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REVIEW ARTICLE

An Insight into the Therapeutic Potential of Pyridopyrimidines as Anticancer Agents

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ABSTRACT:

Cancer is one of the major causes of worldwide human mortality. A wide range of cytotoxic drugs are available on the market, and several compounds are in different phases of clinical trials. In recent years, fused pyrimidine derivatives have been considered as a novel class of cancer chemotherapeutic agents that show promising activity against different tumors. The aim of this article is to comprehensively review focused on recent progressions in chemistry and molecular docking by the description of several applications. This strategy enables the hit identification, lead optimization of the designed pyridopyrimidine scaffolds by proceeding molecular docking studies for targeting protein kinases in anticancer activity and which proves a reliable and fast filter in HT virtual screening, thereby providing a pool of ideas for novel lead molecules. In future research has to be carried to fully explore the anticancer efficacy of insilico succeeded molecules through *in vitro* and *in vivo* animal models.

KEYWORDS: Pyridopyrimidine, Biological activity, Kinase, Molecular docking and Anticancer.

INTRODUCTION:

Pyrimidines have a long and distinguished history extending from the days of their discovery as important constituents of nucleic acids to their current use in the chemotherapy[1]. Among all heterocyclic compounds, pyrimidines are one of the most important heterocycles exhibiting remarkable pharmacological activities because it is an essential constituent of all cells and thus of all living matter. Many simple fused pyrimidines such as purines and pteridines are biologically active by themselves or are essential components of very important naturally occurring substances (i.e., nucleic acids). The presence of a pyrimidine base in cytosine, uracil and thymine, which are the essential building blocks of nucleic acids (DNA and RNA) is one of the possible reasons for their activities[2].

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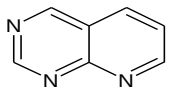
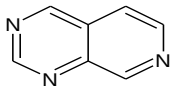
STRUCTURE OF PYRIDOPYRIMIDINES:

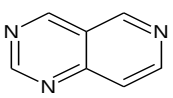
There are four possible isomeric structures for pyridopyrimidines, depending on the position of the nitrogen atom in the pyridine moiety.



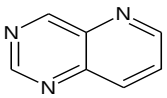
Pyrido(2,3-d)pyrimidine



Pyrido(3,4-d)pyrimidine



Pyrido(4,3-d)pyrimidine



Pyrido(3,2-d)pyrimidine

Activities of pyridopyrimidines:

Pyrimidines are important classes of heterocyclic compounds and exhibit a broad spectrum of biological activities such as anti-inflammatory[3-5], analgesic[6-10], antimicrobial[11-20], anti-HIV[21], antiallergic[22], anti-malerial[23, 24], anticancer activities [25-42] etc.

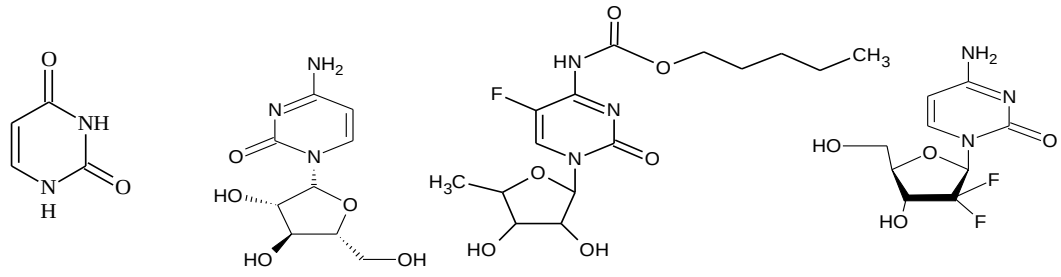


Figure 1: Antitumor drugs

However, the pyridopyrimidine isomers are the least described in the literature because of its difficult and expensive syntheses. Pyrido[3,2-d]pyrimidines have been reported as antimalarial[43], as tyrosine kinase inhibitors[44], as dihydrofolate reductase inhibitors[45], as anti-HCV agents[46] and as immunosuppressive drugs[47]. On the other hand, pyrido[3,4-d]pyrimidines are well known as potential anticancer agents[48], tyrosine kinase inhibitors[49] and they also efficiently inhibit the action of dehydrofolate reductase causing the death of many pathogenic microorganisms[50].

In 2003, Huron *et al*.[51], reported a series of pyrido[2,3-d]pyrimidines as Tyrosine Kinase inhibitors. The compounds exhibited Significant activity against STI-resistant mutant Bcr-abl proteins and they reported that, the Compound was a prototype with picomolar potency and substantial activity against STI571-resistant mutants.

Chemistry of Pyridopyrimidines:

The synthesis of pyridopyrimidine derivatives markedely increased in the literature. Synthesis of some pyrido[2,3-d]pyrimidine ring system Synthesis of pyrido[2,3-d]pyrimidine derivatives was performed according to the following general strategies:

Fusion of the pyridine ring onto the pyrimidine ring system:

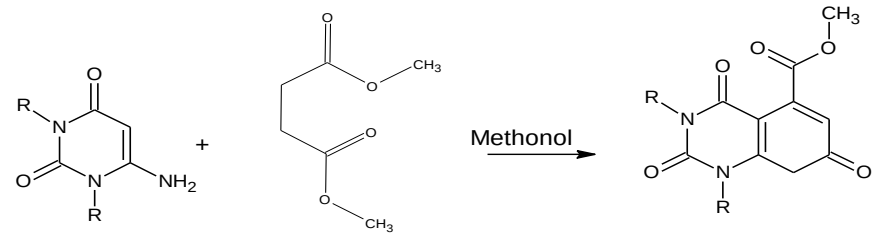
There are four general approaches for the synthesis of pyrido [2, 3-d]pyrimidines starting from pyrimidine ring system, all of which utilize an appropriately substituted 4-aminopyrimidine. The pyridine ring may be formed via the addition of three carbon atoms (route I), or two carbon atoms (route II), or by the intramolecular cyclization of propionyl derivative (route III) or recently by one -pot reaction of three-component including 4-aminopyrimidine (route IV).

Route I synthesis:

The reaction consists of an electrophilic attack on the 5-position of the pyrimidine ring and thus only those Pyrimidines that are activated toward electrophilic substitution by the presence of electron donating substituents at the 2 and 4 positions undergo cyclization. 6-aminouracil, 6-amino-1,3-dimethyluracil, 2,4-diamino-pyrimidin-6(1H)-one, 6-amino-1-ethyl-1H-pyrimidine-2,4-dione and 6-amino-2-thiouracil have all been converted into pyrido[2,3-d]pyrimidines. A wide variety of reagents have been used in this reaction.

a. Dimethyl acetylenedicarboxylate (DMAD):

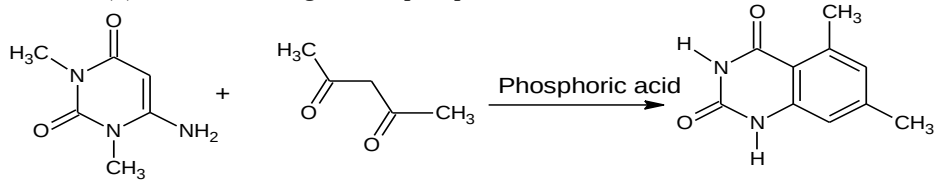
Recently unsubstituted 6-aminouracil **(1a)** [52] and its N-alkyl derivative **1b** [53, 54] have been found to react with dimethyl acetylene dicarboxylate (DMAD) in protic media to give 5-carboxamido-7-oxopyrido [2, 3-d]pyrimidines and the probable mechanism has been described.



a : R = H
b: R = CH_3

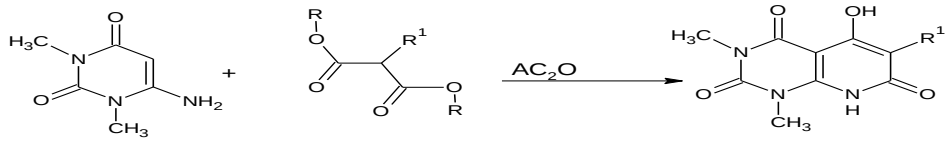
b. 1, 3-Dicarbonyl compounds:

6-Aminopyrimidlnes also react readily with 1, 3 diketones to yield various 5, 6 and **7** substituted pyrido[2,3-d]pyrimidines. Acetyl acetone **(4)** and 6-aminouracil **(1a)**, for example yielded 5, 7-dimethylpyrido[2,3-d]pyrimidine-2,4-dione **(5)** when heated together in phosphoric acid[55].



1a 4 5

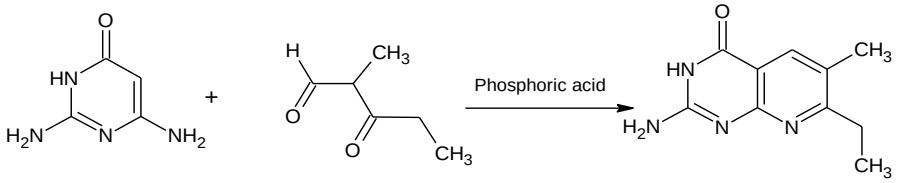
Another example for symmetrical diketones, the reaction of malonic acid or ethyl malonate derivatives 6with 6-amino-1, 3-dimethyluracil **(1b),** for the synthesis of 6-substituted-5-hydroxypyrido[2,3-d]pyrimidin-7-ones **(7)**[56, 57].



1b 6 7

R = H, C_2H_5
R^1 = H, CH_3

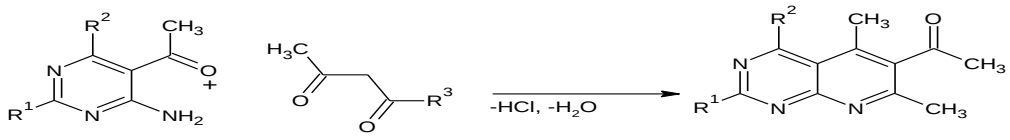
Also, treatment of 2, 4-diaminopyrimidine-6-one (**8**) with 2-methyl-3-oxopentanal (**9**) in phosphoric acid 85% afforded 2-amino-7-ethyl-6-methylpyrido[2,3-*d*]pyrimidin-4-(3*H*)-one (**10**) as the only isolated product which resulted from reaction of the aldehyde function with the 5-position of the pyrimidine ring. Whereas, with unsymmetrical diketones the orientation of the reaction is controlled by the reaction of the most reactive carbonyl group with the 5- position of the pyrimidine ring.



8 9 10

Route II synthesis:

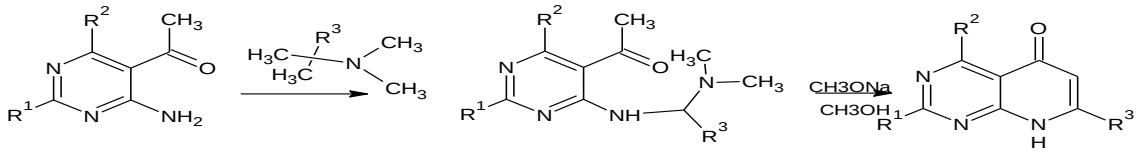
This type of synthesis involves the reaction of an active methylene compound containing an adjacent functional group capable of cyclization with 5-acyl, 5-formyl, or 5-cyano-4-aminopyrimidines. Reaction of 2, 6-disubstituted-5-acetyl-4-aminopyrimidine
(11) with acetylacetone **(12)** or benzoylacetone afforded new substituted 6-acyl pyrido[2,3-*d*]pyrimidines (**13)**[58].



11 12 13

R^1 = Ph, Ph.Cl(p)
R^2 = Me
R^3 = Me,Ph

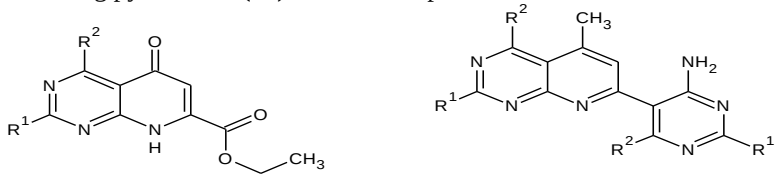
Also, 7-substituted-pyrido[2,3-*d*]pyrimidin-5-ones (**16)** were synthesized by the interaction of 2,6-disubstituted-4-amino-5-acetylpyrimidines **(14)** with *N*,*N*-dimethylformamide dimethyl acetal or N, N-dimethylacetamide dimethyl acetal to give N, N-dimethylacetamide 2,6-disubstituted-4-amino-5-acetylpyrimidines **(15)** followed by cyclization under the action of sodium methoxide in methanol[59].



14 15 16

R^1 = Me, Ph
R^2 = Me, Ph
R^3 = Me,H

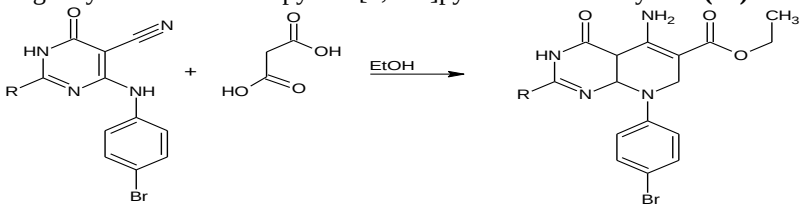
Similarly, ethyl 5-oxo-pyrido[2,3-*d*]pyrimidine-7-carboxylate **(17)** could be obtained *via* condensation of 5-acetyl-4-aminopyrimidines **(14)** with ethyl oxalate in the presence of sodium ethoxide. The products **(18)** of the Friedlander self condensation of the starting pyrimidines**(14)** have been reported [60] .



17 18

R^1 = Me, Ph, Ph(p-NO_2), Ph(p-Cl)
R^2 = Me, Ph, SCH_3

Similarly, 4-(halo phenyl amino)-pyrimidine-5-carbonitrile derivatives **(19)** treated with malonic acid **(20)** in ethanol to give the corresponding ethyl 5-amino-6-oxo-pyrido [2, 3-*d*]pyrimidine carboxylates **(21)**[61] .

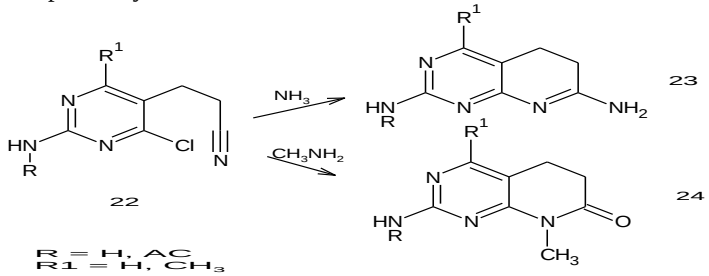


19 20 21

R = CH_3, NH_2

Route III synthesis:

In contrast to the previous synthesis, pyrido[2,3-*d*]pyrimidines prepared by this route are not completely aromatic compounds. Instead they are reduced pyridopyrimidines are obtained by cyclization of an aliphatic propionyl derivative. So, the propionitrile derivative **(22)** yielded the pyrido[2,3-*d*]pyrimidines **(23)**and **(24)** when treated with ammonia or methylamine respectively [62, 63].



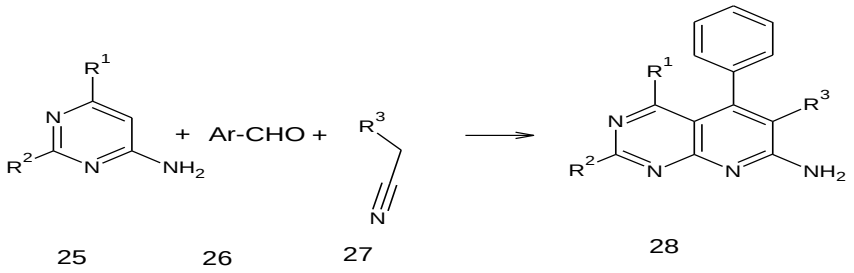
22 23 24

R = H, Ac
R1 = H, CH_3

Route IV synthesis:

A series of pyrido[2,3-*d*]pyrimidines and their polycyclic derivatives have been prepared by one-pot three component reaction of 4-aminopyrimidines, aromatic aldehydes and active methylene compounds (alkyl nitriles, cyclic ketones, cyclic diketones). This efficient synthesis was done thermally with or without using catalysis, under microwave irradiation conditions or under ultrasonic irradiation as a recent trend. The reaction occurs *via* an initial formation of the arylidene, from the condensation of benzaldehyde and active methylene compounds, which suffer nucleophilic attack to give the Michael adduct. Then cyclization and auto-oxidation took place to afford the fully aromatized compound.

Gazzar et al. [64], have reported a series of pyrido[2,3-*d*]pyrimidine derivatives**(28)** by the one-pot reaction of the appropriate 2, 6-disubstituted-4-amino-pyrimidine **(25),** aldehyde **(26)** and alkyl cyanide (**27)** in dimethylformamide.



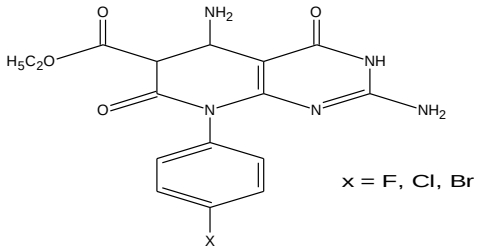
25 26 27 28

Biochemical targets of fused pyridopyrimidines:

Nasser S.M. Ismail et al.[65], have reported synthesis of Pyrazolopyrimidines are fused heterocyclic ring systems which structurally can consider as bioisosteres of adenine, which is fundamental for every aspect of cell life. The Pyrazolo[3,4-d]pyrimidines derivatives have been explored for their inhibitory activity towards various protein kinase enzymes and their role as anticancer agents. Number of studies has explored their SAR, as well as conformation and orientation requirements for kinase binding site through modeling studies. This could provide insight to a medicinal chemist for a comprehensive and target oriented information for development of clinically viable pyrazolo[3,4-d]pyrimidine based anticancer drugs.

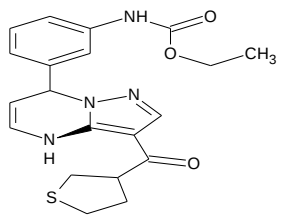
P. Shanmugasundaram et al.[66], have reported Synthesis of pyrido(2, 3-d)pyrimidine-carboxylate derivatives**(29)** and evaluated for cytotoxic activity of using three human cancer cell lines [i.e.colon cancer (HT29), liver cancer (HepG2), cervical cancer (Hela)] with MTT assay. It is seen that all the synthesized compounds showed significant activity. The GI50 of the compound ,ethyl-2,5-diamino-8-(4-chlorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-carboxylate, was found at 18 and 17 µ g/mL on HT29 and HepG2 cell lines, respectively. The GI50 of the compound,ethyl-5-amino-8-(4-flourophenyl)-2-methyl-4,7-dioxo-3,4,5,6,7,8hexahydropyrido(2,3-d) pyrimidine-6-carboxylate was found at 20 µ g/mL on Hela cell lines.

The total growth inhibition (TGI) of the compound, ethyl-2,5-diamino-8-(4-chlorophenyl)-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-carboxylate was found at 35 and 41 µ g/mL on HT29 and HepG2 cell lines, respectively. The TGI of the compound, ethyl-5-amino-8-(4-fluorophenyl)-2-methyl-4,7-dioxo-3,4,5,6,7,8 hexahydro pyrido(2,3-d)pyrimidine-6-carboxylate was found at 49 µ g/ml on Hela cell lines.



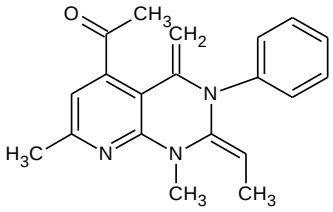
29

Osama M. Ahmed et al.[67], have reported new series of pyrazolo[1,5-*a*]pyrimidine derivatives **(30)**were synthesized and screened for their *in vitro* antitumor activity against three human carcinoma cell lines, namely colorectal carcinoma (HCT116), prostate adeno carcinoma (PC-3) and liver carcinoma (HepG-2) using MTT cytotoxicity assay at 100 µg/ml. Some of the tested compounds displayed good anticancer activities against HCT-116 and PC-3 cells. Whereas, some compounds showed better antitumor activity and the rest of the compounds against both cell lines.



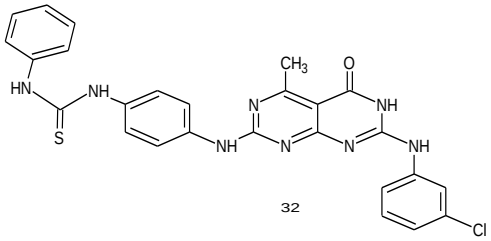
30

Sladowska et al.[68], have synthesized 7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-*d*]pyrimidine-5-carboxylic acid**(31)** and screened for analgesic, anxiolytic, blood pressure activity on mice.



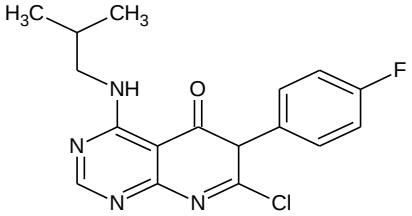
31

Samir M. Moghazy et al[69]., reported a series of 2,5,7-trisubstituted pyrimido[4,5-*d*]pyrimidines **(32)**were designed based rational drug design for cyclin-dependent kinase (CDK2) and synthesized. The coordinate for the protein structure was obtained from the RCSB Protein Data Bank (PDB). Protein Structure was prepared using Discovery Studio (DS 2.0) software package 6-Amino-2-thiouracil is reacted with an aldehyde and thiourea to prepare the pyrimido[4,5-*d*]-pyrimidines. Alkylation and amination of the latter ones give different amino derivatives. These compounds show potent and selective CDK inhibitory activities and inhibit in vitro cellular proliferation in cultured human tumor cells.



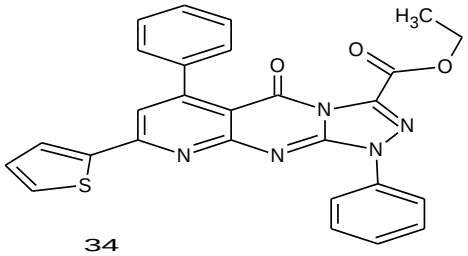
32

Ibrahim DA et al.[70], have synthesized 4-aminopyrido[2,3-d]pyrimidine derivatives **(33)** as CDK2/Cyclin A, CDK4/Cyclin D, EGFR and anti-tumor was evaluated by cytotoxicity studies in A431a, SNU638b, HCT116 and inhibition of CDK2-Cyclin A, CDK4/Cyclin D and EGFR enzyme activity *in vitro.* The anti-proliferative and CDK2-Cyclin A inhibitory activity of these compounds was significantly more active than roscovotine with IC_{50} 0.3 and 0.09 μM respectively. Molecular modeling study, including fitting to a 3D-pharmacophore model, docking into cyclin dependant kinase2 (CDK2) active site and binding energy calculations were carried out and these studies suggested the same binding orientation inside the CDK2 binding pocket for these analogs compared to ATP.



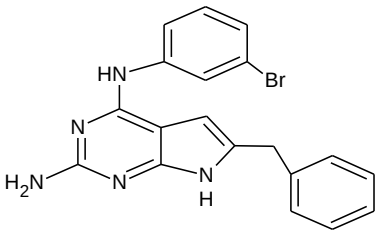
33

Yasser H. Zaki et al.[71], have synthesized a series of pyrido[2,1-*b*]pyrimido [1,3,5]thiadiazinones **(34)** by aminomethylation of pyridopyrimidinethione with formaldehyde solution (37%) and different primary aromatic amines. Another series of pyridopyrimido[2,1-*b*][1,3]thiazinones was prepared by Michael addition reaction of pyridopyrimidinethione to the activated double bond of a number of arylidene malononitrile and 2-(benzo[1,3]dioxol-5-ylmethylene)malononitrile. A comparative study of the biological activity of the synthesized compounds with chloramphenicol and trimethoprim/sulphamethoxazoles and reported synthesized compounds showed adequate inhibitory effects on the growth of Gram-positive and Gram-negative bacteria.



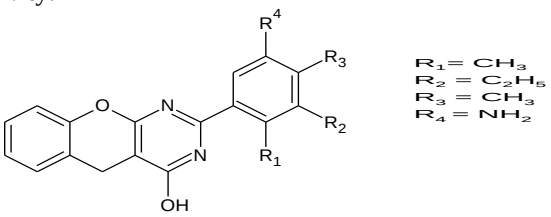
34

Gangjee. A et al.[72], reported *N*4-phenyl substituted-6-(2-phenylethylsubstituted)-7*H*-pyrrolo[2, 3-*d*]pyrimidine-2,4-diamines **(35)** as homologated series of our previously published RTK inhibitors. They reasoned that increased flexibility of the side chain, which determines potency and selectivity, would improve the spectrum of RTK inhibition and remarkable inhibitory activity against epithelial growth factor receptor (EGFR), vascular endothelial growth factor receptor-1 (VEGFR-1), platelet-derived growth factor receptor-b (PDGFR-b).



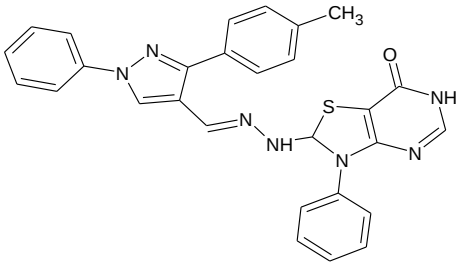
35

Hadfield JA et al.[73], studied on a series of novel substituted bezopyrano[2,3-*d*]pyrimidines derivatives **(36)** and tested for cytotoxic activity against a panel of cancer cell lines including the P388 lymphocytic leukemia cell line. In the series unsubstituted parent compound, some methoxylated derivatives and a cyclohexyl derivative all exhibited potent cytotoxic activity.



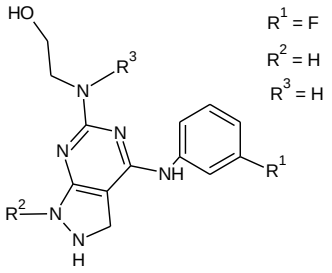
36

Bekhit A et al.[74], 74 reported a series of 1*H*-pyrazolyl derivatives of thiazolo[4,5-*d*]pyrimidines **(37)** were examined for their *in-vivo* anti-inflammatory activity and also evaluated for *in-vitro* antimicrobial activity.



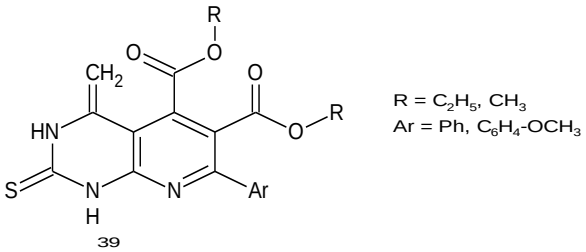
37

Kim DC et al.[75], reported a series of 1,4,6-trisubstituted pyrazolo[3,4-*d*]pyrimidines **(38)** capable of selectively inhibiting CDK2 activity by derivatization at C-4, C-6 and N-1 with various amines and lower alkyl groups. In this series, 4-anilino compounds exhibited better CDK2 inhibitory activity and antitumor activity. The compounds having a 3-fluoroaniline group at C-4 showed comparable or superior CDK2 inhibitory activity to those of olomoucine and roscovitine as reference compounds. In general, the unsubstituted compounds at N-1 possessed higher potency than the substituted compounds for the CDK2 inhibitory activity. The compounds exhibited potent cell growth inhibitory activity against human cancer cell lines, but their CDK2 inhibitory activities were slightly poorer than olomoucine.



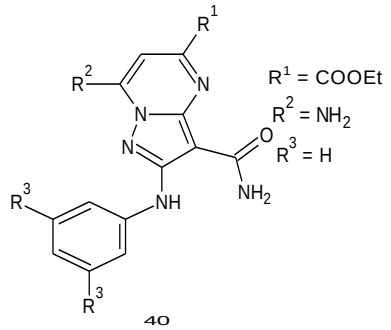
38

Mohamed NR et al.[76], reported condensation of 6-amino-2-thiouracil with aromatic aldehydes afforded azomethine derivatives. The formed azomethines underwent [4+2] cycloaddition with enaminones and enaminonitrile to form the corresponding condensed pyrimidines respectively. On the other hand, the interaction of azomethine derivatives with acetylene derivatives afforded the corresponding pyrido[2,3-*d*]pyrimidines**(39)** and screened for the antimicrobial and antitumor activity.



39

Mukaiyama H et al.[77], have reported a series of novel pyrazolo[1,5-*a*]pyrimidines **(40)** as c-Src kinase inhibitors to improve the *in vitro* potency of the c-Src inhibitor and to address its hERG liability focusing on two regions of the lead compound. The blockade of the delayed cardiac current rectifier K^+ channel was overcome by replacing the ethylenediamino group with an amino alcohol group at the 7-position. In addition, modifying the substituent at the 5-position and the side chain groups on the amino alcohols at the 7-position enhanced the intracellular c-Src inhibitory activity and increased central nervous system (CNS) penetration.



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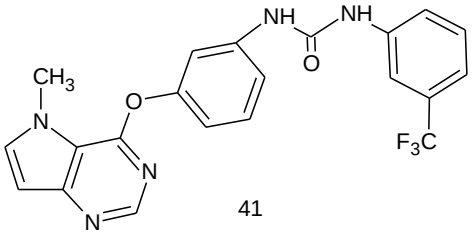
Aisha A.K. Al-Ashmawy et al.[78], have synthesized a series of 2-substituted-4-morpholino-pyrido[3,2-*d*]pyrimidine and pyrido[2,3-*d*]pyrimidine analogs as PI3Kα/mTOR inhibitors. Unlike many PI3Kα/mTOR dual inhibitors, these compounds displayed selectivity for PI3Kα. Based on its potent enzyme inhibitory activity, selectivity for PI3Kα and good therapeutic index in 2D cell culture viability assays, compound 4h was chosen to be evaluated in 3D culture for its IC_{50} against MCF7 breast cancer cells as well as for docking studies with both enzymes.

Oguro Y et al.[79], have synthesized a series of pyrrolo[3,2-*d*]pyrimidine derivatives **(41)** and evaluated their application as type-II inhibitors of vascular endothelial growth factor receptor 2 (VEGFR2) kinase. Incorporation of diphenyl urea moiety at the C4-position of the pyrrolo[3,2-*d*]pyrimidine core *via* an oxygen linker resulted in compounds that were potent inhibitors of VEGFR2 kinase as antitumor activity.



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MOLECULAR DOCKING STUDIES:

Docking has become an essential tool in structure-based ligand design. It is widely applied and meets very heterogeneous demands. Of course a major task remains the identification of new active compounds for a particular target protein. Docking proves a reliable and fast filter in HT virtual screening[80], thereby providing a pool of ideas for novel lead structures, and can show several success stories[81]. Many research groups apply docking and scoring methods, when synthesis and experimental testing have already been performed, in order to correlate the scores with the biological activity[82]. Often the explanation or affirmation of a binding mode for a (structurally new) class of compounds is desired[83]. Many docking approaches aim to find out, whether a specific docking methodology and/or which scoring functions are best suited for a particular target system. Therefore it is tested, if correct binding orientations can be reproduced[84]. Furthermore, databases seeded with active molecules are screened to investigate, if as new descriptors. The latter are based on solvent accessible surface areas and account for conformational entropy changes and desolvation effects of both ligand and receptor upon binding, which are crucial but nevertheless often neglected aspects of the binding process. A training set of 100 and attest set of 24 protein-ligand complexes are used and confirm accurate affinity prediction and therefore applicability of the new functions as filters to guide compound selection after docking.

Anticancer activity by different biochemical targets:

The biological investigations of pyrazolo[3,4-d]pyrimidines have revealed that substitution of various groups on the scaffold imparts anticancer activity through inhibition of different target enzymes.

1. Mitogen-activated protein kinases (MAPK) inhibitors:

p38 Kinases are members of the mitogen-activated protein kinase family that transduce signals from various environmental stresses, growth factors, and steroid hormones. Four p38 MAP kinases have been identified. Increased levels of activated p38 are markers of poor prognosis. In 2007, Dhillon et al.[85], explored the role of MAPK pathways in cancer. Cancerous mutations in MAPK pathways are frequently affecting Ras and B-Raf in the extracellular signal-regulated kinase pathway. Stress-activated pathways, such as Jun N-terminal kinase and p38, largely seem to counteract malignant transformation.

2. Src kinase inhibitors:

Src kinases are a family of nine different PTKs, including c-Src, c-Yes, Fyn, Lck, Lyn, Hck, Frk, Blk, and c-Fgr of which Src is the pro-totype[86]. Src Ks can regulate a number of signaling pathways that impact on the behavior of tumor cells, including proliferation, survival, migration, invasion, and angiogenesis. Src tyrosine kinase expression is frequently elevated in a number of epithelial tumors including colon, breast, prostate, lung, ovary, and pancreas compared with the adjacent normal tissues. Interestingly, Src kinase can be considered as key modulator of cancer cell invasion and metastasis. In 2011, Kumar et al[87]. explored the anti-proliferative and proapoptopic activities of fused[3,4-d]pyrimidines as Src kinase inhibitors in human ovarian adenocarcinoma (SK-Ov-3), breast carcinoma (MDA-MB-361), and colon adenocarcinoma (HT-29).

3. Cyclin-dependent kinase 2 inhibitors:
The cyclin-dependent kinases (CDKs) are a family of serine threonine protein kinases, which play a crucial role in the cell cycle progression[88, 89]. In2003, Kim et al[90] introduced a new series of 1, 4, 6-trisubstituted pyrazolo[3,4-d]pyrimidines capable of selectively inhibiting CDK2 activity. The synthesized compounds were evaluated for their inhibitory activity against CDK2 and EGFR enzymes.

4. Abl-tyrosin kinase inhibitors:
Bcr-Abl is regarded as highly attractive target for drug intervention since the Bcr-Abl fusion gene encodes a constitutively activated kinase. Drug discovery that specifically targeted the ATP binding site of a single kinase is regarded as quite a challenging task since hundreds of protein kinases were known in the human genome. In 2008, Schenone et al[91] reported synthesis of new 4-amino substituted pyrazolo[3,4-d]pyrimidines along with their activity in cell-free enzymatic assays on Src and Abl tyrosine kinases. Some compounds emerged as good dual inhibitors of the two enzymes, showed antiproliferative effects on two Bcr-Abl positive leukemia cell lines K-562 and KU-812, and induced apoptosis [92].

5. EGFR tyrosine kinase inhibitors:
EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase that exists on the cell surface and is activated by binding with specific ligands, including epidermal growth factor and transforming growth factor α (TGF a). EGFR signaling pathway is one of the most important pathways that regulate growth, survival, proliferation, and differentiation in mammalian cells. Over expression or constant activation of EGFR results in uncontrolled cell division which has been associated with a number of cancers, including lung cancer, anal cancers and glioblastoma multiform[93]. In 1997, Traxler et al.[94] optimized a class of the pyrazolo [3, 4-d]pyrimidines producing a series of 4-(phenylamino)-1H- pyrazolo[3,4-d]-pyrimidines as highly potent inhibitors of the EGF-R tyrosine kinase.

6. kinases and CDK1 inhibitors:
Aurora kinase is a serine/threonine kinase involved in the regulation of various stages of mitosis. Therefore, it is an important regulator in maintaining normal cell cycle process[95]. Aurora A isexpressed from the late S phase, peaking at G2/M phase and declining at the G1 phase and is involved in centrosome maturation and separation, bipolar spindle assembly, and mitotic entry[96].

7. Antitumor activities against HL-60 (human leukemia cancer cell):
Song and his coworkers[97] illustrated the synthesis of novel fluorinated pyrazolo[3,4-d]pyrimidine derivatives containing 1,3,4-thiadiazole. The antitumor activities of the synthesized compounds evaluated against HL-60 by an MTT assay[98].

CONCLUSION:
Based on all literature findings, can design many different pyridopyrimidine scaffold derivatives followed by molecular docking studies for different target kinases was reported for cancer chemotherapy. Among all fused pyrimidine derivatives pyridopyrimidines were least focused. Hence, in this review helpful to design and novel pyridopyrimidine derivatives, proceeding molecular docking studies for anticancer activity which proves a reliable and fast filter in HT virtual screening, thereby providing a pool of ideas for novel lead structures. In future research has to be carried to fully explore the anticancer efficacy of synthesized derivative *in vitro* and *in vivo* animal models.

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