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RECENT ADVANCES IN PYRIMIDINE-BASED EGFR INHIBITORS: STRUCTURE–ACTIVITY RELATIONSHIPS, BIOLOGICAL EVALUATION AND MOLECULAR DOCKING

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ABSTRACT:
Although conventional chemotherapy has proven to be the most effective treatment for the majority of cancers, even though the growing problems of chemotherapy's systemic toxicity and emergence of drug resistance have boosted the advancement of targeted therapeutic approaches to cancer. Based on the validated molecular targets, the EGFR is a notably attractive target to study. EGFR have its key functions in regulating the cell proliferation, survival, migration and tumor progression. The capacity of Pyrimidine based scaffolds to adopt essential interactions with the ATP binding domain of the receptor coupled with their beneficial structural features has made them privileged pharmacophores in the design of inhibitors against the EGFR. This review highlights the recent development of Pyrimidine based EGFR tyrosine kinase inhibitor with focus on structural diversity, synthesis, biological evaluation, molecular docking and structure–activity relationship (SAR) studies.The review presents representative compounds with nanomolar inhibitory activity against both wild-type and clinically relevant EGFR mutants (T790M and C797S) with the critical molecular interactions involving the hinge-region residues, hydrogen bonding, lipophilic interaction like MET793, LYS745, CYS797 ,ASP855 and more.The review discuss about the approaches to overcome acquired resistance with mutant-selective and multi-target inhibitors. The review highlights importance of scaffold hybridization, linker optimization, electronic substituent effects and fused heterocyclic structures in the kinase selectivity and anticancer activity. Overall, these findings strengthen the current rationale for further optimization of Pyrimidine frameworks as a viable platform for the development of next generation anti-cancer drugs targeting the EGFR in order to achieve greater potency, selectivity and therapeutic potential.

KEYWORDS: Anticancer agents, Epidermal growth factor receptor, Tyrosine kinase inhibitors, Pyrimidine derivatives, Structure–activity relationship.

1. INTRODUCTION

Cancer is a fatal disease, and a top-2 cause of death globally [1]. Cancer remains a leading cause of mortality globally, posing a substantial economic and societal burden. It is increasing because population growth, aging and the growing number of well-known risk factors such as smoking, obesity, lack of physical activity and changes in reproductive patterns associated with urbanization and economic development. As cancer becomes a more common disease, the need to investigate, prevent and treat cancer has grown [3]. One example among many of data-driven epidemiology recent estimates of the cancer burden in China with information from 919 cancer registries throughout the country that served to identify trends in cancer incidence and mortality in various parts of the country. Of these, 106 registries also provided continuous surveillance data from 2010–2019, allowing for a good longitudinal population and a high consistency for trend analyses. The current methodology used for estimating cancer burden in 2024 was consistent with the method used in the cancer burden assessment (CBA) 2022, allowing easy comparison with the preceding years whilst maintaining analytical consistency, representativeness and data validity [4]. Estimates are made for 36 types of cancer for each territory/country for each age group and gender using the most reliable local data sources: National vital cancer death registration and National population based cancer registers for incidence data. In 2022, lung cancer is the most diagnosed cancer in male with 1.57, million new cases (95% UI: 1.56–1.58), followed by prostate cancer at 1.47 million cases (1.46–1.48). The highest numbers of new cases were found for breast cancer (0.91 million [0.90, 0.91 million]), followed by lung cancer (0.66 million [0.66, 0.67 million]) and cervical cancer (0.30 million [0.29, 0.30 million]). Lung cancer in males (1.23 million [1.22-1.24]) and breast cancer in females (0.67 million [0.66-0.67]) were the top two cancer-related causes of death. [5].
Its complex etiological mechanisms attribute it to a combination of genetic, environmental and lifestyle causes and make it a major cause of death around the world. India has a distinct cancer disease spectrum – breast, oral,

cervical and lung cancers represent a significant share of cancer burden. Considering the demographics and the prospect of rapid population ageing and demographic transition, there will be significant increases in cancer patients and deaths throughout the Asian continent. Cancer is projected to be more than double in number of cases and deaths in older people by 2050 using just socio-demographic estimates, with the highest growth rates forecast in Southern and Western Asia. These patterns seem to highlight the importance of developing an effective preventive strategy, the use of appropriate early diagnostic measures, and the development of targeted therapy in these rapidly increasing oncological burden regions [7].Most treatments to date focus on chemotherapy, although this can be effective, it is also associated with development of resistant patients and significant side effects. [8]. The discovery and development of anticancer drugs is a very complex, multi-step and resource intensive process. Once an identify or rationally designed lead compound has been determined essential for the project, extensive preclinical work is needed to prove that it has the pharmacological efficacy, safety properties and drug like characteristics necessary for advancement to clinical trials. Those candidates with favorable characteristics then enter a multi-stage clinical development process that includes Phase I, Phase II and Phase III clinical trials designed to evaluate tolerability, therapeutic benefit and clinical benefits in patients. This is a prolonged and expensive process that is still one of the biggest hurdles in the development of oncology drugs, and new methods to speed up this process are badly needed to continue to transfer promising molecules into impactful cancer drugs [9]. Traditional therapies, such as chemotherapy and radiotherapy, are important adjuncts to cancer treatment, but often they are restricted in their clinical applications due to the lack of tumor selectivity and undesirable side effects on normal tissues. These interventions are often nonspecific, and a wide range of side effects such as nausea, hair loss, fatigue, and organ dysfunction can result, potentially reducing the quality of life and adherence to therapy among patients [10].The epidermal growth factor receptor (EGFR) is one of the major cancer promoting proteins heavily modified in many cancers by epigenetic modifications and activating mutations. In addition to genetic abnormalities, abnormal DNA methylation has a significant impact on the regulation of EGFR expression and hypomethylation of EGFR has been reported in a variety of cancer types. [11].The EGFR is express widely in numerous cell types inside the body including vascular endothelial cells, HeLa cells, conjunctiva cells, uterine smooth muscle cells, keratinocytes, amniotic cells, and placental membranes [12]. The expression of the Epidermal Growth Factor Receptor (EGFR) is generally raised in several types of cancer and leads to poor prognosis and cancer progression. Also the heterogeneity of cancer is influenced by various mutant versions of EGFR. [13].

2.History of EGFR-

This discovery of growth factors as extracellular signal molecules that control cell proliferation was a critical leap forward in modern biomedical research. One of the ‘well known’ discoveries was that of Epidermal growth factor (EGF) which formed the basis for understanding of growth factor–mediated signaling pathways in normal physiology and disease. The pioneering work of Rita Levi-Montalcini and Stanley Cohen, who identified these molecules in 1962, paved the way to the current development of targeted therapeutic approaches and was awarded with the 1986 Nobel Prize in Medicine and Physiology[14]. It was formerly referred to as "tooth-lid factor" and now known as EGF due to its ability to stimulate the growth and proliferation of epithelial cells. Amino acid sequence in EGF was determined in 1972 and the EGF receptor was localized in 1975 by monitoring the binding of I-labeled EGF to the surface of the fibroblasts. In 1978 EGFR was observed as a 170 KDA protein and in 1980, EGFR was identified as a tyrosine kinase receptor, similar to the v-src protein of Rous sarcoma virus which was found to be tyrosine phosphorylated on receptor binding to EGF. With the discovery of human EGFR cDNA in 1984 and its characterization, high amino acid sequence homology was found with v-ERBB, an oncogene from avian erythroblastosis virus [15]. In April 1998, the first human trial of the EGFR tyrosine kinase inhibitor (TKI) Gefitinib (also named ZD1839) started, marking the beginning of clinical development of drugs targeting this drug target. Pharmacokinetic properties, toxicity, and early anti-cancer activity were evaluated during the early development phases of advanced solid malignancies, laying the groundwork for the further development of EGFR-targeted agents in oncology.' [16].

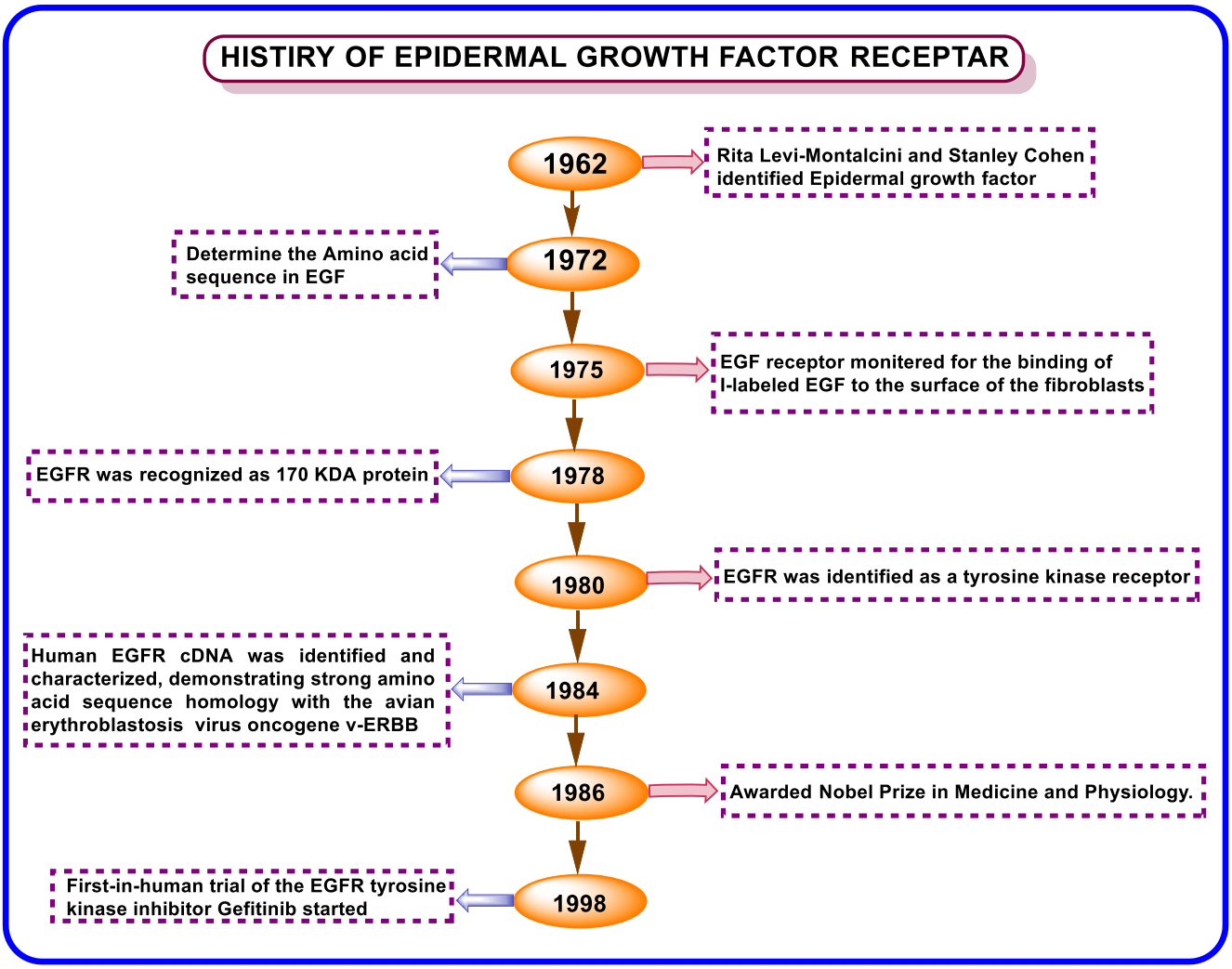


Fig.1 The History of Epidermal growth receptor from identification of EGFR to first human trial of EGFR KI's.

2.1. ERBB Receptor Family

EGFR is part of the ErbB receptor family, the four members of which are: ErbB4 (HER4), ErbB3 (HER3), ErbB2 (HER2) and EGFR (ErbB1/HER1), all of which are important targets for the development of targeted therapeutics against cancer.The transmembrane glycoprotein EGFR, which weighs 170 kDa, has an extracellular ligand-binding domain containing multiple glycosylation sites, an intracellular tyrosine kinase domain and one transmembrane section [17]. The EGFR and ERBBs are control the cell proliferation, survival, migration, angiogenesis, tumorigenesis, and metastasis by binding to ligands, including epidermal growth factor (EGF), and have been associated with the development of various types of cancer [18].

2.2 The mechanism of signaling , activation EGFR and Inhibition of EGFR by TKI's

EGFR is one of the four receptors that make up the ErbB family (ErbB1-4) serving as key mediators of cellular signalling pathways controlling cell growth, death, vascular growth and cell dissemination into metastatic sites [19].EGFR consists of three structural domains: an extracellular ligand-binding domain, a transmembrane domain, and an intracellular domain with a tyrosine kinase. Tyrosine kinase activated by seven different ligands such as transforming growth factor α, epidermal growth factor, etc. Ligand binding brings about clustering of receptors, resulting in autophosphorylation of some of the molecules at specific tyrosine residues, providing sites for docking of molecules in the steps of intracellular signalling cascade. EGFR receptors become hyperactive when the gene is mutated, resulting in an excessive amount of receptors, This activation allows the receptors to stimulate uncontrolled cell growth that can lead to the development of tumors [20]. This activates a multitude of downstream signaling cascades, mainly the RAS/Raf-1/MAPK, PI3K/AKT, and PLC-γ/PKC pathways, which are propagated via adaptor proteins. Phosphorylated receptors are able to directly activate the STAT pathways (Fig 3). In addition, ligand-bound EGFR can be endocytosed and eventually translocated to the nucleus where it directly regulates the transcription of various genes such as Cox-2, iNOS, Aurora kinase A, and Cyclin D1. They coordinately regulate transcription to govern cellular invasion, metastasis, survival, and proliferation [21]. EGFR facilitates invasion through various mechanisms, such as tissue barriers, survival of

circulating tumor cell clusters, and colonization of distant organs through paracrine loops between tumor and stromal cells. [22].

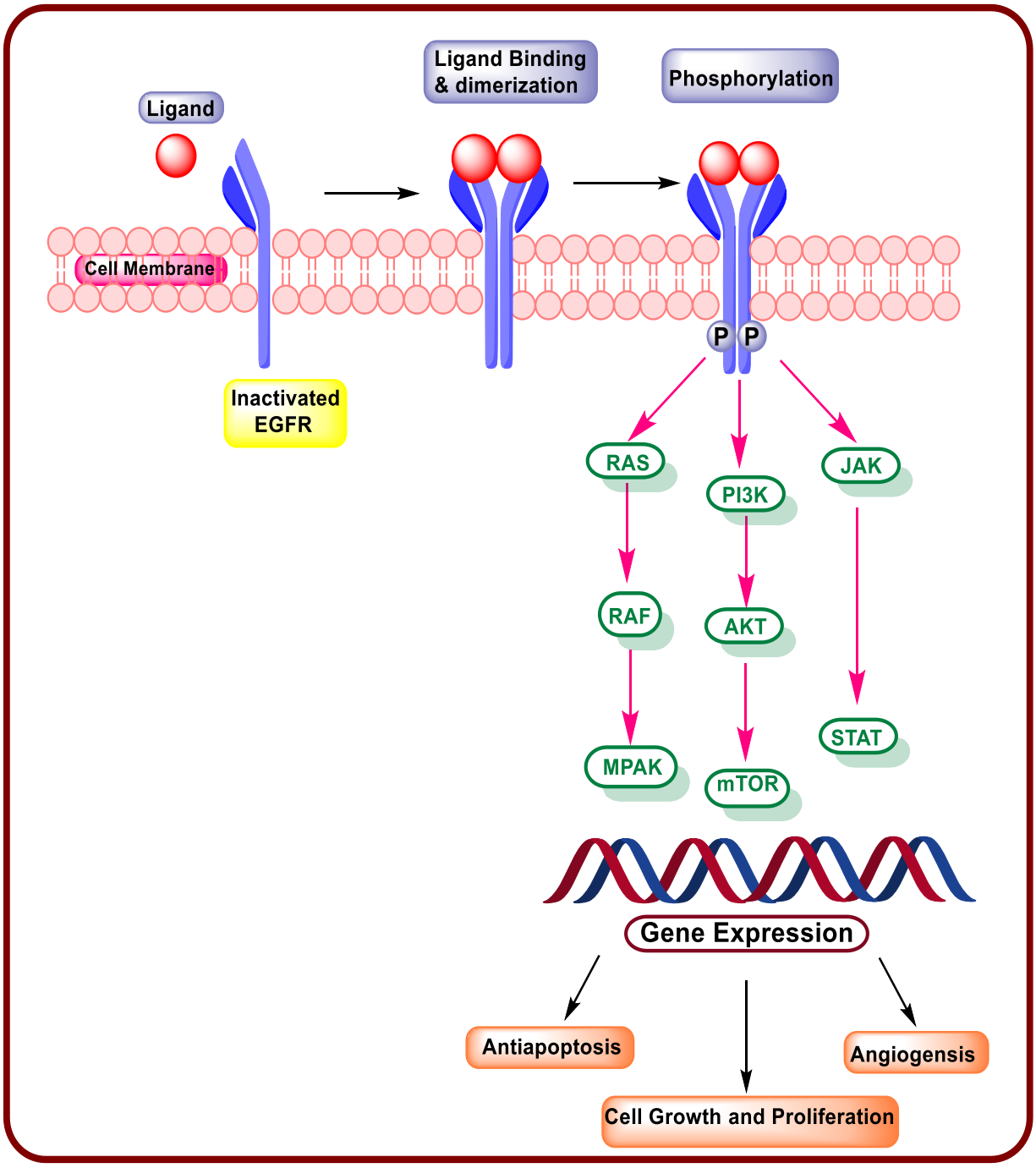


Fig.2 The signaling and activation pathway includes EGFR ligand binding to EGFR, dimerization, autophosphorylation which stimulates downstream signaling pathways.

EGFR overexpression is associated with multiple types of cancer, hence targeting EGFR with small-molecule inhibitors is a promising approach for cancer therapy therefore small-molecule inhibitors has been a major research focus over the past 20 years. [23]. There are some small molecule tyrosine kinase inhibitors like Gefitinib inhibit the ATP-binding pocket of EGFR. These blockers act to competitively inhibit binding the ATP site and inhibit activation of downstream signalling pathways of EGFR after autophosphorylation [24].This competitive inhibition doesn't allow the phosphorylation cascade to go through, damping tumour development and proliferation [25]. (Fig.4)

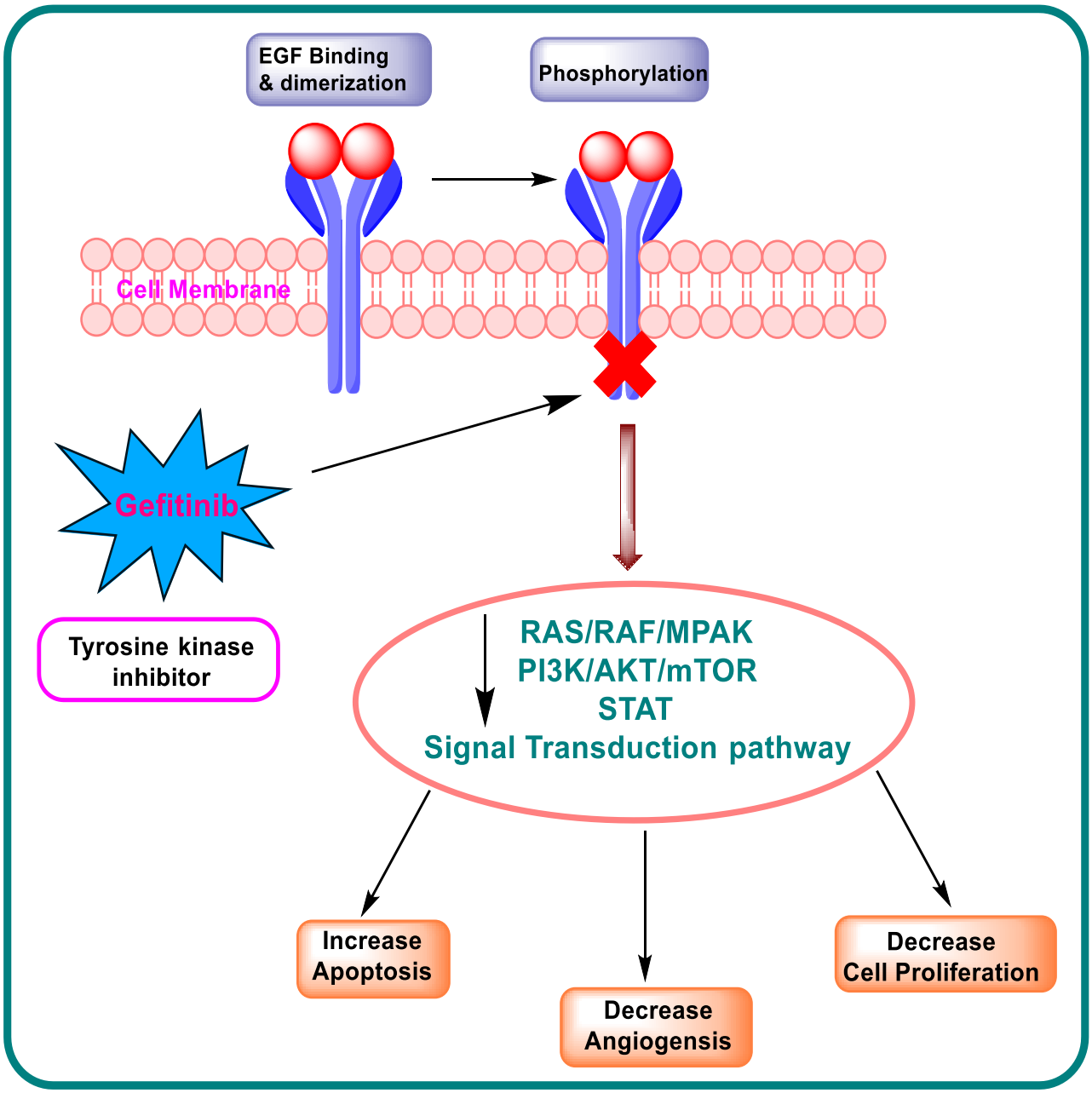


Fig.3 The TKIs (Gefitine) block the ATP binding site of EGFR to stop its autophosphorylation and downstream signaling to help stop the growth of tumor.

EGFR dysregulation is observed in many tumour types, such as NSCLC, colon, head & neck, breast and ovarian cancer. EGFR protein expression (often due to gene amplification or overexpression) can occur in wild-type EGFR. It is critical to explicitly differentiate between EGFR-mutant NSCLC (which is the specific target for TKIs) and EGFR-wild-type disease, as this distinction defines clinical eligibility for TKI therapy.Thus, EGFR has been identified as a potent and logical target for cancer therapy [26]. The current treatment technique such as chemotherapy and radiations creates multidrug resistance and become major obstacle which suffer significant setbacks [27].Clinical evidence highlight that pyrimidines have many biological functions which use as an anticancer pharmacophore. The pyrimidine nucleus is the building block of many medicinal compounds. Pyrimidine derivatives have attracted interest due to its potent anticancer effects on a variety of cancer cell lines. [28]

3.Pyrimidine based TK inhibitor

Heterocycles are Essential in many biochemical and physiological domains such as amino acids, DNA bases, vitamins, endogeneous neurotransmitter, many more. Nitrogen containing heterocycles are also have great source of biological and pharmaceutical significance [29]. Pyrimidines are the nitrogen containing heterocyclic ring and large number of medicinal compounds are containing heterocyclic ring [30].Pharmacological studies have revealed that pyrimidines possess a wide spectrum of biological activities such as antibacterial, antifungal, anti-viral, anti-tumoral and kinase inhibitory functions [31].

3.1 Evolution of EGFR Tyrosine Kinase Inhibitors: From First to Fourth-Generation Therapies

The last 20 years have witnessed the regulatory approval of tyrosine kinase inhibitors targeting the EGFR as clinically approved therapies for the treatment of various cancers. The efficacy and tolerability are different in each drug, thereby categorizing them into first, second, third generation and Fourth TKIs. [32].

3.1.1 First Generation of EGFR Tyrosine Kinase Inhibitors

Gefitinib (Iressa®, ZD1839) was a major breakthrough in cancer targeted therapy as the first epidermal growth factor receptor (EGFR-TKIs) approved by the regulators. Since its approval in 2003 it has become clinically proven that EGFR inhibition is a successful therapeutic approach to non-small cell lung cancer (NSCLC) [33]. Gefitinib works by preventing the epidermal growth factor receptor from signaling through the binding of adenosine triphosphate (ATP) [34]. Lapatinib belongs to the 4-anilinoquinazoline class of from first generation kinase inhibitors. Lapatinib (LAP) developed by GlaxoSmithKline has received FDA approval March 13, 2007, It exhibits tyrosine kinase activity on both HER1 and HER2 by interacting to the ATP binding site in the intracellular part of the receptor and prevents the proliferation of tumor cell [35].

3.1.2 Second Generation of EGFR Tyrosine Kinase Inhibitors

Dacomitinib (Vizimpro®, 45 mg/tablet manufactured by Pfizer Europe) is an irreversible ErBb (HER1, HER2 and HER4) inhibitor. Dacomitinib is a Second Generation of EGFR-TKIs received its first US Food and Drug Administration (FDA) global approval in September 2018. These EGFR-TKIs used to treat patients with metastatic non-small cell lung cancer (NSCLC) who have either an EGFR exon 19 deletions or an exon 21 L858R replacement mutation [36]. Afatinib (BIBW 2992; GilotrifTM U.S., Giotrif©) is second Generation of EGFR-TKI. It is a non-reversible inhibitor of the tyrosine kinase belongs ErbB family. Afatinib binds to EGF receptors covalently and thus blocks the autophosphorylation of the tyrosine kinases and transphosphorylation of the HER3. Afatinib was used in clinical treatment of NSCLC and some relevant and complex EGFR mutations [37,38].

3.1.3 Third Generation of EGFR Tyrosine Kinase Inhibitors

Osimertinib, developed by AstraZeneca, is an orally bioavailable third-generation tyrosine kinase inhibitor used for the treatment of advanced NSCLC. Osimertinib showed inhibiting activity against both EGFR-TKI's-sensitizing and EGFR T790M resistant mutations [39]. Novartis' Nazartinib, is a third-generation EGFR-TKI that specifically inhibits EGFR-TKI sensitizing mutations and Thr790Met resistance mutations. It is used as first-line treatment for NSCLC patients with Thr790Met-positive mutations [40].

3.1.4 Forth Generation of EGFR Tyrosine Kinase Inhibitors

CH7233163 (Formulate by Roche Pharma) is forth generation novel EGFR-TKI exhibit strong anticancer effects against tumors with EGFR-Del19/T790M/C797S both in vitro and in vivo. The recent study observed that CH7233163 more selectively suppresses different types of EGFR mutants (e.g L858R/T790M/C797S, L858R/T790M, Del19/T790M, Del19, and L858R) over wild type, in addition to EGFR-Del19/T790M/C797S.[41]

Table No. I Different generations of EGFR inhibitor and their limitations.[42,43]

Generations of EGFR inhibitors	Drugs	Selectivity	Limitations
First Generation	Gefitinib	WT EGFR	Resisted due to the mutation of EGFR T790M and mutated EGFR downstream signaling.
	Lapatinib	WT EGFR	
Second Generation	Dacomitinib	WT EGFR	Drug-resistance phenomena specially with T790M EGFR mutation
	Afatinib	WT EGFR	
Third Generation	Osimertinib	Mutant-selective with residual wild-type activity	Resistance due to C797G or C797N EGFR mutations
	Nazartinib	Mutant-selective with residual wild-type activity	
Fourth Generation	CH7233163	Mutant EGFR C797S.	Under vigilance

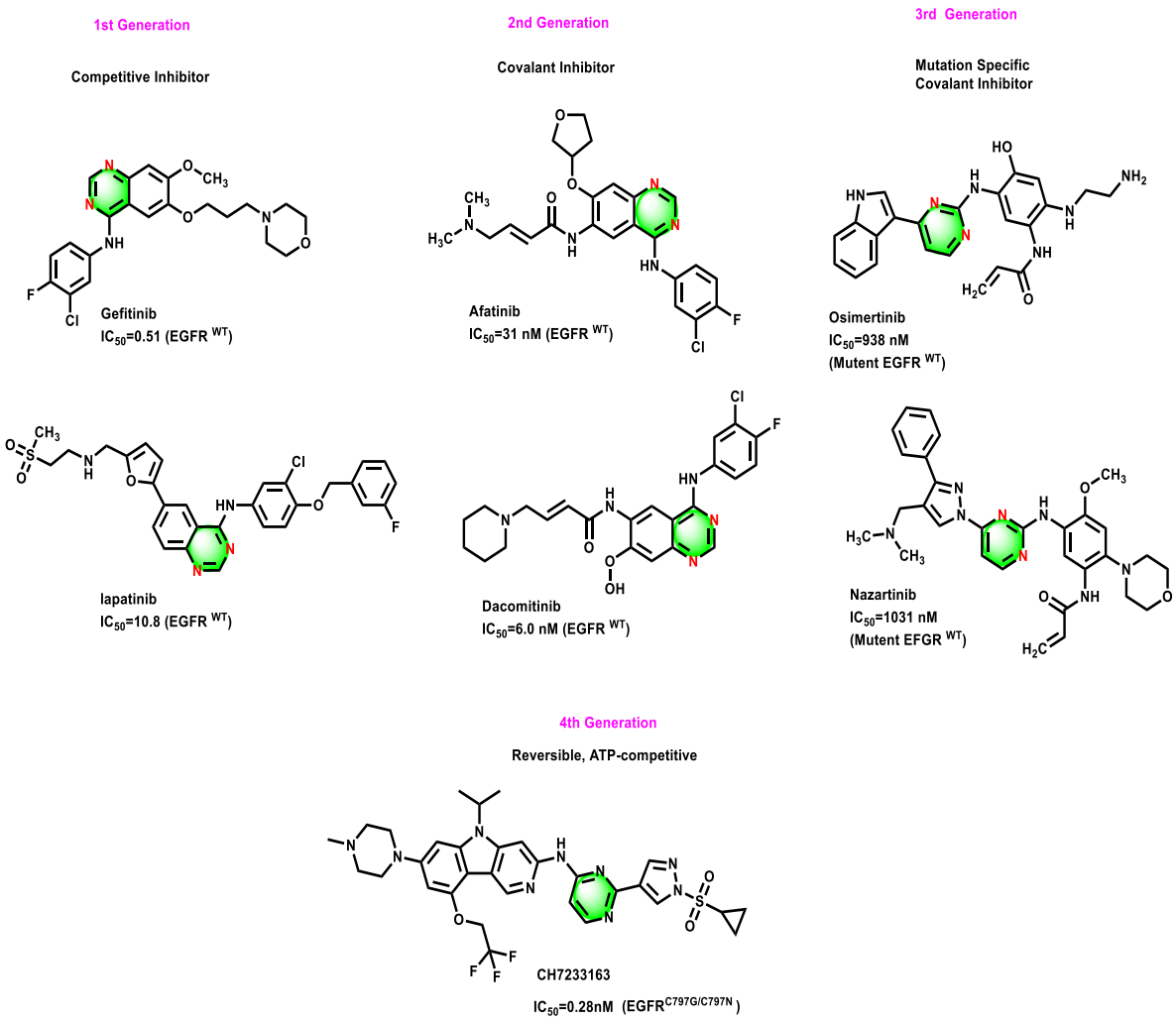


Fig.4 The Approved First to fourth generations of EGFR Inhibitors with IC50 value.

Table II - Ongoing clinical trials of patients with EGFR inhibitors.[44]

Sr. No.	Study title Status	Clinical Trials.gov Identifier	Intervention (Structure)	Sponsor	Status
1	Study of Anti-CD3 x Anti-EGFR Bispecific Antibody (EGFRBi) Armed Fresh Peripheral Blood Mononuclear Cells (EGFR FPBMC) in Metastatic or Unresectable Pancreatic Cancer	NCT06479239	EGFR FPBMC	University of Virginia	Phase I/II Study
2	Dose-finding Trial: Evaluating Safety and Biomarkers Using DK210 (EGFR) for Inoperable Locally Advanced and/or Metastatic EGFR+ Tumors With Progressive Disease Failing Systemic Therapy	NCT05704985	Biological: DK210 (EGFR) Radiation: Radiation therapy Biological: Immune checkpoi	DEKA Biosciences	Phase 1

			nt blockers Drug: Chemotherapy		
3	Implementing Circulating Tumor DNA Analysis at Initial Diagnosis to Improve Management of Advanced Non-small Cell Lung Cancer Patients (NSCLC)	NCT04912687	Diagnostic Test: EGFR gene mutation analysis on liquid biopsy	InstitutBergonié	Not Applicable
4	Postoperative EGFR-TKI Therapy forContralateral Pulmonary Nodules in Patients With EGFR-Mutant NSCLC（ARMOR2501） (ARMOR2501)	NCT06924398	Postoperative EGFR-TKI Therapy	Sun Yat-sen University	Phase 2
5	Open-label Study Evaluating the Safety and Feasibility of CART-EGFR-IL13Ra2 Cells in Patients With EGFR-Amplified Recurrent Glioblastoma	NCT05168423	CART-EGFR-IL13Ra2 Cells	University of Pennsylvania	Phase 1
6	Evaluation of an Ultrasensitive Next Generation Sequencing Method for the Detection of EGFR Gene Mutations in the Plasma of Patients With Lung Cancer	NCT06595498	Plasma-SeqSensei™ Solid Cancer IVD Kit (Sysmex), cobas® EGFR Mutation Test v2 (Roche)	Istituto Oncologico Veneto IRCCS	Not Applicable
7	Clinical Study on the Safety, Pharmacokinetic Characteristics and Preliminary Efficacy of JY016 Injection in Patients With Advanced Solid Tumors Expressing EGFR	NCT07510841	JY016	Biotech Pharmaceutical Co., Ltd.	Phase 1,Phase 2
8	Study of Nintedanib Plus EGFR TKI In EGFR-mutated Non-small Cell Lung Cancer Patients	NCT06071013	Nintedanib, Gefitinib, Erlotinib,	China Medical University Hospital	Phase 1,Phase 2

			Afatinib, Osimertinib		
9	A Single-arm, Open, Exploratory Clinical Study Evaluating the Safety and Efficacy of Epidermal Growth Factor Receptor/B7 Homolog 3 Chimeric Antigen Receptor T-cell therapy in Patients With EGFR/ B7H3-positive Advanced Solid Tumors (Lung and Triple-negative Breast Cancer)	NCT05341492	B7 Homolog 3 Chimeric Antigen Receptor T-cell therapy	Second Affiliated Hospital of Guangzhou Medical University	Early Phase 1
10	A Study of Armed, Activated T-Cells in Patients With Advanced Pancreatic Cancer	NCT04137536	Anti-EGFR-bispecific antibody armed activated T-cells	Memorial Sloan Kettering Cancer Center	Phase 1

Pyrimidines and their derivatives have played a pivotal role in cancer prevention and treatment. However, many cancer therapies are associated with substantial side effects that compromise patient's quality of life, with certain treatments inducing severe complications. According to a WHO report, Nearly 18 million people were diagnosed with cancer in 2018, resulting in 9 million deaths, primarily attributable to suboptimal treatment efficacy [45].Despite the swift onset of resistance that diminishes the sustained efficacy of these inhibitors, research efforts persist undeterred in developing more potent pyrimidine-based EGFR inhibitors.

4. Pyrimidine derivatives as Promising Anticancer Agents.

Gao and co worker were synthesized thieno[2,3-d]pyrimidine derivatives as targeted antitumor agents. All compounds were tested against Anti-hepatoma HepG2 cell line and compound 1 was found to be most active with IC50 = 0.17 μM, compared with the reference Gefitinib, with IC50 is 1.4 μM. The molecular docking study of 1 showed the satisfactory binding free energy for the ATP-binding pocket with EGFR (ΔGcal up to −8.19 kcal/mol). Compound 1 showed binding to hinge region (Q767 & M769) and also by hydrogen bond with K721 and D831 as well as an aromatic π–π interaction with F699, and a Lipophilic interaction with L764, V702 & M742. [46]

Yılmaz and Uluçam et al. designed and developed", "novel group dihydrofuro[3,4-d] pyrimidine as an EGFR inhibitor. They perform cytotoxic activity against DU145 cells and results confirmed that compound 2 (IC_{50} = 17.4 μM) with moderate potency. The molecular modeling results identified Compound 2 had high binding affinity with EGFR active site, -7.4 kcal/mol, where the scaffold formed important hydrogen bonding interactions with ASP813 and ARG817 residues in the active site. [47]

New thiophene-pyrimidine derivatives were synthesized by Zhen Xiao et al. All compounds were tested for EGFRT790M, A549 and A431 cell lines and compound 3 proved to be a potent inhibitor of against EGFRT790M (IC_{50} = 3.79 ± 0.57 μM) and A549 and A431 (IC_{50} = 3.79 ± 0.57 μM and 4.34 ± 0.60 μM respectively). Docking simulations of compound 3 showed a U-shaped binding mode where Met793 generally acts as a key residue (H bonds found at very favourable distances of 1.9–2.2 Å) that is indispensable and where an extra interaction is established with ARG841, generating higher affinity over Olmutinib [48].

Narasimhachar et al. designed and synthesized novel pyrimidine-thio-triazoles as selective EGFR inhibitors. All synthesized compounds were evaluated against MCF-7 cells line and compound 4 was identified as a promising compound with IC50 = 29.62 μM (MCF-7). The docking studies shows good binding energy, −7.27 kcal/mol within the ATP-binding pocket of EGFR for Compound 4, which suggested stable complexes between the ligand and receptor. Compound 4 reveled that hydrogen bond interactions of phenolic hydroxyl to ASP855, π–cationic interaction from the triazole and aryl groups to LYS875 and large hydrophobic contacts to CYS797, PHE723, VAL726, LEU844 and LEU858, which stabilize the binding [49].

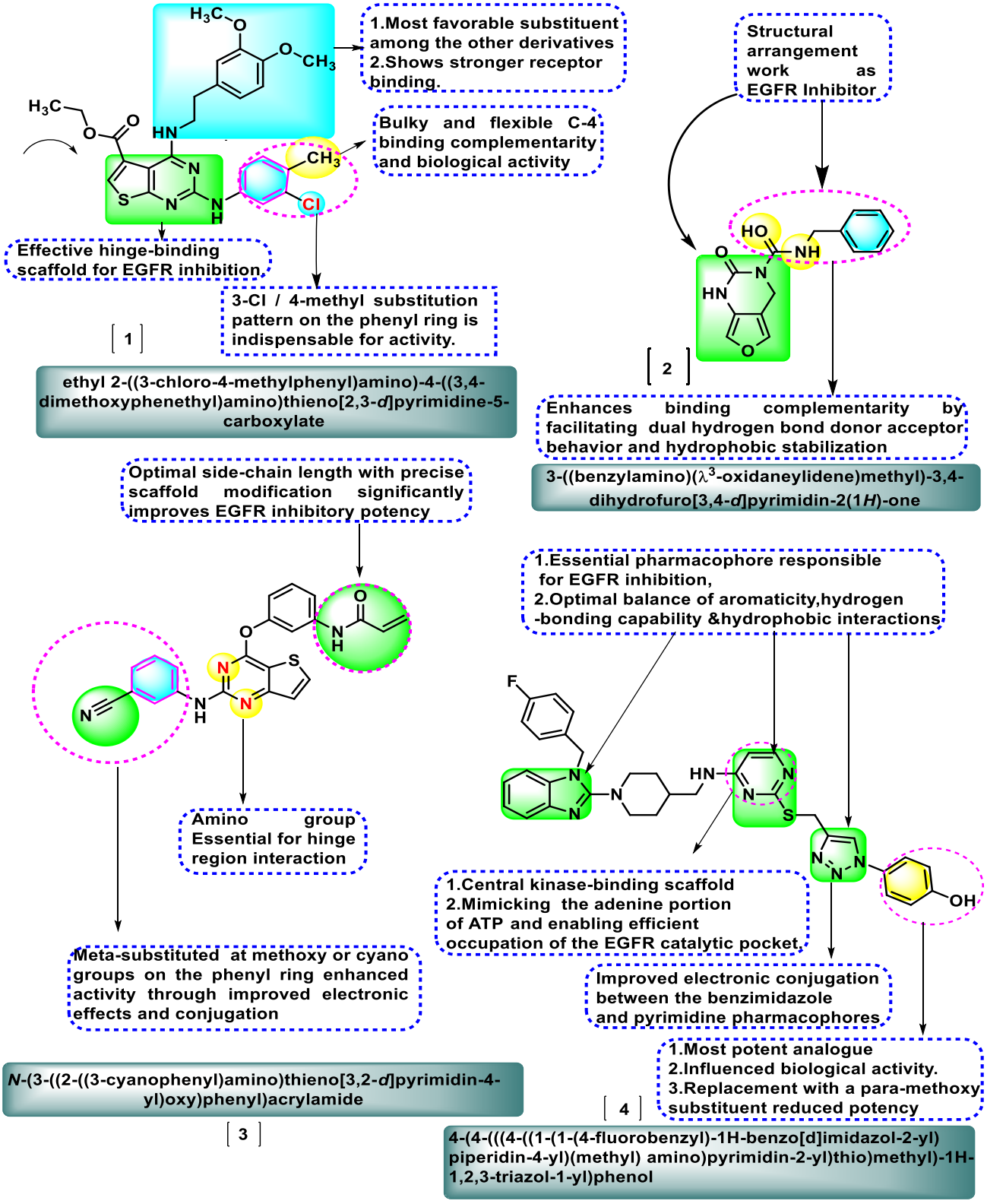


Fig.5 Chemical structure and SAR of compounds 1–4.

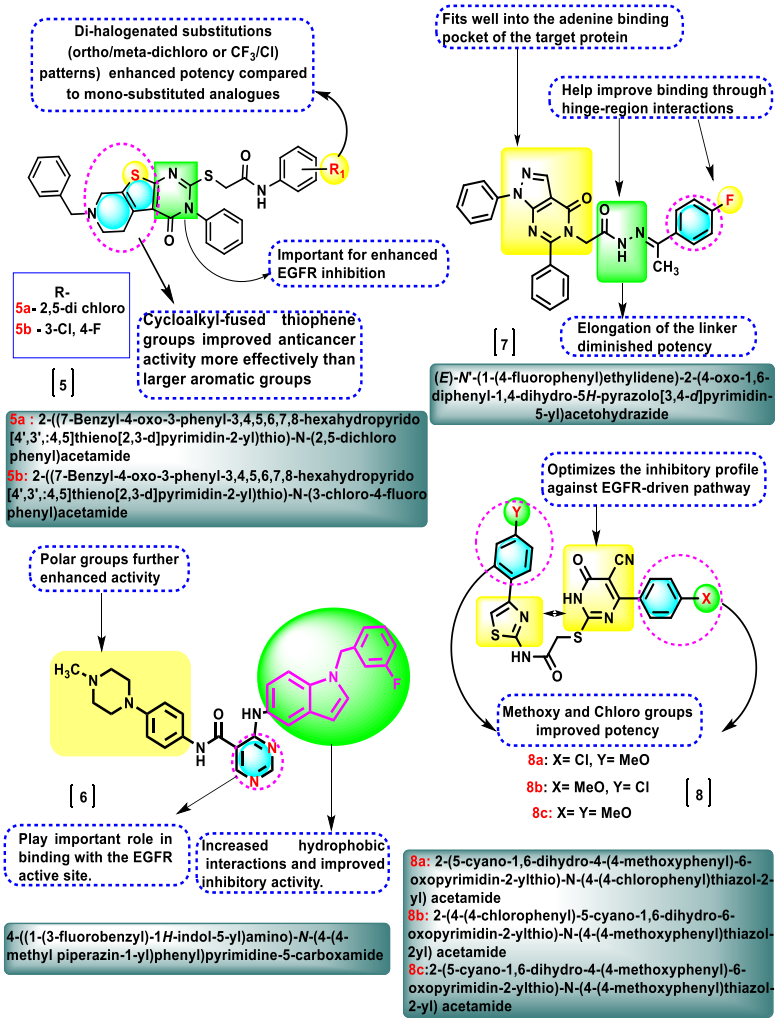
Elsebaie et al. reported a series of novel thieno pyrimidines as an anti-cancer agent. All compound were screened for their anti cancer activity against MCF7 cells & A549 cell line and result revealed that 5a and 5b exhibit good activity with MCF-7 cell line (5a IC50=0.05 µM; 5b IC50= 0.08 µM) and A549 cell line (5a IC50=0.07 µM 5b IC50= 0.07 µM). Also 5a &5b it shows highest inhibition with dual EGFR inhibitors (IC50=67 nM and 41 nM, respectively). Binding affinities were found to be favorable with compound 5b - 9.6 kcal/mo in side EGFR and 8.1 kcal/mol inside STAT3 active sites.The docking study of compound 5a & 5b demonstrated an extensive docking of hydrophobic interactions and π–sulfur interactions with key residues including Met742 and Cys773, with occasional hydrogen bonding. [50]

The anticancer activity of numerous pseudo-bicyclic pyrimidine derivatives was synthesized by Farag et al. All synthesized compound were evaluated and compound 6 showed submicromolar activity (GI50 = 0.52–0.92 µM) in various cancer cell lines showing better efficacy against melanoma, colon, and blood cancers, than Lapatinib.

The molecular docking for 6 showed that the Ligand binding energy was -10.87 kcal/mol with a critical hydrogen bond interaction from pyrimidine N1 with the Met793 hinge residue. Also shows nonpolar interactions with Phe856 and Met766 residues [51].

A novel group of EGFR tyrosine kinase inhibitors pyrazolo pyrimidine derivatives was synthesized by Ahmed A. Abdel Gaber et al. All synthesized Compound were screen againsts human breast cancer cell line, MCF-7 and compound 7 emerged as a highly active EGFR-TK inhibitor with IC50 of 2.89 µM. On docking studies, the Compound 7 accommodates itself within EGFR ATP-binding pocket with H-bond interaction with CYS797 and dual H-arenes with LYS745 and MET793 with highly favourable binding affinity of -6.82 kcal/mol .The Compound 7 fits well into EGFR ATP-binding cavity and forms H-bond interaction with CYS797 and dual H-arene interactions with LYS745 and MET793, which yielded a favourable binding affinity of -6.82 kcal/mol [52].

Salah A. Abdel-Aziz et al. developed new anti-inflammatory EGFR inhibitors pyrimidine-5-carbonitrile derivatives containing 1,3-thiazole moiety .The compounds were tested against COX-1/COX-2.Compunds 8a, 8b and 8c were found most potency with IC_{50}=0.33, 0.22 and 0.19 µM respectively, and reached the activity level of Erlotinib. The molecular docking of 8a, 8b and 8c showed that the methoxy pyrimidine phenyl possesses favorable binding energies (ΔG ≈ −13.20 to −9.82 kcal/mol).Also observed crucial binding interactions of 8a, 8b and 8c for hydrogen-bonding of the pyrimidine phenyl with MET769 and cyano-mediated interactions with Thr766 through water-bridging. Along with hydrophobic interactions with LEU694, GLY722 and MET769 [53].



Top of FormFig.6 Chemical structure and SAR of compounds 5–8.Bottom of Form

Zhang C et al. reported a novel Pyrimidine derivatives to treat as selective inhibitors of EGFR. They performed anti tumor activity against EGFRL858R/T790M, EGFRL858R/T790M/C797S mutation and H1975 & results demonstrated that compound 9 showed potent activity with IC50=5.0 nM (EGFRL858R/T790M), 2.9 nM (EGFRL858R/T790M/C797S) and 0.054 ± 0.016 µM (H1975) . Compound 9 also demonstrated inhibitory activity of against triple mutant. The U-shaped binding conformation for 9 supported by dual hydrogen bonds and halogen bond with the Met793 hinge residue, and with the specific hydrogen bond with Cys797, hydrophobic alkyl π sigma interactions with Val726 and Leu844 [54].

Bandi SR. et al. prepared novel triazolo-pyrrolo pyrido pyrimidines. Compounds were evaluated for cytotoxicity against MCF-7 cell line . The result reported Compound 10a (IC50= 0.33 ± 0.02 µM) and 10b (IC50= 0.41 ± 0.06 µM), shows the most potent activity. Compound 10a and 10b observed better than Erlotinib (IC50= 0.43 ±

0.03 μM). The Compound 10a and 10b showed favorable binding affinity ranging from −8.94 to −9.80 kcal/mol that suggests stable stability in the ligand-receptor complexation in the ATP binding site. Notable interactions for 10a & 10b included hydrogen bonding to LYS721, and sometimes with CYS773. The hydrophobic interactions for 10 & 10b observed via ALA719, VAL702, LEU694, and LEU820 which contributed to the stability of the binding. [55].

New pyrimidine-based pyrene/benzochromene hybrids compounds were prepared by Mohammed YIA et al. 'All synthesized compounds were subjected to cytotoxicity assay against human colorectal carcinoma cells. Compound 11a and 11b proved to be the most potent EGFR-TKIs with the IC50=77.03 nM and 94.9 nM, respectively and also comparable with Erlotinib (72.3 nM). The docking tests revealed best score (ΔG ≈ −21.81 kcal/mol) and it indicated interactions via hydrogen bonds between the pyrimidine–thione core and EGFR residues LYS721 & ASP831. The 11a and 11b noted the lipophilic interaction among the pyrimidine–thione core and residues like PHE699, LEU694, LEU764, LEU820 and PRO770 within the ATP-binding pocket [56].

Naglaa Ahmed et al. discovered novel indolyl-pyrimidine EGFR-targeting anticancer agents. All synthesized compounds were screened for the anticancer activity against Michigan Cancer Foundation-7, Human hepatocellular carcinoma and Human colorectal carcinoma cell lines. Compound 12 shows good activity with IC50=5.10 μM (Michigan Cancer Foundation-7), 5.02 μM Human hepatocellular carcinoma and 6.60μM (Human colorectal carcinoma cell), similar to Erlotinib (IC50=2.96μM). The docking studies were performed shows favorable binding affinity (−9.6 kcal/mol) for compound 12 and showed noteworthy results with a cluster of critical hydrogen bonding interactions with the residues ASP831, LYS721 and THR766. Also the strong lipophilic interaction with LEU694, PHE699 and VAL702 in EGFR ATP site [57].

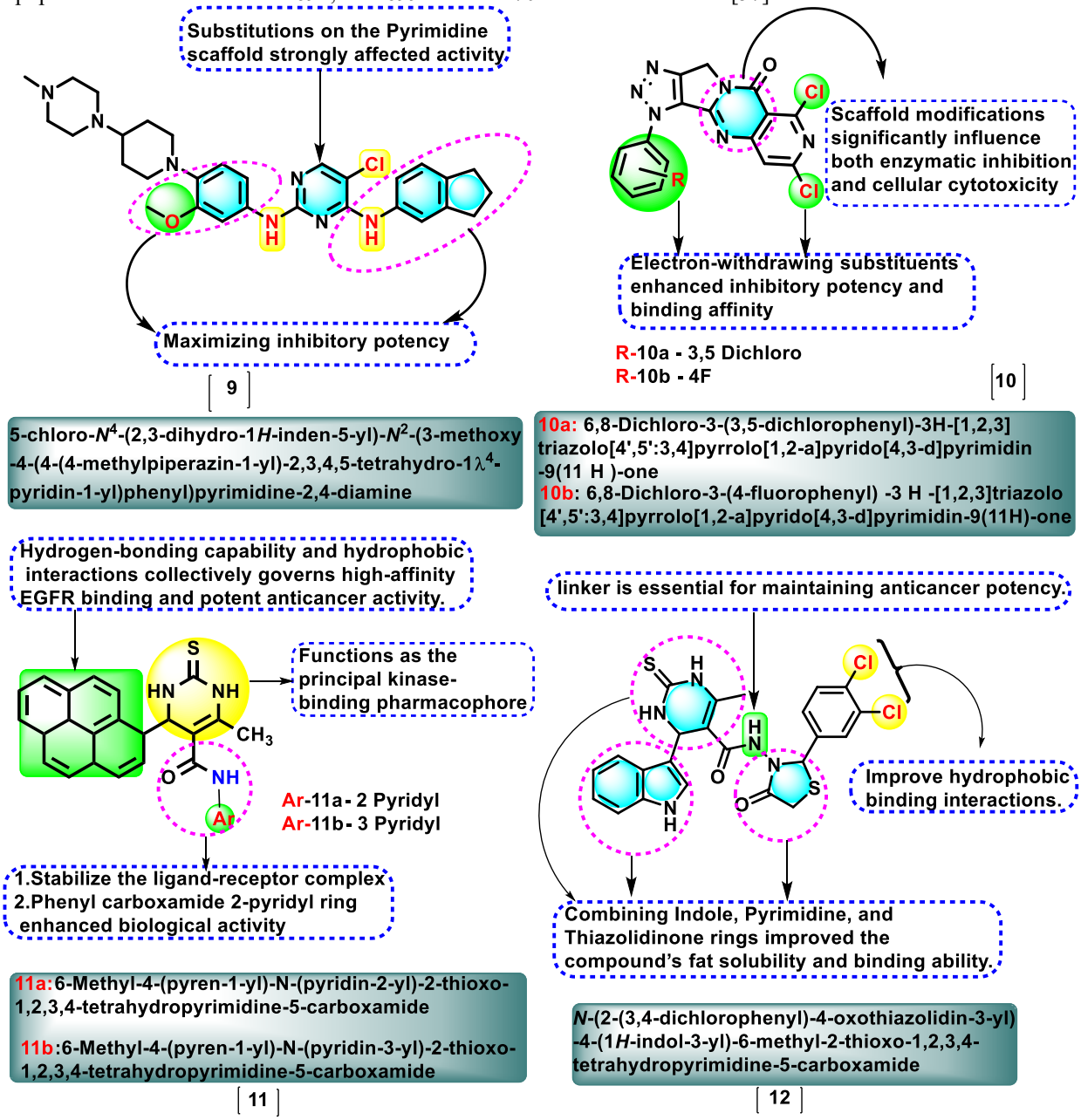


Fig.7 Chemical structure and SAR of compounds 9–12.Bottom of Form

Eman A. Sobh and coworkers designed and synthesized a series of thieno[2,3-dpyrimidine derivatives as new Epidermal Growth Factor inhibitors. Compound 13 shows potent activity with IC50 =37.19 nM and 204.10 nM against Wild-type Epidermal Growth Factor Receptor and EGFRT790M mutant. The docking studies explained that compound 13 reported highest binding affinity of −17.22 kcal/mol, further study indicates MM-GBSA binding free energy of −23.94 kcal/mol, which is stabilized by a critical hydrogen bond with MET769. Also compound 13 observed with multiple lipopholic interactions with LYS721, VAL702, AND LEU820 [58].

Jiadai Liu et al. discovered a new series of quinoxalinamine pyrimidine-2,4-diamine derivatives, All synthesized compounds were evaluated for their anti-proliferative activity against EGFR L858R/T790M/C797S mutant cell lines. Compound 14 exhibited good activity with nanomolar IC50 = 8–9 nM .Docking analysis for Compound 14 based on the 6LUD crystal structure suggests robust binding affinity. 14 indicate the ligand forming critical hydrogen bonds with MET-793 and LYS-745 & stabilizing salt bridge with GLU-804 [59].

Sivaiah and colleagues developed a new series of pyrazolo[1,5-a]pyrimidine derivatives as dual Epidermal Growth Factor Receptor (EGFR)/Human Epidermal Growth Factor Receptor 2 (HER2). They performed enzyme Inhibition studies for all synthesized compounds against HER2 and EGFR and it identified that compound 15 exhibited good inhibitory activity with IC_{50}= 0.126 µM (EGFR) & 0.083 µM (HER2) . Cytotoxicity study carried out against HepG2, MCF7, and HCT116 cells and compound 15 showed the most potency with IC_{50} = 3.26µM, 3.19µM, and 5.01µM respectively . Docking analysis revealed compound 15 had a favorable binding affinity of –9.03 kcal/mol within the EGFR ATP-binding site, stabilized by critical hydrogen-bonding interactions with CYS-797, LEU-798, and TYR-801, complemented by hydrophobic interactions with Val-845 and Trp-817 [60].

Bandi SR et al. developed a group of new pyrido[2,3-d]pyrimidine-thiazolidine-1,2,3-triazole analogues targeted as anticancer agent. All the synthesized compounds are subjected to cell line study against MCF-7 cells. Compound 16a and 16b exhibiting superior kinase activity (IC50= 0.39 ± 0.03 and 0.53 ± 0.02 µMagaint MCF-7 cells and strong antiproliferative effects (IC50= 3.52 ± 0.12 and 4.19 ± 0.24 µM). In silico docking investigations revealed a robust binding affinity of –108.68 kcal/mol for 16a and -105.89 kcal/mol for 16b. The binding affinity stabilized by critical interactions with THR830, THR766, CYS773 and THR830, ASP831, MET742, LYS721, CYS773 within the EGFR ATP-binding site for 16a & 16b [61].

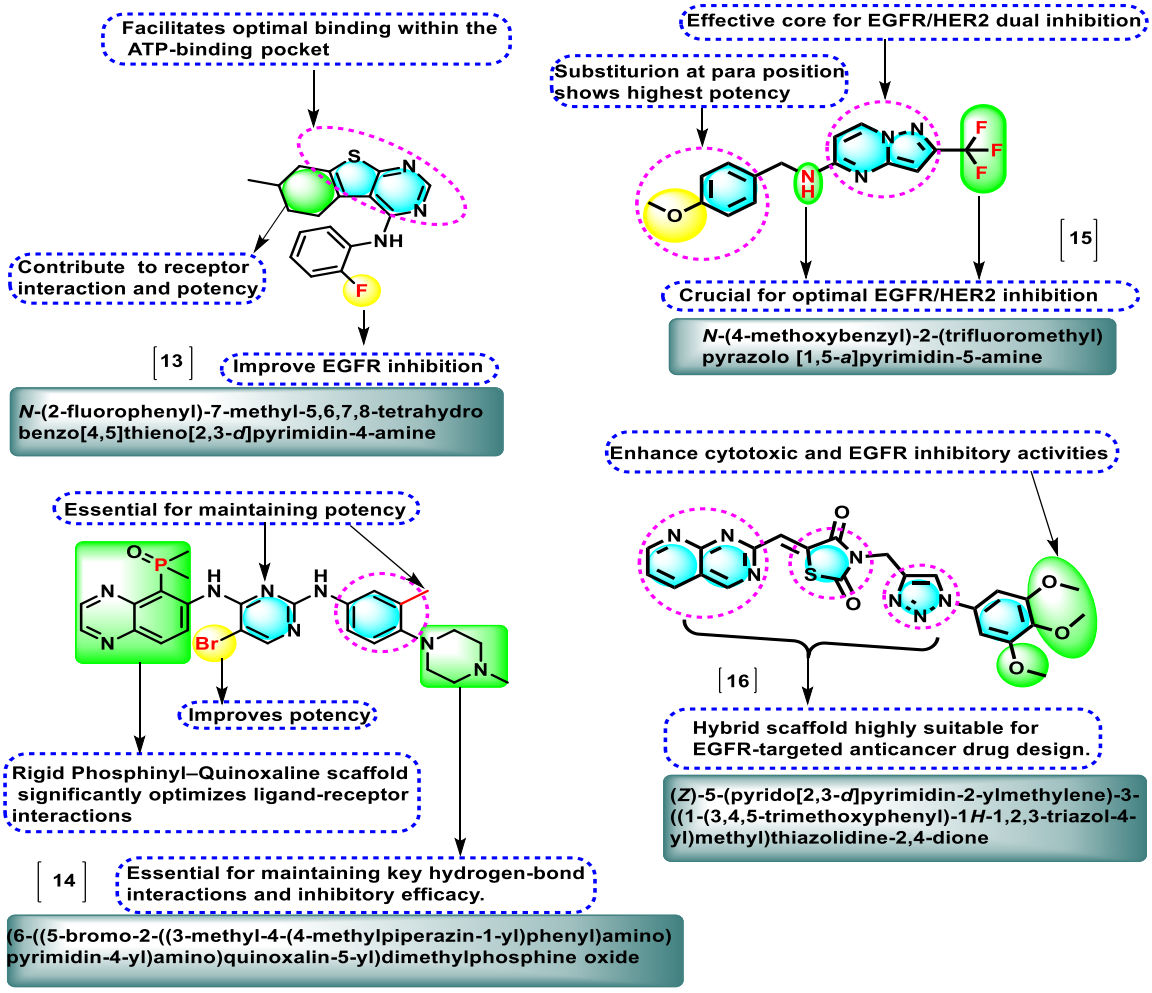


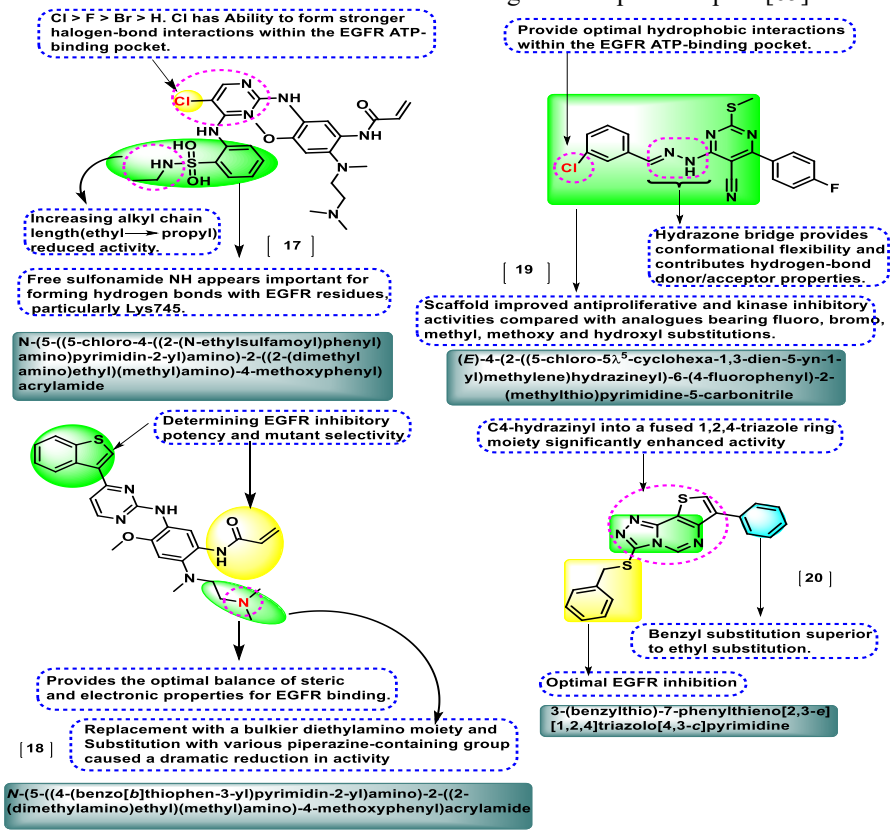
Fig.8 Chemical structure and SAR of compounds 13-16.Bottom of Form

Han Yao et al. designed and synthesized a series of amino pyrimidine derivatives containing sulfonamide as EGFR inhibitors. They performed antiproliferative activity against H1975 cells on all synthesized compounds. Results demonstrate that compound 17 had the highest activity against with nanomolar (IC50 = 0.6 nM in H1975 cells and 0.22–0.35 nM at the kinase level) this indicating strong inhibition of mutant EGFR. The docking studies explained that the 17 noted favorable binding energies (ΔG ≈ −8.29 kcal/mol), with the aminopyrimidine core forming key hydrogen-bonding interactions with MET793 and LEU844 in the kinase hinge region. The results specified that 17 containing sulfonamide group interacted with LYS745 & the acrylamide group covalently targeted CYS797.Additional hydrophobic contacts with Leu718, Val726, Ala743, and Thr854.[62].

Baijiao and colleagues developed new group of pyrimidine-based EGFR- TKIs targeting EGFR T790M mutation. Cell inhibition assay was performed against H1975 cells and EGF T790M/L858R for all synthesized compounds. The result revealed that compound 18 had good activity with IC_{50} = 0.0022 μM against H1975 cells & 0.26 nM targeting EGFT790M/L858R against the wild-type EGFR. Molecular docking studies were evaluated to examine the plausible binding configurations of compound 18 and revealed a favorable binding free energy (ΔG ≈ −67.37 kcal/mol), surpassing the reference inhibitor. The results revealed that compound 18 formed critical hydrogen bonding interactions with MET793 and ASP800, alongside π–H interactions with LEU718 and extensive lipophilc contacts stabilizing the compound within the ATP-binding pocket 18 [63].

A series of pyrimidine-5-carbonitrile analogs as dual EGFR/ Phosphoinositide 3-Kinase (PI3K) inhibitors developed by Nada Reda et al. All synthesized derivatives were evaluated by DTP-NCI against all NCI60 cell lines. Compound 19 identified as most potent analogue, with selective inhibition of EGFR T790M mutant, EGFRWT with IC50= 0.086 μM and 0.132 μM and PI3K-δ with IC_{50} = 0.64 μM. Compound 19 also managed to show the highest docking score (ΔG = −6.13 kcal/mol). In EGFR ATP-binding pocket, 19 established a key hydrogen-bonding interaction with the hinge residue MET793 and additional π–hydrophobic interactions with LEU718 and VAL726, thereby stabilizing the ligand–receptor complex [64].

Ahmed M. Farghaly and co-worker synthesis new thieno[3,2-d]pyrimidine and thienotriazolopyrimidine derivatives as EGFR-targeted anticancer agents. All synthesized compounds tested against enzyme inhibition and MDA-MB-231 breast cell line. Results demonstrated that compound 20 showed the good enzyme inhibition with IC50= 146.8 nM, comparable with erlotinib (IC50= 166.9 nM), and MDA-MB-231 breast cell line with (IC50= 0.89 μM). The docking analysis revealed favorable docking score -5.1685 Kcal/mol. Compound 20 displayed interactions around the heteroaromatic thienotriazolopyrimidine MET793 via hydrogen-bonding interactions with the EGFR ATP-binding site. Compound 20 interacts with active-site residues, complemented by hydrophobic and π-associated contacts that stabilized the ligand–receptor complex [65].



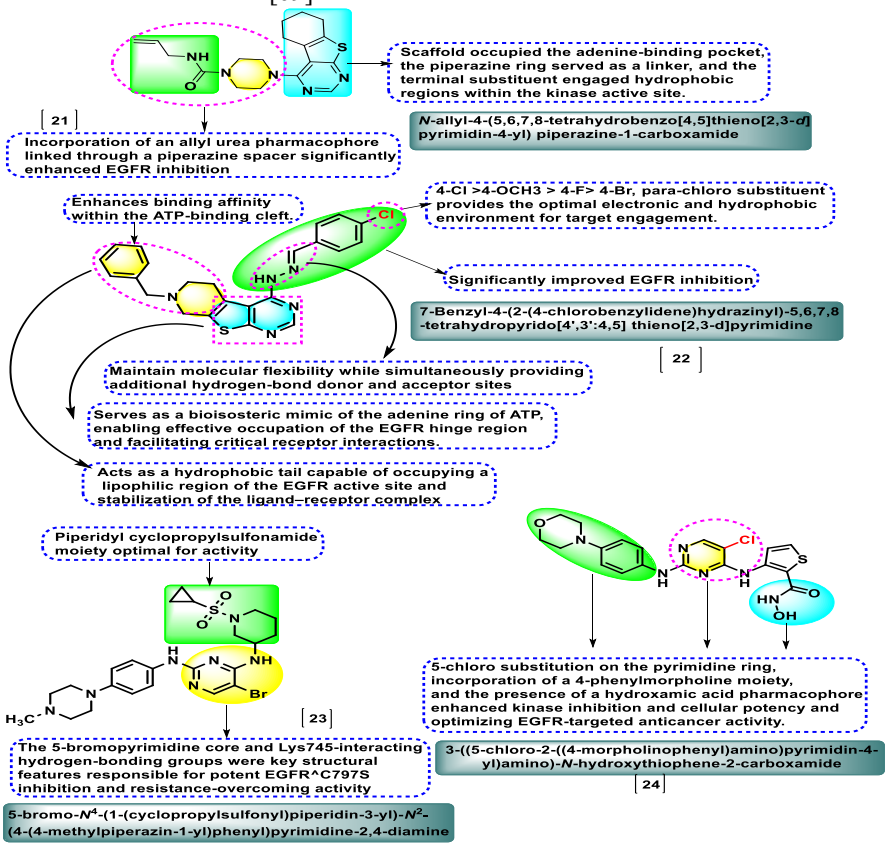
Top of FormFig.9 Chemical structure and SAR of compounds 17-20.

Reda G. Yousef et al. developed a new thieno[2,3-d]pyrimidine analogs as Dual-target EGFR/VEGFR-2 inhibitors. Compounds were tested for invitro cytotoxicity against MDA-231 & MCF-7 and WI-38 cell line and result demonstrated that compound 21 was identified as the most active analogue, with 8.13 ± 0.6 µM (MDA-231), 11.72 ± 0.9 µM (MCF-7), 53.66 ± 3.1 µM (WI-38) with surpassing the reference drug erlotinib (IC_{50} = 7.72 ± 0.58 nM). Compound 21 also demonstrate inhibition of EGFR (IC_{50} = 5.42 ± 0.28 nM) and VEGFR-2 (IC_{50} = 50.31 ± 2.0 nM), suggesting its potential as a dual-target kinase inhibitor. Molecular docking studies revealed that compound 21 showed stable accommodation via an essential hydrogen bond inside the EGFR ATP-binding pocket between the pyrimidine nitrogen and Met769. Compound 21 was able to display hydrophobic interactions involving the cyclohexene and allyl moieties occupied hydrophobic regions I and II. Compound 21 indicated π–π interactions with ALA719, LYS721, and VAL702 further reinforced binding [66].

Sayed MTM et al. reported group of thieno[2,3-d]pyrimidine analogs as EGFR-targeted anticancer agents. All synthesized compounds were evaluated against multiple cancer cell lines and results revealed that compound 22 emerged as the most potent inhibitor (IC_{50} = 0.096 ± 0.004 µM). compared to erlotinib with IC_{50} = 0.037 ± 0.002. Molecular docking studies demonstrated docking at −6.6607 Kcal/mol and favorable accommodation of compound 22 within the EGFR ATP-binding pocket. The thieno[2,3-d]pyrimidine nucleus occupied the adenine-binding region, while the hydrazone-linked 4-chlorophenyl moiety enhanced hydrophobic interactions and overall binding stability [67].

Yu-Ze Mao et al. prepared a series of 2-aminopyrimidine that target the EGFR^del19/T790M/C797S mutant. The enzyme inhibition assay result demonstrated that compound 23 showed the most potent activity against KC-0116 and KC-0122 cells with IC_{50} = 0.20 and 0.21 µM, respectively. Docking analysis of compound 23 revealed that 23 adopted a binding mode similar to Brigatinib, forming a conserved bidentate hydrogen-bond network with MET793. The cyclopropylsulfonyl oxygen group of 23 established an additional hydrogen bond with LYS745. The results displayed that compound 23 shows Mutant selectivity was improved by the 5-bromo substituent's advantageous van der Waals interactions with the gatekeeper Threonine 790 [68].

Yaqing Zuo et al. synthesized novel 4-arylamine-substituted pyrimidine derivatives as noncovalent EGFR inhibitors. Compound 24 showed impressive antiproliferative activity against HCC827 cells (IC50=24.6 nM). Compound 24 demonstrated high and notable inhibitory effect against EGFR^19del/T790M/C797S and EGFR^L858R/T790M/C797S mutants (IC_{50}= 16.06 nM and 11.81 nM, respectively). Based on docking study it report that 24 displayed hydrogen bonding with MET793, LYS745, ASP855, THR854, and Asn842, as well as halogen bonding with MET790 and extensive alkyl, π-alkyl, and π–π interactions.Molecular docking studies of 24 demonstrated stable accommodation within the EGFR ATP-binding pocket, indicating favorable binding affinity toward resistant EGFR mutants [69].

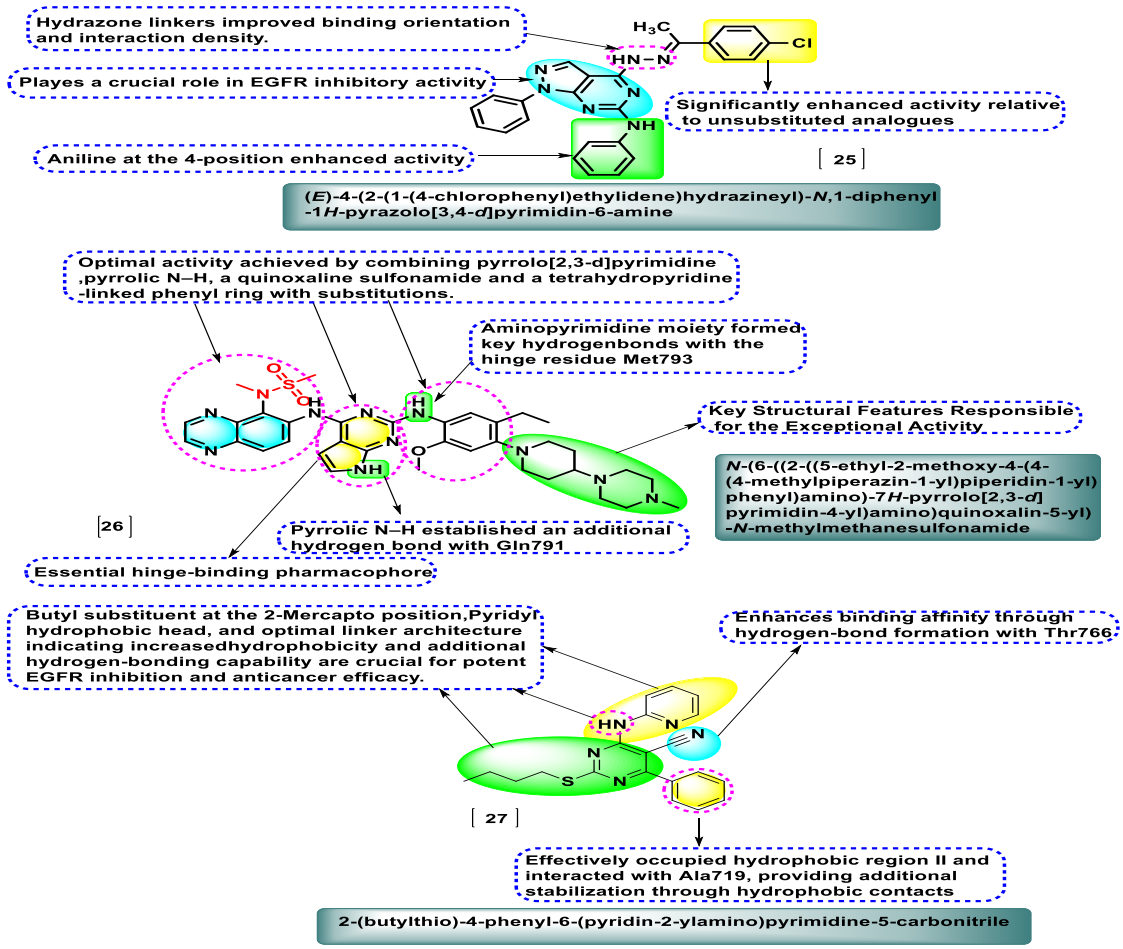


Bottom of Form Top of Form Fig.10 Chemical structure and SAR of compounds 21-24.

A Novel group of 1H-pyrazolo[3,4-d]pyrimidine derivatives synthesized by Ahmed A. Gaber and Coworker . All compounds were tested against A549 human lung adenocarcinoma cell line and HCT-116 human colorectal carcinoma cell & compound 25 found to be the active with IC_{50} = 8.21 & 19.56 µM. Compound 25 exhibit inhibition of Wild-type Epidermal Growth Factor Receptor (EGFR^WT) and Epidermal Growth Factor Receptor T790M mutant (EGFR^T790M) kinases (IC50 = 0.016 and 0.236 µM). The docking analysis revealed that compound 25 demonstrate strong binding affinities toward EGFR^WT and EGFR^T790M with docking scores of −23.09 and −20.59 kcal/mol, respectively. The pyrazolo[3,4-d]pyrimidine core of 25 established key hydrogen bonds with MET769/LYS721 in EGFR^WT and MET793 in EGFR^T790M.The p-chlorophenyl moiety of 25 formed extensive hydrophobic interactions with residues including LYS745, MET790, LEU788, ILE759, LEU764, AND LEU834 [70].

Zhenhua Wu et al. developed a new series of pyrrolo[2,3-d]pyrimidine-based fourth-generation EGFR inhibitor. All the synthesized compounds were evaluated against Ba/F3 EGFR^19del/T790M/C797S and EGFR^L858R/T790M/C797S cells and Compound 26 showed significant activity with IC50 = 0.001 and 0.04 nM, respectively. Crystallographic and molecular modeling studies revealed that 26 adopts a U-shaped conformation within the EGFR ATP-binding pocket, establishing three critical hydrogen bonds with MET793 and GLN791. The compound 26 displayed lipophilic interactions with MET790, CYS775, LEU844, and PHE856, π–π stacking with PHE728, and water-mediated hydrogen bonding involving ASP800 and SER797 collectively contribute to its remarkable affinity and mutant selectivity [71].Top of Form
Bottom of Form

Ahmed A. Nasser et al. designed and developed new pyrimidine-5-carbonitrile analogs as EGFR-targeted anticancer agents. They performed antiproliferative activity against HCT-116 human colorectal carcinoma, HepG2 human hepatocellular carcinoma, Michigan Cancer Foundation-7 (MCF-7) human breast adenocarcinoma, and A549 human lung adenocarcinoma cell lines. The result demonstrated that Compound 27 showd good activity with IC_{50} = 3.37 (HCT-116), 3.04 (HepG2), 4.14 (MCF-7), and 2.40 µM (A549), in that order. Compound 27 gives significant inhibition activity agaist wild-type Epidermal Growth Factor Receptor (EGFR^WT) and the Epidermal Growth Factor Receptor T790M mutant (EGFR^T790M) (IC_{50} = 0.09 and 4.03 µM, respectively). Docking analysis of 27 stated favorable binding within the EGFR ATP-binding pocket with a binding energy of −7.22 kcal/mol [72].

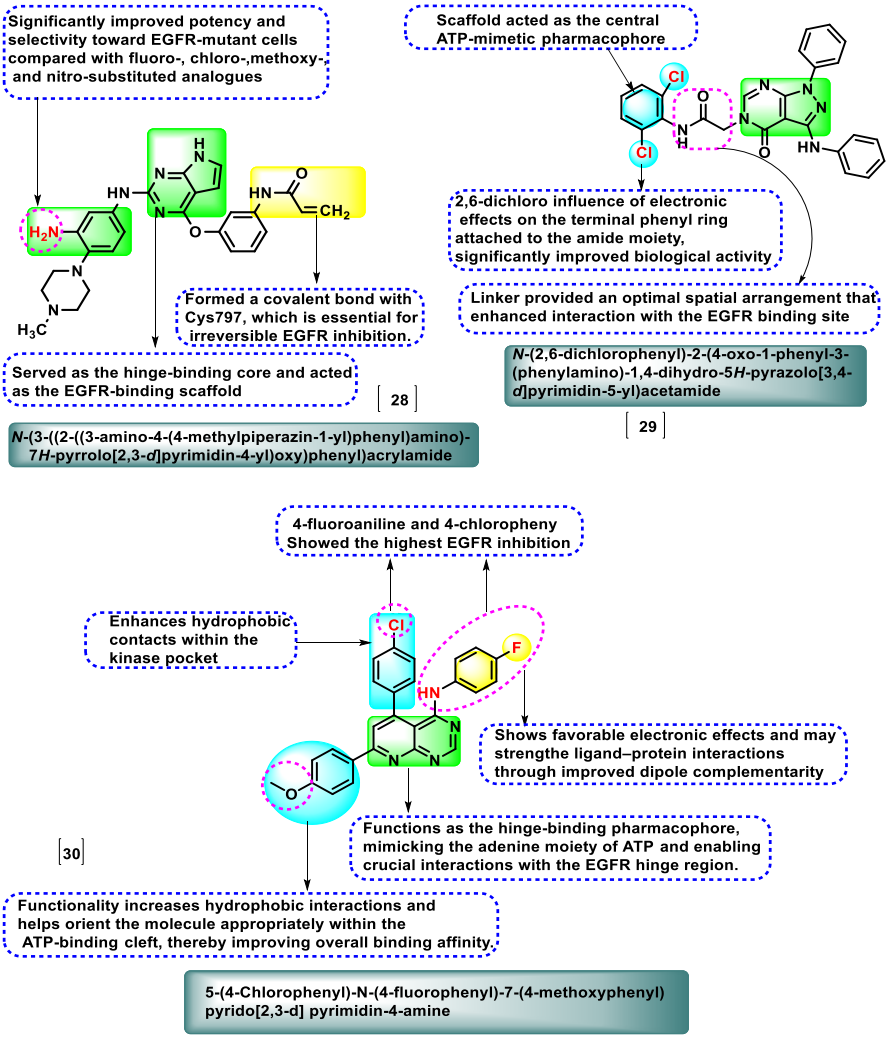


Top of FormFig.11 Chemical structure and SAR of compounds 25-27.

Zhenqiang Xia et al. carried out synthesis of pyrrolo[2,3-d]pyrimidine-based EGFR-TKIs. The compounds were screened for anticancer activity against HCC827 cells and compound 28 exhibit potent results with IC50 = 0.046 μM. The compound 28 further showed potent inhibition of EGFR^T790M (IC50 = 0.21 nM) and EGFR^delE746–A750 (IC50 = 2.2 nM). Molecular docking studies revealed that compound 28 established critical hydrogen-bonding interactions with CYS797, PRO794, MET793, MET788, and CYS792 within the ATP-binding cleft. Also the covalent engagement of the acrylamide warhead with the catalytic cysteine residue contributed to enhanced binding stability and mutant selectivity of compound 28[73].

Farag F. Sherbiny et al. developed a new series of pyrazolo[3,4-d]pyrimidine derivatives as Epidermal Growth Factor Receptor targeted anticancer agents. All synthesized compounds were evaluated and 29 demonstrated the most potent lead compound against HepG2 human hepatocellular carcinoma, HeLa human cervical adenocarcinoma, HCT-116 human colorectal carcinoma, and Michigan Cancer Foundation-7 (MCF-7) human breast adenocarcinoma cell lines with IC50=4.28μM(HepG2), 5.18μM(HeLa) 3.97μM(HCT-116) and 9.85μM (MCF-7), respectively. Compound 29 also shows significant EGFR inhibition (IC50 = 56.02 ± 1.38 ng/mL). Docking analysis 29 indicate revealed a favorable binding energy (ΔG = −60.28 kcal/mol) Within the EGFR ATP-binding pocket, the acetamide NH established a hydrogen-bonding interaction with ASP855. The 29 exhibit extensive hydrophobic interactions with LEU718, GLY719, MET790, LEU792, CYS797, LEU799, and LEU844 stabilized the ligand–receptor complex [74].

Amal A.M. Eissa et al. reported the new series of 4-anilinopyrido[2,3-d]pyrimidine derivatives as potent EGFR inhibitors. All synthesized compounds were screened and compound 30 emerging as the most active analogue, exhibiting an EGFR inhibitory IC50 = 31.72 ± 0.69 nM, surpassing gefitinib (IC50 = 52.37 nM). Compund 30 demonstrated cytotoxicity against MCF-7 and MDA-MB-361 cell lines with IC_{50} = 4.82 ± 0.20 μM and 4.43 ± 0.23 μM, respectively. A docking analysis report that 30 demonstrate the maximum docking score −7.96 kcal/mol and indicated favorable binding within the EGFR ATP-binding region, according to docking studies. The compound 30 established key hydrogen-bonding interactions with MET793 and a water-mediated hydrogen bond with ASP855.While the non-coplanar 4-chlorophenyl moiety occupied the hydrophobic pocket surrounded by ARG841, GLY721, AND ASP855, enhancing binding stability [75].



Top of Form Fig.12 Chemical structure and SAR of compounds 28-30.

Ismail M.M. Othman et al. reported the series of substituted pyrimidine-based EGFR inhibitors. All the synthesized compound were evaluated for anticancer study against Michigan Cancer Foundation-7 (MCF-7) human breast adenocarcinoma and HepG2 human hepatocellular carcinoma cell lines and the result revealed compound 31 (6-phenylpyrimidin-2-one derivative) showed most potent lead with IC_{50} = 0.01 ± 0.03 (MCF-7) and 0.01 ± 0.01 µM (HepG2), respectively. Compound 31 also revealed inhibition of EGFRWT, EGFRL858R, and EGFRT790M with IC50= 0.087, 0.044, and 0.026 µM, in that order. Docking studies showed 31 displays most favorable binding affinities toward EGFRWT (ΔG = −13.22 kcal/mol) and EGFRT790M (ΔG = −12.65 kcal/mol), mediated by multiple hydrogen-bond interactions involving the pyrimidin-2-one core in 31 with key hinge-region residues MET769, GLN767, GLN766, and THR766 in EGFRWT, and MET793 and GLN791 in the mutant receptor [76].

Abdel-rahman et al. synthesized the library of triazolo- &thiazolo-pyrimidine derivatives as EGFR-targeted anticancer agents. Compound tested against Michigan Cancer Foundation-7 (MCF-7) human breast adenocarcinoma and HCT-116 human colorectal carcinoma cell lines. The result identified that compound 32 was most active EGFR inhibitor, exhibiting superior antiproliferative activity with IC50 = 7.21 (MCF-7) and 9.15 (HCT-116) µM , respectively. Docking analysis result revealed compound 32 shows favorable binding affinity of −8.10 kcal/mol, attributed to four key hydrogen-bonding interactions involving MET793, ASP855. Compound 32 shows dual interactions observed with LYS745, where the carbonyl functionalities, pyridine nitrogen, and nitrile moiety acted as critical hydrogen-bond acceptors within the ATP-binding pocket[77].

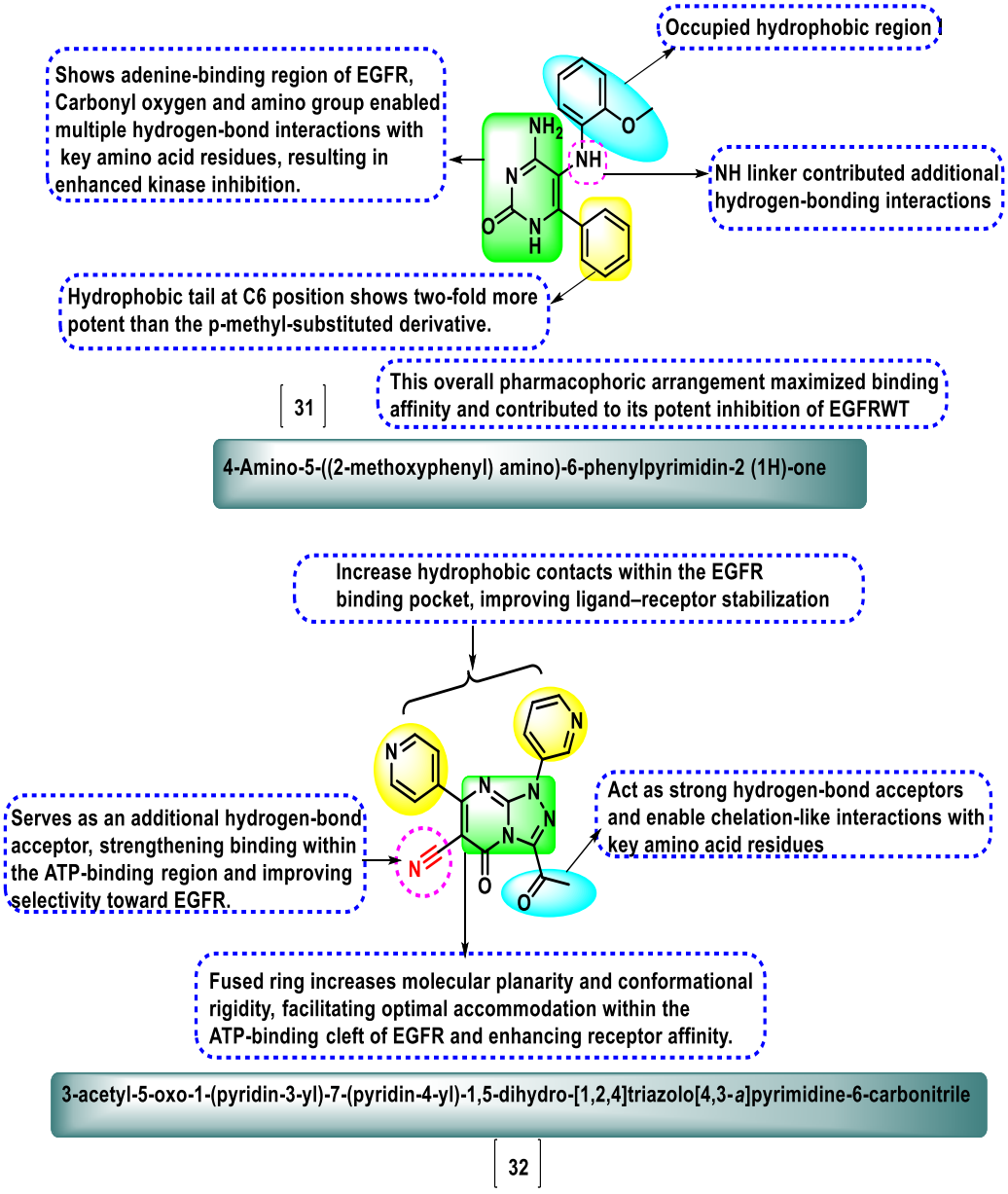


Fig.13 Chemical structure and SAR of compounds 30-32.

Table No.III Detailed summary of substituted Pyrimidine derivatives as Promising Anticancer Agents.

Comp. No.	Author name& year of publication	Chemical Scaffold	IC_{50} Value Against Cell Line	Docking Value (kcal/mol) & PDB ID	Amino Acid Interactions	Ref.
1	Gao et al. 2024	Thieno[2,3-d]pyrimidine	HepG2: 0.17 µM Superior antiproliferative activity compared with Gefitinib 1.4 µm.	−8.19 ID: 2ITY	Hydrogen bonds with Lys721, Gln767, Met769, Asp831; π–π interaction with Phe699; Hydrophobic interactions with Leu764, Val702, Met742.	[46]
2	Yılmaz and Uluçam et al. 2023	Dihydrofuro[3,4-d]pyrimidine	DU145: 17.4 µM Moderate cytotoxicity and EGFR inhibitory potential.	−7.40 ID:1M17	Hydrogen bonding interactions with Asp813 & Arg817 within the EGFR active site.	[47]
3	Zhen Xiao et al. 2020	Thiophene-pyrimidine	EGFR 790M : 3.79 ± 0.57 µM A549: 3.79 ± 0.57 µM A431:4.34 ± 0.60 µM Potent inhibition comparable to reference inhibitors.	- ID:3IKA	U-shaped binding mode; key hydrogen bond with Met793 (1.9–2.2 Å); Additional interaction with Arg841, contributing to enhanced binding affinity.	[48]
4	Narasimhachar et al. 2025	Pyrimidine-thio-triazole	MCF-7 : 29.62 µM Selective EGFR inhibitor with moderate antiproliferative activity.	−7.27 ID 3W33	Hydrogen bond with Asp855; π-cation interaction with Lys875; Hydrophobic interactions with Cys797, Phe723, Val726, Leu844, and Leu858.	[49]

5a, 5b	Elsebaie et al. 2024	Thienopyrimidine	MCF-7 5a :0.05 µM; 5b: 0.08 µM . A549 5a : 0.07 µM 5b: 0.07 µM Both compounds exhibited potent anticancer activity.	5b: -9.6 Inside EGFR & 5b : -8.1 Inside STAT3 active sites. EGFR receptor-ID:1M17 & STAT3 receptor-ID:6NJS	Hydrophobic interactions and π–sulfur interactions with Met742 and Cys773, along with occasional hydrogen bonding within the ATP-binding site.	[50]
6	Farag et al. 2022	Pseudo-bicyclic pyrimidine	GI_{50} = 0.52 to 0.92 µM Against multiple cancer cell lines, including melanoma, colon, and blood cancers	−10.87 ID: 1xkk	Hydrogen bond between pyrimidine N1 and Met793 (hinge region) Hydrophobic interactions with Phe856 and Met766.	[51]
7	Ahmed A. Abdel Gaber et al. 2021	Pyrazolo[3,4-d]pyrimidine	MCF-7: 2.89 µM Identified as a potent EGFR-TK inhibitor.	−6.82 ID: 4ZAU	Hydrogen bond with Cys797; dual H-arene interactions with Lys745 and Met793, stabilizing binding within the ATP-binding pocket.	[52]
8a, 8b, 8c	Salah A. Abdel-Aziz et al. 2021	Pyrimidine-5-carbonitrile containing 1,3-thiazole	EGFR enzyme: 8a = 0.33 µM, 8b = 0.22 µM, 8c = 0.19 µM Potency comparable to Erlotinib.	−13.20 to −9.82 ID:1m17	Hydrogen bonding with Met769; Cyano-mediated water-bridged interaction with Thr766; hydrophobic interactions with Leu694, Gly722, and Met769.	[53]
9	Zhang C. et al. 2025	Pyrimidine derivative	EGFRL858R/T790M: 5.0 nM, EGFRL858R/T790M/C797S: 2.9 nM, H1975 : 0.054 ± 0.016 µM	- EGFRT790M/C797S ID: 7VRE &	U-shaped binding conformation; Dual hydrogen bonds and halogen bond with Met793; hydrogen	[54]

			Potent inhibition of resistant EGFR double and triple mutants.	EGFRT790M-ID:3IKA	bond with Cys797; Hydrophobic π–sigma interactions with Val726 and Leu844.	
10a, 10b	Bandi S.R. et al. 2023	Triazolo-pyrrolo-pyrido pyrimidine	MCF-7 : 10a : 0.33 ± 0.02 µM, 10b: 0.41 ± 0.06 µM. Both compounds more potent than Erlotinib (0.43 ± 0.03 µM).	−8.94 to −9.80 ID:4HJO	Hydrogen bonds with Lys721 and occasionally Cys773; Hydrophobic interactions with Ala719, Val702, Leu694, and Leu820.	[55]
11a, 11b	Mohammed Y.I.A. et al. 2025	Pyrimidine-based pyrene/benzochromene hybrid	EGFR inhibition: 11a :77.03 nM; 11b: 94.9 nM Comparable to Erlotinib (72.3 nM). HCT-116 : 11a :1.34 µM 11b : 1.90 µM Comparable to Erlotinib with an 1.32 µM.	−21.81 ID:1M17	Hydrogen bonds with Lys721 (3.83 A°) and Asp831 (1.69 A°) ; lipophilic interactions with Phe699, Leu694, Leu764, Leu820, and Pro770 in the ATP-binding pocket.	[56]
12	Naglaa Ahmed et al. 2021	Indolyl-pyrimidine	MCF-7: 5.10 µM, HepG2: 5.02 µM HCT-116: 6.60 µM Comparable to Erlotinib (2.96 µM).	−9.60 ID: 1M17	Hydrogen bonds with Asp831, Lys721, and Thr766; lipophilic interactions with Leu694, Phe699, and Val702.	[57]
13	Eman A. Sobh et al. 2023	Thieno[2,3-d]pyrimidine	EGFRWT: 37.19 nM; EGFRT790M : 204.10 nM. Potent inhibition of both wild-type and mutant EGFR.	−17.22 EGFRWT - ID:4HJO & EGFRT790M ID:3W2O	Hydrogen bond with Met769; lipophilic interactions with Lys721, Val702, and Leu820.	[58]

14	Jiadai Liu et al. 2025	Quinoxalinamine pyrimidine-2,4-diamine	EGFRL858R/T790M/C797S and EGFR Del19/T790M/C797S kinases mutant: 8–9 nM. Exhibited potent antiproliferative activity against resistant EGFR triple-mutant cell lines.	- ID: 6LUD	Hydrogen bonds with Met793 and Lys745; salt bridge interaction with Glu804, contributing to stable ligand binding.	[59]
15	Sivaiah et al. 2023	Pyrazolo[1,5-a]pyrimidine	Enzyme inhibition – EGFR: 0.126 µM HER2: 0.083 µM HepG2: 3.26µM MCF7: 3.19µM HCT116 : 5.01µM.	−9.03 EGFR-ID: 1M17 & HER2-ID: 3RCD	Hydrogen bonds with Cys797, Leu798, and Tyr801; hydrophobic interactions with Val845 and Trp817.	[60]
16a, 16b	Bandi S.R. et al. 2023	Pyrido[2,3-d]pyrimidine-thiazolidine-1,2,3-triazole	EGFR kinase-16a :0.39 ± 0.03 µM, 16b: 0.53 ± 0.02 µM. MCF-7-16a :3.52 ± 0.12 µM 16b : 4.19 ± 0.24 µM.	−108.68 (16a) −105.89 (16b) ID:1M17	16a: Thr830, Thr766, Cys773. 16b: Asp831, Met742, Lys721, Cys773; interactions stabilize binding within the EGFR ATP-binding pocket.	[61]
17	Han Yao et al. 2025	Aminopyrimidine–sulfonamide	H1975 : 0.6 nM; EGFR kinase : 0.22–0.35 nM. Potent inhibition of mutant EGFR.	−8.29 ID:6JWL	Hydrogen bonds with Met793 and Leu844; sulfonamide interaction with Lys745; covalent interaction with Cys797; hydrophobic contacts with Leu718, Val726, Ala743, and Thr854.	[62]

18	Baijiao et al. 2022	Pyrimidine-based EGFR-TKI	H1975 : 0.0022 ± 0.001 μM PC9 cells : 0.0048 ± 0.001 μM EGFRT790M /L858R : 0.26 nM. Excellent potency against the resistant EGFR mutant.	−67.37	Hydrogen bonds with Met793 and Asp800; π–H interaction with Leu718; extensive lipophilic interactions within the ATP-binding pocket.	[63]
19	Nada Reda et al. 2024	Pyrimidine-5-carbonitrile	EGFRT790M : 0.086 μM EGFRWT: 0.132 μM; PI3K-δ: 0.64 μM. As a dual EGFR/PI3K inhibitor with selective activity toward mutant EGFR.	−6.13 EGFRT790M ID: 3W2O & -9.16 PI3K-delta ID: 6G6W	Hydrogen bond with Met793; π–hydrophobic interactions with Leu718 and Val726; lack of interaction with Thr790 contributed to mutant selectivity.	[64]
20	Ahmed M. Farghaly et al. 2021	Thieno[3,2-d]pyrimidine and thienotriazolopyrimidine	EGFR enzyme: 146.8 nM (Erlotinib: 166.9 nM) MDA-MB-231 : 0.89 μM. Displayed potent EGFR inhibitory and antiproliferative activities.	-5.1685 ID:1M17	Hydrogen-bond interaction with Met793 in the ATP-binding site, supported by hydrophobic and π-associated interactions that stabilized the ligand–receptor complex.	[65]
21	Reda G. Yousef et al. 2025	Thieno[2,3-d]pyrimidine	MDA-231 : 8.13 ± 0.6 μM MCF-7 : 11.72 ± 0.9 μM WI-38 : 53.66 ± 3.1 μM EGFR :5.42 ± 0.28 nM VEGFR-2 : 50.31 ± 2.0 nM.	- VEGFR- ID: 4HJO & EGFR- ID: 2OH4	Hydrogen bond between the pyrimidine nitrogen and Met769; hydrophobic interactions through cyclohexene and allyl moieties; π–π interactions with Ala719, Lys721, and Val702.	[66]

22	Sayed M.T.M. et al. 2023	Thieno[2,3-d]pyrimidine	Multiple cancer cell lines: 0.096 ± 0.004 µM compared with Erlotinib 0.037 ± 0.002 µM Showed Potent antiproliferative activity	PDB:1M17 −6.6607	Thieno[2,3-d]pyrimidine nucleus occupied the adenine-binding region; hydrazone-linked 4-chlorophenyl moiety enhanced hydrophobic interactions and binding stability within the EGFR ATP-binding pocket.	[67]
23	Yu-Ze Mao et al. 2023	2-Aminopyrimidine	KC-0116 : 0.20 µM KC-0122 : 0.21 µM. Potent inhibitor targeting EGFRdel19/T790M/C797S mutant.	- ID:7VRE	Conserved bidentate hydrogen bonds with Met793; additional hydrogen bond with Lys745; favorable van der Waals interaction between the 5-bromo substituent and Met790, enhancing mutant selectivity.	[68]
24	Yaqing Zuo et al. 2024	4-Arylamine-substituted pyrimidine	HCC827 : 24.6 nM EGFR19del/T790M/C797S 16.06 nM& EGFRL858R/T790M/C797S: 11.81 nM. Displayed potent activity against resistant EGFR mutants.	T790M-mutant ID: 3IKA, & T790M/C797S-mutant ID:5XGN & L858R/T790M/C797S-mutant ID: 6LUD	Hydrogen bonds with Met793, Lys745, Asp855, Thr854, and Asn842; halogen bond with Met790; extensive alkyl, π–alkyl, and π–π interactions within the ATP-binding pocket.	[69]

25	Ahmed A. Gaber et al. 2022	1H-Pyrazolo[3,4-d]pyrimidine	A549: 8.21 µM HCT-116 : 19.56 µM. EGFRWT : 0.016 µM EGFRT790M : 0.236 µM Potent inhibition of wild-type and mutant EGFR.	−23.09 (EGFRWT) ID: 4HJO & −20.59 (EGFRT790M) ID: 3W2O	Hydrogen bonds with Met769 and Lys721 (EGFRWT) and Met793 (EGFRT790M); hydrophobic interactions with Lys745, Met790, Leu788, Ile759, Leu764, and Leu834.	[70]
26	Zhenhua Wu et al. 2025	Pyrrolo[2,3-d]pyrimidine	Ba/F3 EGFR19del/T790M/C797S: 0.001 nM Ba/F3 EGFRL858R/T790M/C797S: 0.04 nM Identified as a fourth-generation EGFR inhibitor with remarkable potency against resistant mutants.	- ID: 6LUB	Three hydrogen bonds with Met793 and Gln791; lipophilic interactions with Met790, Cys775, Leu844, and Phe856; π–π stacking with Phe728; water-mediated hydrogen bonds involving Asp800 and Ser797.	[71]
27	Ahmed A. Nasser et al. 2020	Pyrimidine-5-carbonitrile	HCT-116 : 3.37 µM HepG2 : 3.04 µM MCF-7 : 4.14 µM A549 : 2.40 µM EGFRWT : 0.09 µM EGFRT790M : 4.03 µM.	−6.77 to −9.55 ID:3W2O	Lipophilic interactions with Val702 and Lys721; hydrogen bond with Thr766; interaction with Thr830; additional hydrophobic contacts with Leu764 and Ala719.	[72]
28	Zhenqiang Xia et al. 2021	Pyrrolo[2,3-d]pyrimidine	HCC827: 0.046 µM EGFRT790M : 0.21 nM EGFRdelE746–A750 : 2.2 nM Demonstrated potent EGFR-TKI activity against resistant	- EGFRWT - ID: 6VHP & EGFRT790M mutant ID: 3IKA	Hydrogen bonds with Cys797, Pro794, Met793, Met788, and Cys792; covalent interaction of the acrylamide warhead with Cys797,	[73]

			mutants.		enhancing binding stability and mutant selectivity.	
29	Farag F. Sherbiny et al. 2021	Pyrazolo[3,4-d]pyrimidine	HepG2 : 4.28 µM HeLa : 5.18 µM HCT-116 : 3.97 µM MCF-7 : 9.85 µM EGFR enzyme : 56.02 ± 1.38 µM . Potent antiproliferative and EGFR inhibitory activities.	−60.28 ID:3W2O	Hydrogen bond between the acetamide NH and Asp855; hydrophobic interactions with Leu718, Gly719, Met790, Leu792, Cys797, Leu799, and Leu844, stabilizing the ligand–EGFR complex.	[74]
30	Amal A.M. Eissa et al. 2021	4-Anilinopyrido[2,3-d]pyrimidine	EGFR enzyme : 31.72 ± 0.69 nM, Superior to Gefitinib : 52.37 nM. MCF-7: 4.82 ± 0.20 µM MDA-MB-361: 4.43 ± 0.23 µM.	−7.96 ID:1XKK	Hydrogen bond with Met793; water-mediated hydrogen bond with Asp855; hydrophobic interactions involving Arg841, Gly721, and Asp855 surrounding the 4-chlorophenyl moiety, enhancing binding stability.	[75]
31	Ismail M.M. Othman et al. 2021	Substituted pyrimidine (6-phenylpyrimidin-2-one)	MCF-7: 0.01 ± 0.03 µM HepG2: 0.01 ± 0.01 µM EGFRWT : 0.087 µM EGFRL858R : 0.044 µM EGFRT790M : 0.026 µM. Potent antiproliferative activity and broad-spectrum inhibition of wild-type and mutant EGFR.	−13.22 EGFRWT ID: 1M17 & −12.65 (EGFRT790M) ID : 6JX0	Hydrogen-bond interactions with Met769, Gln767, Gln766, and Thr766 in EGFRWT; hydrogen bonds with Met793 and Gln791 in EGFRT790M.	[76]

32	Abdel-Rahman et al. 2025	Triazolo-&thiazolo-pyrimidine	MCF-7: 7.21 µM HCT-116 : 9.15 µM. Most active EGFR-targeted antiproliferative analogue in the series.	−8.10 ID: 3W32	Four hydrogen-bond interactions involving Met793, Asp855, and dual interactions with Lys745. The carbonyl groups, pyridine nitrogen, and nitrile moiety served as key hydrogen-bond acceptors within the ATP-binding pocket.	[77]

CONCLUSION

Targeting EGFR receptor suppression has revolutionized the treatment of non-small cell lung cancer and other cancers that are driven by the receptor. The Pyrimidine scaffold is a favored pharmacophore for developing inhibitors of EGFR tyrosine kinase as it has showed a high structural diversity, synthetic convenience, and ability to make key contacts within the ATP-binding pocket as shown in this review. Recent advances in scaffold modifications, linker optimization, fused heterocyclic systems, and covalent warheads have enabled the development of several derivatives with good nanomolar activity against the wild-type and clinically relevant resistant mutations of EGFR, such as T790M and C797S.Despite these advances, key challenges remain, such as acquired resistance, tumor heterogeneity, pharmacokinetic limitations, and toxicity that hinder the effective clinical translation. Although there are a number of Pyrimidine derivatives that show very promising preclinical results, further pharmacokinetic studies and safety testing are still needed.The rational scaffold design, combined with the prediction of ADMET properties, molecular dynamics simulations, the use of biomarkers for precision medicine, and the application of AI for drug discovery, should be included in future studies. In addition, there are several novel methods to overcome resistance including allosteric modulators, PROTAC degraders, reversible covalent inhibitors, and multitarget inhibitors. Overall, Pyrimidine-EGFR inhibitors remain an extremely promising platform for the development of targeted therapeutics for cancer with improved long-term clinical efficacy, safety and selectivity.

AuthorContributions -

Sapana Nagare: Conceptualization, original draft preparation, and collection. Dr.Vinayak Deshmukh: Supervising the work,conception,anddesign. **R.B.Pandhare:** Formal analysis, data curation. Ganesh Andhale: Writing-review, Secondary editing Resources, project administration.

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Abbreviations	
Å	Angstrom
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
AKT	Protein Kinase B (AKT)
ATP	Adenosine Triphosphate

BATs	Bispecific Antibody-Armed Activated T Cells
B7-H3	B7 Homolog 3 (CD276)
CAR-T	Chimeric Antigen Receptor T-cell
C797S	Cysteine-to-Serine substitution at position 797
CBA	Cancer Burden Assessment
COX-2	Cyclooxygenase-2
ctDNA	Circulating Tumor DNA
ΔG	Binding Free Energy
DHFP	Dihydrofuro[3,4-d]pyrimidine
DNA	Deoxyribonucleic Acid
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
EGFR-TKI	Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor
EMA	European Medicines Agency
ErbB	Erythroblastic Oncogene B Receptor Family
FDA	Food and Drug Administration
FPBMC	Fresh Peripheral Blood Mononuclear Cells
GI_{50}	50% Growth Inhibitory Concentration
HER1	Human Epidermal Growth Factor Receptor 1
HER2	Human Epidermal Growth Factor Receptor 2
HER3	Human Epidermal Growth Factor Receptor 3
HER4	Human Epidermal Growth Factor Receptor 4
HCC	Hepatocellular Carcinoma
HCC827	Human Non-Small Cell Lung Cancer Cell Line
HCT-116	Human Colorectal Carcinoma Cell Line
HepG2	Human Hepatocellular Carcinoma Cell Line
IC_{50}	Half-Maximal Inhibitory Concentration
IL13Rα2	Interleukin-13 Receptor Alpha 2
iNOS	Inducible Nitric Oxide Synthase
MAPK	Mitogen-Activated Protein Kinase
MCF-7	Michigan Cancer Foundation-7 Breast Cancer Cell Line
MDA-MB-231	Human Triple-Negative Breast Cancer Cell Line
MM-GBSA	Molecular Mechanics-Generalized Born Surface Area
NSCLC	Non-Small Cell Lung Cancer
PI3K	Phosphoinositide 3-Kinase
PKC	Protein Kinase C
PLC-γ	Phospholipase C Gamma

PROTAC	PROteolysisTArgeting Chimera
QSAR	Quantitative Structure–Activity Relationship
RAF	Rapidly Accelerated Fibrosarcoma
RAS	Rat Sarcoma Viral Oncogene
RNA	Ribonucleic Acid
SAR	Structure–Activity Relationship
Src	Proto-oncogene Tyrosine-Protein Kinase Src
STAT	Signal Transducer and Activator of Transcription
STAT3	Signal Transducer and Activator of Transcription 3
T790M	Threonine-to-Methionine substitution at position 790
TKI	Tyrosine Kinase Inhibitor
VEGFR	Vascular Endothelial Growth Factor Receptor
VEGFR-2	Vascular Endothelial Growth Factor Receptor 2
WHO	World Health Organization
WT	Wild Type
ZD1839	Developmental Code of Gefitinib

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