorocyclopentenyl pyrimidine derivatives was carried out along with assay against six human cancer cell lines including HCT-116, SNU-638, A-549, PC-3, SK-Hep-1, and MDA-MB-231 via SRB assay. **44a** (IC_{50} =1.10–2.17 μM) and **44b** (IC_{50} = 2.14–15.3 μM) exhibited good activity. The anticancer activity was attributed to arrest of s-adenosylhomocysteine hydrolase which led to inhibition of histone methyltransferase. N6-amino group was essential for anticancer activity whereas deamination, introduction of bulky alkyl group or amino group at C2 position results loss of activity [47]. In simultaneous study, 4-aminopyrazolo [3,4-d] pyrimidines were reported as the potent inhibitors of both IGFIR/Src and as well as an anticancer agent with minimum toxicity to normal cells. **45** inhibited the kinase enzymes efficiently as well as induced apoptosis in the cancer cell lines MCF-7 and A549 with the IC_{50} range = 9.7 to 15 μM. In vivo toxicity of the compound was evaluated on xenograft tumors and mutant Kras-driven lung tumorigenesis [48].

Pyrazolo [3,4-d] pyrimidines were reported as transmembrane RET inhibitors. **46** inhibited the RET phosphorylation and downstream signaling in BaF3/CCDC6-RET cells being a potent RET inhibitor in the various biochemical assays conducted. It had an IC_{50} = 61 nM

which makes it 6-folds more potent than the second best compound which was moderately efficacious against BaF3/CCDC6-RET cells with IC_{50} = 433 nM in the cellular assays. Flexible side chain on the isoxazoline moiety like hydroxymethyl turned out to be most potent RET kinase inhibitor. Extending the carbon chain, capping the free hydroxyl group or addition of esteric substituent/alkyl substituents bearing a terminal pyrrolyl/morpholinyl moieties led to decrease in activity [49]. In a parallel study, in vivo and in vitro anticancer activity of pyrazolo[3,4-d]pyrimidines, **47(a–b)** were reported in the form of prodrug. The compounds were evaluated against U-87 cancer cell line (in vitro), against Src and Abl kinase enzyme and also in vivo [50].

A series of novel N-4 substituted benzylidene acetohydrazide pyrazolo[3,4-d] pyrimidine was assessed for in vitro cytotoxicity against MCF-7, A549, and HT-29 cell lines. The compounds exhibited potent antitumor activity with **48** being the most potent. Molecular docking studies against the EGFR-TK revealed the compound was most effective inhibitor with docking score of – 28.8 Kcal/mol [51]. Pyrazolo[3,4-d] pyrimidine-3-carbonitriles were evaluated for activity against Hep 2 cancer cell line using MTT assay. Compound **49a** (IC_{50} =36.9 μM) and compound **49b** (IC_{50} = 21.3 μM) were found to be more active compared to the standard anticancer drug 5-FU (IC_{50} = 41.5 μM). The presence of NH-C-S moiety and N-tosyl group in the pyrazolo-pyrimidine may be accounted for the activity [52].

Pyrazolo[3,4-d]pyrimidinones derivatives **50 (a–e)** containing 1,2,3-triazole displayed good anticancer activity against the MCF-7 and HCT-116 cell lines using MTT assay. At the concentration of 100 μM, the inhibition percent of the cancer cell growth by these compounds is of the range 43–75% with tamoxifen as positive control. The major anticancer activity in this series of compounds is observed due to the presence of triazole group linked to the pyrazolo-pyrimidine ring through the methylene spacer linker [53]. Previously, novel pyrazolo[3,4-d]pyrimidine derivatives having anticancer potential were evaluated using MTT assay. **51** was tested against a panel of various cancer cell lines had the best potential against the A549 cells with IC_{50} = 2.49 μM which is comparatively better than the positive control DOX [54].

In a study on cross-docking simulation of pyrazolo-[3,4-d]pyrimidine on Bcr-Abl T315l mutant, **52a** and **52b** exhibited the best activity. 4-Bromo atom in paraposition of the N1 side chain of phenyl ring was responsible for interaction with the T315l mutant. The antitumor activity was tested in vivo using a mouse model xenograft [55]. Interestingly, a series of pyrazolo[3,4-d] pyrimidine was formed via substituting different polar moieties in C4 and C-6 positions of a promising anti

leukemia lead. The compounds exhibited Src/Abl inhibitory activity and potent antiproliferative activity in leukemia cell lines (KU-812, MEG-01, and K-562). **53a** and **53b** showed good in vitro ADME properties as well as active in hypoxic leukemia cells [56].

Pyrazolo [3,4-d] pyrimidines with benzylidene hydrazinyl group were tested for their cytotoxic activity against MCF-7 cell line. 1-Phenyl substituted derivatives exhibited better activity compared to 1-(4-methoxyphenyl) derivatives compound **54** (IC_{50} = 7.5nM) was the most potent [57]. One pot synthesis of pyrazolo [3, 4-d] pyrimidine derivatives along with their antiproliferative activity was reported. **55 (a–d)** showed the most potency due to the presence of p-Me-Ph, p-Cl-Ph, or p-OMe-Ph group at C-3 position and phenyl or 2-quinolinyl groups at N-1 [58]. The green synthesis of pyrazolo [3, 4]-pyrimidine-thiones was carried out and **56 (a–b)** were most potent with IC_{50} = 66 and 35 mg/ml against EAC cell lines (Fig. 17) [59]. Compound 57 was reported to be most active among the series of substituted pyrazolo [3, 4-d] pyrimidines with the in vitro evaluation against MCF-7 cancer cell lines. The level of hydrogen peroxide and activity of superoxide dismutase were noticed to increase significantly whereas catalase activity and glutathione peroxidase levels were lowered. SAR is illustrated in Fig. 18 [60].

Pyrazolo [4,3-d]-pyrimidine

Pyrazolo [4, 3-d] pyrimidines with substitutions at 3, 5, 7 positions were reported as the inhibitors of CDK and also tested for in vivo and in vitro anticancer activity. **58** was the most potent, inhibited CDK 2, 5 and 9 (IC_{50}= 0.002 μM) as well as exhibits both activities, and was better than the positive control CR8. When tested against 60 panels of cancer cancer cell lines, apoptosis was induced due to activation of caspases, dephosphorylation of CDK substrates, downregulation of XIAP, and MCL-1 and cleavage of PARP-1. In vivo activity was evaluated against various xenograft models. It was suggested that the activity was due to the modification of 2-aminoethylthio group [61]. Previously, pyrazolo [4,3-d] pyrimidine a bioisoester of roscovitine was evaluated for the dual property of both CDK inhibition and antiproliferative activity. **59** was the bioisoster responsible for both the activities of CDK inhibition being the primary one. The anticancer activity of the compound was tested against a cancer cell line panel of 60 (IC_{50} range = 3.6 ± 0.3 to 11 ± 1.8 μM) [62].

In vitro activity of substituted pyrazolo[4,3-d]-pyrimidine derivatives were reported against MCF-7, HT-29, and HepG2 tumor cell lines. Tetrazole ring fused to pyrazole [4,3-d]-pyrimidine **60** was most potent against all the cell lines with IC_{50} range = 0.13 ± 0.03 to 0.36 ± 0.04 μmol/L respectively. The presence of tetrazole
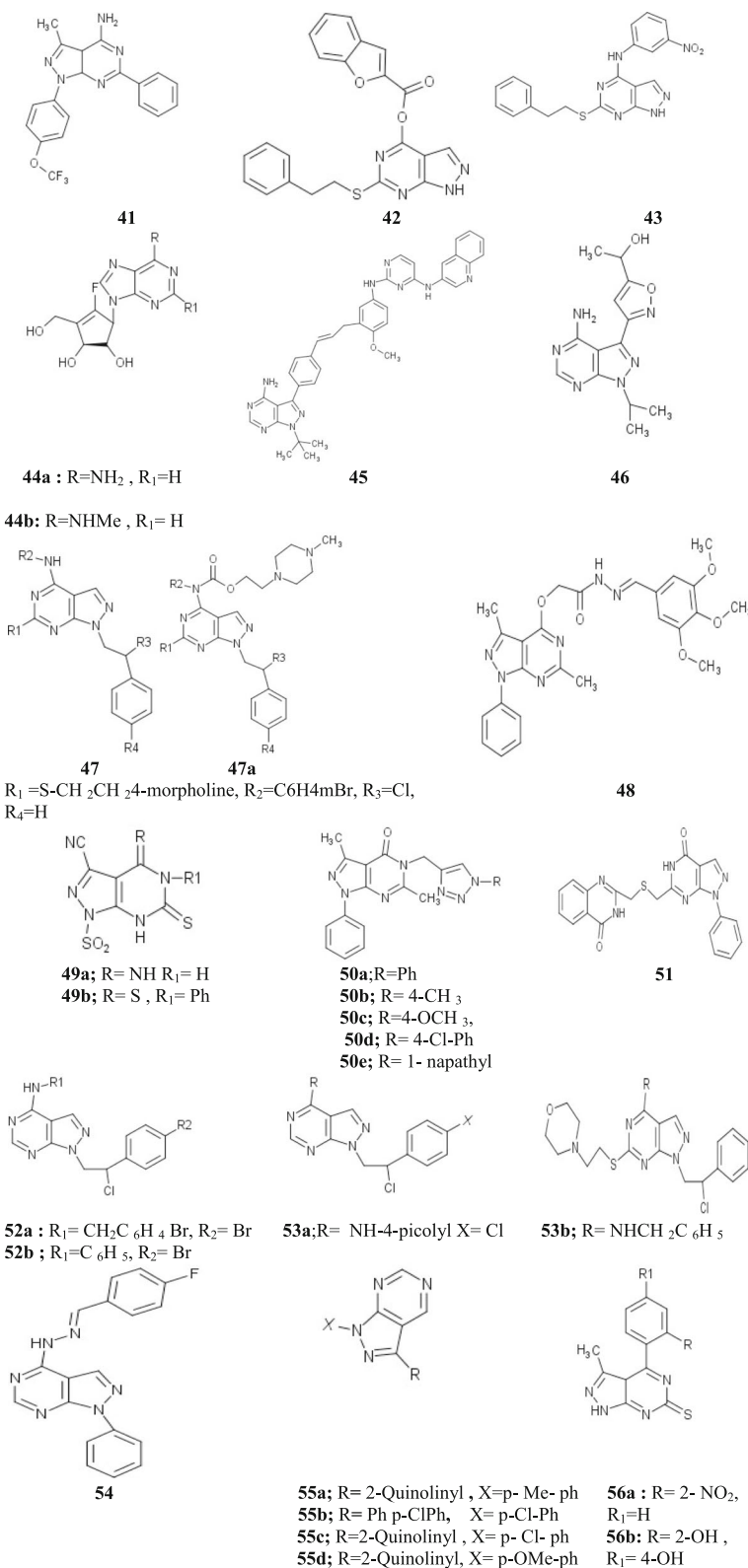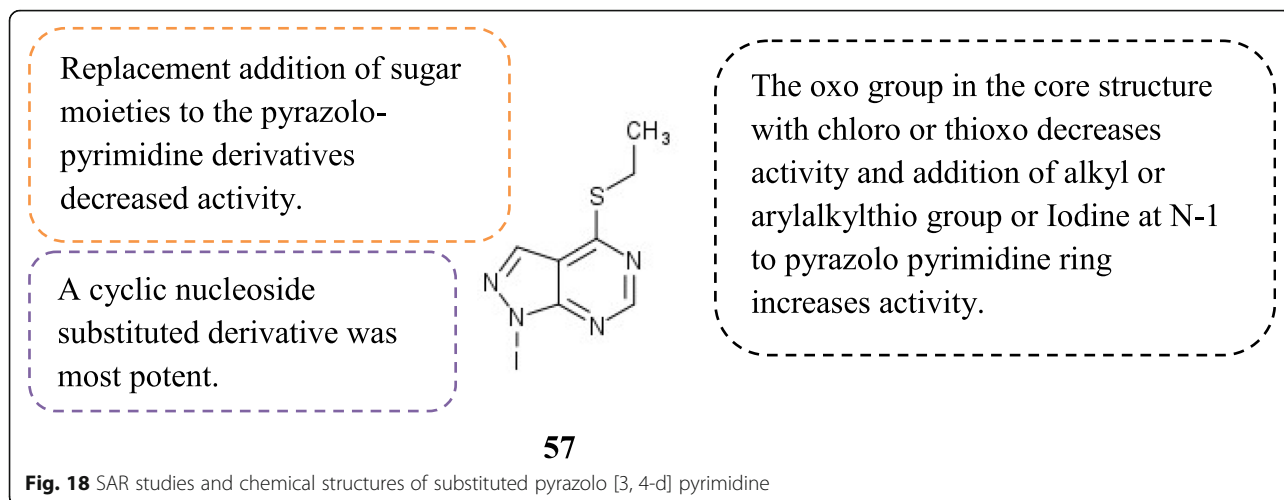
41

42

43

44a : R=NH$_2$, R$_1$=H

44b: R=NHMe , R$_1$= H

45

46

47   **47a**

R$_1$ =S-CH$_2$CH$_2$4-morpholine, R$_2$=C6H4mBr, R$_3$=Cl,
R$_4$=H

48

49a; R= NH R$_1$= H
49b; R= S , R$_1$= Ph

50a;R=Ph
50b; R= 4-CH$_3$
50c; R=4-OCH$_3$,
50d; R= 4-Cl-Ph
50e; R= 1- napathyl

51

52a : R$_1$= CH$_2$C$_6$H$_4$ Br, R$_2$= Br
52b ; R$_1$=C$_6$H$_5$, R$_2$= Br

53a;R= NH-4-picolyl X= Cl

53b; R= NHCH$_2$C$_6$H$_5$

54

55a; R= 2-Quinolinyl , X=p- Me- ph
55b; R= Ph p-ClPh,   X= p-Cl-Ph
55c; R=2-Quinolinyl , X= p- Cl- ph
55d; R=2-Quinolinyl, X= p-OMe-ph

56a : R= 2- NO$_2$,
R$_1$=H
56b: R= 2-OH ,
R$_1$= 4-OH

Fig. 17 Chemical structures of pyrazolo [3, 4-d] pyrimidines

Replacement addition of sugar moieties to the pyrazolo-pyrimidine derivatives decreased activity.

A cyclic nucleoside substituted derivative was most potent.

The oxo group in the core structure with chloro or thioxo decreases activity and addition of alkyl or arylalkylthio group or Iodine at N-1 to pyrazolo pyrimidine ring increases activity.

57

Fig. 18 SAR studies and chemical structures of substituted pyrazolo [3, 4-d] pyrimidine

moiety fused to the pyrazolo-pyrimidine ring and also the nature of the substituted groups at the aryl ring-linked carbohydrazide with the pyrazole unit led to increased cytotoxic activity [63]. In a simultaneous study, a series of 5-substituted 3-isopropyl-7-[4-(2-pyridyl) benzyl] amino-1(2) H-pyrazolo [4,3-d] pyrimidine derivatives were reported and it was concluded that compound **61** was the most potent inhibitor of both CDK2 (IC_{50} = 21n M) and CDK5 (IC_{50} = 35 n M). The activity was mainly due to the presence of hydroxyalkylamines at the 5 position of the pyrazolo-pyrimidine ring. The

cell cycle was arrested at S and G2/M phase which resulted in induction of apoptosis [64].

In study selective inhibitors of CDK2, CDK5, and aurora A kinase with in vitro anti-angiogenic activity were reported. **62** was the most potent in the series and was known to inhibit all the three kinase enzymes as well as cause the downregulation of cyclins A and B, the dephosphorylation of histone H3 at Ser10, and the induction of mitochondrial apoptosis in the HCT-116 cancer cell line. It also reduced cell migration in human endothelial cells. The anti-angiogenic property of the

58   **59**   **60**

61   **62**   **63**

Fig. 19 Chemical structures of substituted pyrazolo [4, 3-d] pyrimidine
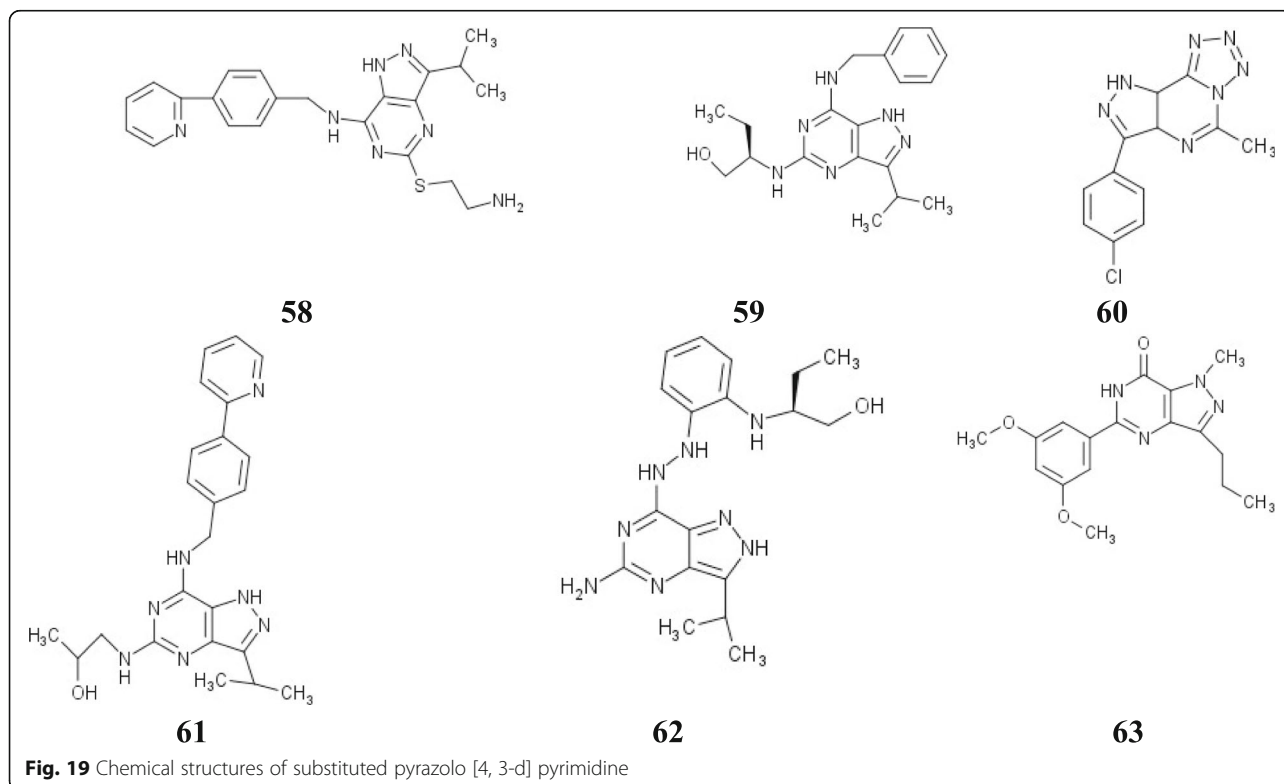
compound is linked to CDK5 inhibition [65]. The microwave assisted synthesis of 1H-pyrazolo [4,3-d] pyrimidin-7(6H)-ones and their anticancer activity were reported. The compounds were tested against HeLa, CAKI-I, PC-3, MiaPaca-2, and A549 human cancer cell lines by MTT assay. **63** was the most potent as it exhibits anticancer activity against all cell lines and the IC_{50} range = 14 to 38 μM, with apoptosis mechanism along with mTOR inhibition at nM potency (Fig. 19) [66].

Pyrrolo-pyrimidine derivatives

7H-pyrrolo [2,3-d] pyrimidine derivatives were reported as potential FAK inhibitors. **64** suppressed the FAK enzymatic action at IC_{50} = 5.4 nM and inhibited the MDA-MB231 and A549 cancer cell lines with the IC_{50} range = 3.20 ± 0.41 to 17.41 ± 1.3 μM respectively. The compound had activity against a panel of 26 kinases and was less cytotoxic against the HK2 cell line. It led to induction of apoptosis and suppression of migration in lung cancer cell line in a dose dependently. The SAR studies are illustrated in Fig. 20 [67].

Previously, some Src tyrosine kinase family selective novel pyrrolo [2, 3-d] pyrimidine derivatives were reported. The inhibitory activity was tested against the Fyn, Lyn, Hck, and c-Src members of the Src kinase family. **65** was known to show the most potent activity Fyn, Lyn, and c-Src though it was non selective and not show activity against the Hck. PP2 and CGP77675 were used as positive control [68]. A novel series of pyrrolo-pyrimidine and pyrrolo-pyridine compounds bearing pyridazinone moiety were studied as c-Met kinase inhibitors, evaluated for in vitro and in silico molecular docking activity against the A549, HepG2, PC-3, and MCF-7 cancer cell lines. **66a** was more active than **66b** showed excellent activity against the cell lines with the IC_{50} (μM) range 3.62 ± 1.24 to 9.61 ± 0.95 and 2.73 ± 0.98 to 3.77 ± 1.24 respectively. The compound induced apoptosis in HePG2 cell line. The compounds were screened against 4 tyrosine kinase (Slp-3, VEGRF-2, c-Kit, and EGFR). EWG on the aryl group in the pyridazinone moiety makes the compound increasingly active. The pyridine derivatives containing pyridazinone moiety are better than the pyrimidine moieties comparatively and, thus, a pyridine derivative was selected as c-Met kinase inhibitor [69].
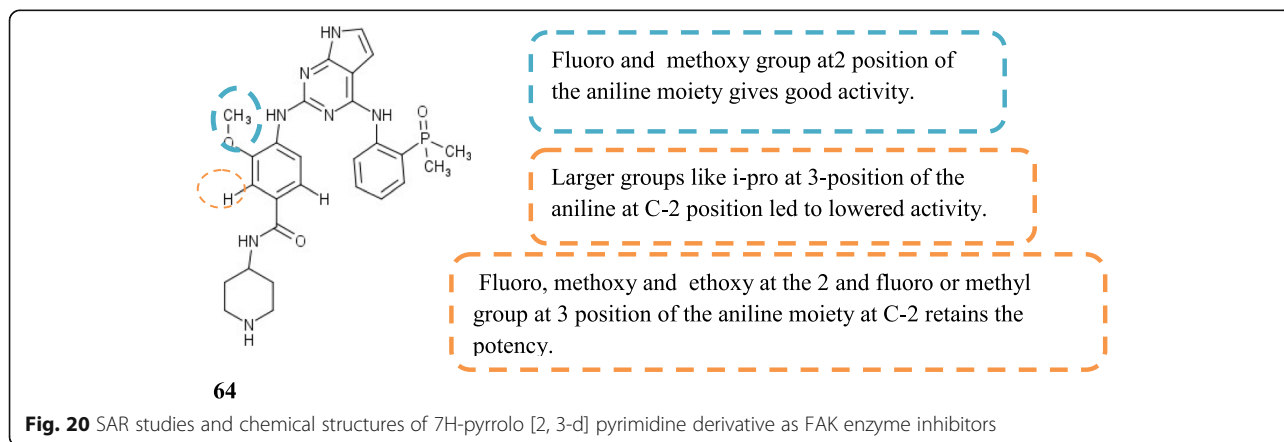
A series of novel sulfonamide bearing pyrrolo-pyrimidine derivatives were screened against the MCF-7 cancer cell line. **67a** and **67b** (IC_{50} = 8.30 and 8.39 μM) exhibited comparable activity to the positive control DOX. For the study of the mechanism of action, a molecular docking study on the compounds was conducted for the Src kinase enzyme and it was suggested that the inhibition of this enzyme is the reason for the anticancer potential of the compounds (Fig. 21) [70].

Triazole pyrimidine derivatives
Triazolo [1, 5-a] pyrimidines

Recently [1,2,4]triazolo[1,5-a]pyrimidine derivatives were screened for in vitro antiproliferative activities against HeLa, HCT116, and A549, via MTT assay. Compound **68** efficiently inhibited the growth of A549 and HeLa, cell lines with IC_{50} = 1.02 and 0.75 μM respectively. HeLa cells arrested in G2/M phase of cell cycle and had a detrimental effect on the cell morphology and microtubule networks. The SAR is depicted in Fig. 22 [71].

In a study based on the cytotoxicity of 1, 2, 3-triazoles and 1, 2, 4-triazolo fused with [1, 5-a] pyrimidines in MCF-7 cancer cell line. **69** showed better activities compared to other compounds in the series. VEGF and its receptor VEGFR are essential for the neovasculature; it is also associated with the progression of various malignancies which make this a significant target for the cancer treatment. The effect of the compounds on the MCF-7 cancer cell line was tested with the help of immunoblotting assay. 1, 2, 3-triazoles cause the inhibition of VEGFR1 giving a lead to evaluate the effect of EFT of
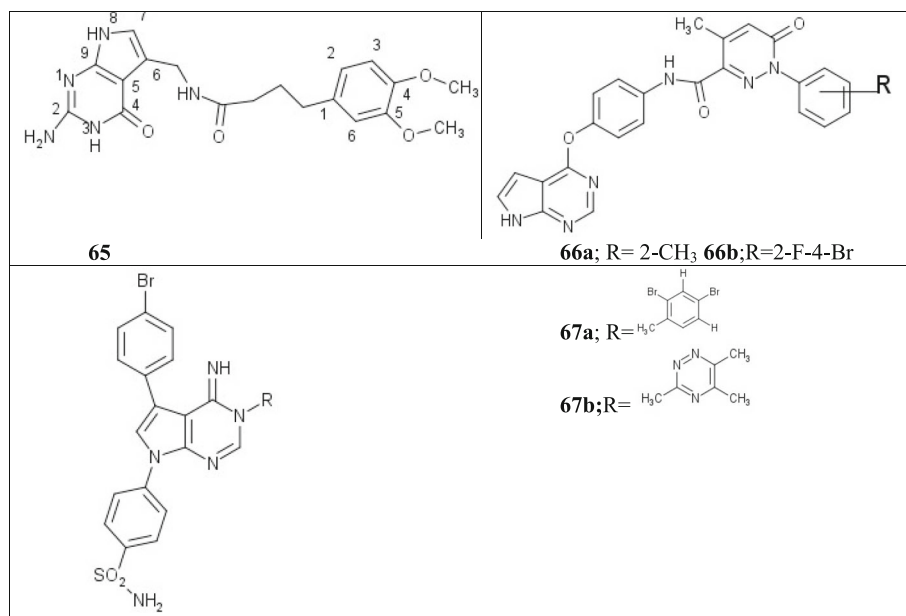

Fluoro and methoxy group at2 position of the aniline moiety gives good activity.

Larger groups like i-pro at 3-position of the aniline at C-2 position led to lowered activity.

Fluoro, methoxy and ethoxy at the 2 and fluoro or methyl group at 3 position of the aniline moiety at C-2 retains the potency.

64

Fig. 20 SAR studies and chemical structures of 7H-pyrrolo [2, 3-d] pyrimidine derivative as FAK enzyme inhibitors

Fig. 21 Chemical structure of pyrrolo-pyrimidine derivatives

VEGFR1. Results concluded that the compounds are non-toxic to normal human cells and EFT downregulates the expression of VEGFR1 thus, imparting activity by targeting VEGFR1 in breast cancer cells [72]. In a report [1, 2, 4] triazolo fused with [1,5-a]pyrimidines linked with monomeric and dimeric steroids the compounds elucidated activity against a panel of six solid tumor cell lines. The results determined **70a–b** as the most potent against T-47D and WiDr cells, better than positive control cisplatin [73].

Novel steroidal[17,16-d][1,2,4]triazolo [1,5-a]pyrimidines were determined as antitumor agents against PC-3, MCF-7, and EC9706 cancer cell lines. **71(a–c)** were found to show great activity in the evaluation by the cytotoxic assay. The presence of p-isopropyl and o-phenoxy on the phenyl rings at R position increased the anticancer activity whereas the phenyl rings at R when replaced by heteroaromatic rings did not change the activity (Fig. 23) [74].

Triazolo [4, 5-d] pyrimidine

LSD1 is known to play a significant role in lysine methylation and over expression of the LSD1 lead to progression of certain human malignant tumor. In the year 2017, [1–3] triazolo [4, 5-d] pyrimidine derivatives were reported for their LSD1 inhibition properties. **72** with the IC_{50} = 0.564 μM inhibited the LSD1 very efficiently. The compound demonstrated selectivity for LSD1 over monoamine oxidase by acting as a reversible inhibitor. When the MGC-803 cells were treated with compound **72**, the activity of LSD1 was instantly inhibited along with the migration of the cells to a great extent [75]. Previously worked on a series of hydrazone bearing [1,2, 3]triazolo[4,5-d]pyrimidine derivatives and evaluated
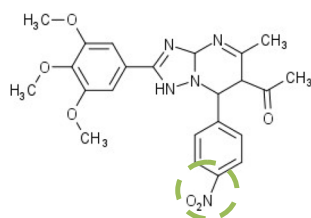
Fig. 22 SAR studies and chemical structures of [1, 2, 4] triazolo [1, 5-a] pyrimidine derivatives
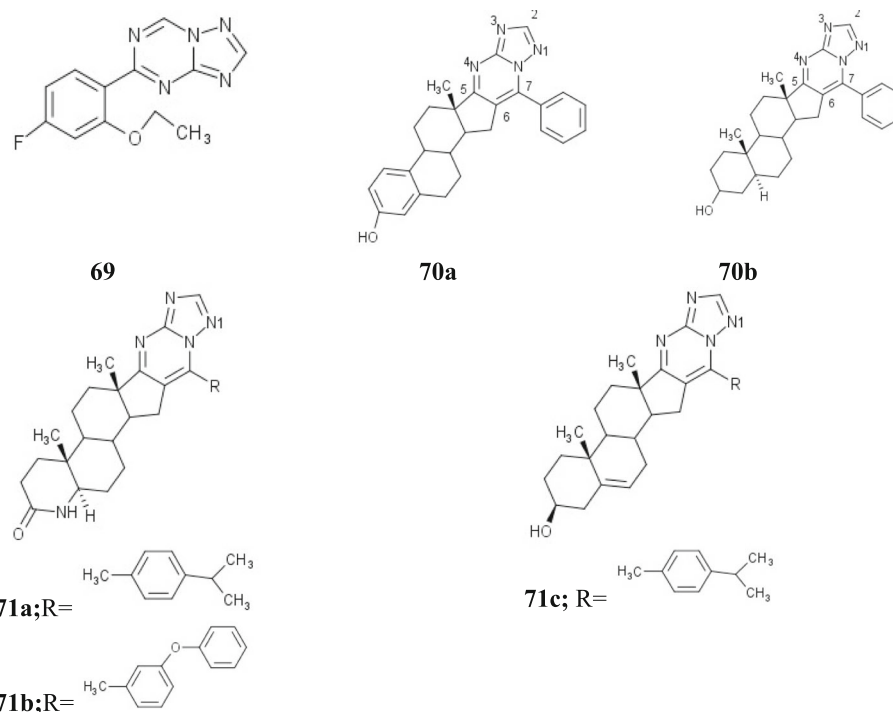
Fig. 23 Chemical structure of triazolo pyrimidines

their activity against different human cancer cell lines via MTT assay. Compound **73** showed the best efficiency by being selective towards normal cell line and the cancer cell line. The compound had an IC$_{50}$ = 0.87 µM against the MGC-803 cell line and IC$_{50}$ = 56.17 µM for the GES1. On further analysis, it was concluded that the compound induces apoptosis by attacking the mitochondrial pathway by decreasing the mitochondrial membrane potential, upregulation of the expression of Bax, Bak, activation of caspase-9/3, and p-53, and downregulation of that of Mcl-1 and Bcl-2. The compound also inhibited formation of MGC-803 cells at concentration of 0.8 µM (Fig. 24) [76].

Other triazolo pyrimidine derivatives

A group of novel pyrimidine-2, 4-dione–1,2,3-triazole and furo[2,3-d]pyrimidine2-one–1,2,3-triazole hybrids derivatives were reported. The evaluation was carried out with a vast range of cell line and 5-FU was used as positive control. **74** exhibited the best cytostatic activity against HepG2 and HeLa cells with an IC$_{50}$ = 2.67 µM and 6.51 µM respectively. In terms of mechanism, cytostatic effect was attributed to its property of inhibition of Wee-1 kinase and finishing of sphingolipid signaling mediated by acid sphingosine kinase 1 and ceramidase. The compound was non-mitochondrial toxic agent (Fig. 24) [77].
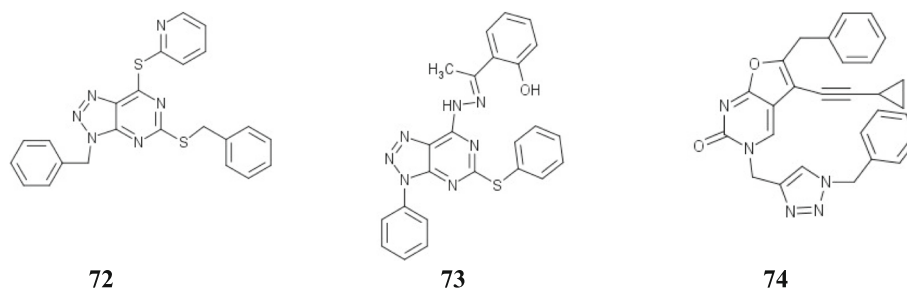

Fig. 24 Chemical structure of Triazolo and other triazolo pyrimidine derivatives

Imidazolo pyrimidine derivatives

Recently, antiproliferative activity of N-9- and N-7-1,2,3 triazole analogs of 2,6-di-substituted purines was reported. They were tested against HCT-1, THP-1, IMR-32, and A-549 cancer cell lines. **75** was the most potent against the THP-1 and A-549 cell lines with the IC_{50} = 0.08 and 0.4 μM respectively. The activity was mainly due to C6 position substitution with amines like aminoethnol and benzyl amine, and C2 position was substituted with cyclic secondary amines like piperidine and pyrolidine [78]. Earlier, the anticancer activity of newly synthesized imidazo[1,2-a]pyrimidine mannich bases was studied. In a series of 29 compounds **76(a–c)** were found to be the most potent giving similar activity to DOX. The compounds were evaluated against the human lung, pancreatic, cervical, and breast adenocarcinoma cell lines. The compound **76a** inhibited the growth of all the four cancer cell lines. The two series of compounds were evaluated by the SRB assay. From the SAR analysis, it was concluded that the main activity of the compounds is due to the C-2 and C-3 substitutions [79].

(RS)-9-(2,3-dihydro-1,4-benzoxaheteroin-2-ylmethyl)-9H-purines were reported for anticancer activity and tested against the MCF-7 cancer cell line. **77(a–c)** are known to show the best activity with the IC_{50} range 2.75 ± 0.03 to 7.64 ± 0.03 μM. Compound **77b** showed 3.3-fold higher activity than their bioisosters. The compounds inhibited eIF2α phosphorylation and induced apoptosis in the p53-independent manner (Fig. 25) [80].

Pyrido pyrimidine derivatives

Pyrido [1,2-a]pyrimidine derivatives

For the 3-carboxamide derivatives of pyrido[1,2-a]pyrimidine, the compounds were tested against DU145, A549, SiHa, and MCF-7 using 5-FU as positive control. **78b** and **78a** showed relatively higher activity with IC_{50} = 3.6 ± 0.11 and 3.2 ± 0.12 μg/mL against A549 respectively. In the SAR, it was established that thien-2-yl group in place of phenyl group at 6th position of the compounds showed better activity (Fig. 26) [81].

Pyrido[2,3-b]pyrimidine derivatives

Novel pyrido[3′,2′:4,5]furo[3,2-d]pyrimidines were reported. The compounds were screened against Hela, neuro-2a, Colo 205, and A549 tumor cell lines using the MTT assay. **79a** and **79b** were the most potent with IC_{50} range = 5.8 to 3.6 μM. Pyrimidinone ring had better activity than furo [2, 3-b] pyridine ring system. Nitrogen substitution on N3 resulted in marginal reduction the cytotoxicity (Fig. 26) [82].

Pyrido[2,3-d]pyrimidine derivatives

In a parallel study HepG-2 and HCT-116 cancer cell lines were screened by novel pyrido[2,3-d]pyrimidine derivatives and **80(a-d)** were found to be most active (Fig. 26) [83]. Pyrido[2,3-d] [1,2,4]triazolo[4,3-a]pyrimidine derivatives and pyrido [2,3-d]pyrimidines were evaluated against two cancer cell lines, namely PC-3 and A-549. **81** (IC_{50} = 0.36 μM) showed activity against both the cell lines with 5-FU (positive control) but was more sensitive on cell line PC-3. The cell cycle was arrested at G1 phase and induction of apoptosis in PC-3 cell line was due to caspase-3 dependent pathway. SAR is illustrated in Fig. 27 [84].

Pyrido[2,3-d]pyrimidine derivatives were screened for their cytotoxic properties against PC-3 cell line using the MTT assay. **82** (IC_{50} = 7.0 μM) exhibited strong activity
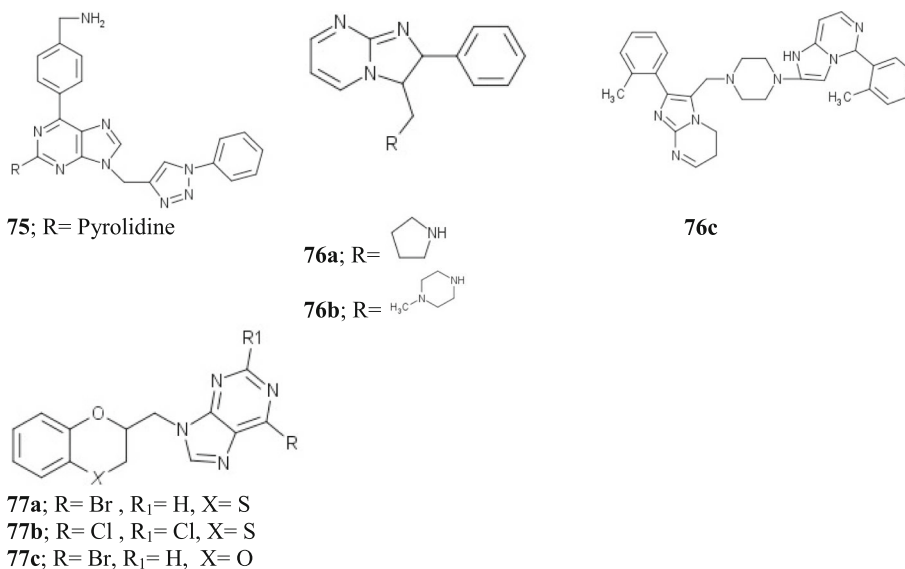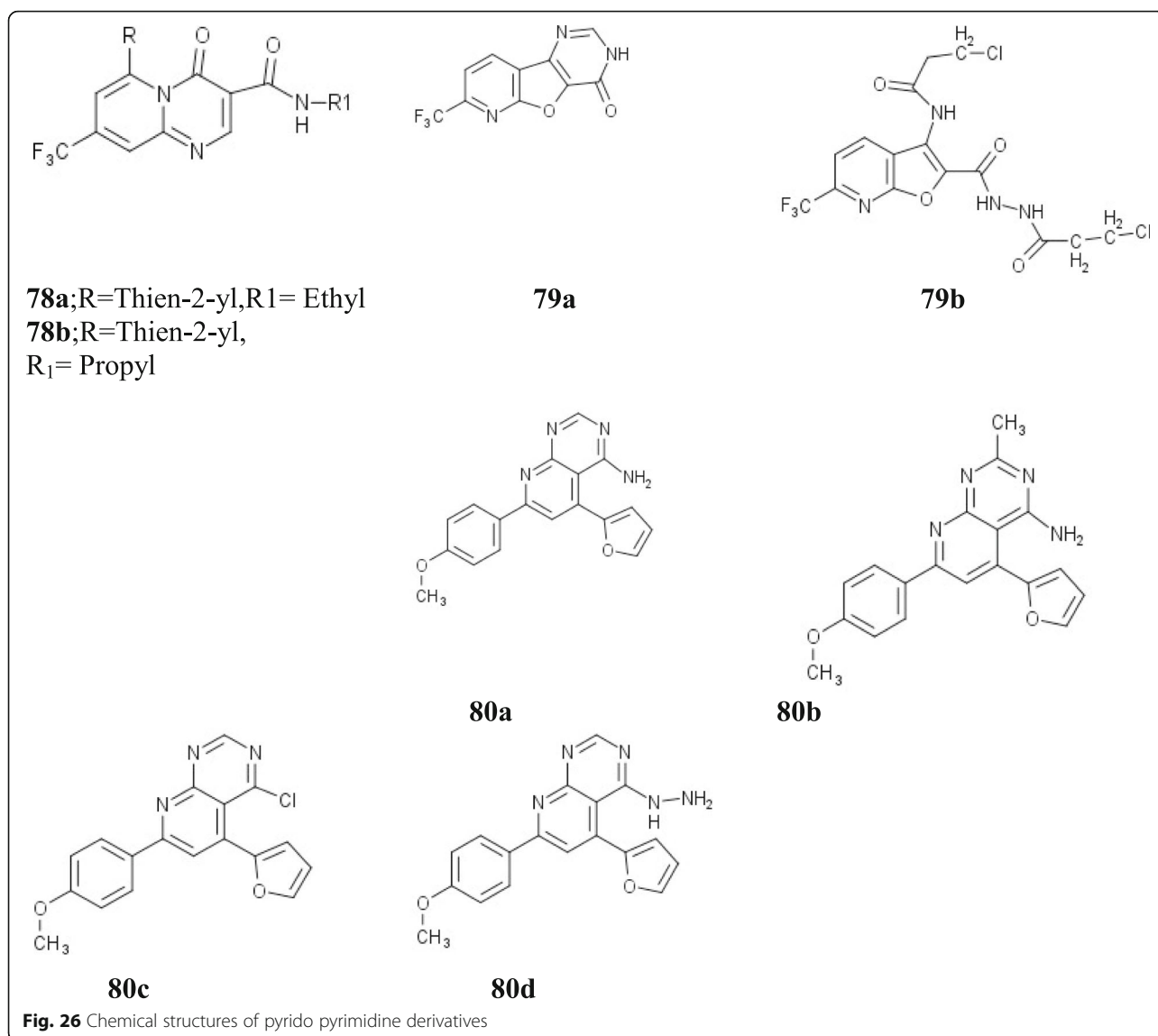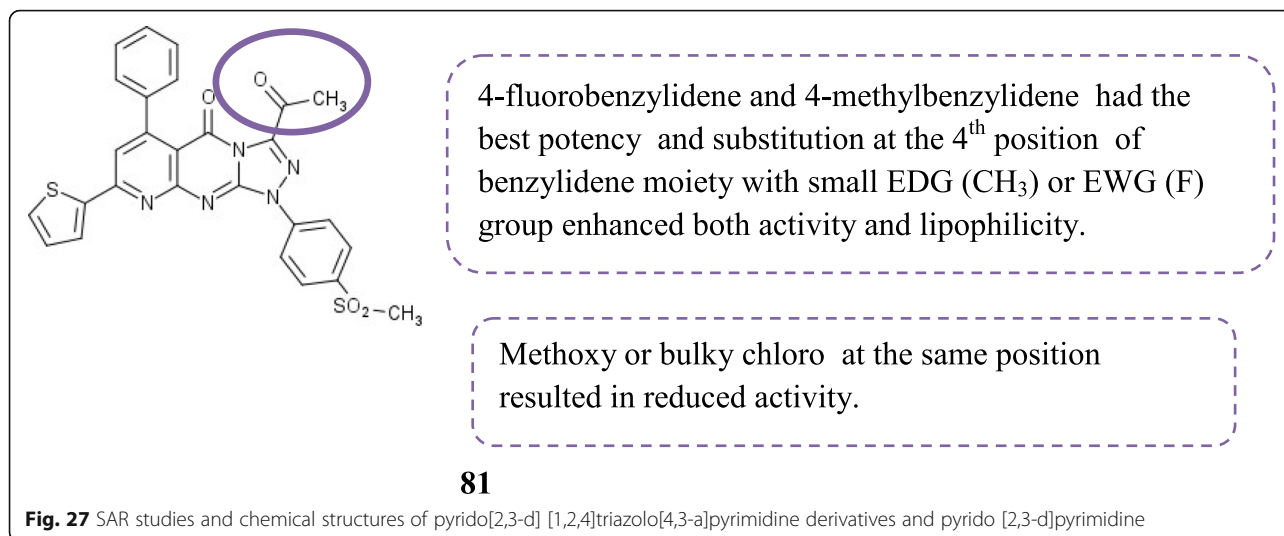

Fig. 25 Chemical structure of imidazolo-pyrimidine derivatives

78a;R=Thien-2-yl,R1= Ethyl
78b;R=Thien-2-yl,
R_1= Propyl

79a

79b

80a

80b

80c

80d

Fig. 26 Chemical structures of pyrido pyrimidine derivatives

compared to the positive controls due to the ability of increasing capase-3 levels leading to dose dependent cell cycle arrest. The screening reveals that the pyridopyrimidine nucleus is better than the corresponding quinazoline, mainly when the substitutions in 2 and 4 positions are different. Substitutions SCH_3 or $SeCH_3$ located at paraposition of the aryl moiety improved the activity [85]. Simultaneously, the 1,8-naphthyridines substituted isosters of 2,4,5,7-tetrasubstituted pyrido[2,3-d]pyrimidines were reported. The cytotoxic activity was investigated against MCF7 cancer cell line where **83a** and **83b** (IC_{50} = 7.70 and 7.54 µM respectively) were efficacious compared to DOX (IC_{50} = 8.48 µM), the positive control [86].

A study of potential in vitro cytotoxic activity of selenium and sulfur derivatives of pyrido[2,3-d]pyrimidine were screened against four cancer cell lines, HTB-54, HT-29, MCF-7, and CCRF-CEM. **84 (a–c)** exhibited cytotoxic effects in all cell lines tested where **84c** was selectively potent against MCF-7 with cisplatin and etoposide as positive control. It was concluded that the cell death was induced without affecting cell cycle phases. The derivatives with a SeH group in the 4th and 2nd positions usually have better activity than the analogs with selenoalkyl group [87]. In later researches, cancer cell lines HeLa, A549, PANC 1, and MDA MB-231 at < 10 µM concentration were used to test the anticancer activity of new triazole/isoxazole functionalized 7-(trifluoromethyl) pyrido [2,3-d]pyrimidine derivatives. **85a** against PANC1 and **85b** against A549 had the best activity better compared to positive control nocodazole. The high anticancer activity of the compound was due to the presence of ethyl chain at C-2 position and the long alkyl/perfluoroalkyl chain at R on triazole ring. Many

Fig. 27 SAR studies and chemical structures of pyrido[2,3-d] [1,2,4]triazolo[4,3-a]pyrimidine derivatives and pyrido [2,3-d]pyrimidine

structures showed activity regardless of the substitutions on the C-2 position or the triazole ring [88].

Interestingly, some pyrido[2,3-d]pyrimidine derivatives inhibiting EGFR were reported for elucidating potency against A549, MCF-7, BT-474, MDA-MB-231, and SK-BR-3. **86a** and **86b** were found to have potent inhibition activity against the EGFR tyrosine kinase with the IC_{50} = 2.97 nM and 3.58 nM respectively and also showed good anti-proliferative activity against the SK-BR-3 cells IC_{50} = 3.10 (**86a**) and 5.87 μM (**86b**). Similar binding pattern to the EGFR was observed standard drug gefitinib (Fig. 28) [89].

Compounds **87 (a–b)**, pyrido[2,3-d]pyrimidine derivatives with alkyl triazole, were reported for exhibiting anticancer activity with IC_{50} range = 8.16 ± 0.68 to 19.25 ± 1.46 and 15.01 ± 1.54 to 6.20 ± 0.68 μg/ml respectively. They were active against cancer cell lines THP-1, U937, and COLO 205 as screened by MTT assay. Activity of the abovementioned compounds was better compared to positive control etoposide, against U937 cell lines. SAR is illustrated in Fig. 29 [90].

Pyrido[3,4-d]pyrimidines
Potential anticancer agents in the form of new pyrido [3, 4-d]pyrimidine derivatives were evaluated against the panel of 60 cancer cell lines resulting to be selective towards the renal and breast cancer. **88a** had good activity and selectivity towards both breast and renal cancer cell lines. **88b** had selective for breast cancer cell line due to the expression of certain kind of kinase enzymes namely, PDGFR, ErbB-1, HER2/Neu (ErbB-2, over expressed in 15–20% of breast cancer), EGFR, and VEGFR [91].

Other pyrido pyrimidine derivatives
Pyrido[20,30:3,4] pyrazolo[1,5-a]pyrimidine derivatives were tested against cancer cell lines MDA-MB-231

(HTB-26), HeLa (CCL-2), Hep G2 (HB-8065), PC-3 (CRL-1435), and normal HUVEC (CRL-1730). **89(a–c)** displayed prominent antitumor activity with the IC_{50} (μM) range = 5.22 to 93.2 ± 0.14 with 5-FU was used as control. **89a** and **89c** inhibited topoisomerase I (TopI) activity, which was comparable to camptothecin thus making it a significant target for the anticancer activity. The compounds were also tested on the normal cell line HUVEC and had an IC_{50} more than 80 μM making it non-toxic for the normal cells [92]. Subsequently, substituted pyrido pyrimidines derivatives were evaluated for against cancer cell lines, PC-3, HepG-2, MCF-7, HCT-116, and A-549. **90** showed the best activity against the cell lines with the IC_{50} = 0.5 to 7 μM with DOX as positive control. **90** also exhibited promising activity against three kinases CDK4/cyclin D1, EGFR, and PDGFR β, at two different concentrations of 100 μM and 50 μM at a mono measurement. In further assessment of the compound, molecular docking studies were conducted to check the binding of the compound with EGFR and CDK4/cyclin D1 kinases. Overall, the potency of the compound was credited to remarkable kinase inhibitory activity (Fig. 30) [93].

Thieno pyrimidine derivatives
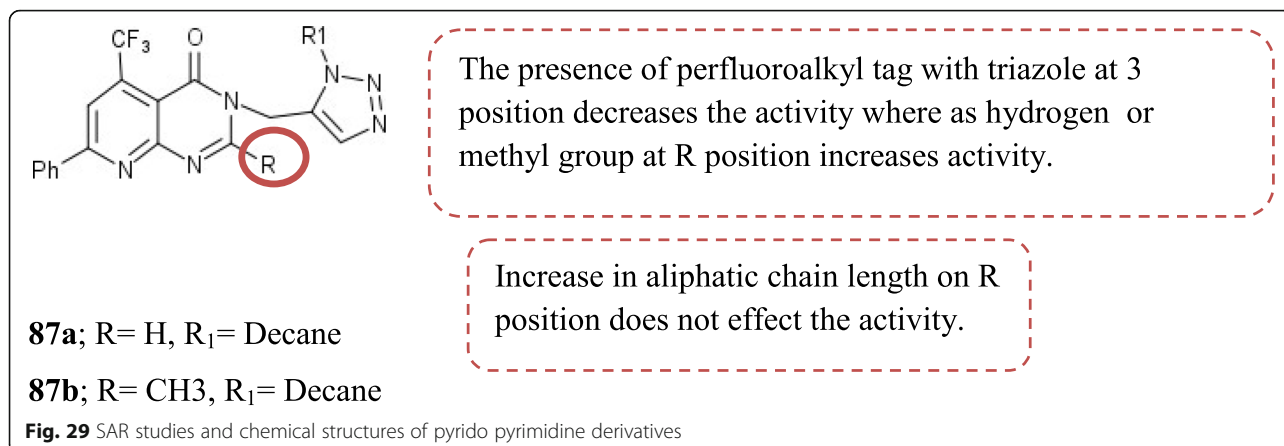#### Thieno[2,3-d]pyrimidines
Novel 2,3-disubstituted-4-oxo-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidines were studied against breast and liver cancer cell lines with DOX a positive control. **91** showed the best activity in the series with the IC_{50} = 0.19 μM (breast cancer cell line). The SAR is illustrated in Fig. 31 [94]. Some novel thieno[2,3-d]pyrimidines with aminophosphonate were studied anticancer activity against the HepG2, MGC-803, and EC109 by MTT assay. **92** was the most potent with the inhibitory percent of 91.2 and 94.4 at 50 μg/ml concentration

Fig. 28 Chemical structures of pyrido pyrimidine derivatives
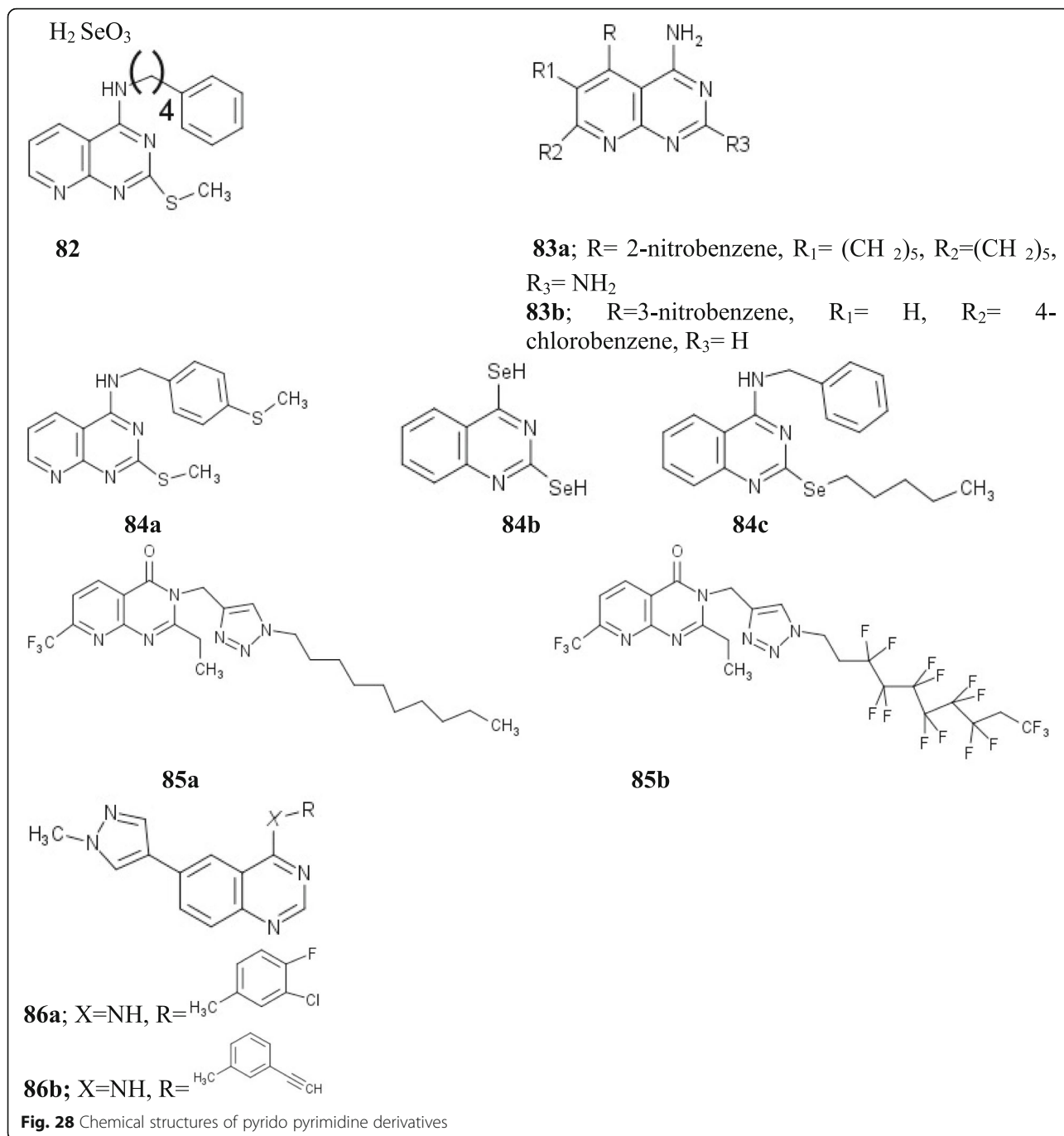
specifically against the HepG2 and MGC-803 respectively. SAR is depicted in Fig. 32 [95].

Antitumor activity of some 3-ethyl-2-mercaptothieno [2,3-d]pyrimidin-4(3H)-ones was reported and screened against HT-29, MDA-MB231, HeLa, and HepG2 as well as Lep-3. **93a** was active against the HepG2 cell line with the (IC_{50} = 0.99 μM), **93b** was potent against the HeLa cell line (IC_{50} = 0.83 μM), and **93c** with the (IC_{50} = 0.001 μM) against the HT-29 cell lines. In the theinopyrimidine derivative linked to the thiadiazole, activity was

due to the presence of oxygen 4th position of the theinopyrimidine ring [96]. A series of thieno [2,3-d]pyrimidine derivatives were reported to initiate apoptosis and EGFR/HER2 arrest. Targeting a tyrosine kinase-related entity makes a drug important in cancer treatment regime. The compounds were tested in vitro for the inhibition of the EGFR and those which passed the test were further evaluated for the mutant EGFR and HER2 kinase inhibition. MCF-7, HCT-116, A431, and HepG2 cell lines were used to screen compound **94** (IC_{50} range =
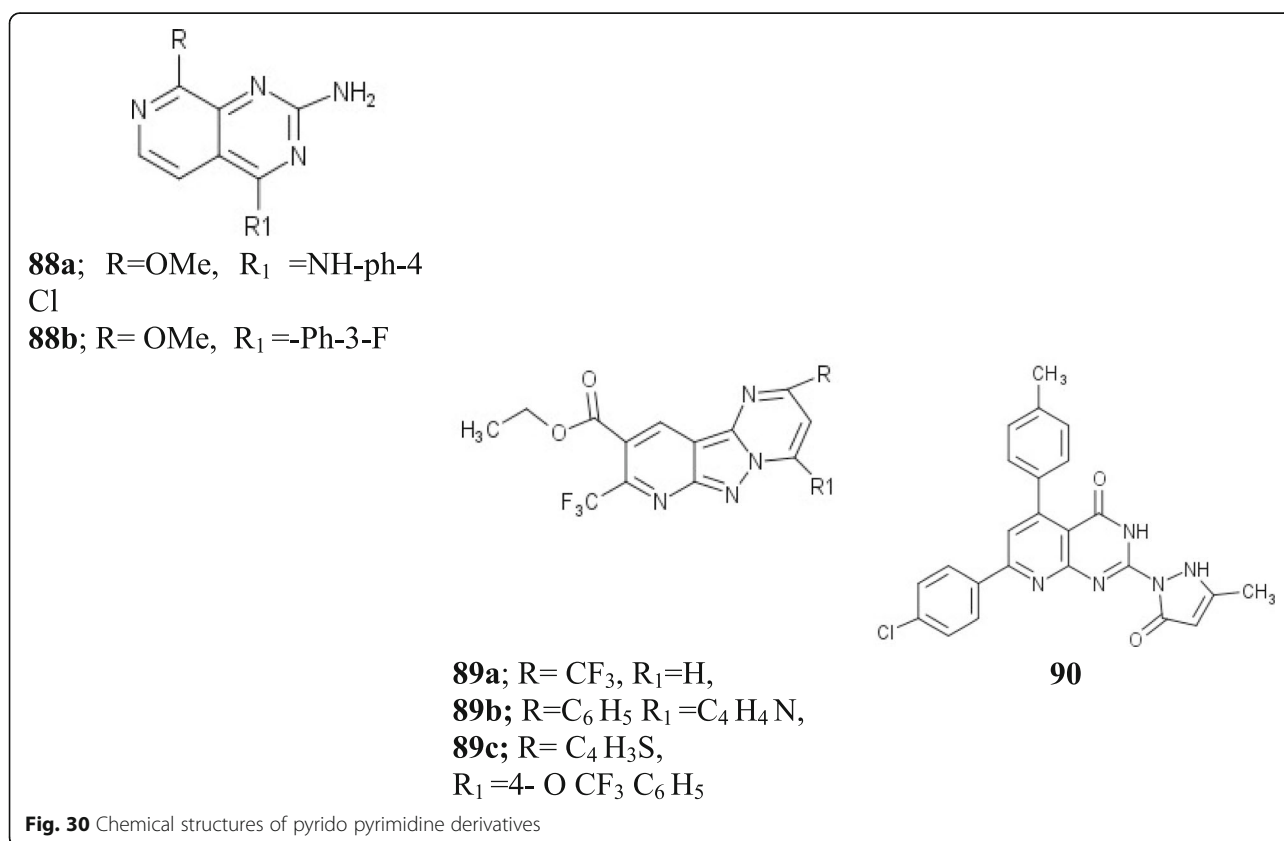
The presence of perfluoroalkyl tag with triazole at 3 position decreases the activity where as hydrogen or methyl group at R position increases activity.

Increase in aliphatic chain length on R position does not effect the activity.

87a; R= H, R₁= Decane

87b; R= CH3, R₁= Decane

Fig. 29 SAR studies and chemical structures of pyrido pyrimidine derivatives

7.5 ± 0.32 to 16.06 ± 0.02 μM) compared to erlotinib as reference. The compound arrested the cell cycle at G2/M phase and induced apoptosis in the breast cancer cell lines; it also had a binding affinity for EGFR as concluded in the docking studies [97].

Recently, anticancer evaluation of the thieno [2,3-d]pyrimidine derivatives in the inhibition of the enzyme toposiomerase II was reported. **95(a–d)** were designed and tested against the HepG2 and MCF-7 cell lines giving the IC_{50} range = 4–10 μM. Compounds with higher

IC_{50} = > 60 μM showed selectivity towards the cell lines. The major mechanism of action in the whole process was that the active compounds upregulated the expression of p-53, downregulated the expression of caspase-3, led to the cell cycle arrest, and further apoptosis leading to cancer cell destruction. These compounds increased the p-53 expression by 3–4-folds compared to the 2-folds of DOX [98].

Thieno[2,3-d] pyrimidine derivatives were active against 60 human cancer cell line panel by the primary
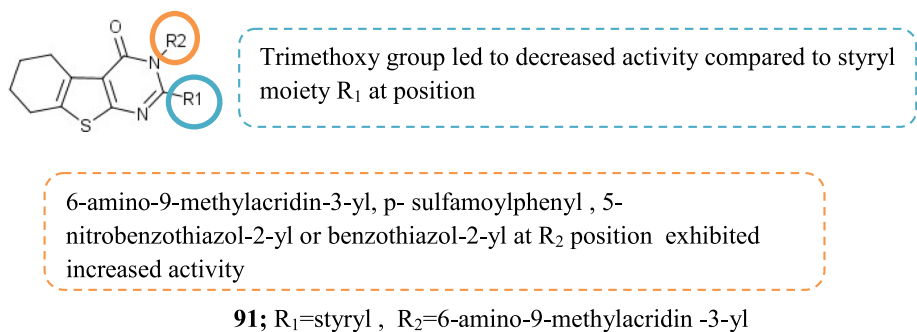

88a; R=OMe, R₁ =NH-ph-4 Cl

88b; R= OMe, R₁ =-Ph-3-F

89a; R= CF₃, R₁=H,

89b; R=C₆H₅ R₁ =C₄H₄N,

89c; R= C₄H₃S, R₁ =4- O CF₃ C₆H₅

90

Fig. 30 Chemical structures of pyrido pyrimidine derivatives

Trimethoxy group led to decreased activity compared to styryl moiety R_1 at position

6-amino-9-methylacridin-3-yl, p- sulfamoylphenyl , 5-nitrobenzothiazol-2-yl or benzothiazol-2-yl at R_2 position exhibited increased activity

91; R_1=styryl , R_2=6-amino-9-methylacridin -3-yl

Fig. 31 SAR studies and chemical structures of 2,3-disubstituted-4-oxo-5,6,7,8-tetrahydrobenzo [4,5]thieno[2,3-d]pyrimidines

anticancer assay as a result **96** was found to have the best activity. Although the compound **96** exhibited a wide spectrum of activity, it was not selective towards any particular group of human cancer cell lines [99].

Evaluation for in vitro antiproliferative activity of new thieno [2, 3-d] pyrimidine and thiophene derivatives was conducted and **97(a–e)** were the best compounds, with the IC$_{50}$ (μM) = 4.30 ± 0.3 to 23.57 ± 1.8 when tested against five cancer cell lines namely, HepG-2, Hep-2, MCF-7, PC-3, and HeLa by the standard MTT assay. **97d** was efficient than the positive control DOX. The abovementioned compounds were also tested for DNA binding and enzyme inhibition activity, the order of inhibition: DNA polymerase> thymidylate synthase >tyrosine kinase [100]. In a parallel study, some novel thieno [2, 3-d] pyrimidine derivatives were tested for antitumor activity against the human breast and cervical cancer cell line. **98b** was highly active with the IC$_{50}$ = 18.87 ± 0.2 μg/ml, and the **98a** was active against cervical cancer cell line with the IC$_{50}$ =40 ± 1.7 μg/ml both better than the positive control, Paclitaxel [101].

Recently, urea derivative based thieno [2,3-d]pyrimidine-based were screened for in vitro antitumor activity against the tamoxifen sensitive and resistant breast cancer cell lines. **99** a sorafenib analog and a theinopyrimidine-based urea derivative inhibited VEGF R2 tyrosine kinase activity up to 65% and also showed

anticancer activity against its parental MCF7 BC cells, LCC2 and TAM-resistant. The compound was also sensitive to the LCC2 due to the upregulation of key enzymes for apoptosis, LDH in media downregulated the mRNA expression of Ki-67, survivin, and Akt and reduced levels of ROS and glucose uptake led to cell death of proteins like caspases 8, 9, 3, p53, Bax/Bcl-2 ratio [102]. Some novel thieno [2, 3-d] pyrimidines were evaluated in 2012 and compounds **100(a–b)** showed the best antitumor activity against the HCT-116 with the IC$_{50}$ = 15.92 and 25.85 μM and 19 to 2.26 times more efficacious than imatinib (IC$_{50}$ = 34.40 μM). SAR suggested that N-ethyl substitution imparted comparable activity to the positive control whereas N-phenyl or N-substituted phenyl gave moderate activity in case of thienopyrimidine derivatives (Fig. 33) [103].

Tetrahydrobenzo [4,5]thieno [2, 3-d] pyrimidine-4-yl)-pyrolidine-2-carboxylic acid derivatives were studied for in vitro anticancer activity against MCF-7 and HCT-116. **101 (a–b)** demonstrated decent activities against the MCF-7 in contrast to the standard positive control DOX. In the SAR studies, it was concluded that aniline derivatives show good anticancer activity, **101a** being a 4-fluro aniline derivative showed the most potent activity. SAR is depicted in Fig. 34 [104].

A series of thieno [2, 3-d] pyrimidine derivatives were reported and evaluated for the in vitro activity against
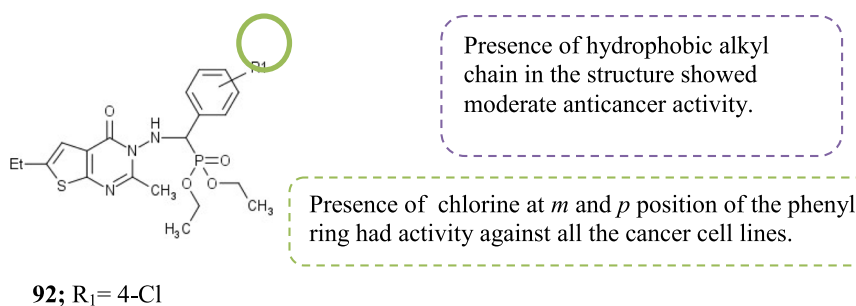

Presence of hydrophobic alkyl chain in the structure showed moderate anticancer activity.

Presence of chlorine at *m* and *p* position of the phenyl ring had activity against all the cancer cell lines.

92; R_1= 4-Cl

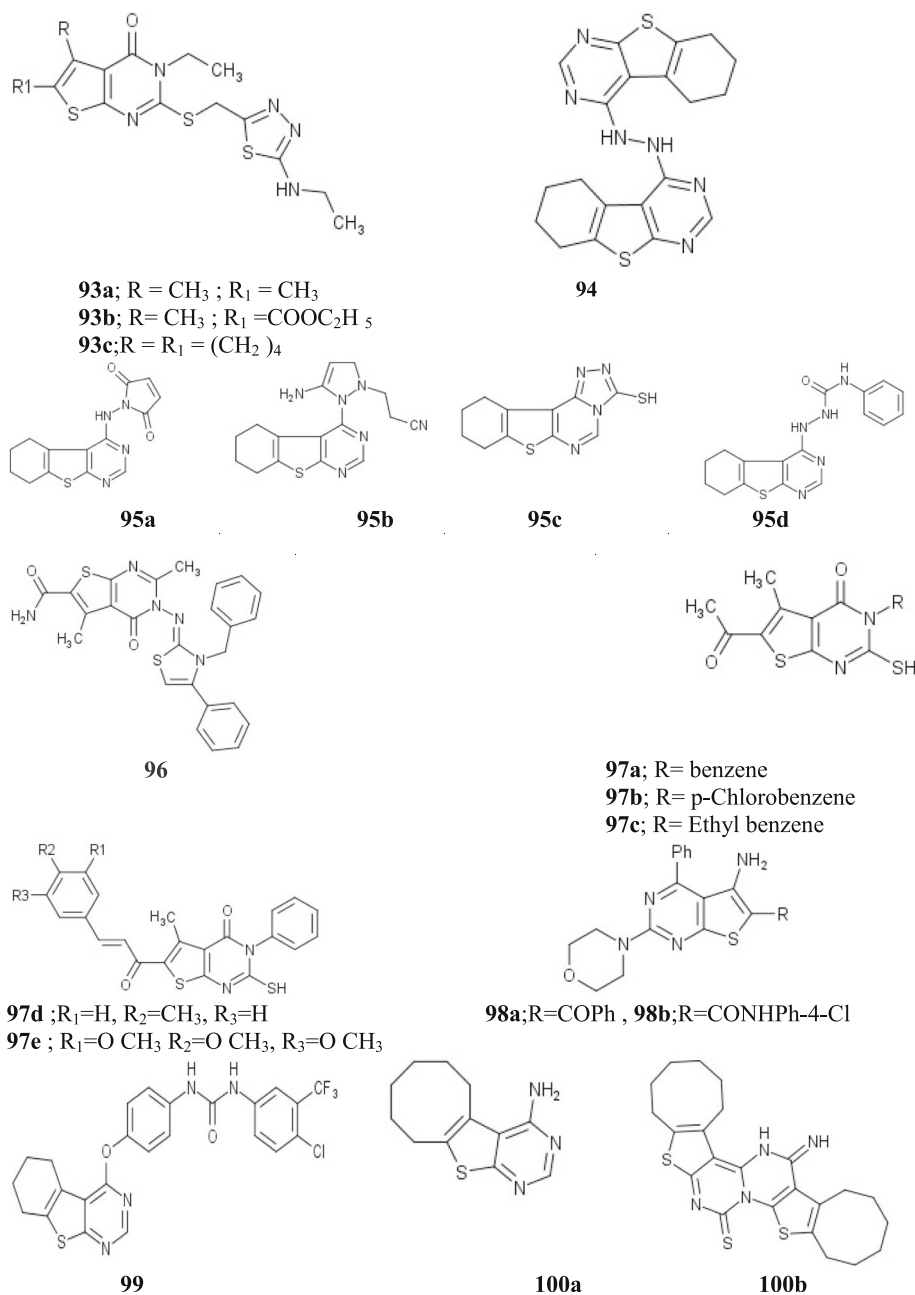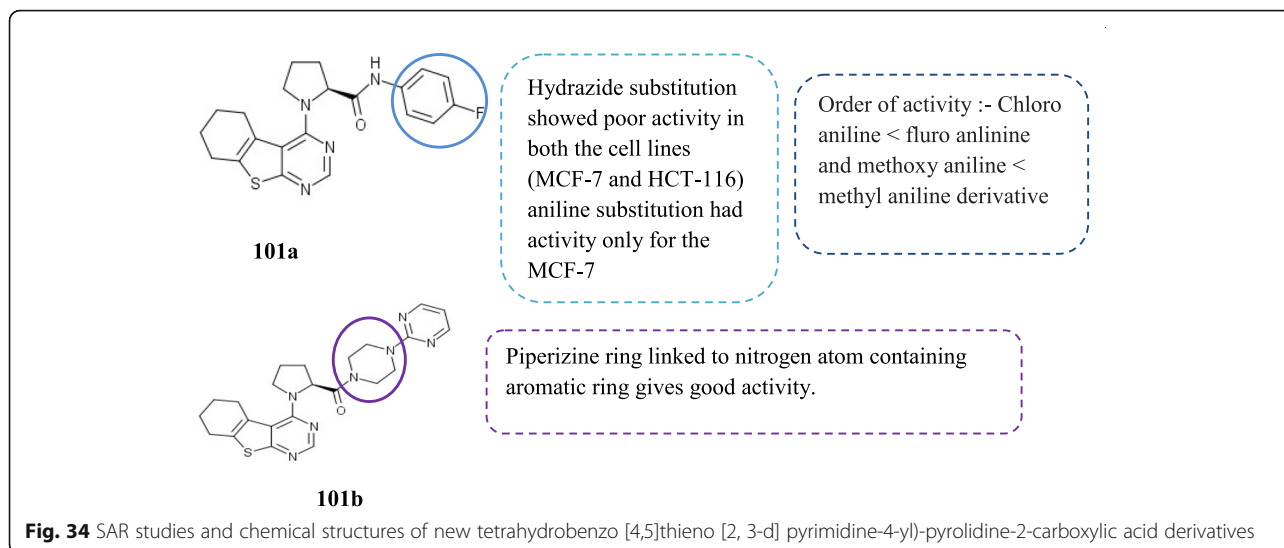**Fig. 32** SAR studies and chemical structures of thieno [2, 3-d] pyrimidines having a-aminophosphonate

Fig. 33 Chemical structures of thieno pyrimidine derivatives

the MCF-7 cancer cell line. **102(a–f)** elucidated good oncogenic activity with the IC$_{50}$ range = 37.78 to 22.12 μM. Compounds **102a**, **102d**, **102e**, and **102f** showed better activity than DOX with the IC$_{50}$ = 30.40 μM and **(102b** and **c)** showed nearly same activity as the positive control. SAR study is summarized in Fig. 35 [105].

Amide containing thieno[2, 3-d] pyrimidines were studied for their growth inhibition effects in different cancer cell lines MDA-MB-453, MCF-7, DU-145, A549 HCT-116, HT-29, HCT-15, and BT-549. **103(a–c)** were

tested for growth inhibition and **103c** exhibited selectivity towards MDAMB-453 and MCF-7 cell and arrested cell cycle at G0/G1 phase in MCF-7 cells. This potential was attributed to the presence of cinnamide moiety and trifluoromethyl group. **103(d–e)** was selective towards A549 and MDA-MB-453cell lines, respectively, with ZSTK474 as positive control [106]. Previously, the varied substitution of groups at the R position of the thieno [2, 3-d] pyrimidine derivatives led to properties like c-Met and VEGFR-2 inhibition which is an important target

Fig. 34 SAR studies and chemical structures of new tetrahydrobenzo [4,5]thieno [2, 3-d] pyrimidine-4-yl)-pyrolidine-2-carboxylic acid derivatives

for cancer therapies. BaF3-TPR-Met cells were used to test **104(a–b)**, which elucidated nearly equivalent inhibition percent compared with the cabozantinib (positive control). **104a** was most potent against HUVEC and BaF3-TPR-Met with IC$_{50}$ = 0.086 μM and 0.051 μM, respectively, and displaying the most efficient inhibitory activity against VEGFR-2 and c-Met with IC$_{50}$ = 0.048 μM and 0.025 μM [107].

Recently, condensed thieno [2, 3-d] pyrimidines were evaluated for their cytotoxic potential against A431 and H9c2 cancer cell lines through MTT assay. Most of the synthesized compounds except 1**05** had 85% cell death in both rat and H9c2 cell lines. The presence of ethyl, methyl, and ethyl-fused five-membered rings in the structure showed better activity in A431 than in H9c2 cells and in case of the six-membered structures, methyl-substituted cyclohexyl compounds inhibited

A431 cell growth, although dimethyl substituted thiophene derivatives displayed better activity in H9c2 cells. Nitromethyl, cyclohexyl, and dimethyl substitution lowered activity compared to chloro substitution fused with seven membered rings and the methyl-substituted structures had better potency than the aryl substituted structures [108].

Anticancer activity, apoptosis-inducing ability, and cell cycle profiling of hexahydrocyclooctathieno [2, 3-d] pyrimidines was reported using DOX as positive control. **106(a–b)** were tested for antiproliferative activity against 56 cancer cell lines and **106b** was potent against a broad range of cell lines. **106a** and **106b** led to the induction of S-phase and G2/M-cell cycle arrest in MCF7 and HCT116 cell lines with a significant increase in the pre-G phase cell population in a time-dependent way; **106b** also increased cleaved caspase-3 as a marker of apoptosis

a; R= sulfanilamide, **b**;R= sulfathiazole, **c**;R=sulfadiazine, **d**=sulfamerazine, **e**;R=sulfa-dimethoxazine, **f**;R= sulfadoxine

Fig. 35 SAR studies and chemical structures of new tetrahydrobenzo [4,5]thieno [2, 3-d] pyrimidine-4-yl)-pyrolidine-2-carboxylic acid derivatives
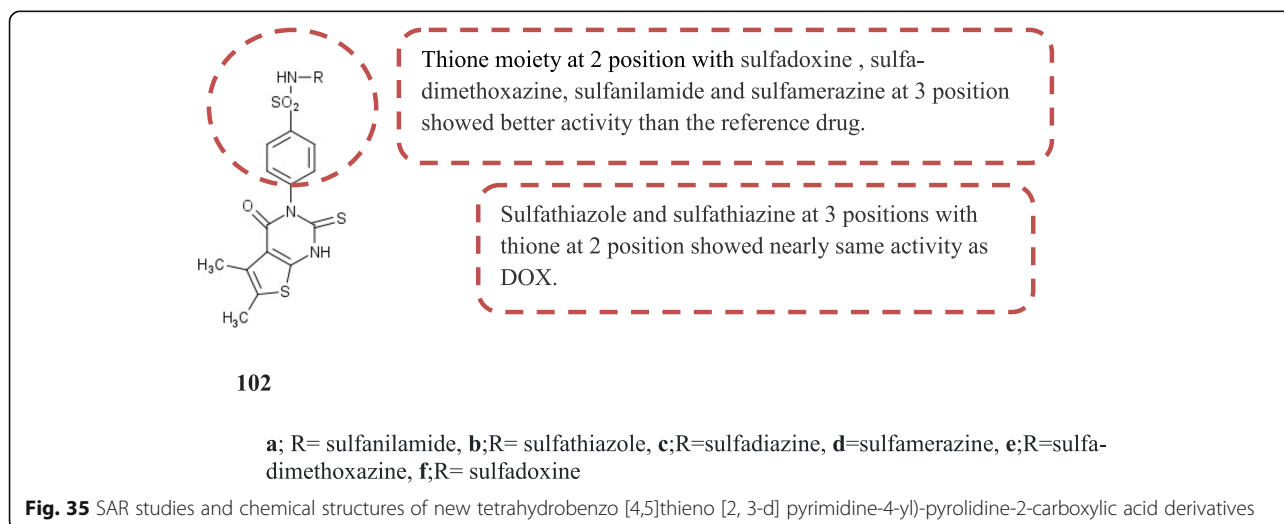
[109]. A series of new 2-pyridyl hexahydrocycloocta [4, 5]thieno [2, 3-d] pyrimidine derivatives were evaluated with DOX as positive control. **107 (a–e)** manifested the most potent activity due to the substitution of 2-pyridyl group at C-2, amino group at C-4 increases activity, and chlorine group at C-4 leading to an inactive compound and the bulkiness of the amino at C-4 effects the activity [110].

Thieno [3, 2-d] pyrimidine

N-substituted thieno [3, 2-d] pyrimidine and thiophene derivatives were studied for the antitumor activity and **108(a–b)** had more potency than standard drug DOX in MTT assay against the cancer cell lines of liver, colon, and lung. According to the SAR the pyrrolo-pyrimidine moiety and triazolopyrimidine moiety at the C-3 position of the theinopyrimidine ring and EWG at the para-position of the phenyl ring contributed to the high activity, on the contrary an EDG at the same position gives moderate activity [111]. Screening of antitumor activity of 4-morpholinothieno [3, 2-d]-pyrimidine derivatives bearing aryl methyl hydrazine moiety were reported. The activity was mainly due to heteroaryl methylene hydrazinyl moiety and various substitutions on the benzene ring when screened against H460, HT-29, and MDA-MB-231 cell lines. **109** bearing 3,4-methylenedioxy phenyl group was most efficacious against all the three abovementioned cell lines with the IC_{50} = 0.003 μM, 0.42 μM, and 0.74 μM respectively with more selectivity towards lung cancer cell line and was 1.6- to 290-folds more active than GDC0941 [112].

Diaryl urea moiety containing thieno [3,2-d] pyrimidine derivatives were tested for their antitumor activity by MTT assay using sorafenib and GDC-0941 (positive control). **110** was tested against various tumor cell lines gave the most potent activity against the MDA-MB-231, MKN-45, HT-29, and H460 and cell lines with the IC_{50} = 0.23 μM, 0.18 μM, 0.058 μM, and 0.081 μM respectively. The SAR of the study concluded that the compounds with the mono halogen group on the phenyl ring were more efficient than the one's with methyl group or double groups and presence of chlorine atom at the 6,7-position of thieno[3,2-d]pyrimidine moiety lead to detrimental activity but had good selectivity towards HT-29 and H460 cell lines. In the enzymatic evaluation, it was found that the compound was active against the PI3Kα kinase (IC_{50} = 0.13 μM). The apoptosis in the cancer cell was induced by the compound in concentration dependent manner with the increase in protein expression of Clv-PARP [113]. Diaryl semicarbazone linked to thieno [3,2-d] pyrimidine derivatives as potential antitumor agent were studied in the same year. **111** was the most potent compound of the series with the IC_{50} = 0.23 to 0.57 μM against the tumor cell lines

H460, MKN-45, MDA-MB-231 and HT-29 using MTT assay and sorafenib and GDC-0941 as positive control. The compound also showed promising activity against the PI3Kα better than the standard reference GDC-0941 indicating that the compound will make this particular kinase enzyme a potential target in future. By the help of the western blot method, it was determined that the compound can be used for induction of apoptosis in a concentration dependent manner [114].

Recently, the biological activity of the new series of novel thieno[3,2-d]pyrimidine derivatives were reported along with the test for the fak inhibition. These compounds were designed in a way that it mimics the conformation of the diaminopyrimidine moiety. **112** most potently inhibited the enzyme with the IC_{50} (28.2 = nM) and also acted against the proliferation in the U-87MG, A-549, and MDA-MB231 cancer cell lines with the IC_{50} = 0.16, 0.27 and 0.19 μM respectively and was less toxic to HK2 cell line. It arrested the cell cycle at G0/G1 phase in the MDA-MB231 cancer cell line, also preventing the migration and induced apoptosis [115]. HDAC was inhibited by novel thieno [3, 2-d] pyrimidines and for their anticancer activity was evaluated on three cell lines including, MCF-7, HeLa, and HCT-116 by using MTT assay. The HDAC inhibition in the compound was studied using fluorimetric assay. **113** inhibited HDAC potently with the IC_{50} = 0.38 μM by inducing apoptosis in HCT-116 cell line and cell cycle arrest at G2/M phase with SAHA as positive control [116].

Thieno [2, 3-d] pyrimidine and thieno [3, 2-e] triazolo [4, 3-c] pyrimidine

Thieno [3, 2-e] triazolo [4, 3-c] pyrimidine and thieno [2, 3-d] pyrimidine derivatives were reported for antiproliferative activity. **114** elucidated the most potent results against the 56 tumor cell lines with higher selectivity for breast cancer cell lines. Compound **114** arrested the cell cycle at the G2/M phase and the accumulation of the cells from the degradation and fragmentation of the genetic material started from the pre G1 phase of the cell cycle in the MDA-MB-468 cell line leading to induction of apoptosis in the cancer cells [117]. Previously, the thieno [3, 2-e] triazolo [4, 3-c] pyrimidines and thieno [2, 3-d] pyrimidine-4-ones were studied for activity on the HCT-116 tumor cell line, using SRB assay. **115(a–e)** with IC_{50} = 11.90, 12.43, 15.91, 25.80, and 32.11 μM respectively showed potent antitumor activity against the said cell line. The compounds had 2.89–1.07-fold more activity than positive control imatinib (IC_{50} = 34.4 μM). In the SAR study, it has been summarized that substitution at C-2 position highly affects the potency and presence of methylene between electronegative moieties and pyrimidin-4-one ring, Cl, and arylamino increases the

activity. Arylcarbothioyl derivative did not affect the activity, whereas the 2-nitrophenyl group increased the activity by 2.89 folds. Molecular docking was used to study the binding and affinity of the compound towards the CDK2 receptor [118].

Other thieno pyrimidine derivatives

In silico method was used to determine the anticancer activity of new annelated thieno [2, 3-e][1–3] triazolo [1, 5-a] pyrimidines, using 60 human cancer cell lines. Compounds 116(a–b) were the most potent and showed high antiproliferative activity at sub micromolecular concentration with low toxicity to normal cells and highly potent towards cancer cells. The basic chain histamine or methylpiperazine was important for potency enhancement of the activity and the isosters pyridothieno were less effective than the benzothieno bicycle [119]. Using the unusual dimroth rearrangement in an in silico research study conducted simultaneously led to synthesis of annelated thieno[3,2-d][1,2,3]triazolo[1,5-a]pyrimidines. 117a (angular isomers) showed excellent anticancer activity against the 60 cancer cell line panel at sub nM concentration. Compound 117b (linear isomer) displayed good antiproliferative activity. In the preliminary in vivo test of compound 117b, it showed high potency (Fig. 36) [120].

Thiazolopyrimidine derivatives
Thiazolo [1, 2-a] pyrimidine

3-carboxylate derivatives of benzo [4,5]thiazolo [1, 2-a] pyrimidines were reported and 118(a–b) displayed potency against MDA-MB-231 and MCF-7 cancer cell lines. Compound 118a showed activity against both with the IC$_{50}$ = 0.58 and 1.58 μM respectively, and 118b had the best activity against MDA-MB-231 with the IC$_{50}$ = 5.01 μM. The activity was imparted to the MCF-7 and MDA-MB231 due condensation of methyl piperazine and piperidine with carboxylic acid at 3′-position of the benzothiazopyrimidine ring. Condensation of β-alanine at the third position of benzothiazopyrimidine ring led to increase in chain length subsequently decreasing the activity compared to piperidine and methylpiperazine moieties [121].

Thiazolo [3,2-a]pyrimidines

Recently, thiazolo [3, 2-a] pyrimidine derivatives were tested for anticancer activity against MCF-7, HeLa, A549, and SKNSH by MTT assay. 119a with IC$_{50}$ = 2.2 ± 0.6 μM against A549 and compound 119b with IC$_{50}$ = 5.6 ± 0.4 μM against HeLa exhibited better effects than positive control DOX. The presence of methoxyphenyl groups and naphthyl on thiazolopyrimidine demonstrated enhanced activity [122]. Novel thiazolo [3, 2-a] pyrimidines were screened for in vitro antiproliferative
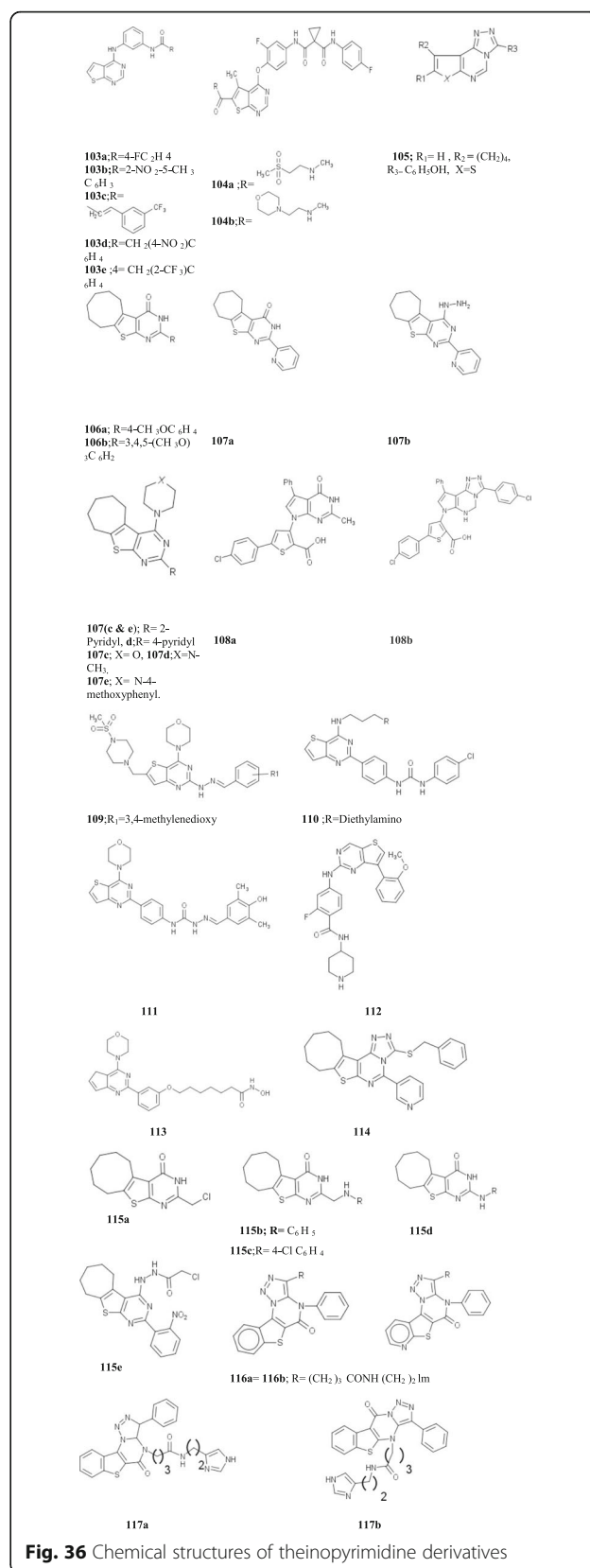

Fig. 36 Chemical structures of theinopyrimidine derivatives

118a;R= CH$_2$;
118b;R= N-CH$_3$

119a;R$_1$=2-Naphthyl, R$_2$= 3,4,5-trimethoxyphenyl
119b; R$_1$= 2-Naphthyl, R$_2$= 3-Bromophenyl

120a; R$_1$=4-Cl, R$_1$=4- NH$_2$, R$_3$=H
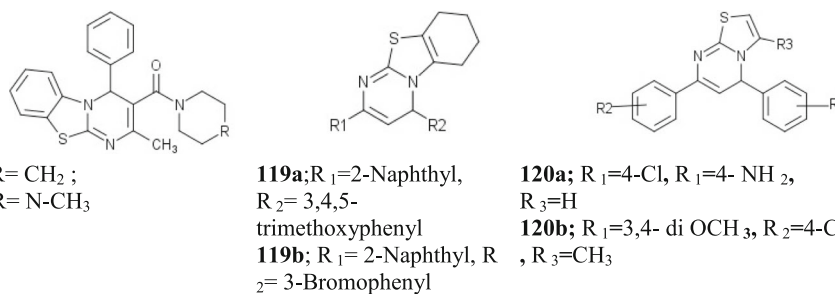**120b;** R$_1$=3,4- di OCH$_3$, R$_2$=4-Cl , R$_3$=CH$_3$

Fig. 37 Chemical structures of thiazolopyrimidine derivatives

activity, **120(a–b)** showed the best activity against 60 cancer cell line panel. Three cancer cell lines Hop-92, SK-MEL-2, and IGROV1 exhibited some potency towards the compounds out of the 60 cancer cell lines during the in vitro anticancer activity assay. In the molecular docking studies, it was summarized that some compounds of the series fit into the minor groove and bind to the AT base pairs and thus had shown anticancer activity (Fig. 37) [123].

Novel pyrido [4, 3-d] fused with thiazolo [3, 2-a] pyrimidine analogs were tested for anticancer activity against the panel of 60 cancer cell lines. **121(a–b)** were 9-folds more active with the IC$_{50}$ = 2.4 and 9.1 μM and compounds **121(c–d)** were 7-fold more active with the IC$_{50}$ = 3 and 16.3 μM better than the standard reference drug 5-FU (IC$_{50}$ range = 36 to 54 μM). SAR is illustrated in Fig. 38 [124].

Thiazolo[3,2-a] pyrido[4,3-d]pyrimidine containing derivatives were evaluated against the 60 different human tumor cell lines with **122(a–m)** being the most potent. The different activity between the compounds was due to variation of substitutions in the phenyl group of the molecule and the presence of sulfur and nitrogen atom subsequently enhanced the activity. Thiopyrimidine moieties fused to N-methylpipredine ring boosted the activity [125].

Thiazolo [5, 4-d] pyrimidines

Apoptosis-inducing and antiproliferative activity of thiazolo [5,4-d]pyrimidines were reported using the SRB assay against cancer cell lines A549, A431, T98G, T47D, PC-3, HL-60, NCI-H322, and MIAPaCa-2. **123** showed the induction of apoptosis in A549 cells at the concentration of 10 μM determined by the high sub –G1 population. In the western blotting assay, the compound demarcated cleavage in PARP-1 and lead to procaspase-3 inhibited apoptosis [126]. The study of some thiazolo [5,4-d]pyrimidine derivatives were carried out by the
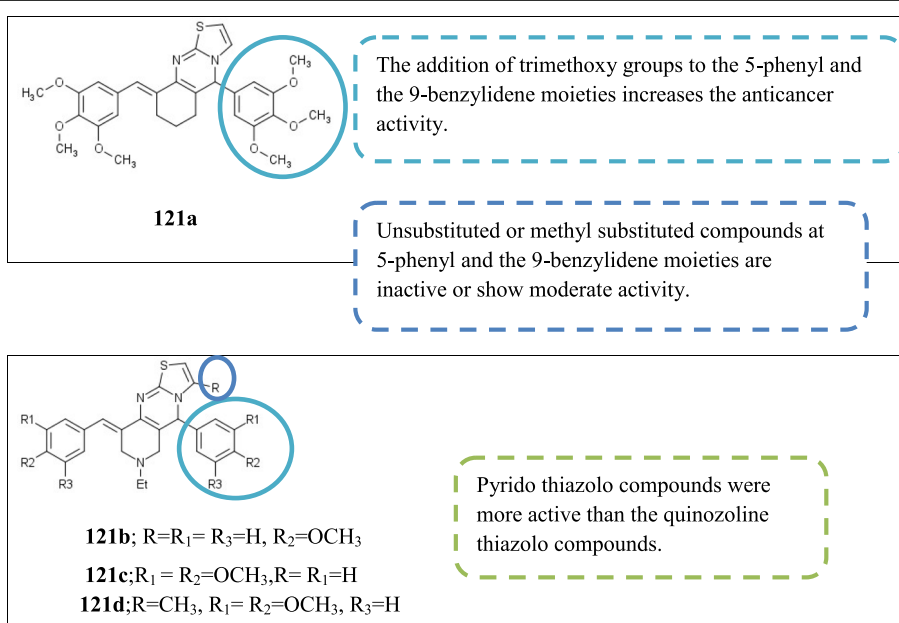

121a

The addition of trimethoxy groups to the 5-phenyl and the 9-benzylidene moieties increases the anticancer activity.

Unsubstituted or methyl substituted compounds at 5-phenyl and the 9-benzylidene moieties are inactive or show moderate activity.

121b; R=R$_1$= R$_3$=H, R$_2$=OCH$_3$
121c;R$_1$ = R$_2$=OCH$_3$,R= R$_1$=H
121d;R=CH$_3$, R$_1$= R$_2$=OCH$_3$, R$_3$=H

Pyrido thiazolo compounds were more active than the quinozoline thiazolo compounds.

Fig. 38 Chemical structure and SAR studies of novel pyrido [4, 3-d] fused thiazolo [3,2-a]pyrimidine analogs

drug repurposing strategy and evaluated against three cancer cell lines. **124** showed the best anti-cancer activity with the IC_{50} = 1.03 and 38.95 µM against the MGC803 and GES1 cell lines respectively, with efficient selectivity between cancer cell and normal cells. The compound also inhibited the formation and migration of MGC803 cells and also led to apoptosis by expression of some changes in the apoptosis concerned proteins by upregulation of Bax and caspase-3/9 and downregulation of Bcl-2, confirmed by the western blot assay [127].

The antiproliferative activity of novel thiazolo [5,4-d]pyrimidines were evaluated. **125** exhibited efficacious selectivity between the human cells and cancer cells which was confirmed as it had IC_{50} = 1.22 µM against HGC-27 cell line and low toxicity towards the GES-1 cells. The induction of apoptosis was due to upregulated expression of Bax, downregulation of Bcl-2, and cleavage in caspased-3/9. It also prevented the migration and formation of new HGC-27 cell proving it as an efficacious lead for future anticancer drugs [128].

Other thiazolopyrimidine derivatives
Novel 1-thia-4-azaspiro [4.5] decane and their derived thiazolopyrimidines were studied for activity against the HepG-2, PC-3 and HCT116 cancer cell lines. **126(a–b)** showed good anticancer activity against the HCT116 cell line with the IC_{50} range = 92.2 to 120.2 µM and showed some activity against the HepG-2 cell line. The MTT assay was conducted for the cytotoxicity with compound dosage of 100 ppm and DOX was used as positive control (Fig. 39) [129].

Oxazolo-pyrimidine derivatives
Recently, work on the anticancer activity of some 4-(Oxazolo [5,4-d]pyrimidine derivatives linked with 1,2,4-oxadiazole was reported. Compounds were tested against the human breast, lung, colon, and ovarian cancer cell line using the MTT assay with etoposide as the positive control. **127a** showed activity against all the cancer cell lines with the IC_{50} range = 0.046 to 0.12 µM and 127b showed activity against cell lines namely breast, lung, and ovarian cancer with the IC_{50} range = 0.012 to 0.63 µM. In the SAR studies, it was concluded that the compound with an EDG had the most potent activity and compounds with 3,5-dimethoxy, 4-methoxy group depicted low activity. Compounds with one nitro group had moderate activity whereas the ones with bromo di nitro group had good activity. A weak EDG group with the compound showed considerable activity [130].

New series of oxazolo [5,4-d]pyrimidines were reported as efficacious VEGFR-2 inhibitors. The compounds were tested against HUVEC and VEGFR-2 with **128** being the most potent in the series with the IC_{50}= 0.33 µM for VEGFR-2 and IC_{50}= 0.29 µM for HUVEC.

Compounds of this series were also moderate inhibitors of EGFR and sunitib was used as positive control [131]. In a parallel study, a newly synthesized oxazolo[5,4-d]pyrimidine derivative, **129** was reported. HUVEC was inhibited in a dose dependent manner with the IC_{50} = 9.30 ± 1.24 µM. Reported in other in vitro and ex vivo studies, it also inhibited new micro vessels sprouting from rat aortic ring. The migration and chemotactic invasion was stopped along with the downstream signaling of VEGFR-2 and downregulation of phosphorylation of PI3K, ERK1/2, and p38 MAPK (Fig. 40) [132].

Quinazoline containing derivatives
Anticancer activity of 2,3-disubsituted quinazoline was reported and the compounds were tested using MTT assay against MCF-7 cancer cell line with amphotericin B as positive control. **130(a–c)** exhibited anticancer with an IC_{50} (µM) = 6, 6, and 4 respectively [133]. New quinazoline derivatives were being studied for anticancer activity against the MDA-MB231 and HeLa cancer cell lines, using MTT assay, while using gefitinib as positive control. **131(a–c)** had the lowest (IC_{50} = 1.85 to 2.81 µM) in relation to gefitinib (IC_{50} = 4.3 and 28.3 µM) against MDA-MB231and HeLa cells, respectively. They were deemed as most potent because they acted through EGFR-TK pathway. The addition of heteroalkyl atom led to decrease in cytotoxicity. The NO_2, CN, phthalimido, and morpholino groups alongside the N-benzyl group and the substitution at R_2 position affected the cytotoxic activity to a great extent [134].

2-furano-4(3H)-quinazolinones, diamides (open ring quinazolines), and quinoxalines were evaluated against OVCAR-4 and NCI-H522 cancer cell lines and **132** was found to be the most efficient compound. The potency was due to the blocking of hydrophobic pocket of tyrosine kinase because of 2-chloro benzylideneamine group at 3-position of quinazoline. The presence of OH and benzoic acid at N3 of the ring lowered the activity whereas 2-chloro benzylideneamine group increased activity [135].

Quinazoline derivatives incorporated with chalcone were tested against cancer cell lines MCF-7, A375, A549, and HT-29, using MTT assay. **133a** (IC_{50} = 0.18 to 2.90 µM), **133b** (IC_{50} = 0.10 to 1.34 µM), **133c** (IC_{50} = 0.10 to1.56 µM), and **133d** (IC_{50} = 0.16 to 2.89 µM) showed more potent activity than the control drug, combretastatin–A4 [136]. Previously, in a report on anti-breast cancer activity of novel quinazoline derivatives, they were evaluated against MCF-7 cancer cell line by resazurin reduction method and DOX as positive control. Oxadiazole, pyrazole, and thiazolidinone-linked quinazolin-4-one scaffold, **134(a–d)** (containing thiazolidinone moieties) displayed the best activity with IC_{50} = 3 to 9 nM/mL [137].
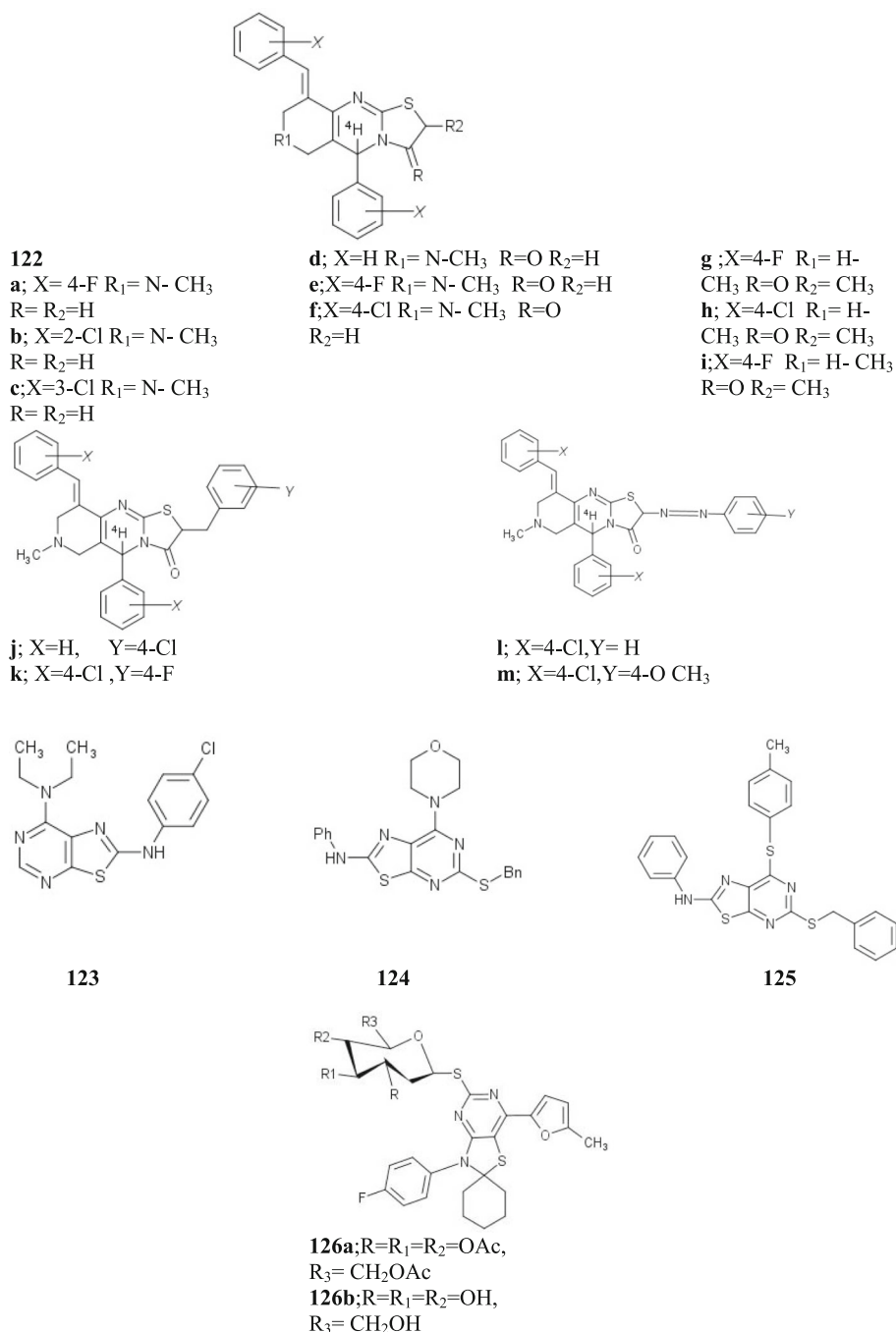
122
a; X= 4-F R$_1$= N- CH$_3$ R= R$_2$=H
b; X=2-Cl R$_1$= N- CH$_3$ R= R$_2$=H
c;X=3-Cl R$_1$= N- CH$_3$ R= R$_2$=H

d; X=H R$_1$= N-CH$_3$ R=O R$_2$=H
e;X=4-F R$_1$= N- CH$_3$ R=O R$_2$=H
f;X=4-Cl R$_1$= N- CH$_3$ R=O R$_2$=H

g ;X=4-F R$_1$= H- CH$_3$ R=O R$_2$= CH$_3$
h; X=4-Cl R$_1$= H- CH$_3$ R=O R$_2$= CH$_3$
i;X=4-F R$_1$= H- CH$_3$ R=O R$_2$= CH$_3$

j; X=H,   Y=4-Cl
k; X=4-Cl ,Y=4-F

l; X=4-Cl,Y= H
m; X=4-Cl,Y=4-O CH$_3$

123

124

125

126a;R=R$_1$=R$_2$=OAc, R$_3$= CH$_2$OAc
126b;R=R$_1$=R$_2$=OH, R$_3$= CH$_2$OH

Fig. 39 Chemical structures of some thiazolo pyimidine derivatives

Novel amine substituted quinozoline-linked benzimidazole compounds were tested for antitumor activity. **135** elucidated the best activity against the prostate and colon cancer cell lines. In the SAR studies, it was revealed that the substituents at C-2 and C-4 position of the quinozoline ring affected the activity of the compounds and with benzimidazole at the C-4 position it also possessed growth inhibition property

[138]. A regioisomeric series of quinazoline and benimidazole hybrids were studied as anticancer agents and screened against a panel of 60 cancer cell lines. **136** was most potent of all, better than 5-FU (positive control) [139].

A series of quinozoline derivatives were reported in a simultaneous study for their activity against human tumor cell lines derived from 9 different
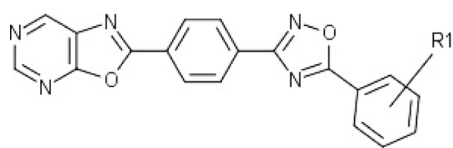
127a; R₁=3,4,5-trimethoxy

127b ; R₁=3,5-dinitro
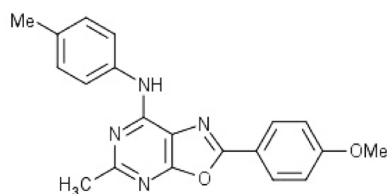
128
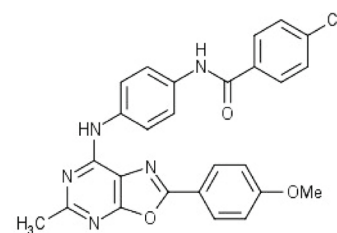
129

Fig. 40 Chemical structures of oxazolo pyrimidine derivatives
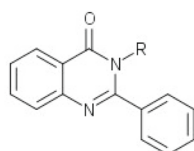
130a; R= Benzene
130b; R= 3-Chlorobenzene
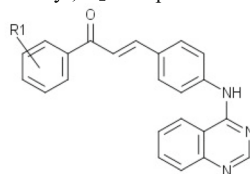**130c**; R= p-toluene
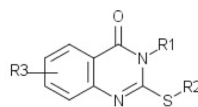
131a; R₃= Methyl, R₁=Benzyl, R₂=3-(Phthalimido-2-yl) propyl
131b; R₃=Methoxy, R₁= Benzyl, R₂=3-(Phthalimido-2-yl) propyl
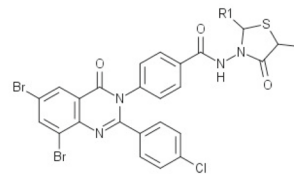**131c**; R₃=Methoxy, R₁= Benzyl, R₂=Morphilinoethyl
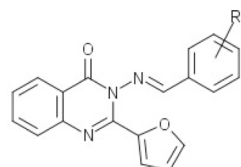
132; R₁=2-chloro

133a; R₁=2-fluoro-4-trifluoromethy
133b; R₁=4-trifluoromethyl
133c; R₁=3,4-dimethoxy
133d; R₁=4-methoxy

134a;R=H , R₁= p- toluene
134b ;R=H, R₁= 2-hydroxyPh

134c; R=
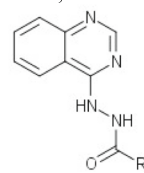R₁= benzene

134d;R=   , R₁=p-Toluene
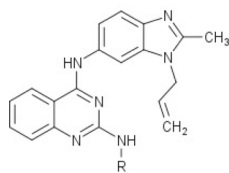
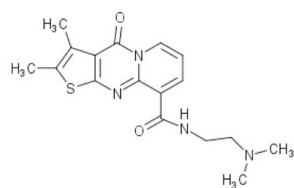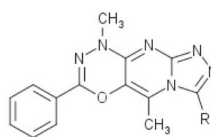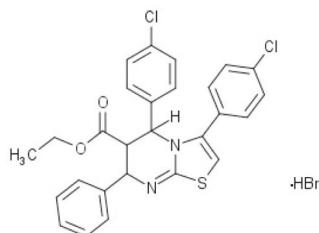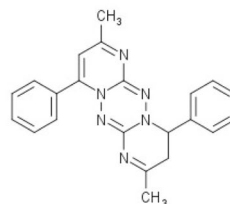**135**;RNH₂=1-Allyl-2-methyl-1H-benzimidazol-5-ylamine
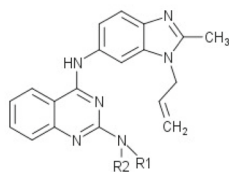
136; N R₁ R₂= Pyrrolidin-1-yl

137; R= 3,4,5-Trimethoxybenzene

Fig. 41 Chemical structures of some quinazoline derivatives

138   **139a** ; R= CH₃ , **139b**; R= SCH₂ CN

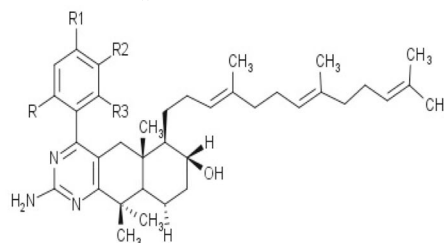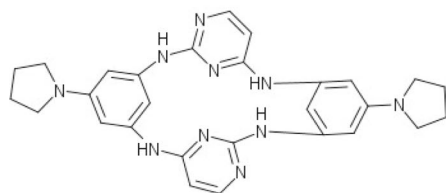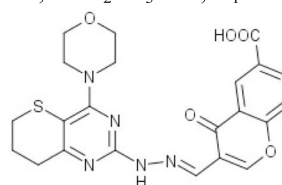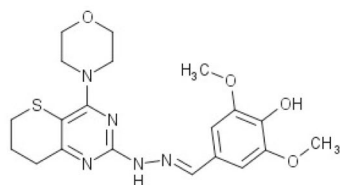**140**   **141a**
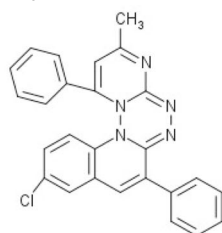
141b   **142a**; R=R₂=R₃ =H , R₁ =Br
            142b; R=R₂=R₃ =H , R₁ = Cl

143   **144a**

144b

Fig. 42 Chemical structures of some miscellaneous fused ring containing pyrimidine derivatives

sources using the SRB assay. **137** showed antiproliferative potency against all the cell lines due to the presence of the phenylvinylic substituent at 2 position. Other compounds with methylene, trichlorophenoxymethylene, and benzyl displayed moderate activity against specific cell lines (Fig. 41) [140].

Miscellaneous fused ring pyrimidine derivative

Some novel pyrido-thieno-pyrimidine derivatives were responsible for the induction of phosphorylation and acetylation which led to the activation of p53 in the colorectal cancer cells. **138** was responsible for the p53-mediated activity which led to activation of downstream

Fig. 43 SAR and chemical structure of novel anilino pyrimidine derivative

Presence of the ester moiety linking aniline to the benzene ring makes the compound more hydrophobic and it can easily penetrate through the cell membrane thus, increasing activity.
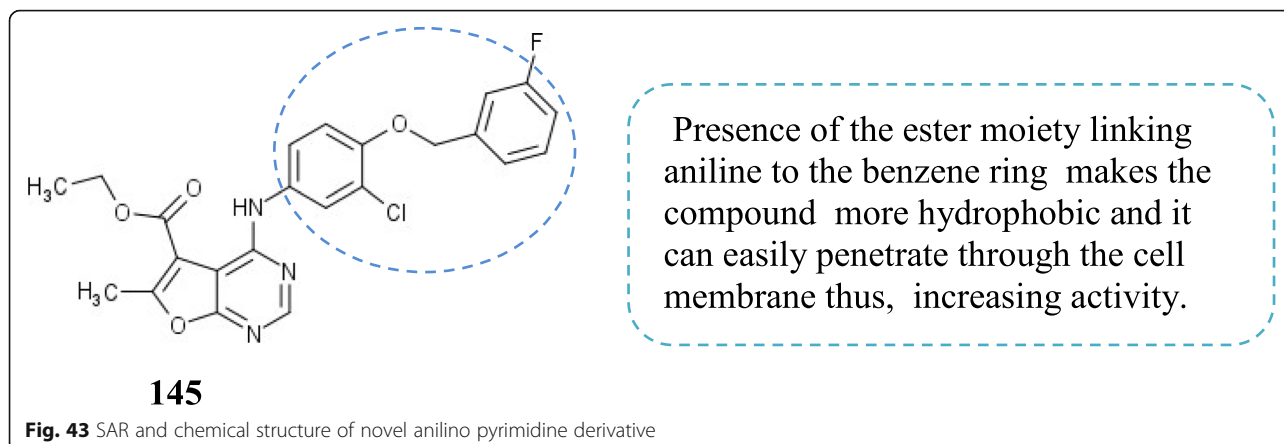
genes like p51 and PUMA (which resulted towards delay in growth, cell cycle stoppage at G1, cell senesce, and cell death). Controlled interaction between p53 and MDM2 led reduced degradation of p53 and due to these attributes, **138** was considered a potential anticancer agent [141]. Pyrimidooxadiazine and triazolopyrimidooxadiazine derivatives were reported as anticancer agents. **139(a–b)** elucidated the best potency against the A549, MCF7, and HepG2 cancer cell lines because of the change in substitutions at triazole moiety fused to pyrimido[4,5e][1,3,4]oxadiazine heterocyclic ring and C-7 position. Compounds with fused triazole ring were more active compared to the chlorine or pyrrolidine moiety at the C-7 position. Induction of cell death by apoptosis was caused by various cellular modifications such as biochemical changes like protein cleavage, protein cross-linking, DNA fragmentation, and phagocytic recognition [142].

Recently, a novel series of thiazolopyrimidine hydrobromides and triazolopyrimidines which act as topoisomerase II inhibitors was reported. The compounds were screened against 60 human cancer cell lines by the MTT colorimetric assay. Results elucidated **140** as the most significant inhibitor against the A498 (IC_{50}= 3.5 μM) cell line. The cell cycle was arrested at the G2/M phase leading to inhibition of cell proliferation and induced apoptotic activity. **140** also elucidated potent topoisomerase II inhibitory activity (IC_{50}=2.89 μM) versus DOX (IC_{50}= 2.67 μM) which was used as a positive control [143].

Fused pyrimidine hybrids were tested using MTT assay against the B-16, A-549, MCF-7, ACHN, and COLO-205 cancer cell lines. **141a** (IC_{50}= 9.5 μM) and **141b** (IC_{50}= 7.7 μM) showed the best activity against A-549 cancer cell line due to induction of apoptosis at the G0/G1 phase of the cell cycle. SAR suggested that 4-chloro and 4-bromo pyrimidine hybrid have 5–6 times more activity against the A-549 whereas 2–3 times more activity against the other abovementioned cell lines than

the parent compound [144]. Novel azacalix[2]aren e[2] pyrimidines were screened against the MCF-7 cancer cell line. **142(a–b)** were the best with anticancer activity with the IC_{50} = 0.58 and 1.82 μM respectively. Phenyl ring containing pyrrolidine group shows good ctytotoxic activity against A549, MCF7, and SHSY5Y cell lines but not against L02 cells. Compounds methyl and methoxy substituent on phenyl ring instead of no substitutions shows decreased activity. Other substitutions on the phenyl ring gave moderate activity whereas CF_3 group on the phenyl ring showed good activity [145].

Chromone moiety bearing 7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidine derivatives was tested against PC-3, A549, MCF-7, HepG2, and Hela cancer cell lines using MTT assay. Compound **143** showed excellent cytotoxicity and inhibitory activity against PI3Ka kinase, mTOR kinase, and the five cancer cell lines with IC_{50} = 1.1 μM, 0.92 μM, and 8.77 to 14.3 μM respectively. The presence of morpholine group and hydrazinyl group led to the anticancer activity [146]. Previously, antitumor activity of new 4-morpholino-7,8-dihydro-5H-thiopyrano[4,3-d]pyrimidines due to variations in substitutions of the aryl moiety and 4-OH substitution was reported. **144a** and **144b** showed strong antitumor activities against mTOR kinase, PC-3, and H460 cell lines with IC_{50} = 0.80 ± 0.15 μM, 11.90 ± 0.94 μM, and 7.43 ± 1.45 μM respectively (Fig. 42) [147].

Novel anilino pyrimidine derivatives were evaluated in 2018 and compound **145** (IC_{50} = 0.5 μM) depicted the most potent anticancer activity against A549 cell line mainly due to the presence of the linkage via ester moiety. SAR is illustrated in Fig. 43 [148].

Conclusions

Pyrimidine is a significant scaffold due to its presence in naturally occurring nucleotides and is being explored extensively for its anticancer profile. The present manuscript has discussed the anticancer potential of substituted pyrimidine at various positions as well as

pyrimidine fused with other heterocyclic ring. Substitutions at the C-2, C-4, and C-6 position of the pyrimidine core has a great influence on the antitumor activity especially a thio or amino group at C-2 and substituted phenyl group at C-4. Pyrimidine fused with five member rings like pyrazolo, pyrrolo, triazolo, imidazole, oxazolo, thiazolo, and thieno exhibited more distinct anticancer activity compared to the six member rings like pyrido and quinazoline. Pyrimidine analogs act as anticancer agent through diverse mechanism of action including, kinase (erbB2, raf, CDK, Src etc.) enzyme inhibition, cell cycle arrest, activation of oncogenes, reduction of mitochondrial membrane potential, increase of ROS, and induction of apoptosis by upregulation of apoptotic and downregulation of anti-apoptotic proteins. The current manuscript can be valuable to scientists and researchers around the globe to optimize and select specific targets for the development of potent lead molecules as anticancer agents in future.

Abbreviations
SAR: Structure activity relationship; EDG: Electron donating group; EWG: Electron withdrawing group; DNA: Deoxyribonucleic acid; RNA: Ribonucleic acid; IC_{50}: Half minimal inhibitory concentration; DOX: Doxorubicin; 5-FU: 5-Fluorouracil; ROS: Reactive oxygen species; EGFR: Epidermal growth factor receptor; COX-2: Cyclooxygenase-2; PRAP: Poly ADP ribose polymerase; FAK: Focal Adhesion Kinase; Raf: Rapidly accelerated fibrosarcoma; Pim-2: Serine/ threonine-protein kinase; erbB2: Receptor tyrosine protein kinase; VEGFR: Vascular endothelial growth factor receptor; S-Phase: Synthesis phase; G0 Phase: Resting phase; CDK: Cyclin-dependent kinases; Bcl: B-cell lymphoma; PTK: Protein kinase inhibitor; PDGFR: Platelet-derived growth factor receptor; STAT: Signal transducer and activator of transcription; HDAC: Histone deacetylase; VLAK: Virtual Lock-and-Key; PI3K: Phosphoinositide 3-kinase; MAPK: Mitogen-activated protein kinase; ER: Estrogen receptors; EAC: Elrich ascities carcinoma; XIAP: X-linked inhibitor of apoptosis protein; DNMT: DNA-methyltransferase; RET: Receptor tyrosine kinase; LSD-1: Lysine specific demethylase-1

Assays
SRB: Sulforhodamine–B; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; ELISA: Enzyme-linked immunosorbent assay; CAM: Chick Chorioallantoic membrane; LDH: Lactate dehydrogenase

Cancer cell lines
Breast: MCF7, T47D, BT-474, MDA-MB-231, MDA-MB-453, BT-549MCF10A-ras, Bcap-37, MDA361, MDA MB468, SK-BR-3; Colon: Caco-2, HT29, SW620, Colo-205, HCT-8, HCT-15, HCT-116, SW707; Lung: A-549, HOP-92, HOP62, NCI-H460, Hop-92, NCI-H522, NCI-H322, NCI-H23, HTB-54, H 460, H69AR; Leukemia: THP-1, Molt-4, P-388, L1210, HL-60, K-562, MEG-01, KU-812, CLL, U937, MCL-1 (myeloid cell leukemia 1), K-562 (chronic lymphocytic leukemia), CEM-13; Melanoma: B16F10, SK-MEL-2, A375, RPMl-8226; Ovary: IGROV-1, OVCAR-8, SK-OV-3, OVCAR-3, OVCAR-4, A2780; Liver: HEP-2, epG2, SK-Hep-1, Hep3B, BEL-7402; Prostate: DU-145, PC-3, RWPE-1, LNCaP; Stomach: SGC-7901; Huh7, HGC-27; Cervical cancer: HeLa; Gastric cancer cell line: MGC-803, SNU-638; Esophageal: EC-109, EC-9706; Neuroblastoma: SH-SY5Y; Kidney: CAKI-I, A498; Human osteosarcoma: SaOS-2; Connective tissue: HT-1080; Pancreatic: PANC-1; Urinary bladder: T-24

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AM, TP, and TS have equally contributed to this work. The authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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