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REVIEW ARTICLE

Targeting tumor cells with pyrazolo[3,4-*d*] pyrimidine scaffold: A literature review on synthetic approaches, structure activity relationship, structural and target-based mechanisms

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Abstract Pyrazolo[3,4-*d*]pyrimidine had been attracted awesome interest due to its pharmacological potential especially as an anticancer. Several mechanisms of action were accounted for the anticancer potential of this privileged scaffold. Previous researches explained its role in binding with many receptors as cyclin-dependent kinases, epidermal growth factor receptor, Src kinase, m-TOR, and JAK kinase. Nevertheless, there is an incredible demand for the discovery of target-oriented compounds. In this review, we shed the light on the antitumor potential of this important fused heterocyclic system, different mechanisms of actions of this ring, and SAR studies towards many targets in a trial to pave the way for medicinal chemists to optimize this ring system in order to discover new anticancer agents with better selectivity and increased anticancer potential.

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Contents

1. Introduction

Cancer is among the most dangerous diseases characterized by limitless proliferative potential, growth signals, self-sufficiency, in addition to apoptotic and antiproliferative cues resistance (Ruiz-Torres et al., 2017; González et al., 2019; Caon et al., 2020; Rossari et al., 2020; El Azab et al., 2021). It was detected that many risk elements including smoking, consuming alcoholic beverages, insufficient consumption of vegetables and fruits and obesity were the most important causes of cancer (Alsamarrai et al., 2014; Gul et al., 2016; Onvani et al., 2017; Salem and Mackenzie, 2018; Hur et al., 2019; Barrea et al., 2021; Entwistle et al., 2021; Feng et al., 2021). In addition, in low-income nations, cervical cancer in women occurred due to infection with papilloma virus through sexual transmission (Randall and Ghebre, 2016; Habtu et al., 2018; Cohen et al., 2019; Hull et al., 2020). Cancer receives assistance from the neighboring cells, forming new blood vessels to obtain oxygen and nutrients, metastasizes by spreading *via* blood vessels and lymphatic system to other organs especially lung, liver, and bones (Popper, 2016; Abdelgawad et al., 2017; Fares et al., 2020; Follain et al., 2020; Steinbichler et al., 2020). It has been established that neither radiation nor surgery nor the combination of both strategies can effectively manage metastatic cancer (Garbe et al., 2020; Cornford et al., 2021; Witjes et al., 2021). Furthermore, conventional chemotherapy is being used to cure cancer, but this kind of therapy does not distinguish between cancer cells and normal cells causing many adverse effects (Egeblad and Werb, 2002; Kerbel and Kamen, 2004; Jackson and Bartek, 2009; Abdellatif et al., 2014b). In an attempt to avoid these side effects, new strategies using molecular targeting agents had been developed to make treatments more tumor-specific (Tietze et al., 2015; Chester et al., 2018; Mooney et al., 2018; Park et al., 2019). This strategy uses medications that inhibit the growth of tumor cell through interfering with specific receptors and signaling pathways, so this strategy is more selective against cancer cells (Ho et al., 2014; Fan et al., 2015; Wang et al., 2019; Pottier et al., 2020; Zhang et al., 2020).

Literature survey recorded that pyrazolo[3,4-*d*]pyrimidine fused ring drew great attention as a result of its diverse and versatile pharmacological potential due to the chemical similarity between this scaffold and purines (Schenone et al., 2004; Akbari, 2008; Bakr et al., 2012b; Abdellatif et al., 2014a; Bakr and Mehany, 2016; Falsini et al., 2017; Li et al., 2019). It exhibited many pharmacological activities including antiviral (Wang et al., 2018), anti-inflammatory (Yewale et al., 2012; Yadava et al., 2013; Bakr et al., 2016), antimicrobial (Abdel-Megid, 2020; Greco et al., 2020; Hassaneen et al., 2020), antimycobacterial (Kulkarni et al., 2021), antihypertensive (Xia et al., 1997; El-Hamouly et al., 2006), radioprotective (Ghorab et al., 2009; Ghorab et al., 2010; Ghorab et al., 2012), antioxidant (Hassan et al., 2011; Rashad et al., 2011; Kostić et al., 2021).

This scaffold had been documented in literature to exhibit anticancer activity through many different mechanisms (Abdelall et al., 2014; Abdellatif et al., 2014a; Bakr and Mehany, 2016; Bakr et al., 2017; Abdellatif and Bakr, 2021). This review discusses the efforts of researchers in medicinal chemistry in the discovery of novel pyrazolopyrimidines with anticancer potential focusing on the mechanism of action of these candidates with particular emphasis on their structure activity relationship (SAR) and the synthetic procedures for the preparation of the major pyrazolo[3,4-*d*]pyrimidines.

2. Synthetic approaches

A lot of strategies had been used for the synthesis of pyrazolo [3,4-*d*]pyrimidines depending on the starting materials.

2.1. From pyrazole derivatives

o-Aminoamide pyrazole **I** was documented as starting material for preparing many pyrazolopyrimidine derivatives through different pathways (Fig. 1). Treating the aminoamide pyrazole **I** with diethyl oxalate afforded pyrazolylaminoxoacetate intermediate, which cyclized to the corresponding pyrazolopyrimidine in 73% yield using reflux in glacial acetic acid (Pathway A) (Harb et al., 2005). Also, refluxing *o*-aminoamide **I** with the appropriate aromatic aldehydes employing acidic catalyst as Keggin type heteropolyacid $H_3PW_{12}O_{40}$ and Pressler type, $H_{14}[NaP_5W_{30}O_{110}]$ for 0.5–3 h afforded novel set of pyrazolopyrimidines (Pathway B) (Heravi et al., 2006). Davoodnia et al. (2006) reported the

construction of some pyrazolopyrimidines utilizing one-pot two components reaction of orthoesters and *o*-aminoamide pyrazole **I** utilizing silica gel under microwave irradiation [Pathway C]. In addition, cyclizing *o*-aminoamide **I** with the appropriate aldehydes using equimolar amount of iodine as oxidizing agent in acetonitrile yielded the corresponding pyrazolopyrimidines (Pathway D) (Bakavoli et al., 2010). On the other hand, reacting *o*-aminocyanopyrazole **II** with trimethyl orthoformate in acetic anhydride gave pyrazolylimidoformate derivative. Reacting the latter with hydrazine hydrate followed by heating the product with dry benzene afforded the corresponding pyrazolopyrimidines (Pathway E) (Harb et al., 2005). Besides, refluxing *o*-aminocyanopyrazole **II** for 6 h with formic acid produced the corresponding pyrazolopyrimidine (Pathway F) (Yang et al., 2008). In 2019, a group of coworkers designed novel series of pyrazolopyrimidines employing [3 + 2]cycloaddition reaction (Rahmouni et al., 2019). First, refluxing *o*-aminocyanopyrazole **II** with acetic anhydride for 6 h followed by thermal intramolecular cyclization of the intermediate with piperidine in ethanol for 4 h (Pathway G). Reaction of *o*-aminocyanopyrazole **II** with *p*-nitrobenzoyl chloride followed by refluxing the pyrazole intermediate with H_2O_2/NaOH resulted in the pyrazolopyrimidine (Pathway H) (Kaplan et al., 2010). In addition, pyrazolopyrimidine ring could be obtained from *o*-aminoesterpyrazole **III** either by reaction with hydrazine hydrate and trimethyl orthoformate

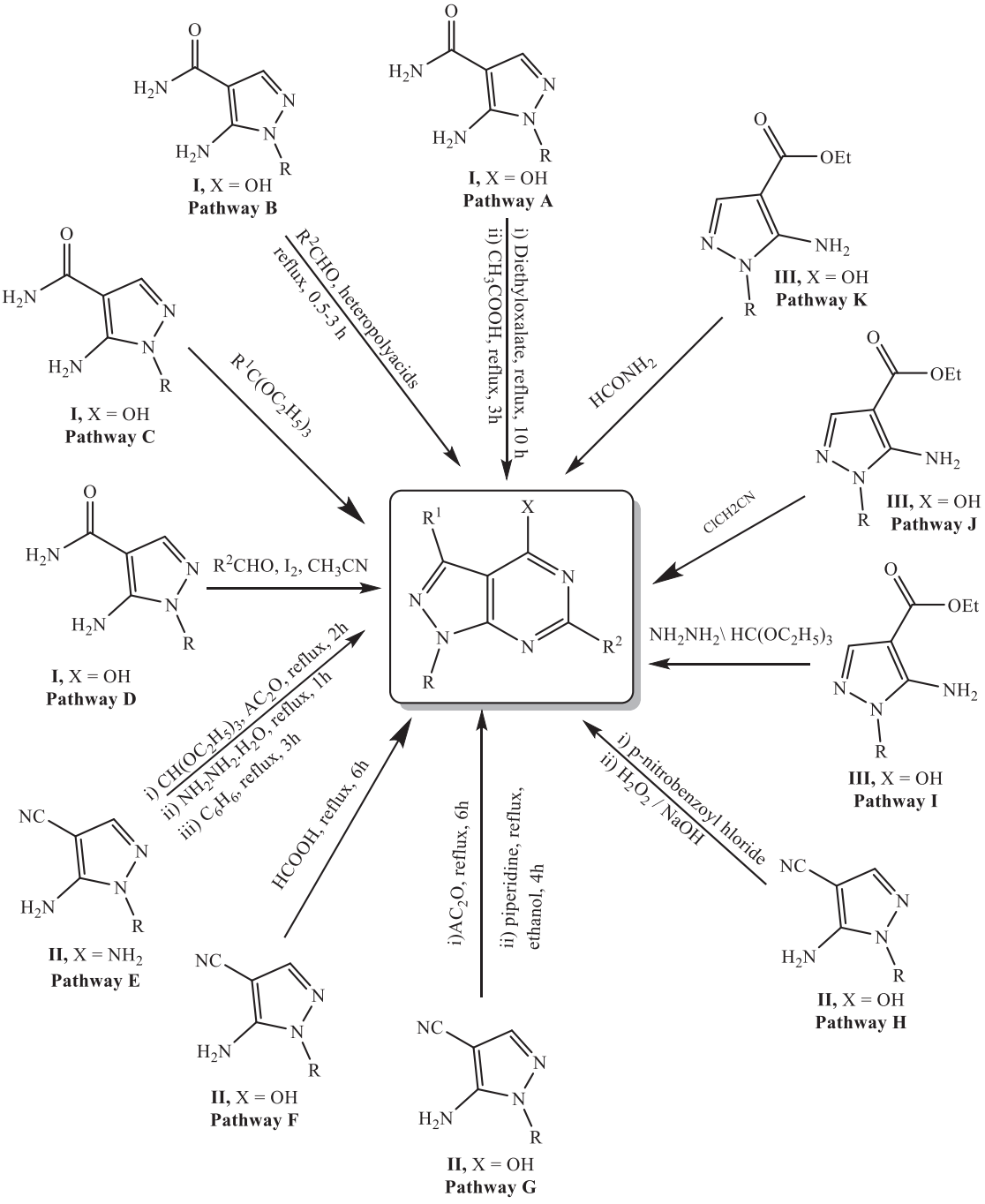


Fig. 1 Synthetic strategies for pyrazolopyrimidines starting from substituted pyrazoles.

(Pathway I) (Ghorab et al., 2010) or chloroacetonitrile in dioxane (Pathway J) (Ogurtsov and Rakitin, 2021) and/or formamide (Pathway K) (Bakr et al., 2012a).

The mechanism of pyrazolopyrimidine formation from *o*-aminocyanopyrazole **II** using acetic anhydride (Pathway G) is demonstrated in Scheme 1 (Abdellatif and Bakr, 2018).

2.2. From pyrimidine derivatives

Pyrimidine derivatives were reported in many publications as starting material for the construction of pyrazolopyrimidines. Refluxing 1,3-dimethyl-6-hydrazinouracil (**III**) with α-ketoalkynes in ethanol yielded the corresponding pyrazolopyrimidine (Pathway L) (Prajapati et al., 2006) (Fig. 2). In 2017, novel pyrazolopyrimidines were constructed from cyclizing 4-amino-6-chloropyrimidine-5-carbonitrile **IV** with isopropylhydrazine hydrochloride (Wang et al., 2017) (Pathway M).

In addition, pyrazolopyrimidines could be obtained from reacting trichloropyrimidine **V** with 1-benzyl-4-hydrazinylpiperidine dihydrochloride at 78 °C in presence of trimethyl amine (Pathway N) (Gilbert et al., 2010). Moreover, treating 4-chloro-5-cyanopyrimidine **VI** with *tert*-butyl carbazate in ethyl amine produced pyrazolopyrimidine (Pathway O) (Soth et al., 2011). Reacting *o*-aminocyanopyrazole **VII** with isocyanates in THF yielded pyrazolopyrimidines in one pot synthesis (Pathway P) (Chauhan and Kumar, 2013). In addition, refluxing 3-cyano-2-methylthiopyrimidine derivative VIII with hydrazine hydrate in dry DMF for 4 h afforded the pyrazolopyrimidine (Pathway Q) (Khobragade et al., 2010). Finally, treating pyrimidine derivatives **IX** with hydrazine hydrate in ethanol yielded the target pyrazolopyrimidines (Pathway R).

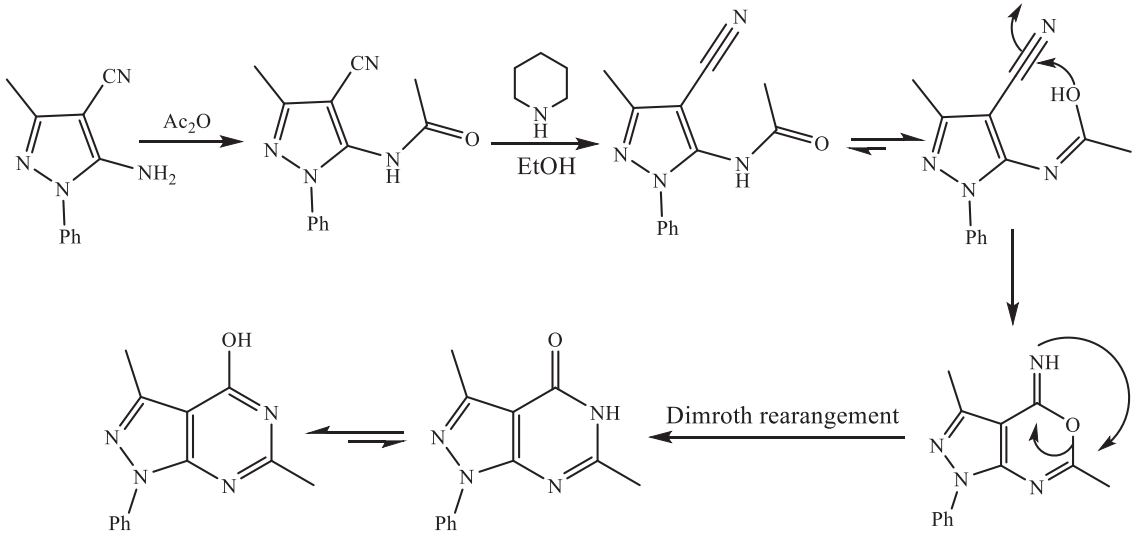
3. Mechanism of action of pyrazolopyrimidines as antitumor agents

3.1. Cyclin dependent kinase (CDK) suppressors

CDKs are kinases belonging to serine threonine kinases, which are responsible for transcription and cell cycle regulation, and the differentiation of cell (Kreis et al., 2019; Ding et al., 2020; Leal-Esteban and Fajas, 2020). The regulatory protein known as cyclin is bound by CDK and the human cdk2 structure has an altered ATP binding region, which modified by cyclin binding (Alexander et al., 2015; Henriques and Lindorff-Larsen, 2020). In absence of cyclin, CDK has little activity as T-loop (activation loop) blocks the cleft, and the position of key amino acid residues is not optimal for binding with ATP (Lu and Schulze-Gahmen, 2006; Liao, 2007). These CDK enzymes are overexpressed in cancer, and targeting CDK by many selective inhibitors like pyrazolopyrimidine derivatives is one of the strategies in curing cancer (Collins and Garrett, 2005; Malumbres and Barbacid, 2007; Musgrove et al., 2011).

Kim et al. (2003) prepared two pyrazolo[3,4-*d*]pyrimidine series **1a-d** and **2a-g** and evaluated the activity of these derivatives as CDK2 inhibitors. Structure activity study has been performed on these compounds, which showed that derivatives bearing anilino moiety as in compounds **2a-g** showed better CDK2 inhibitory activity than the corresponding benzyl analogues as shown in derivatives **1a-d.** The compounds **2d** and **2e** incorporating 3-fluoroanilino moiety at C-4 of pyrazolopyrimidine were as active as roscovitine (**3**) that served as the reference. Moreover, the unsubstituted compounds (**2a**, **2d**, **2e**, **2f**, and **2 g**) at N-1 exhibited higher CDK2 inhibitory potential compared to the substituted derivatives (**2b**, and **2c**) (Fig. 3, Table 1).

Le Brazidec et al. (2012) prepared a new set of 1,6-disubsti tutedpyrazolopyrimidines as kinase inhibitors, from this set, the target compound **4** (Fig. 4, Table 2) combined strong potency against Aurora-B kinases (AKA/AKB) and CDK1. Aurora family belongs to serine/threonine kinases, which are essential for mitotic cell division and for controlling the precise partition of the duplicated genome into two daughter cells (Al-Sanea et al., 2020). Aurora kinases overexpression has been implicated in many cancer types so, Aurora kinases are targeted for cancer therapy (Tang et al., 2017). SAR study displayed the influence of substituent lipophilicity at N-1 on potency and metabolic stability as explained in (Fig. 4). Furthermore, this study recorded that replacing the primary amide attached to the pyrrole ring at C-6 with disubstituted pyrrolidine amide moiety afforded the compound **5** with increased



Scheme 1 Mechanism of pyrazolopyrimidine formation.

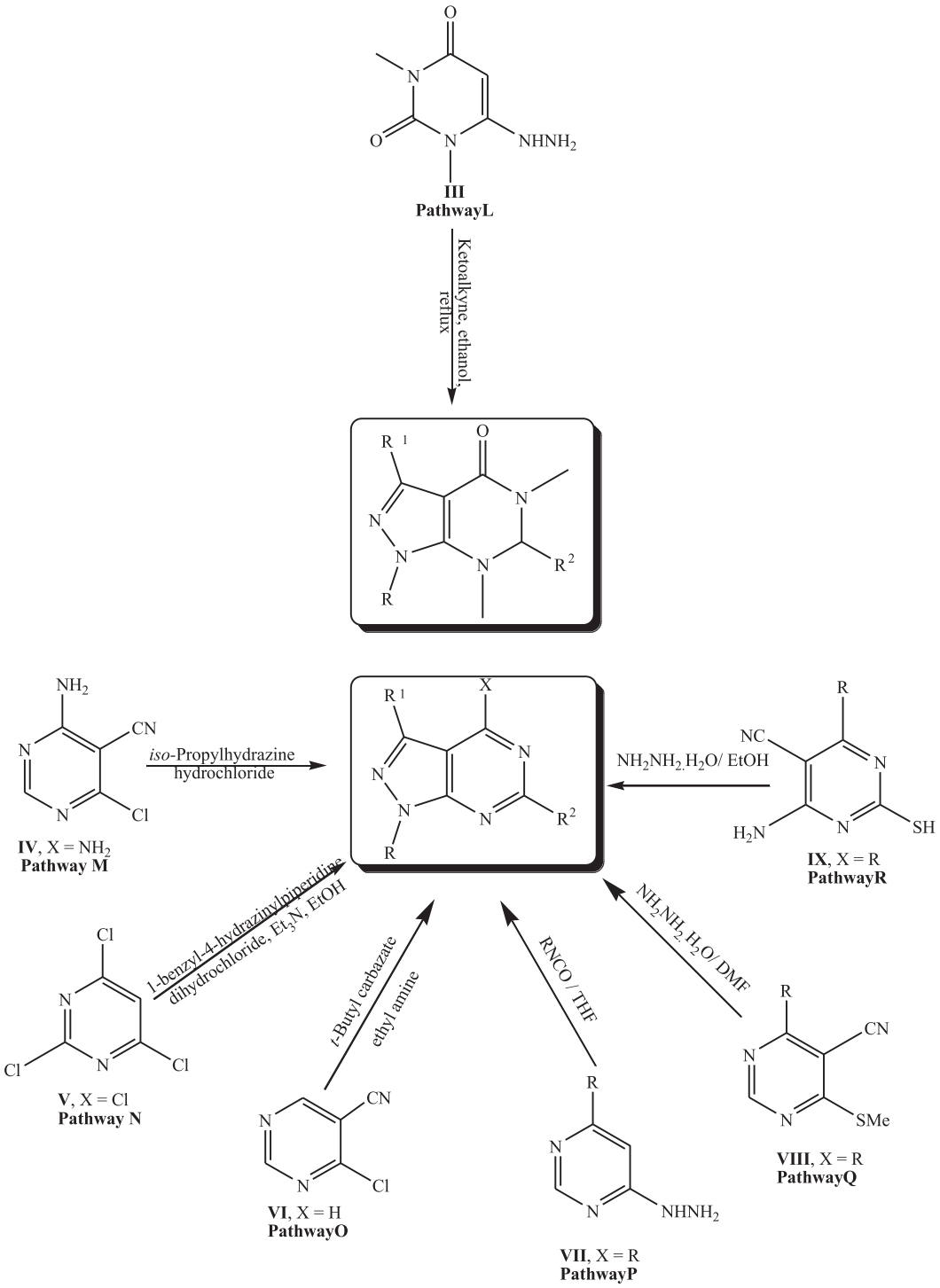


Fig. 2 Synthetic strategies for construction of pyrazolopyrimidine starting from substituted pyrimidines.

AKA/CDK1 inhibitory activity (Fig. 4). Furthermore, compound **6** (Fig. 4) had been designed as a roscovitine bioisostere and the anticancer potential of this compound **6** was screened on a panel of human cell lines (Jorda et al., 2011). The results revealed that this compound (**6**) recorded higher potential over roscovitine towards cancer cell lines, in addition, X-ray crystal structure of this pyrazolopyrimidine **6** binded with CDK2 recorded similar binding mode to that shown by roscovitine.

In addition, 4-amino-6-hydroxy-pyrazolopyrimidinisoxazolyl benzenesulphonamide derivative (**7**) (Fig. 5, Table 3) was documented to exhibit CDK2 inhibitory activity with IC_{50} = 0.24 μM and displayed excellent docking score (Ibrahim et al., 2009).

In 2012, Yang et al. (2012) prepared 3-aminopyrazolopyrimidinylaminophenylurea derivative (**8**) (Fig. 5, Table 3) which exhibited CDK1 inhibitory activity with IC_{50} = 78 nM. Recently, in 2018, new derivatives of 4,6-disubstituted pyrazolopyrimidines were constructed and screened for their inhibitory potential against CDK2 (Cherukupalli et al., 2018). SAR displayed that introducing thiophenethyl group at C6 as in **9a**

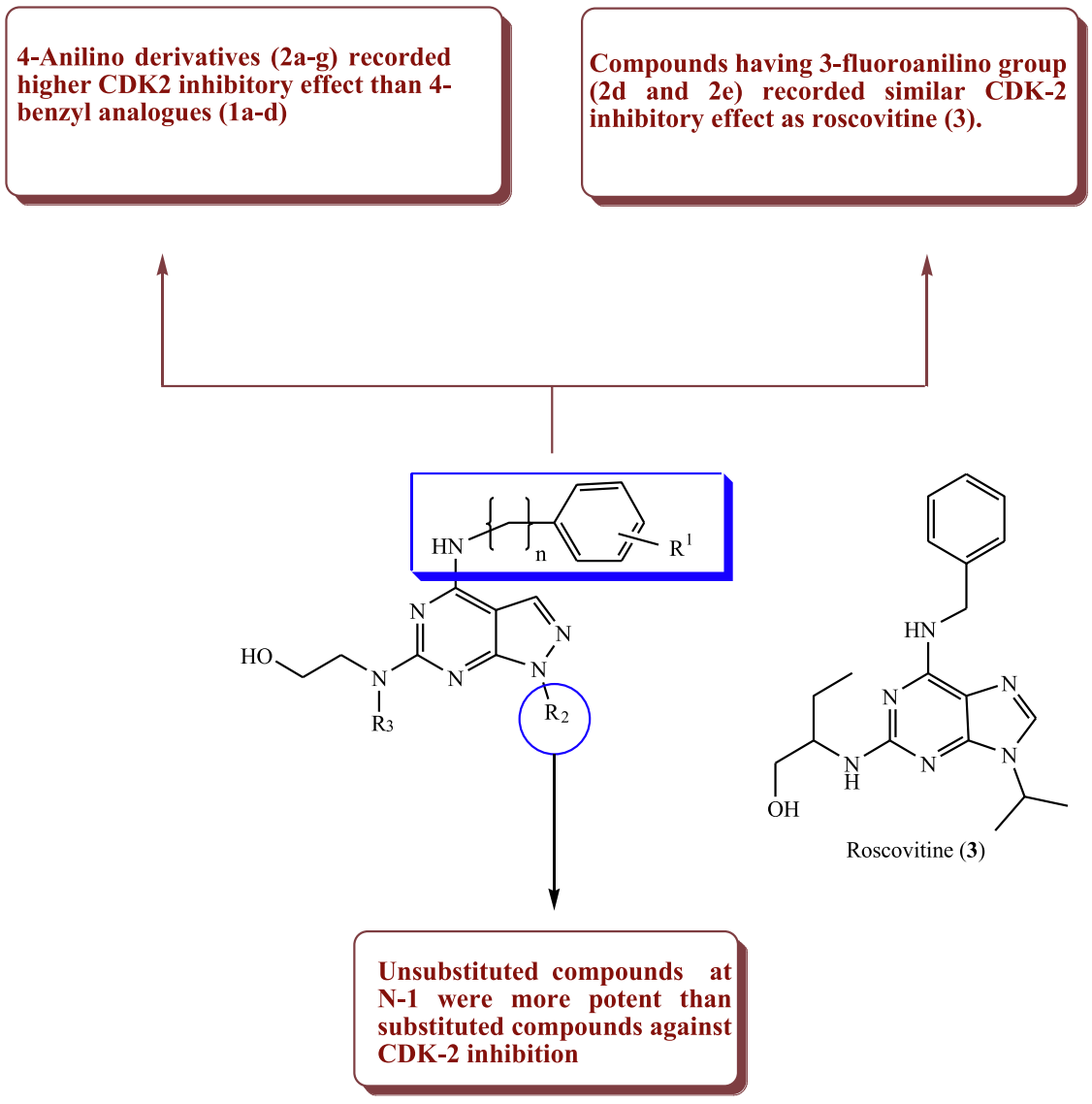


Fig. 3 Substitution effect and SAR of some pyrazolopyrimidines with CDK-2 inhibitory potential.

Table 1 IC_{50} of compounds **1a-d**, **2a-g** and roscovitine **(3)** on CDK2.

C.N	R^1	R^2	R^3	n (number of carbons)	CDK2 (IC_{50}, μM)	C.N	R^1	R^2	R^3	n (number of carbons)	CDK2 (IC_{50}, μM)
1a	H	H	H	1	29.5	**2c**	3-Br	CH_3	$(CH_2)_2OH$	0	>100
1b	H	CH_3	$(CH_2)_2OH$	1	>100	**2d**	3-F	H	H	0	0.5
1c	H	$CH(CH_3)_2$	H	1	>100	**2e**	3-F	H	$(CH_2)_2OH$	0	0.9
1d	4-NO_2	$CH(CH_3)_2$	H	1	46.8	**2f**	3-F	CH_3	H	0	21.4
2a	3-Br	H	$(CH_2)_2OH$	0	3.8	**2 g**	3-F	CH_3	$(CH_2)_2OH$	0	36.3
2b	3-Br	CH_3	H	0	>100	**3**	–	–	–	–	0.5

(IC_{50} = 5.1 μM) recorded better inhibitory activity than thiopentane group as in **9c** (IC_{50} = 17.7 μM). In addition, monosubstitutedphenyl moiety at C-4 exhibited higher CDK2 inhibitory effect than the disubstituted phenyl group which is obvious upon comparing target candidate **9a** (IC_{50} = 5.1 μM) with **9b** (IC_{50} = 13.4 μM) (Fig. 5, Table 3).

Moreover, a set of 5-substituted-3-isopropylpyrazolo[3,4-*d*] pyrimidine derivative **10** substituted at C-5 was prepared and screened for their inhibitory effect towards CDK2 and CDK5. From the obtained outcomes, most of the prepared compounds were more potent (IC_{50} < 1 μM) than the standard purine compound CR8 (**11**) towards CDK2/CDK5 inhi-

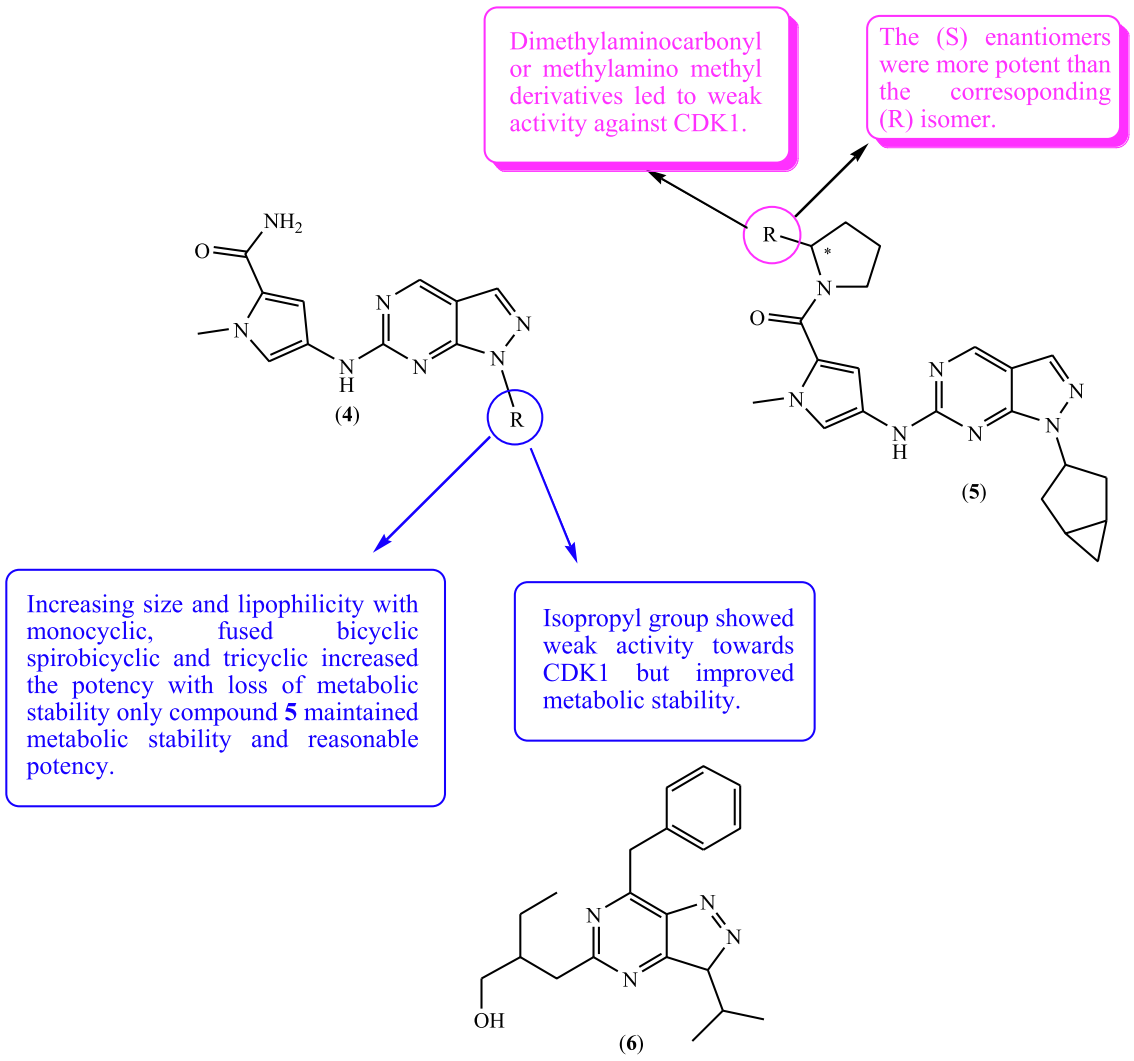


Fig. 4 SAR and substitution effect of pyrazolopyrimidines on CDK1 inhibitory potential.

Table 2 IC_{50} of compounds **4-5a,b** on CDK1 enzyme and HCT116 cell line.

C.N.	R	CDK1 IC_{50} (μM)	HCT116 IC_{50} (μM)	cLog*P*
4		0.024	0.079	1.6
5a	(*S*)CH_2OCH_3	0.022	0.01	–
5b	(*R*)CH_2OCH_3	0.15	0.06	–

bition (Vymětalová et al., 2016). The optimized SAR reveled that substitution at C-5 markedly improved the activity as depicted in Fig. 6, Table 4.

In addition, novel pyrazolo[3,4-*d*]pyrimidines incorporating benzenesulfonamide moiety were constructed and screened for their antiproliferative potential against MCF-7 and HepG2 cell lines (Hassan et al., 2017). Candidate **12a** was the most active cytotoxic agent on MCF-7 cell lines with IC_{50} = 1.4 μM, while **12b** was the most active on HepG2 with IC_{50} = 0.4 μM, respectively. Moreover, the mechanism of action of compound **12b** was postulated to inhibit CDK2 enzyme with IC_{50} = 0.19 μM. SAR study showed that substitution at positions 4 and 6 was essential for the cytotoxic potential as illustrated in Fig. 7, Table 5

3.2. Epidermal growth factor receptor (EGFR) inhibitors

EGFR belongs to *trans*-membrane growth factor receptor protein tyrosine kinases. EGFR has four members HER2 (known also as erbB2), HER1, HER3, and HER4. EGFR overexpres-

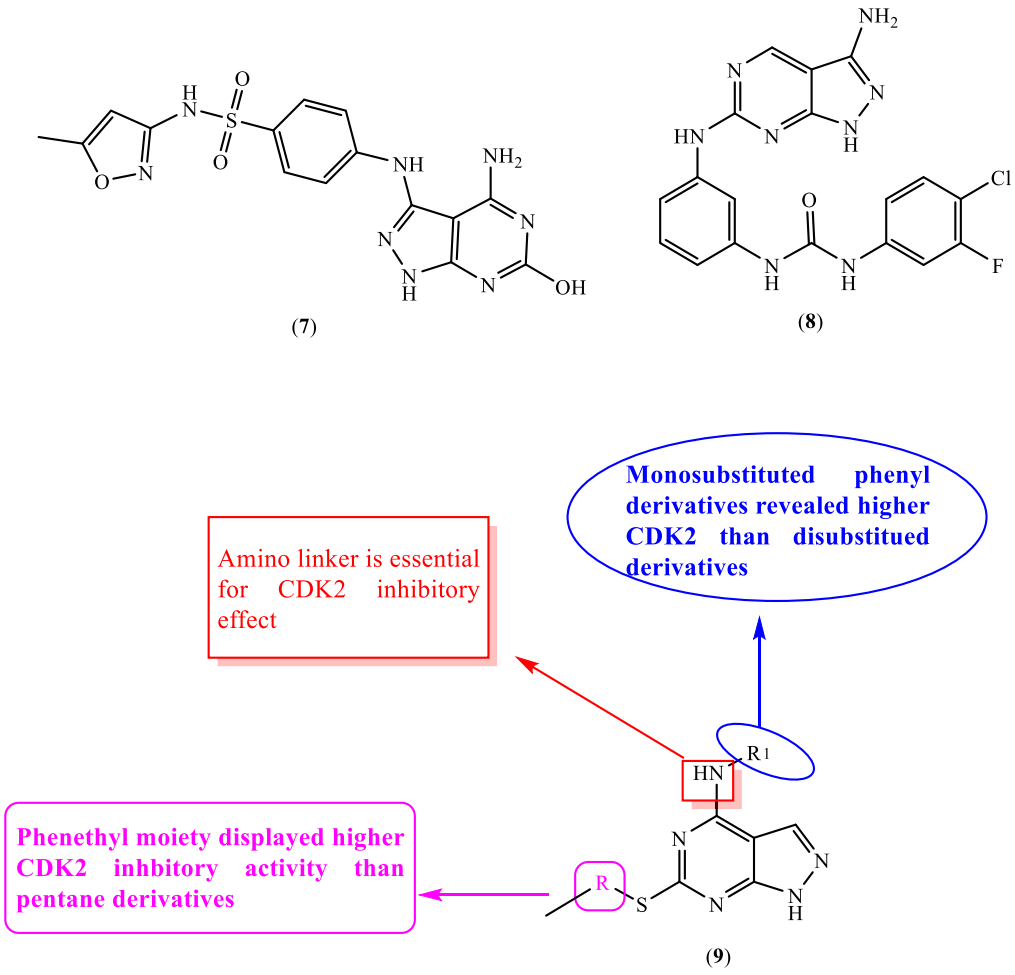


Fig. 5 SAR and substitution effect on CDK2 inhibition.

Table 3 CDK2 and anticancer inhibitory potential of some novel pyrazolopyrimidines.

C. N	R	R^1	CDK2 IC_{50}	Ab1 (μM)	K-526 (μM)	MCF-7 (μM)
7	–	–	0.24 μM	–	–	–
8	–	–	78 nM	–	–	–
9a	SCH_2CH_2Ph	3-NO_2-Ph	5.1 μM	> 25	> 12.5	> 12.5
9b	SCH_2CH_2Ph	4-Cl-3F-Ph	13.4 μM	> 25	> 6.25	> 6.25
9c	$S(CH_2)_3$	3-NO_2-Ph	17.7 μM	> 25	> 12.5	> 12.5
Roscovitine	–	–	0.1 μM	> 100	42	11

sion has been implicated in many cancer of epithelial origin (Rayego-Mateos et al., 2018).

In 2005, three series of pyridopyrazolopyrimidine **13a-c**, **14**, and **15a-f** (Fig. 8, Table 6) were constructed and tested for their activity against EGFR, erbB2 kinases (Alberti et al., 2005). SAR study of these derivatives demonstrated that the primary amino-substituted derivative **14** showed no activity against all the tested kinases. While, introducing an anilino moiety instead of the free amino group afforded potent inhibition towards erbB2 and/or EGFR. Substitution on the anilino moiety determines the potency towards erbB2 and/or EGFR for example, **13a** having a halogen disubstituted anilino group, showed more selectivity for EGFR than erbB2. While compound **13b** incorporating benzyl ether substituted anilino moiety exhibited potent dual EGFR/erbB kinase inhibitory activity. In addition, replacing the anilino moiety with aryl urea diminished the activity against erbB2 or EGFR as in **15a-f**. This diminished activity is attributed to inability of the 4-urea derivatives to bind with Thr830 as a result of the forming intramolecular H-bond between the tricyclic core nitrogen and the urea NH (Alberti et al., 2005).

In 2006, *N*-(1,3-benzodioxolylmethylphenylpyrazolopyrimidin-4-amine (**16**) (Fig. 9, Table 7) was reported by Cavasotto et al. (2006) as a low-micromolar EGFR suppressor. Furthermore, a novel set of pyrazolo[3,4-*d*]pyrimidin-yloxy-4-substitutedbenzylidene acetohydrazide were prepared and assayed for

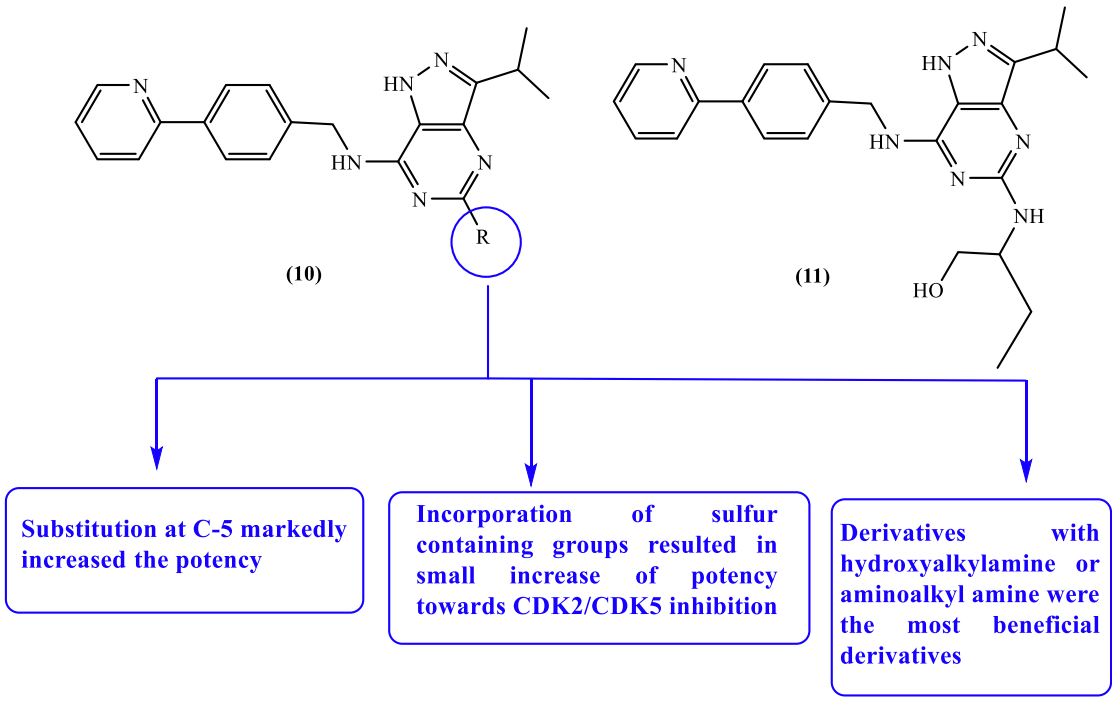


Fig. 6 SAR studies of some pyrazolopyrimidines with CDK2/CDK5 inhibitory potential.

Table 4 CDK inhibitory potential and cytotoxicity of some pyrazolopyrimidines.

C.N	R	CDK2 IC_{50} (μM)	CDK5 IC_{50} (μM)	K-562 IC_{50} (μM)	MCF-7 IC_{50} (μM)	G361 IC_{50} (μM)	HCT-116 IC_{50} (μM)
10a	H	0.197	0.183	1.000	1.190	0.875	1.237
10b	SO_2CH_3	0.070	0.165	0.640	0.460	0.793	1.140
10c	$NHCH_2C(CH3)_2$	0.009	0.001	0.029	0.024	0.048	0.085
11 (CR8)	–	0.062	0.225	0.175	0.160	0.503	0.350

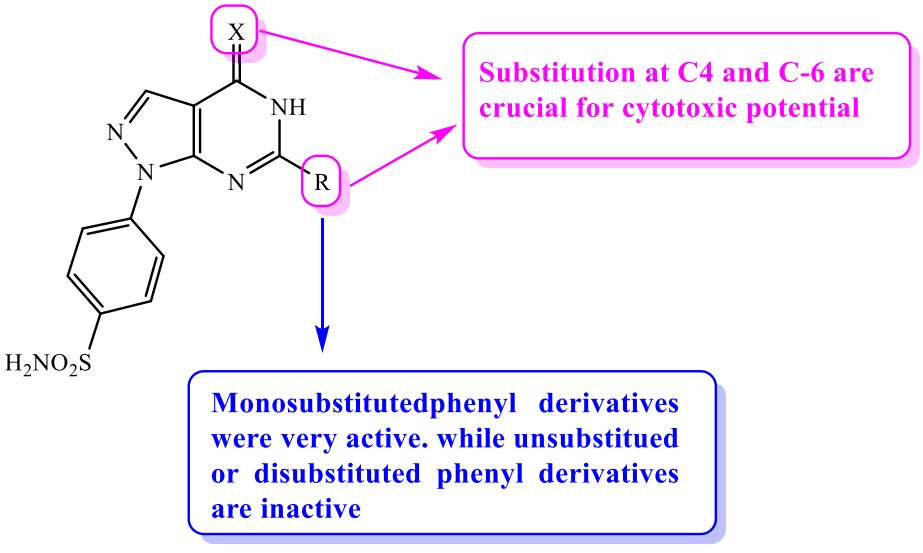


Fig. 7 SAR analysis of some pyrazolopyrimidines.

Table 5 Cytotoxicity and CDK inhibitory potential of compounds **12a,b** and DOX.

C.N.	R	X	MCF-7 IC_{50} (μM)	HepG2 IC_{50} (μM)	CDK2 IC_{50} (μM)
12a	4-OCH_3—C_6H_4	O	1.4	> 100	–
12b	C═O	NH	> 100	0.4	0.19
DOX	–	–	1.02	0.90	–

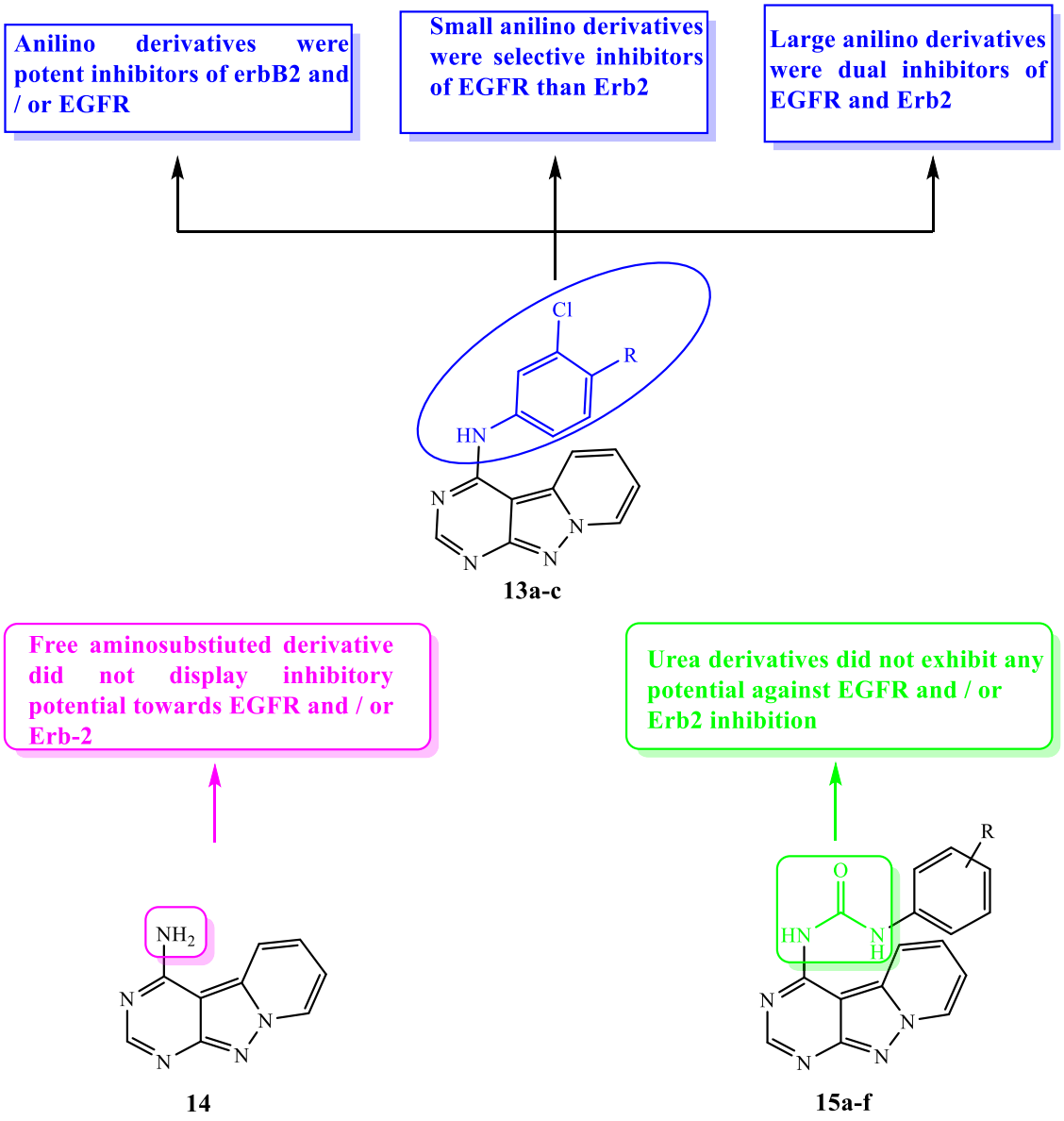


Fig. 8 SAR and substitution effect of pyrazolopyrimidines as EGFR inhibitors.

Table 6 EGFR and ErbB-2 inhibitory potential of some pyrazolopyrimidines.

C.N.	R	ErbB-2 IC_{50} (μM)	EGFR IC_{50} (μM)	C.N.	R	ErbB-2 IC_{50} (μM)	EGFR IC_{50} (μM)
13a	F	> 10	0.20	**15c**	3-NO_2	> 10	> 10
13b	OCH_2-3-F—C_6H_4	0.063	0.032	**15d**	4-Br	> 10	> 10
13c	OCH_2—C_6H_5	0.200	0.050	**15e**	4—CF_3	> 10	> 10
15a	3—CN	> 10	> 10	**15f**	4F	> 10	> 10
15b	3—CF3	> 10	> 10				

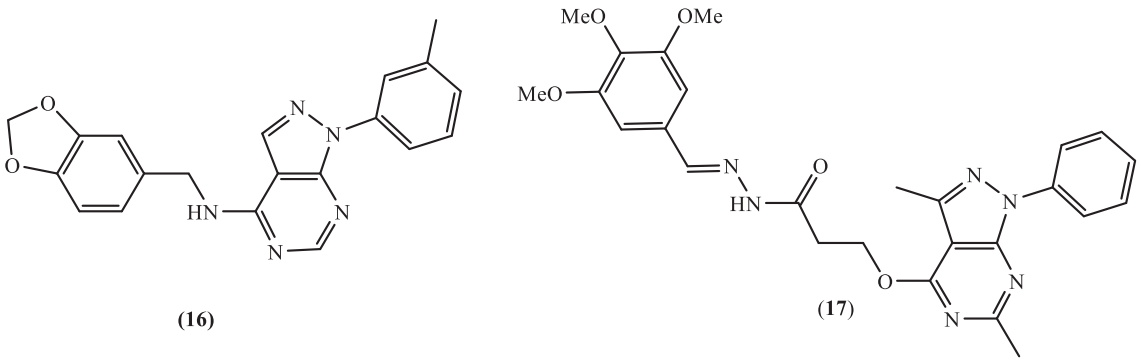


Fig. 9 Chemical structures of pyrazolopyrimidines **16** and **17**.

Table 7 Cytotoxicity and EGFR inhibitory potential of compounds **16** and **17.**

C.N.	MCF-7 IC_{50} (μM)	A549 IC_{50} (μM)	HT-29 IC_{50} (μM)	EGFR IC_{50} (μM)
16	–	–	–	15
17	6.14 ± 2.13	9.09 ± 2.36	5.36 ± 1.98	4.18
DOX	2.01 ± 0.87	1.17 ± 0.35	ND	ND

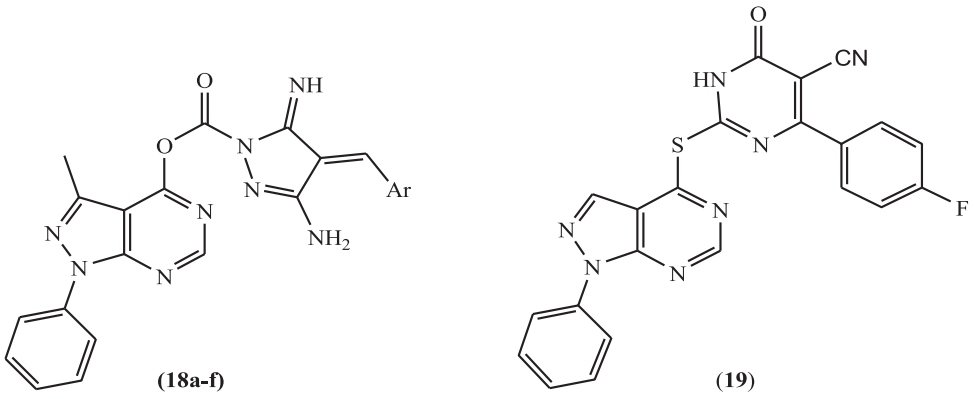


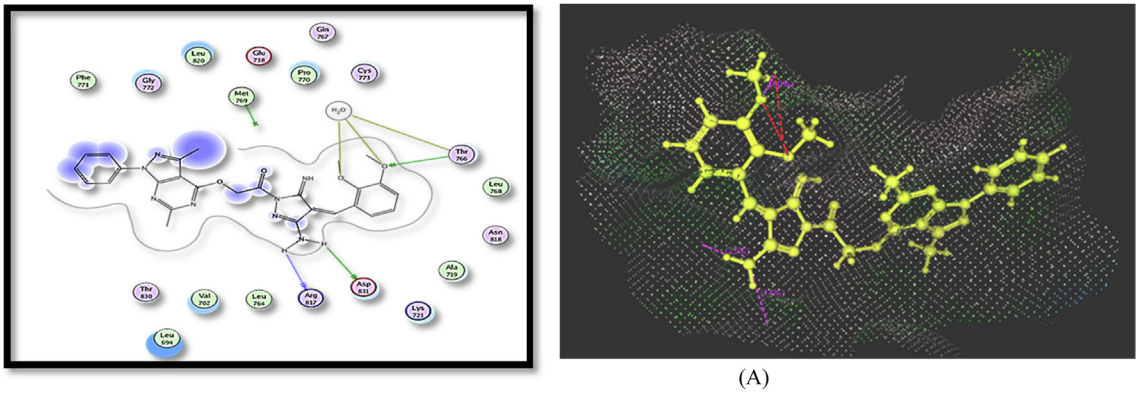
Fig. 10 Structure of pyrazolopyrimidine derivatives **18a-f** and **19** as EGFR inhibitors.

Table 8 Cytotoxicity and EGFR inhibitory potential of **18a-g.**

C.N.	Ar	HEPG-2 IC_{50} (μM)	MCF-7 IC_{50} (μM)	HCT-116 IC_{50} (μM)	EGFR IC_{50} (μM)
18a	3-OCH_3—C_6H_4	6.86 ± 0.08	8.17 ± 0.04	7.96 ± 0.62	10.91
18b	4-OCH_3—C_6H_4	5.52 ± 0.05	6.80 ± 0.02	5.87 ± 0.57	13.12
18c	4—Cl—C_6H_4	39.98 ± 0.42	54.19 ± 1.00	50.6 ± 1.52	18.82
18d	4-NO_2—C_6H_4	3.65 ± 0.02	1.45 ± 0.03	2.00 ± 0.32	14.34
18e	2,3-$(OCH_3)_2$—C_6H_3	10.88 ± 0.09	16.81 ± 1.01	17.27 ± 0.91	8.27
18f	2,3,5-$(OCH_3)_3$—C_6H_2	11.01 ± 0.10	15.69 ± 0.07	11.58 ± 0.81	3.71
DOX	–	5.66 ± 0.12	2.60 ± 0.02	8.48 ± 0.32	–

antitumor activity towards breast carcinoma (MCF-7), non-small cell lung cancer (A549) and human colorectal adenocarcinoma (HT-29) cell lines (Abdelgawad et al., 2016). This study showed that compound **17** recorded the highest anticancer potential with (IC_{50}) between 5.36 and 9.09 μM. Exposing this candidate to a small panel of kinases displayed that this candidate **17** was the most potent suppressor against EGFR with IC_{50} = 4.18 μM.

In 2016, Bakr et al. (2017) prepared hybrids of pyrazolopyrimidine incorporating pyrazole scaffold **18a-f** (Fig. 10,

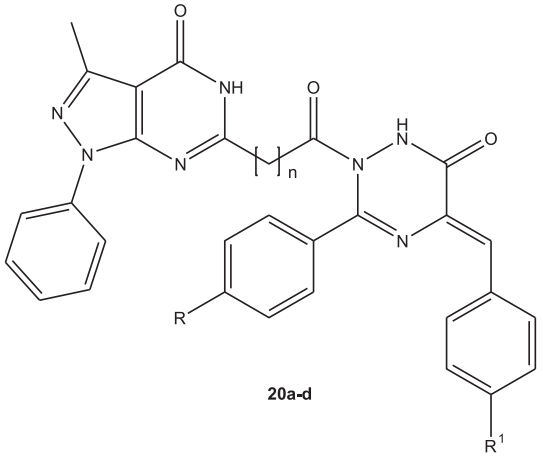


(A)

(B)

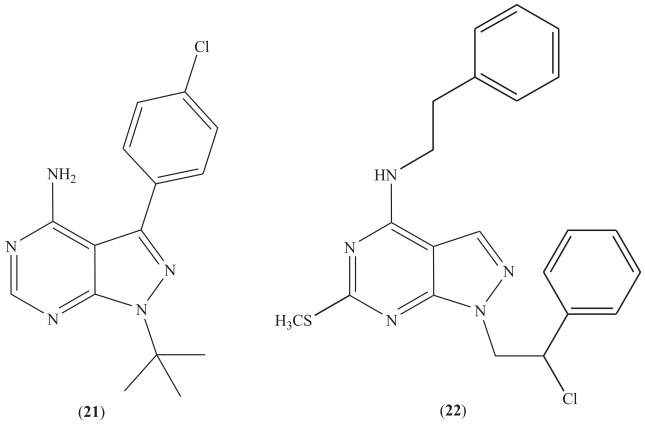
A) The interactions of **18e** with EGFR. B) 3D interactions of **18e** with EGFR.

Fig. 11 A) The interactions of **18e** with EGFR. B) 3D interactions of **18e** with EGFR.



20a-d

Fig. 12 Chemical structure of pyrazolopyrimidines **20a-d** as mutant EGFR inhibitors.



(21) **(22)**

Fig. 13 Structure of pyrazolo[3,4-*d*]pyrimidines **21** and **22** as Src kinase suppressors.

Table 8). These candidates were tested for their anticancer potential towards HCT-116, MCF-7 and HEPG-2 cell lines. All the tested compounds demonstrated good anticancer potential, especially **18d** was the most potent with IC_{50} = 3.65, 1.45, and 2.00 μM towards HEPG-2, MCF-7 and HCT-116 cell lines, respectively. These compounds (**18a-f**) were screened for their inhibitory activity against EGFR and this study detected that all the tested derivatives exhibited inhibitory activity with IC_{50} = 8.27–18.82 μM.

Furthermore, *in-silico* docking study had been done on compound **18e** which detected that this compound exhibited the energy score = -29.50 kcal/mol and demonstrated five H-bonds interactions between OCH_3 with Thr766, NH_2 with Asp831 and NH_2 with Arg817 (Fig. 11) (Bakr et al., 2017).

Furthermore, in 2015, pyrazolo[3,4-*d*]pyrimidin-4-yl)thio]d ihydropyrimidine-5-carbonitrile (**19**) (Fig. 10) was found to inhibit EGFR (91%) comparing to the standard drug gefitinb (100%) (Abbas et al., 2015).

Table 9 Cytotoxicity and inhibitory potential against EGFR-T790M and HER2.

C.N.	n	R	R^1	HCT-116 IC_{50} (nM)	MCF-7 IC_{50} (nM)	W138 IC_{50} (nM)	EGFR-T790M (% inhibition)	HER2 (% inhibition)
20a	0	CH_3	Cl	5.60 ± 0.25	59.30 ± 2.20	26.40 ± 0.59	65.70%	54.49%
20b	0	Cl	Cl	68.90 ± 2.18	6.90 ± 1.40	47.20 ± 1.87	76.35%	83.61%
20c	1	CH_3	Cl	4.80 ± 0.10	49.80 ± 1.70	29.40 ± 1.13	76.97%	72.61%
20d	1	Cl	OCH_3	12.90 ± 0.54	6.50 ± 0.10	54.90 ± 2.16	81.81%	86.66%
Lapatinib	–	–	–	12.00 ± 0.48	21.00 ± 0.54	45.20 ± 0.96	77%	94%

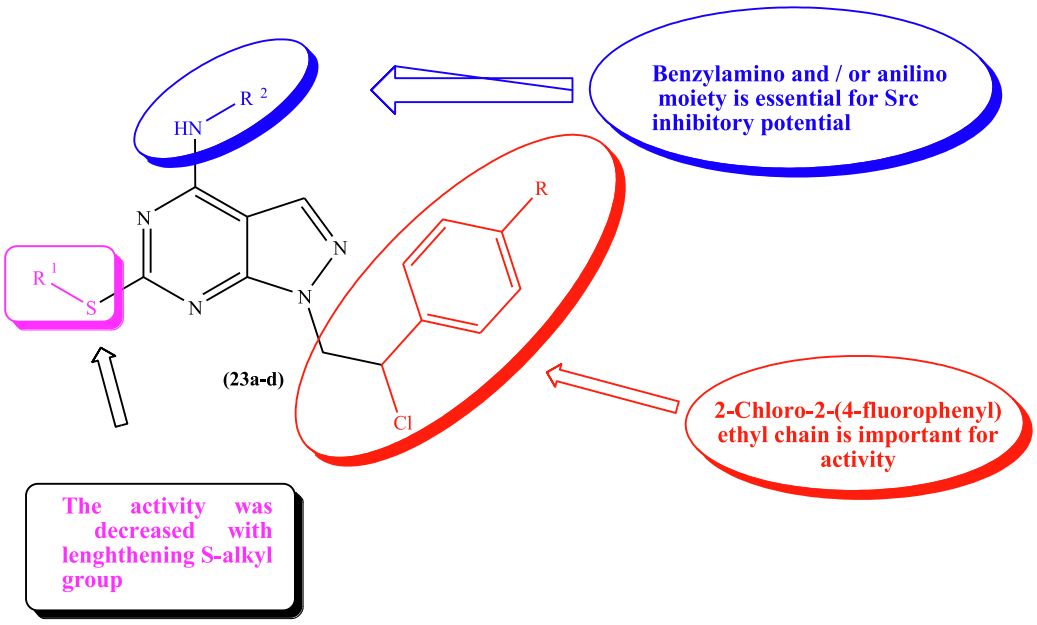


Fig. 14 SAR and substitution effect of pyrazolo[3,4-*d*]pyrimidines on Src inhibitory effect.

Table 10 Ki values of pyrazolopyrimidine derivatives **23a-d.**

C.N.	R	R^1	R^2	Src Ki (μM)
23a	F	CH_3	CH_2-4-F—C_6H_4	0.31 ± 0.07
23b	F	CH_3	CH_2-2-F—C_6H_4	0.30 ± 0.06
23c	F	CH_3	3-F—C_6H_4	0.21 ± 0.02
23d	H	C_3H_7	CH_2—C_6H_5	0.51 ± 0.1

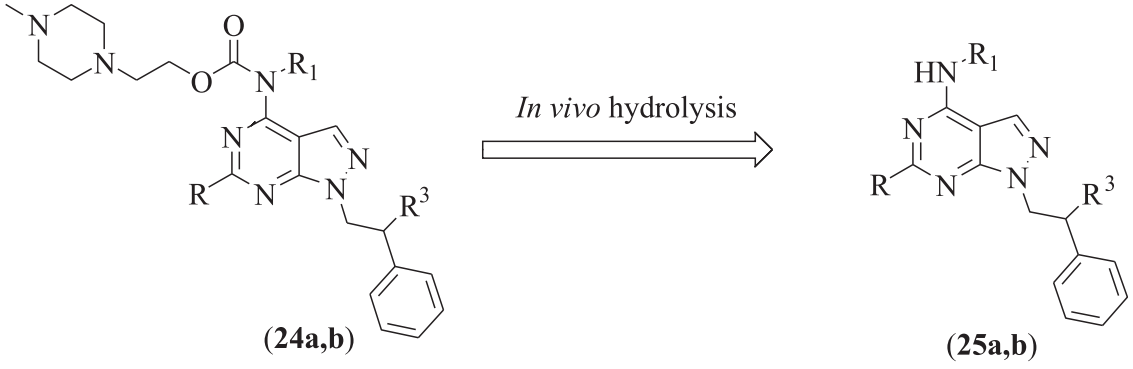


Fig. 15 Structure of pyrazolopyrimidines **24a,b** and **25a,b**.

Table 11 Cytotoxicity and Ki values of pyrazolopyrimidines **24a,b** and **25a,b.**

C.N.	R	R^1	R^2	*Ki* (μM)		IC_{50} (μM)	
				c-Src	C-Ab1	32D-p210	32D-T315I
24a	SC_2H_5	*n*Bu	Cl	0.60	0.32	3.5	6.7
24b	SCH_3	*m*BrC_6H_4	CH_3	0.02	1.07	6.2	5.8
25a	SC_2H_5	*n*Bu	Cl	NA	NA	1.2	2.4
25b	SCH_3	*m*BrC_6H_4	CH_3	NA	NA	2.8	2.6

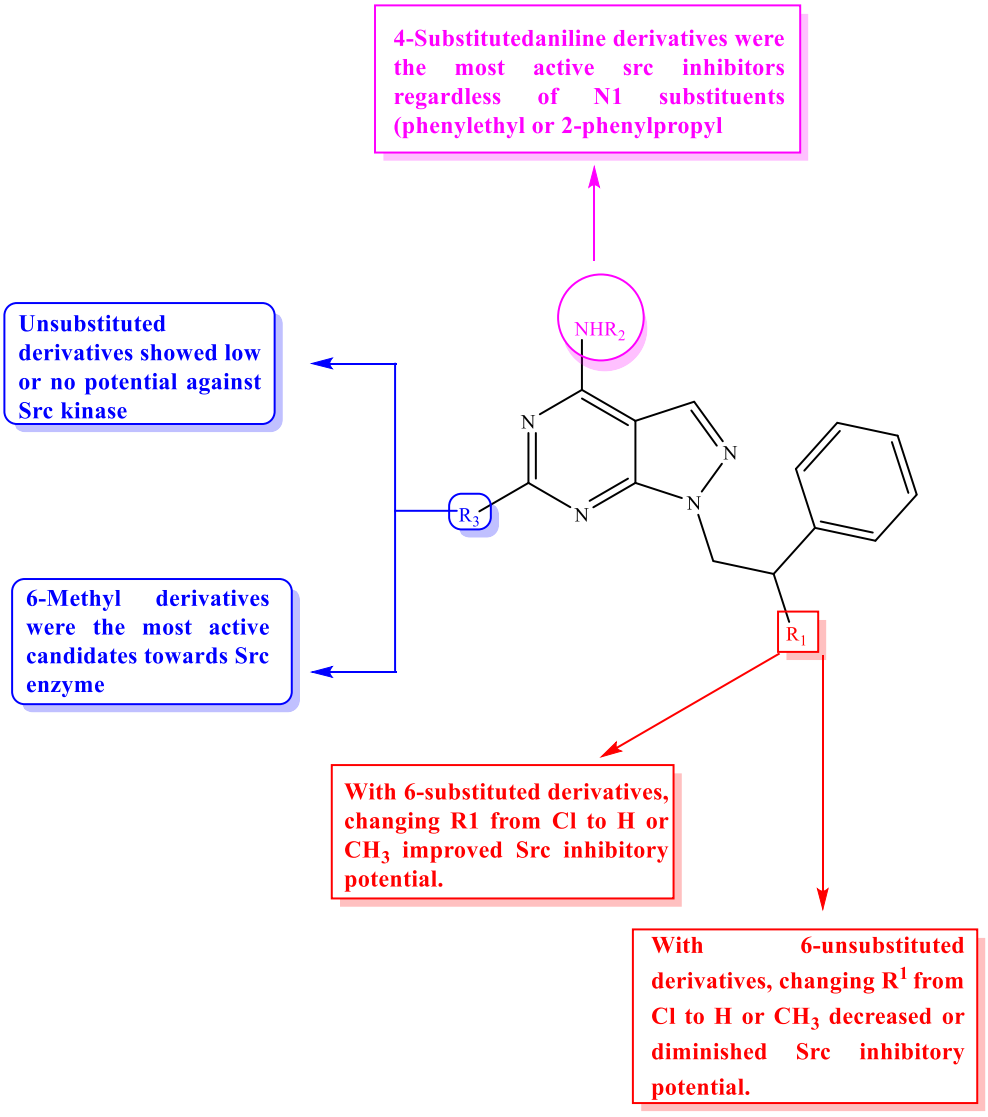


Fig. 16 SAR and substitution effect of pyrazolopyrimidines to Src inhibition.

Table 12 Ki values of pyrazolopyrimidine derivatives **26a-f**.

C.N	R^1	R^2	R^3	c-Src (Ki) μM	C.N	R^1	R^2	R^3	c-Src (Ki) μM
26a	H	3—Cl—C_6H_4-	SCH_3	0.09	40 g	CH_3	3—Cl—C_6H_4-	SCH_3	0.025
26b	CH_3	$CH_2C_6H_5$	SCH_3	3.7	40 h	CH_3	3-Br—C_6H_4-	SCH_3	0.018
26c	CH_3	CH_2-2-FC_6H_4	SCH_3	2.16	40i	H	CH_2-2-FC_6H_4	H	0.51
26d	CH_3	CH_2-4-FC_6H_4	SCH_3	2.81	40j	H	3—Cl—C_6H_4-	H	0.48
26e	CH_3	CH_2-2—ClC_6H_4	SCH_3	2	40 k	CH_3	CH_2-3-FC_6H_4	H	0.081
26f	CH_3	C_6H_5	SCH_3	0.025	40 l	CH_3	CH_2-2-FC_6H_4	H	0.151

In 2021, novel set of pyrazolopyrimidine was designed as mutant-EGFR inhibitors (Lamie et al., 2021). These derivatives were assessed for their anticancer activity against MCF-7, HCT-116 and W138 cell lines. It was found that compound **20d** (Fig. 12, Table 9) was the most potent deivative towards MCF-7 (IC_{50} = 6.50 nM) while, **20c** derivative was the most potent aginst HCT-116 (IC_{50} = 4.80 nM) comparing with the standard drug, lapatinib (IC_{50} = 21 nM, 12 nM, on

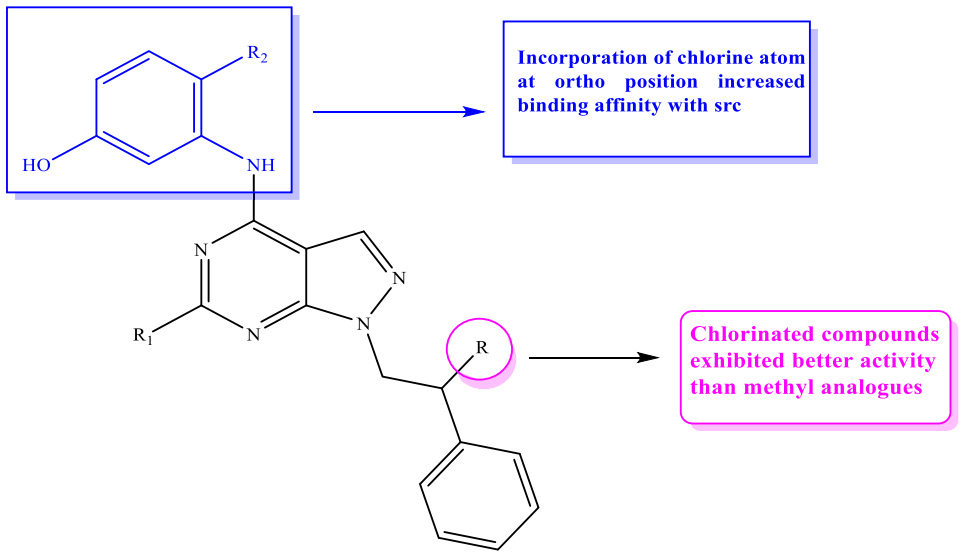
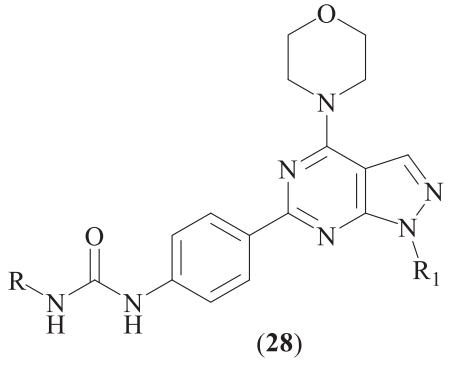


Fig. 17 SAR studies of pyrazolopyrimidines **27a-f** as Src Kinase suppressors.

Table 13 Cytotoxicity and Ki values of pyrazolopyrimidines **27a-f.**

C.N.	R	R^1	R^2	c-Src (K_i) μM	SH SY5Y (IC_{50}) μM
27a	CH_3	H	Cl	0.12	8.63 ± 0.7
27b	Cl	H	Cl	0.017	8.63 ± 0.7
27c	CH_3	SCH_3	Cl	0.05	6.7 ± 1.9
27d	Cl	SCH_3	Cl	0.0035	6.7 ± 1.9
27e	CH_3	$NHCH_2CH_2OH$	H	0.3	3.1 ± 0.3
27f	Cl	SCH_2CH_2-4-morpholino	Cl	0.0035	6.6 ± 1.8



(**28**)

Fig. 18 Structure of pyrazolopyrimidines **28**.

MCF-7 and HCT-116, respectively). In addition, derivatives **20a-d** were subjected to assessment towards EGFRT790M/HER2 inhibitory potential.the obtained data displayed that these candidates exhibited 81.81–65.70% and 86.66–54.49% inhibition to EGFRT790M and HER2, sequentially (Fig. 12, Table 9).

3.3. Src kinase suppressors

Src kinases family represents a major set of nonreceptor tyrosine kinases, and its members play important roles in signal transduction and so are of basic importance for many biological functions, including proliferation, differentiation, migration, apoptosis, and angiogenesis (Sridhar and Miranti, 2006; Guarino, 2010). Literature survey studies afforded evidence that c-Src enzymes are involved in the generation and metastasis in many tumors, including prostate, head, colon, breast, neck, lung, and pancreatic cancer (Coleman et al., 2005).

4-Amino-5-(4-chlorophenyl) 7 (dimethylethyl)pyrazolo [3,4 *d*]pyrimidine (PP2) (**21**) (Fig. 13) is one of the most prominent compounds used to suppress c-Src (Saito et al., 2010). Inhibiting Src by PP2 caused over-expression of markers representative of both epithelial polarization and intestinal terminal differentiation events, leading to up-regulation of Cdx2 and HNF1a and the reduction of polycomb PRC2-related epigenetic repressing activity, so Src family exert a critical role in regulating intestinal epithelial cell terminal differentiation (Seltana et al., 2013).

Preparation of novel pyrazolo[3,4-*d*]pyrimidines along with evaluating their Src and cell line proliferation inhibitory activity (A431 and 8701-BC cells) had been carried out by Carraro

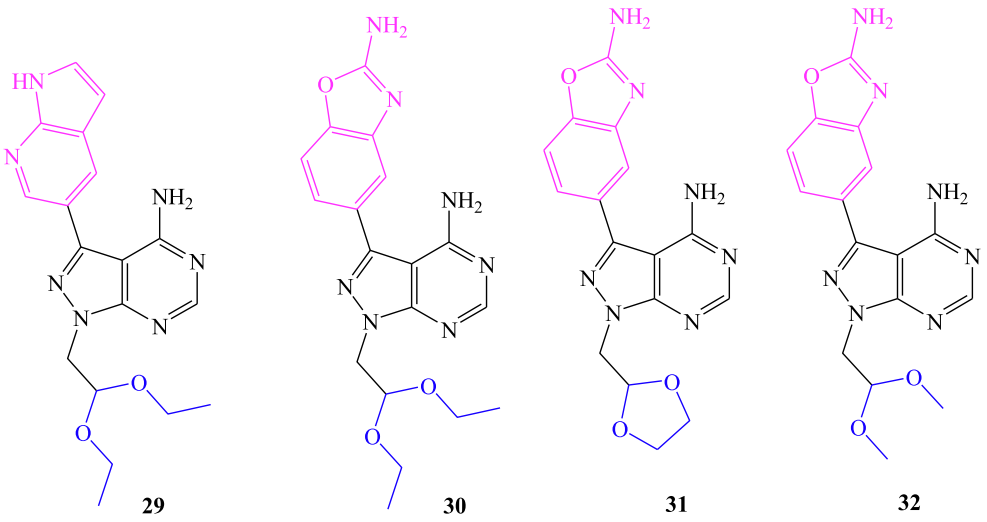


Fig. 19 Structure of pyrazolo[3,4-*d*]pyrimidines **29**–**32** with m-TOR inhibitory potential.

Table 14 Anticancer and m-TOR inhibitory potential of pyrazolopyrimidines **29**–**32**.

C.N	m-TOR IC_{50} (μM)	MCF (EC_{50}, μM)
29	328	320
30	15	8.4
31	59	7.6
32	25	6.7

et al (Carraro et al., 2006). From these derivatives, compound **22** (IC_{50} = 31.2 μM) showed Src inhibitory activity higher than the reference compound (**21**) (IC_{50} = 61.8 μM) by two-folds (Fig. 13). Unfortunately, compound **22** showed very low solubility in aqueous media and to enhance solubility Dreassi and coworkers solubilized this derivative with 30% w/v 2-hydroxypropyl-*b*-cyclodextrin solution. This method increased solubility of pyrazolopyrimidine derivative **22** and improved the antiproliferative potential against osteosarcoma (SaOS-2) and leukemic (K-652,KU-812 and HL-60) cell lines in comparison with the not complexed compounds (Dreassi et al., 2010).

In addition, a novel set of 4-amino substituted pyrazolopyrimidines **23a-d** (Fig. 14, Table 10) having a 2-chloro-2-phenylethylamino at N1 and thio-methyl or -propyl groups at C6 was synthesized and screened for their Src kinase inhibitory activity (Schenone et al., 2008). All of the screened derivatives demonstrated potent inhibitory activity with Ki values = 0.21–0.51 μM.

In addition, to improve pharmacokinetic properties of pyrazolopyrimidine fused ring, Vignaroli *et al* (Vignaroli et al., 2013) designed pyrazolo[3,4-*d*]pyrimidine derivatives as prodrugs (**24a,b**) (Fig. 15), with high water-solubility. *In vitro* studies recorded a significant enhancement of water solubility and plasma stability, proposing predominant in vivo bioavailability. These prodrugs **24a,b** did not exhibit any potential against Src and Ab1 but displayed significant antitumor effect in myeloid cell lines, as a result of hydrolysis of solubilizing moiety and release of the active compounds **25a,b** (Fig. 15, Table 11).

Radi *et al* (Radi et al., 2011) prepared novel pyrazolo[3,4-*d*] pyrimidines **26** and these compounds were able to suppress the growth of SH-SY5Y neuroblastoma cell lines, in particular compound **26a** was the most potent derivative (IC_{50} = 80 μM). In addition, these novel compounds were assayed for their Src inhibitory potential as a mechanism for their antiproliferative potential. SAR analysis described the importance of methylthio group at C-6 and anilino moiety at C-4 of the pyrazolopyrimidine ring as depicted in Fig. 16 and Table 12.

Recently, in 2018, Molinari et al. (2018) designed and constructed novel pyrazolopyrimidines **27** incorporating 3-hydroxyphenylamino moiety at C-4 and added different substituents at C-6 in an attempt to modulate ADME and expand SAR. All the designed derivatives had been assayed for their Src inhibitory activity and cellular viability on SH SY5Y cells. The outcomes obtained displayed that compounds **27d** and **27f** recorded the highest inhibitory potential against Src enzyme with Ki value equal to 3.5 nM. SAR study showed that compounds incorporating 3-amino-4-chlorophenolic moiety are more active than C-4 non halogenated derivatives. This was in accordance with the computational study which recorded a favourable ortho substitution of a hydrogen with a chlorine atom ($\Delta\Delta G$ = 4.8 Kcal/mol) as depicted in Fig. 17 and Table 13.

3.4. m-TOR Inhibitors

Mammalian target of rapamycin (mTOR) is seine/threonine kinase, which acts as the catalytic subunits the catalytic subunit of two essential protein complexes called mTORC1 and mTORC2. These complexes exert fundamental role in signal transduction to regulate proliferation, metabolism, migration, and survival (Chang et al., 2015). Overexpression of mTOR is reported in many cancers and neurodegenerative disorders

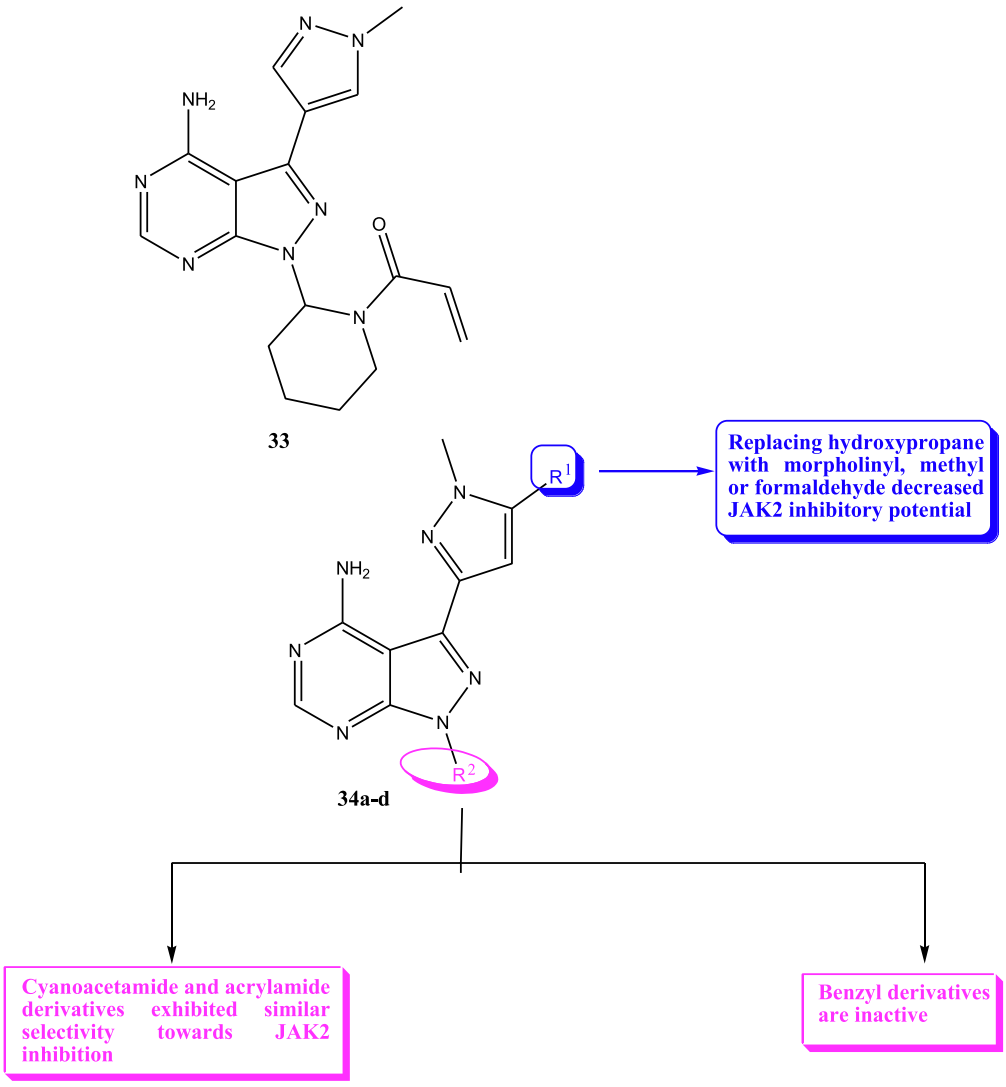


Fig. 20 Structure of pyrazolopyrimidines **33, 34a-d** as JAK2 inhibitors.

(Guo et al., 2011). In 2009, Verheijen and co-workers (Verheijen et al., 2009) hybridized 4-morpholino-1*H*-pyrazolopyrimidines with alkylureidophenyl groups at C-6 of pyrazolo[3,4-*d*]pyrimidine ring to give target compounds **28** (Fig. 18). These targets **28** demonstrated high mTOR inhibitory activity with (IC_{50} < 1 μM).

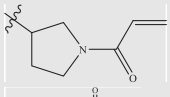
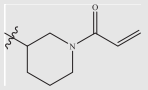
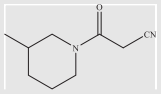
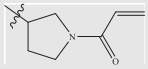
Fraser *et al* (Fraser et al., 2016) discovered the lead pyrazolopyrimidine compound **29** (Fig. 19) as m-TOR inhibitory candidate with EC_{50} = 320 μM. Optimization of the hit compound **29** by introducing 2-amino-1,3-benzoxazole ring at C-3 and adding different acetal groups at N-1 afforded the target compounds **30**–**32**. These compounds are tested for their antiproliferative potential towards MCF-7 and for their kinase inhibitory potential towards panel of kinases. All the new compounds (**30**–**32**) possessed superior antiproliferative effect towards MCF-7 cell line than lead compound **29** with EC_{50} = 8.4, 7.6 and 6.7 μM, respectively. Moreover, these candidates revealed higher inhibition to mTOR than exhibited by lead compound **29** (Fig. 19) (see Table 14).

3.5. JAK kinase suppressor

The Janus kinases (JAKs) and their downstream effectors, signal transducer and activator of transcription proteins (STATs) form a critical cell signalling circuit, which control survival, proliferation, and differentiation of a variety of cell. JAK signalling is of fundamental importance in innate immunity, inflammation, and haematopoiesis, and dysregulation is frequently observed in immune disease and cancer (Yin et al., 2018).

In 2018, Yin et al. (2018) designed new pyrazolo[3,4-*d*] pyrimidines using structure based drug design strategy. This study afforded compound **33** (Fig. 20), which revealed potent JAK3 inhibitory potential (IC_{50} = 6.2 μM) and antiproliferative activity on T-cell with IC_{50} = 9.4 μM. One year later, the same group of investigators prepared novel pyrazolo[3,4-*d*] pyrimidine-4-amino compounds **34** (Fig. 20) trying to develop more active JAK-3 inhibitors (Yin et al., 2019). Unfortunately,

Table 15 Cytotoxicity and JAK2 inhibitory potential for pyrazolopyrimidines **34a-d.**

C.N	R^1	R^2	JAK2 IC_{50} (μM)	HEL ($JAK2^{V617F}$) IC_{50} (μM)	TF-1 (JAK2) IC_{50} (μM)	THP-1 (JAK1/3) IC_{50} (μM)	NK-2 (JAK1/3) IC_{50} (μM)
34a			7.2	4.7	2.0	18.5	22.1
34b			6.5	5.6	2.3	19.8	14.7
34c			8	–	–	–	–
34d			9.7	4.3	5.4	16.0	23.6
Tofacitinib	–	–	4.3	5.2	2.6	0.9	0.6

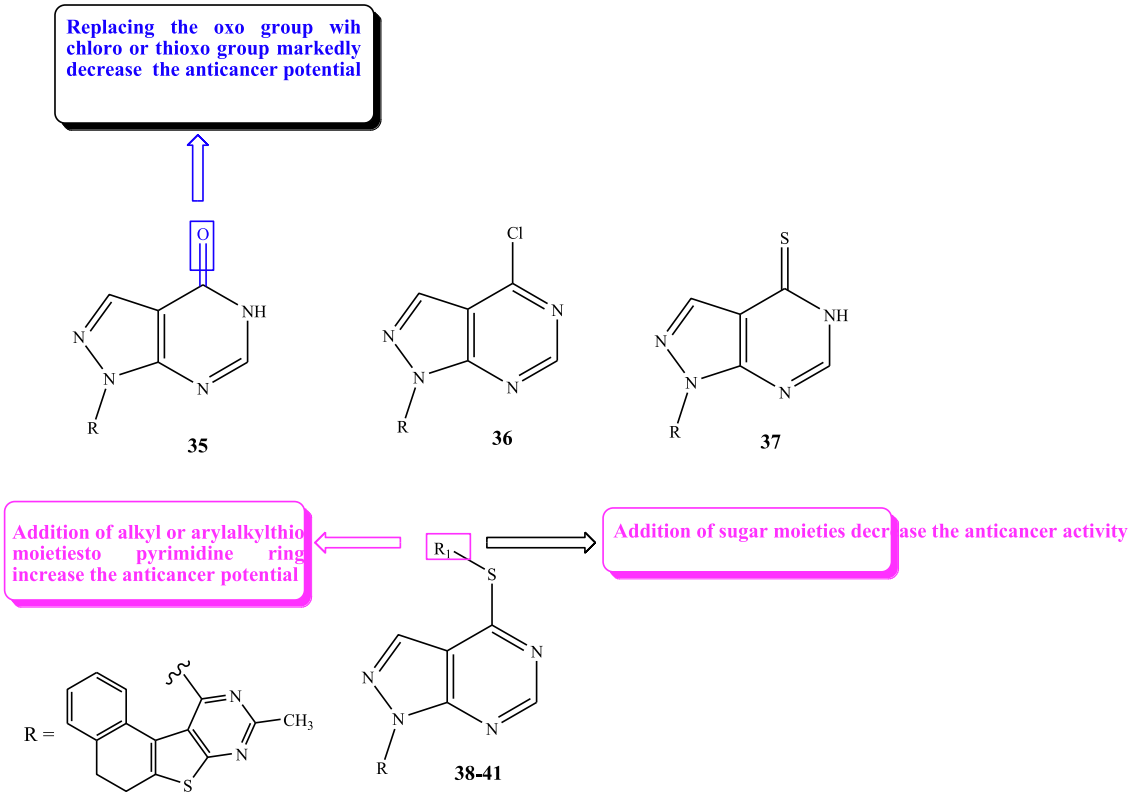


Fig. 21 Structure and SAR of pyrazolopyrimidines **35**–**41**.

these novel candidates exhibited weak Janus inhibitory potential (IC_{50} > 20 μM) but fortunately they revealed exciting inhibitory potential towards JAK-2. From these compounds, **34a-d** were the most active and selective JAK-2 kinase inhibitors revealing IC_{50} range 6.5–9.7 μM. SAR study displayed that 3,5-disubstituted-1-methylpyrazole moiety improved the potency and selectivity of 3-(1-methyl-1*H*-pyrazol-3-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamine as discussed in Fig. 20 and Table 15.

3.6. Reactive oxygen species formation

Reactive oxygen species (ROS) are molecules having oxygen that are chemically reactive (Dickinson and Chang, 2011). ROS play critical roles in homeostasis and cell signalling and formed naturally as by-products of the abnormal oxygen metabolism (Brieger et al., 2012; Bystrom et al., 2014). Cancer cells exhibit greater ROS stress than normal cells (Pelicano et al.,

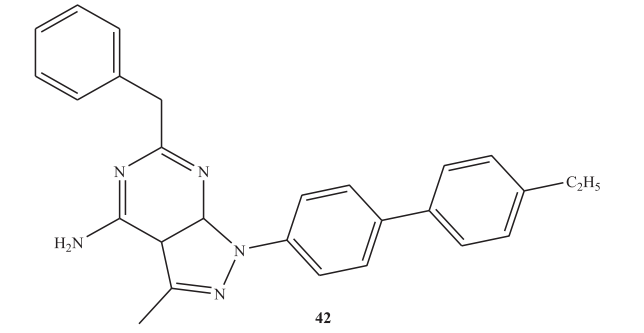
Table 16 Effect of the target pyrazolopyrimidines **35–41** at conc. 20 μg/mL on SOD, CAT, GSH and H_2O_2 in addition to DNA and RNA in MCF-7 cell line.

C.N	R^1	SOD U/mg protein	CAT U/mg protein	GSH nmol/mg protein	H_2O_2 nmol/mg protein	DNA (μg/10^6 cells)	RNA (μg/10^6 cells)
35	–	290 ± 31	2.30 ± 0.27	2.65 ± 0.25	60.30 ± 6.50	2.70 ± 0.28	5.25 ± 0.55
36	–	200 ± 24	3.65 ± 0.25	6.70 ± 0.72	52.33 ± 6.11	3.85 ± 0.41	6.50 ± 0.18
37	–	180 ± 19	6.70 ± 0.72	6.82 ± 0.78	46.37 ± 5.12	4.00 ± 0.41	6.80 ± 0.65
38	CH_2CH_3	385 ± 39	6.82 ± 0.78	2.20 ± 0.24	80 ± 8.60	1.82 ± 0.21	3.61 ± 0.35
39	$CH_2CH_2OCH_3$	310 ± 35	2.10 ± 0.26	2.90 ± 0.42	66.80 ± 7.50	2.50 ± 0.28	4.80 ± 0.50
40	$CH_2CH_2OCH_2CH_3$	250 ± 29	3.20 ± 0.35	5.25 ± 0.60	55 ± 6.25	3.00 ± 0.33	6.20 ± 0.70
41	Ribose	160 ± 18	5.35 ± 0.63	5.40 ± 0.60	40.33 ± 4.13	4.45 ± 0.45	7.22 ± 0.74
Cisplatin	–	470.60 ± 55	1.20 ± 0.11	1.40 ± 0.18	84.80 ± 9.60	1.11 ± 0.14	2.30 ± 0.26

2004), (Boonstra and Post, 2004). Rashad et al. (2011) designed and prepared novel sets of pyrazolopyrimidines (**35–41**) and screened the anticancer potential of these candidates towards MCF-7 using MTT assay. All the screened pyrazolopyrimidines (**35–41**) exerted excellent anticancer effect towards MCF-7 cell lines, especially compound **38**, which recorded higher anticancer effect than the standard drug cisplatin. To elucidate the mode by which the tested derivatives exhibit their anticancer potential, they determined the activities of free radical metabolizing enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), also the levels of glutathione and hydrogen peroxide were also determined. Treating the cells with theses pyrazolo[3,4-*d*]pyrimidines (**35–41**) resulted in an increase of SOD activity and H_2O_2 level, in addition a decrease in both CAT and GSH-Px. These changes were in the following order: **38** > **39** > **35** > **40** > **36** > **37** > **41**, which are in accordance with the anticancer potential so these compounds can act as anticancer through the production of ROS.

SAR study displayed that the substituent at C-4 of the pyrazolo[3,4-*d*]pyrimidine derivatives greatly affects the anticancer activity as explained in Fig. 21, Table 16.

Furthermore, in 2019, novel pyrazolo[3,4-*d*]pyrimidine **42** (Fig. 22) was found to suppress the growth of non-small cell lung cancer (NC-H460), renal cancer (786–0), non-small cell lung cancer (A549), and renal cancer (ACHN). The anticancer effect of this compound was exerted by generation of ROS (Gaonkar et al., 2020). Recently, in 2021, Si306 (**43**) and pro-Si306 (**44**) (Fig. 22) were reported to stimulate ROS production and expression of antioxidant enzymes in primary Glioblastoma cells (Kostić et al., 2021).



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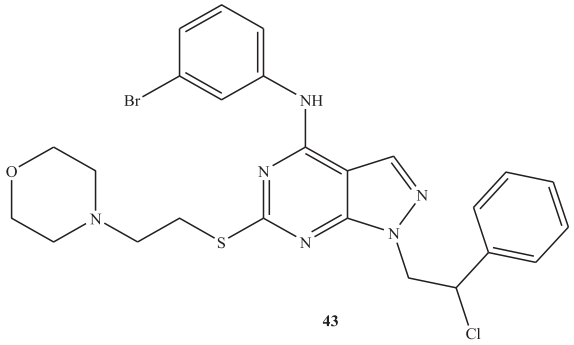

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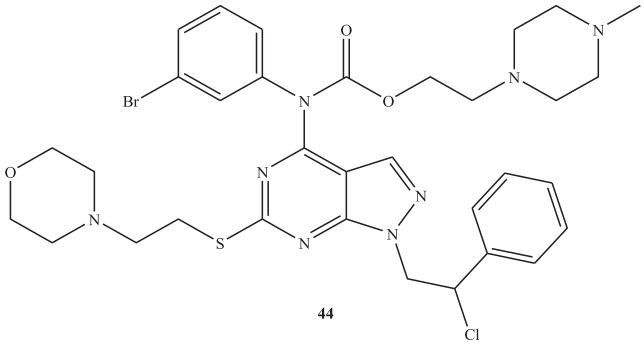

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Fig. 22 Structure of pyrazolopyrimidines **42–44**.

3.7. Bruton's tyrosine kinase inhibitors

BTK is a nonreceptor tyrosine kinase that has a vital role in B cell receptor signaling and controls B-lymphocyte differentiation, signaling and survival (Corneth et al., 2015; Singh et al., 2018). Abnormal BTK activity is a crucial feature of B-cell malignancies, so targetting BTK is a selective strategy to cure B-cell malignancies. Ibrutinib **45** is the first discovered irreversible BTK inhibitor that approved for treating chronic lymphatic leukemia, mantle cell lymphoma and Waldenstrom's macroglobulinemia (Smith, 2015; Ruella et al., 2016; Papanota et al., 2019). In 2019, Ran et al. (2019) performed some modifications on the chemical structure of ibrutinib to yield novel derivatives of pyrazolopyrimidines **46a-g** (Fig. 23, Table 17). These modifications included the replacement of the piperidine moiety with phenyl ring which was linked to

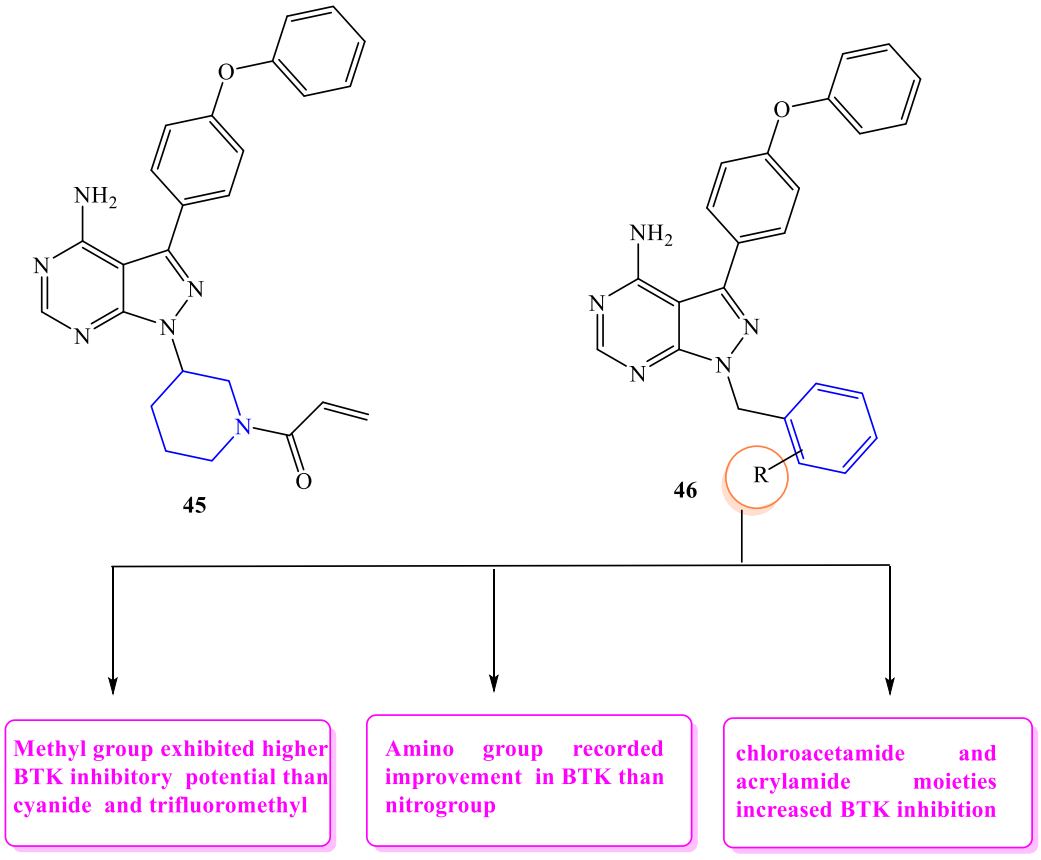

Fig. 23 SAR of pyrazolopyrimidines as BTK inhibitors.

Table 17 BTK inhibitory potential and cell viability assay of ibrutinib and pyrazolopyrimidines **46a-g**.

C.N.	R	BTK inhibitory % at 1 μM	Cell viability assay, IC_{50} μM			
			Mino	Jeko-1	ZI38	Maver-1
46a	4—CH_3	66	–	–	–	–
46b	4—CN	12	–	–	–	–
46c	4—CF_3	27	–	–	–	–
46d	4-NO_2	67	–	–	–	–
46e	4-NH_2	93	13.9	13.2	18.4	18.7
46f	3-$NHCOCH_2Cl$	98	0.9	0.4	0.8	0.4
46 g	3-$NHCOCH{=}CH_2$	97	1.3	1.3	2.8	2.6
IBN (45)	–	100	15.7	1.1	9.7	7.8

N-1 of pyrazolopyrimidine through a methylene group. From these target candidates, **46 g** recorded the highest BTK inhibitory potential (IC_{50} = 27 nM) comparing to the standard drug ibrutinib **45** (IC_{50} = 8 nM).

3.8. Phosphoinositide-3-kinase (PI3-K) suppressors

PI3Ks are lipid kinases that are included in phosphorylation and regulation of cellular metabolism, survival and proliferation (Hoxhaj and Manning, 2020). Abnormal PI3K pathway activation causes PI3Kα isoform overexpression which may suppress PTEN that is essential for converting PIP3 to PIP2 (Jung et al., 2018). Although all PI3K isoforms are included in PTEN-null tumors, PI3K is primarily responsible for malignant cancer transformation (Thorpe et al., 2015).

Parsaclisib (**47**) (Fig. 24) is a highly selective PI3Kδ (IC_{50} < 1 nM) pyrazolopyrimidine derivative with drug like ADME properties that recorded an excellent in vivo profile as shown by pharmacokinetic studies in monkeys, dogs and rats (Yue et al., 2019). Furthermore, the pyrazolopyrimidine **50** was designed depending on some chemical modifications

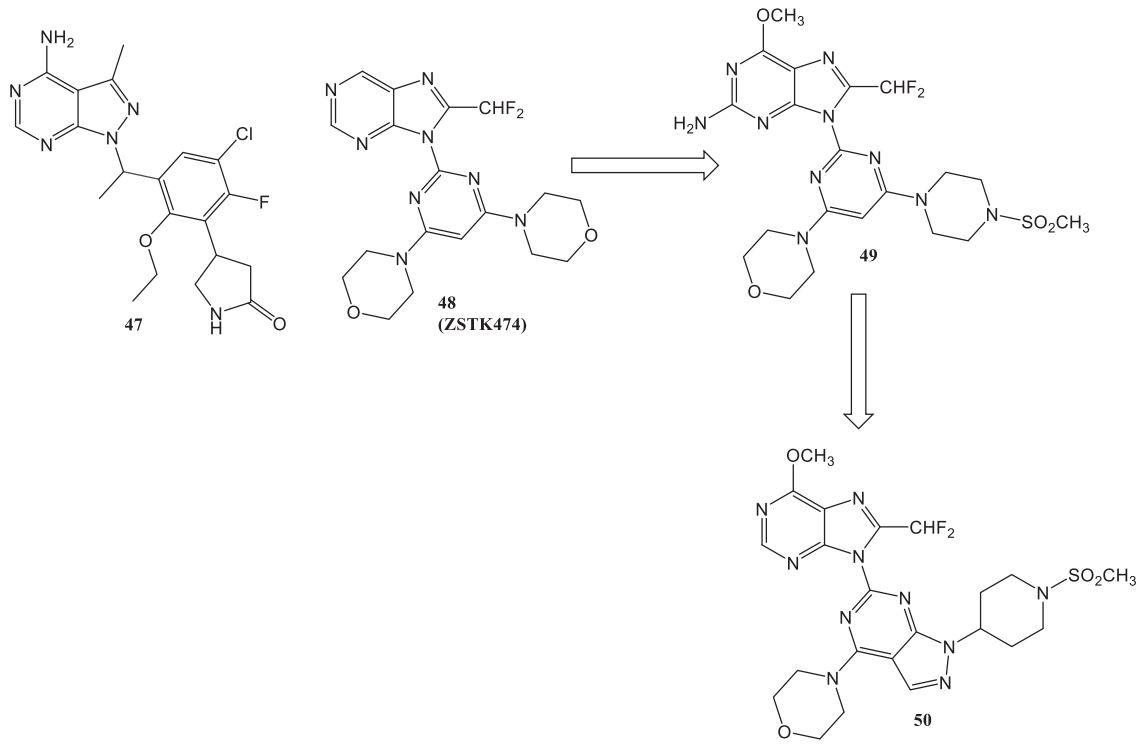

Fig. 24 Some examples of some PIK3 inhibitors **47**–**50.**

Table 18 Biological data for compounds **48**–**50.**

C.N.	Cell (IC_{50}) nM		Enzyme IC_{50} (nM)		
	NZB5	NZOV9	P110α	P110β	P110δ
48	220	287	8.9	58	38
49	74	5	22	116	13
50	114	24	2.6	768	58

on pan-PI3K inhibitor, ZSTK474 (**48)** (Fig. 24, Table 18) (Gamage et al., 2017). Modelling study of compound **48** displayed that one of the two morpholino moieties was not essential for binding with p110δ active region (Berndt et al., 2010). This point encouraged Gamage et al. (2017) to replace one of morphlino ring with piperazinyl sulphonamide group to afford comound **49** that showed dual PI3Kα/δ inhibition. Optimization of compound **49** was done through replacing the pyrimidine ring with pyrazolopyrimidine to produce compound **50** which displayed potent and selective inhibition towards PI3Kα (IC_{50} = 2.6 nM) (Table 18).

3.9. MKK7 inhibitors

Mitogen-activated protein kinase 7 (MKK7) is a part of c-JUN N-terminal kinase (JNK) signaling pathway that is included in many cellular regulation process (Ying et al., 2021). It is associated with many diseases as leukemia, lung cancer, myeloma and Alzheimer's diseases (Ooshio et al., 2021). Development of MKK7 suppressors received little attention and no MKK7 suppressors have included in clinical trials. Wolle et al. (2019a) prepared novel series of pyrazolopyrimidines **51a-d** with MKK7 inhibitory potential. SAR study of these compounds is explained in Fig. 25 (see Table 19).

3.10. BRAF and VEGFR-2 suppressors

RAF kinases are essential regulators in RAS-RAF-MEK-ERK signaling pathway that exert a pivotal role in cell proliferation, survival and differentiation (Roberts and Der, 2007). ARAF, BRAF and CRAF are members of RAF family (Pedersen and Plotkin, 2010). RAF kinases are stimulated via dimerization of their kinase domains (Lavoie and Therrien, 2015). Dysregulation of RAF kinases activation

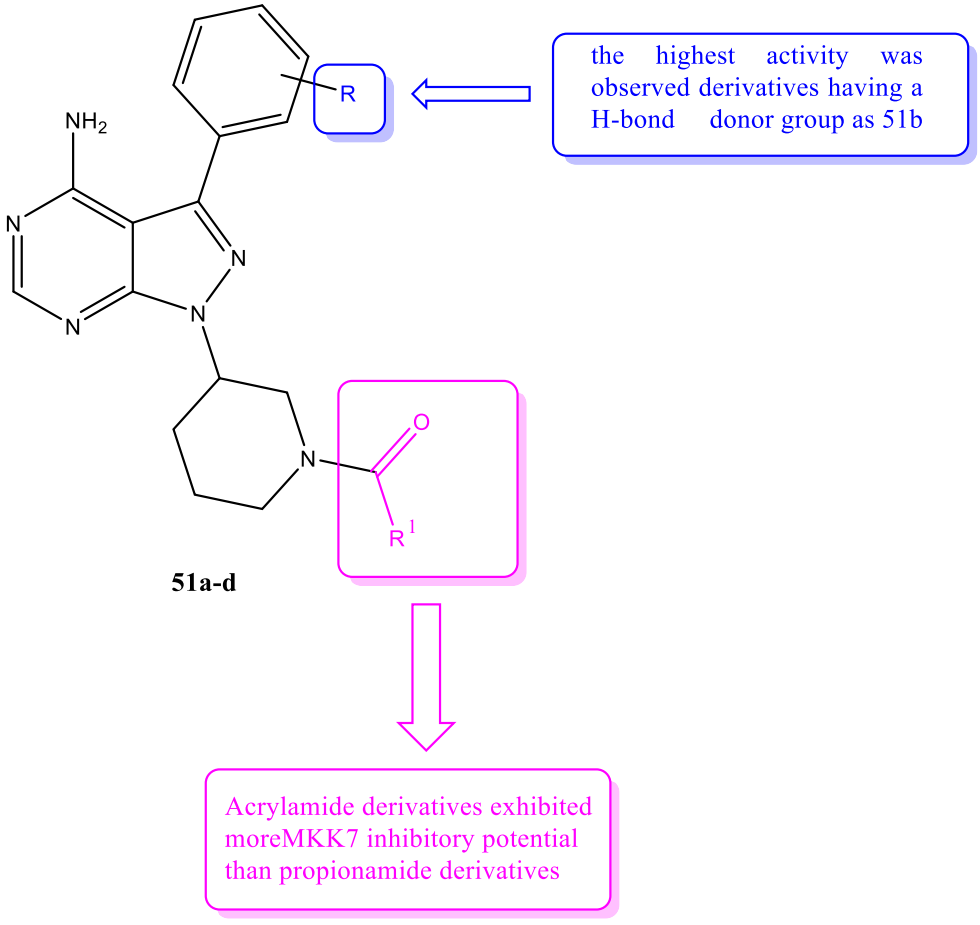

Fig. 25 SAR study of pyrazolopyrimidines **51a-d** with MKK7 inhibitory effect.

Table 19 MKK7 inhibitory effect of pyrazolopyrimidines **51a-d** and ibrutinib.

C.N	R	R^1	MKK7 (IC_{50}, nM)
51a	3—CF_3	—CH=CH	159 ± 117
51b	4-OH	—CH=CH	8.6 ± 2.9
51c	3—CF_3	—CH=CH—CH_2-N(CH_3)$_2$	2611 ± 1503
51d	3—CF_3	—CH_2—CH_3	> 10,000
Ibrutinib	–	–	78 ± 21

is a powerful cause of cancer (Durrant and Morrison, 2018). The cancer tissues secrete vascular endothelial growth factor (VEGF) to enhance angiogenesis from neighboring blood vessels (Pandya et al., 2006). Suppressing VEGFR-2 is considered as a rational approach for cancer curing. A novel series of pyrazolopyrimidines **52a-d** had been constructed and assessed for their inhibitory potential against VEGFR-2 and $BRAF^{V600E}$ (Wolle et al., 2019b). From the obtained results, some SAR was concluded as represented in Fig. 26, Table 20. It is noted that compounds substituted at terminal phenyl ring enhanced the inhibitory potential comparing with the unsubstituted effect, implying that the substituted aryl ring was required for effective $BRAF^{V600E}$ and VEGFR2 suppression.

4. Conclusion

Pyrazolo[3,4-*d*]pyrimidine is one of the most important heterocyclic systems due to its wide applications in medicinal chemistry mainly as anticancer. Many chemistry researches used this ring as a template

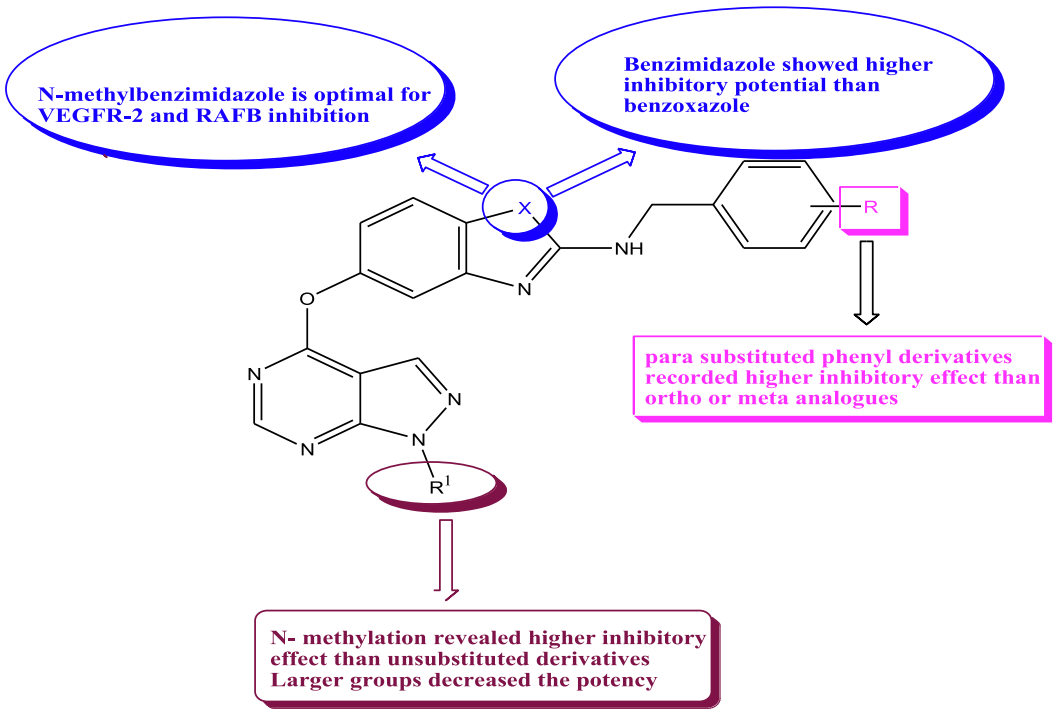

Fig. 26 SAR and substitution effects of pyrazolopyrimidines with RAFB and VEGFR-2 inhibitory potential.

Table 20 Kinase inhibitory potential and *in vitro* anticancer effect of **52a-d**.

C.N	X	R^1	R	Kinase inhibitory potential (IC_{50}, μM)		*In vitro* anticancer activity		
				$BRAF^{V600E}$	VEGFR-2	A375	HT-29	HUVEC
52a	NH	CH_3	4—Cl	82.0	14.6	> 100	22.87	> 100
52b	NMe	CH_3	4—CH_3	90.1	96.6	25.36	6.13	15.71
52c	NMe	CH_3	2—Cl	65.4	84.2	> 100	80.36	78.62
52d	O	CH_3	4-OCF_3	56.1	22.3	> 100	> 100	61.62

for the design and preparation of many anticancer agents. In this review, we have compiled a list of recent studies on pyrazolopyrimidines and their target specific activities in cancer treatment. Moreover, we displayed the new advances in the synthesis of this scaffold and structure–activity relationship to assist in the generation of novel potential candidates in a short time.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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