2.1. Fused pyrimidines

The chemistry of fused pyrimidine emerged in 1776 when Scheele isolated uric acid (Fused imidazolopyrimidine) from kidney stones. However, the search for the systematic investigations on fused pyrimidines began around 1880’s. Further, several research studies took place in the later years when the eminent chemists like, Bischler, Riedel, Niementowski, Gabriel and Bogert contributed on this system extensively in establishing their relevance in the field of chemistry (Adrien, 1982).

2.2. Fused Benzopyrimidines

Development of biologically active benzopyrimidin-4(3H)-ones got initiated only in the last few decades. The discovery of febrifugine, a quinazolin-4(3H)-one derivative, kindled the research on fused benzopyrimidines (Quinazolines). For example, erlotinib, having a benzopyrimidine scaffold is a tyrosine kinase (TK) inhibitor used to treat non-small cell lung cancer. Another such example is Prazosin, an α1-antagonist, used to treat hypertension and benign prostate hypertrophy.

Quinazolines were widely reported for possessing several activities including anticancer activity. Renault et al., 1991, reported that, extensive research on quinazolines is increasing where, compounds like raltitrexed and thymitaq (Compound 7a and 7b) as TS inhibitors have come into the lime light.

![Compound 7a and Compound 7b]
The non-classical antifolate discovery for high selectivity towards the parasitic or the bacterial or even the tumor cell DHFR is a continuous on-going research program. It is worth mentioning that, Shah et al., 1995, synthesized some novel 2,3-disubstituted benzopyrimidines and reported them as potent anticancer agents against 60 cell lines of 9 types of human cancers. The data revealed that, the presence of aryl substitution at the 3rd position (Compound 8) could be responsible for their anticancer activity.

![Structure of Compound 8](image)

**Compound 8**

Gottasova et al., 1998, reported a series of 2,6-disubstituted 4-anilinoquinazolines (Compound 9) and tested for antibacterial activity. The results showed that, all the synthesized compounds exerted a significant effect on the gram positive *Bacillus subtilis* and *Staphylococcus aureus*. However, they did not exhibit any influence on *Escherichia coli*, *Proteus mirabilis* and *Pseudomonas aeruginosa*. Hence, the results revealed that, the compounds having simple or bromine substituted aromatic ring at the 6th position and phenyl, morpholine or piperidine moiety at the 2nd position exerted a considerable antibacterial activity. Further, the activity was encouraged by the presence of unsubstituted or methyl or amino substituted aniline group at the 4th position.

![Structure of Compound 9](image)

**Compound 9**
Skelton et al., 1998 and Skelton et al., 1999 discovered the compound 10, as a potent cytotoxic agent during the development of TS inhibitors. Here, a quinazoline based structural core with a group of pyridine containing compounds was synthesized. Interestingly, although 2-pyridyl and 4-pyridyl compounds exhibited cytotoxic potency consistent with their *in vitro* TS inhibitory activity, surprisingly a 3-pyridyl analogue (*Compound 10*), came out with 100-fold greater cytotoxic effect than expected.

![Compound 10](image)

A series of tetrahydroquinazoline analogues of piritrexim (*Compound 11*) were synthesized by Rosowsky *et al.*, in 1999. These compounds were characterized by six membered carbocyclic β-ring and one Carbon Bridge between the phenyl ring and the heterocyclic moiety. They showed DHFR inhibitory activity at IC₅₀ range of 0.057–0.10 μM, and proved to be active against 13 different tumor cell lines at concentration range between 0.1–1.0 μM.

![Compound 11](image)

Bavetsias *et al.*, 2002, synthesized some benzopyrimidine derivatives (*Compound 12*) and were believed to have folate independent locus of action. They also showed a delayed, non-phase specific cell cycle arrest.
Murugan et al., 2003, synthesized certain 2-substituted benzopyrimidines (Compound 13) and evaluated their anticancer and cytotoxic activities. Increase in the body weight and the mean survival time were considered as main parameters in this study. The compounds containing m-nitrobenzene and phenyl substitutions led to a better activity.

In 2003, Huron et al., had screened a series of pyrido[2,3-d]pyrimidines as TK inhibitors. The compounds exhibited significant activity against STI-resistant mutant Bcr-abl proteins and they reported that, the Compound 14 was a prototype with picomolar potency and substantial activity against STI571-resistant mutants.
Ashok et al., 2003, synthesized a series of 2-(ω-chloroacetonyl)-3-substituted phenyl-6-halo / 6,8-dihaloquinazolin-4(3H)-ones (Compound 15), 2-(ω-hydrazinoacetonyl)-3-substituted phenyl-6-halo / 6,8-dihaloquinazolin-4(3H)-ones (Compound 16) and 1′-[3-substituted phenyl-6-halo / 6,8-dihaloquinazolin-4-(3H)-one-2-acetonyl]-3′-aryl-5′-(2-substituted indol-3-yl)-Δ²-pyrazolines (Compound 17) and were reported with proven antiinflammatory, analgesic, ulcerogenic and cyclooxygenase activities.

Padam and Saksena, 2003, synthesized two series of compounds namely, 2-phenyl-3-p-(2′-methyl-3′-aryl-4′-oxo-thiazolin-2′-yl)phenyl quinazolin-4-ones (Compound 18) and 2-phenyl-3-p-(1′-aryl-3′-phthalimido-4′-methylazetidine-2′-one-4′-yl)phenyl quinazolin-4-ones (Compound 19) by the annulation reaction of 2-phenyl-3-(p-arylideneaminomethyl phenyl)quinazolin-4-ones with thioglycolic acid and phthalimidoacetyl chloride, respectively. The reports showed that, these compounds were inactive towards the bacterial strains; however, they showed moderate antifungal activity.
Compound 18

Murugan et al., 2004, synthesized 2,3,6,7-tetrasubstituted benzopyrimidines and evaluated for their in vitro cytotoxicity and in vivo anticancer studies. The results revealed that, the 2-[2-bis-(2-chloroethyl)aminomethyl]-3-aryl-4(3H)-quinazolinone (Compound 20) was found to be a promising anticancer agent.

Compound 19

Taha, 2005, reported the reactions of Schiff’s base with sulphur nucleophiles namely, o-amino thiophenol and / or thioglycolic acid to afford Michael type adducts. The bioassay studies indicated that, some of the target compounds (Compound 21) were exhibiting selective anticancer activity.

Compound 20

Compound 21
Avinash et al., 2010, synthesized 2-[5-substituted-1-H-benzo(d)imidazol-2-yl sulfinyl]methyl-3-substituted quinazolin-4-(3H)-one compounds and tested their antiulcer activity on animals challenged with pylorus ligation, aspirin and ethanol. All the synthesized compounds were characterized by using IR, MS, 1H NMR spectral and elemental analysis. Compounds 22 and 23 showed higher activity than omeprazole.

![Chemical structure of compounds 22 and 23]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substituent 1</th>
<th>Substituent 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>3,4-dimethoxy phenyl</td>
<td>OCHF₂</td>
</tr>
<tr>
<td>23</td>
<td>2-pyrazine</td>
<td>OCHF₂</td>
</tr>
</tbody>
</table>

Synthesis of 2-phenyl-3-(substituted phenyl)-3H-quinazolin-4-ones was carried out by Sati et al., 2010, using crude 2-phenylbenzo[1,3-d]oxazin-4-one and various substituted anilines in dry toluene. All the compounds were screened for their 5-HT₂ antagonistic activity. It is noteworthy to mention that, compound 3-(2-chlorophenyl)-2-phenyl-3H-quinazolin-4-one (Compound 24) was one of the most potent derivative in this series. Results also showed that, the presence of electron withdrawing groups (Cl and F) at ortho and para positions led to increase in their potency. However, the presence of electron donating group showed the opposite effect.

![Chemical structure of compound 24]
2.3. Fused pyridopyrimidines

Among the bicyclic heterocycles, pyrido[2,3-\textit{d}]pyrimidine scaffold is associated with a wide range of biological activities and hence, have been the subject of detailed investigations.

Werbel and Degnan, 1987, synthesized a variety of analogues of 2,4-diamino-6-[arylthio]quinazolines (\textbf{Compound 25}), wherein, the 4-amino group was replaced by hydrazino and hydroxylamino moieties. Such changes were found to markedly reduce their antimalarial and antitumor properties.

![Compound 25](image1)

\textbf{Compound 25}

Pyrido[2,3-\textit{d}]pyrimidines are well known for their potent and selective inhibition of DHFR. To improve their cell penetration, an attempt was made by Gangjee \textit{et al.}, in 1998. The results showed that, the replacement of the N\textsuperscript{8}-nitrogen of pyridopyrimidine by carbon (\textbf{Compound 26}) led to an unanticipated loss of the selectivity. Alternatively, those compounds showed remarkable antitumor activity.

![Compound 26](image2)

\textbf{Compound 26}

Lee \textit{et al.}, 2001 synthesized certain 6-substituted pyridopyrimidine analogues as potential adenosine kinase (AK) inhibitors. This led to the discovery of 4-amino-5-
(3-bromophenyl)-7-(6-morpholinopyridin-3-yl)pyrido[2,3-d]pyrimidine, a novel and potent non-nucleoside AK inhibitor with oral activity in animal models against pain and inflammation (Compound 27).

![Compound 27](image)

Molina et al., 2001, synthesized a number of pyrido[1,2-c]pyrimidines (Compound 28) on solid phase using the iminophosphorane methodology and tested their antiinflammatory activity by carrageenan-induced paw oedema in rats at a dose of 20 mg/kg body weight. The results showed that, the compounds inhibited prostaglandin-E2 levels in inflamed paw, without affecting the content of eicosanoids in stomach.

![Compound 28](image)

Stuart et al., 2001, described the design and synthesis of indazolyl amino pyridopyrimidines and quinazolines as inhibitors of the class I TK receptor. The results showed that, \(N^4-(1\text{-benzyl-}1H\text{-indazol-5-yl})-N^6,N^6\text{-dimethylpyrido[3,4-d]pyrimidine-4,6-diamine (Compound 29)}\) selectively inhibited EGFR and c-ErbB-2 (Onco protein) enzymes.
In 2001, Guo et al, reported a novel series of pyrido[2,3-d]pyrimidine analogues (Compound 30) as potent AK inhibitors based on their SAR and computational studies. It was reported that, the substitution at the C7 position of the pyridopyrimidino core with C2′ substituted pyridino moiety, increased the \textit{in vivo} potency and enhanced the oral bioavailability of these AK inhibitors.

Earlier, Rajeev and Bhabatosh, reported that, specific pyridopyrimidines (novel CDK inhibitors) caused the cell cycle arrest in mink lung epithelial cells and the arrest was abrogated by over-expression of cyclin dependent kinase (CDK4). In 2001, they also showed that, one of these inhibitors effectively maintained the cell cycle arrest in a leukemic or a breast cancer cell line even after the respective cells over-expressed an oncogene, either B cell lymphoma 2 or cyclin D1. Thus, the results proved that the novel CDK inhibitors could be useful chemical genetic tools
for understanding the underlying mechanisms of growth arrest and/or apoptosis in normal versus tumor cells.

Daqing et al., 2007, furnished a short and facile synthesis of pyrido[2,3-d]pyrimidine derivatives (Compounds 31a,b) in good yield via the three component reaction of aldehydes, alkyl nitriles and amino pyrimidines in water in the presence of triethyl benzyl ammonium chloride (TEBAC).

Compounds 31a,b

In 2007, Shujiang et al., reported the synthesis of a series of fused pyrido[2,3-d]pyrimidin-4,7-diones. A new reaction of 4-arylidene-3-methylisoxazol-5(4H)-one or 4-arylidene-2-phenyloxazol-5(4H)-one with 2,6-diaminopyrimidin-4(3H)-one was described and a number of new pyrido[2,3-d]pyrimidin-4,7-dione derivatives (Compounds 32a,b) were synthesized. The protocol showed good yield, broad substrate scope and simple work-up.
Zhicai et al., 2008, reported a new synthetic route of pyridopyrimidines to facilitate their structural optimization in a library fashion and described the development of pyridopyrimidines (Compounds 33a,b) that had excellent enzymatic and cell potency against Akt1 and Akt2 (Protein Kinase B). This series also showed a high level of selectivity over other closely related kinases and significantly improved caspase-3 activity with the more optimized structures.

![Compound 33a and Compound 33b](image)

**Compound 33a**  **Compound 33b**

Eric et al., 2009, synthesized a series of pyridopyrimidine derivatives (Compounds 34a, b and c) and screened for their ability to inhibit cyclic nucleotide synthesis in the presence of a stable toxin of Escherichia coli. The structure activity relationships around the basic core structure were examined and they showed potentially better pharmacological properties.

![Compound 34a, Compound 34b, and Compound 34c](image)

**Compound 34a**  **Compound 34b**  **Compound 34c**

In 2009, Shanmugasundaram et al., synthesized pyrido[2,3-d]pyrimidine carboxylates through nucleophilic substitution reactions and their structures were confirmed by elemental analysis, IR, $^1$H NMR and MS analysis. Further, these compounds were evaluated for their anticancer activity by MTT assay and the
results showed that, these compounds were significantly cytotoxic. The GI$_{50}$ of the compound 35 was found at 21 and 19 µg/mL on HT29 and HepG2 cell lines respectively. The GI$_{50}$ was found at 24 µg/mL on HeLa cell lines.

**Compound 35**

Ayoob *et al.*, 2009, reported a simple, clean and three-component one-pot cyclo-condensation reaction of barbituric acids, aromatic aldehydes and 6-aminouracils or 1H-pyrazol-5-amines for the synthesis of pyrido[2,3-$d$:6,5-$d$]dipyrimidines (Compound 36) and pyrazolo[4′,3′:5,6]pyrido[2,3-$d$]pyrimidines (Compound 37). Most of these compounds showed good spectrum of antimicrobial activity.

In 2010, Said and Adbulla, reported the synthesis and antiinflammatory activity of phenyl-1,8-napthyridines, phenylpyrido[2,3-$d$]pyrimidines (Compound 38) and their derivatives. The pharmacological screening showed that, many of these compounds had good antiinflammatory activities comparable to Valdicoxib.
In 2011, Shanmugasundaram et al., reported the synthesis of ethyl-5-amino-8(4-halophenyl)-2-methyl-4,7-dioxo-3,4,5,6,7,8-hexahydro-pyrido(2,3-d)pyrimidine-6-carboxylate (Compound 39) and ethyl-5-amino-8(4-halophenyl)-2-amino-4,7-dioxo-3,4,5,6,7,8-hexahydro-pyrido(2,3-d)pyrimidine-6-carboxylate (Compound 40) through nucleophilic substitution reaction with the use of amidines, followed by 4-haloanilines and malonic acid. The proposed structures were confirmed by elemental analysis, IR, $^1$H NMR and MS spectra. In this study, it was concluded, that the halogen substituted compounds, especially fluoro-substituted compound showed good antimicrobial and antitumor activities compared with that of the other counterparts.

2.4. Angularly fused pyrimidines

Fusion of three or more heterocyclic nuclei has not been studied as widely as other heterocyclic systems. Recently, such attempts were tried to demonstrate the fusion
of furopyrimidine and furo[2,3-b]pyridine with pyrimidine; this may give rise to new molecules with different and possibly better pharmacological profile.

Mahadevan et al., 2003, synthesized some 2-acyl-3-aminonaphtho[2,1-b]furans (Compound 41), from 2-hydroxy-1-naphthonitrile and then converted into 2-chloromethyl-4-alkyl/ aryl naphtho[2,1-b]furo[3,2-d]pyrimidine-3-oxides (Compound 42) through corresponding oxime formation. 2-Acyl-3-aminonaphtho[2,1-b]furans (Compound 41) on reaction with acyl chloride and hydrazine hydrate gave 2-alkyl/aryl-3,4-dihydro-3–amino-4-hydroxy-4-alkyl/aryl-naphtho[2,1-b]furo[3,2-d]pyrimidines (Compound 43), which on further treatment with formic acid, produced 2-acyl-3-(3’-alkyl/aryl-1’,2’,4’-triazol-4’-yl)naphtho[2,1-b]furans (Compound 44).

Kumaraswamy et al., 2006, synthesized and evaluated some angularly fused naphtho[2,1-b]furo[3,2-d]pyrimidines (Compound 45). They reported that the compounds containing electron withdrawing group exhibited good activity as compared with the compounds having electron releasing group (-CH₃). Further, the introduction of chloro or hydroxyl group in benzene ring led to increase in their diuretic and antiinflammatory activities, respectively.
In 2007, Hayakawa et al., reported the synthesis and biological evaluation of pyrido[3′,2′:4,5]furo[3,2-d]pyrimidines as novel PI3K (Phosphoinositide kinase) p110α inhibitors. 4-Morpholin-4-yl-pyrido[3′,2′:4,5]thieno[3,2-d]pyrimidine (Compound 46) was discovered as a novel p110α inhibitor with an IC$_{50}$ of 1.4 µM. The structural modification of Compound 46, the 2-aryl-4-morpholinopyrido[3′,2′:4,5]furo[3,2-d]pyrimidine (Compound 47) resulted as a p110α (Sub unit of PI3K) inhibitor with approximately 400-fold greater potency than Compound 46 and also showed anti-proliferative activity in various cell lines, including multi-drug resistant MCF7/ADR-res cells (adriamycin resistant cells), and was also effective against HeLa human cervical tumor xenograft in nude mice.

Yang-Gen et al., 2010, reported that, carbodiimide, obtained by aza-Wittig reaction of iminophosphorane with 4-fluorophenyl isocyanate, reacted with various nucleophiles under mild conditions to give a series of 2-substituted-3-(4-fluorophenyl)-benzofuro[3,2-d]pyrimidin-4(3H)-ones (Compound 48) in good
yield. Their structures were confirmed using NMR, EI-MS, IR, and elemental analysis. The preliminary bioassays indicated that, these compounds showed moderate fungicidal activities against six different kinds of fungi at 50 mg/L.

Joan et al., 2011, reported a series of pyrido[3′,2′:4,5]furo[3,2-d]pyrimidines (PFP; Compound 49) and tested for PDE type 4 inhibitory activity, with the potential to treat asthma and chronic obstructive pulmonary disease. Structural modifications in this series showed the enzyme inhibitory profile of the compounds on PDE-4. Both gem-dimethylcyclohexyl moiety fused to the pyridine ring and the substitution at the 5th position of the PFP scaffold, proved to be the key elements to get a high affinity towards the enzyme.

2.5. Microwave irradiation in the synthesis of fused pyrimidine heterocycles

At present, the usage of microwave irradiation (MWI) is well known tool for the synthesis of a variety of compound; wherein, chemical reactions are accelerated because of the selective absorption of microwave. From the literature search, it was
found that, the synthesis of our target heterocyclic scaffolds were also attempted based on green chemistry approach.

Robb *et al.*, 2003, described a microwave-assisted method for monoacylation of 7-amino-5-aryl-6-cyanopyrido[2,3-\textit{d}]pyrimidines (**Compound 50**) using excess acid chlorides in pyridine. The diacylated intermediate was effectively deacylated to the product amide (**Compound 51**) by a macroporous-Tris resin. A small library of 17 amides was prepared to validate the method and was not easily accessible by conventional synthetic techniques.

A series of novel 5-substituted-8-cyano-4,6,7-triphenyl-3,4-dihydrobenzo[2,3-\textit{d}]pyrimidines (**Compound 52**) were synthesized by Mazaahir and Akkaldeomishra, 2004, through the condensation of 6-substituted-2-amino-1-benzoyl-3-cyano-5-hydroxy-4,5-diphenyl-1,3-cyclohexadiene and formamide, using inorganic solid supports under microwave irradiation for 4-5 min (73-87 % yield). *In vitro* antifungal and antibacterial activities were reported for the synthesized compounds.

**Compound 52**

Gang *et al.*, 2006, developed a simple, efficient and general method for the synthesis of various \textit{N}-aryl heterocyclic substituted-4-aminoquinazolines
(Compound 53) from 4-chloroquinazoline and aryl heterocyclic amines under microwave irradiation using 2-propanol as solvent. The reaction time for the synthesis of compounds was reduced from 12 hour (h) to 20 minutes (min) with the one-step microwave-assisted procedure. The advantages of this method are: faster reaction rates and high yield (79.1 - 96.5 %), while the classical method of formation of N-aryl heterocyclic substituted-4-aminoquinazoline derivatives involved longer reaction time (12 h).

![Compound 53](image)

In 2010, Mishra, synthesized different 3,4-dihydrobenzo[2,3-d]pyrimidine derivatives (Compound 54) by the condensation of substituted 1,3-cyclohexadienes and formamide, using inorganic solid supports under microwaves. Simple and common chemicals were used as starting materials in the reactions. These compounds were tested against different fungal and bacterial strains and were found to be considerably effective. Only 4-5 min was required for completion of the reaction with 87 % yield. However, the time taken was 6-7 h by conventional method to yield 65 % product. These observations demonstrated that, the above method was an expeditious, facile and environmentally benign one for the organic synthesis.

![Compound 54](image)
A series of pyrido[2,3-d]pyrimidine derivatives (Compound 55) have been prepared by Shahrzad and Saeed et al., 2012, through one-pot three-component reaction of 4-aminouracil, malononitrile and aromatic aldehydes. This synthesis was done by two methods; one under microwave irradiation (method A) and another with catalytic amount of diammonium hydrogen phosphate [(NH$_4$)$_2$HPO$_4$, DAHP] in aqueous media (method B). The operational simplicity, simple purification procedure, high yield (82 – 95 %), environmentally friendly character, and high speed synthesis (Method A, 5 - 10 min) were the advantages of this method.

Method A: MWI-Dimethyl formamide (DMF); 250 W, 120 °C
Method B: 10 Mol % Diammonium hydrogen phosphate; H$_2$O:EtOH; 2:1 ratio; reflux)

Recently, in 2012, Yvonnick et al., reported the synthesis of pyrido[2',3':4,5]furo[3,2-d]pyrimidines (Compound 56) substituted by a primary or secondary amino group on position 4 of the pyrimidine ring. Here, it was clearly demonstrated that, the application of microwave irradiation technology allowed fast and convenient procedures with good yield (77 - 97 %).
2.6. Fused pyrimidines as DHFR and TS inhibitors

TS and DHFR inhibitors alone or in combination with other agents may provide a successful antitumor therapy. Anthony et al., 2002, determined the crystal structures of two human dihydrofolate reductase (hDHFR) ternary complexes, each with bound NADPH (Nicotinamide Adenine Dinucleotide Phosphate) cofactor and a lipophilic antifolate inhibitor at atomic resolution. The potent inhibitors, 6-([5-quinolylamino]methyl)-2,4-diamino-5-methylpyrido[2,3-d]pyrimidine (Compound 57) and (Z)-6-([2,5-dimethoxyphenyl]ethen-1-yl)-2,4-diamino-5-methyl pyrido[2,3-d]pyrimidine (Compound 58) were developed at Southern Research Institute against *Toxoplasma gondii* DHFR-TS. The 5-deazapteridine ring of each inhibitor adopted an unusual puckered conformation that enabled the formation of identical contacts on the active site. Conversely, the quinoline and dimethoxybenzene moieties at the 6th position exhibited distinct binding characteristics that accounted for the differences in inhibitory activity.

![Chemical structures of Compounds 57 and 58](image)

In 2004, Rosowsky and Forsch, from the Dana Farber Cancer Institute Inc., reported several lipophilic DHFR inhibitors having an aromatic and a heteroaromatic group linked by a methylene group in the treatment of *P. carinii* infections. Trimethoprim (Compound 60) and piritrexim (Compound 59) are lipid soluble antifolates that have been used clinically for the prophylaxis and treatment of *P. carinii* infections in patients with AIDS.
In 2004, Graffner Nordberg *et al.*, reported a series of DHFR inhibitors where, the methylene amino bridge of non-classical inhibitors was replaced with an ester function and prepared as potential soft drugs intended for inhalation against Pneumocystis carinii pneumonia (PCP). The most potent new ester based DHFR inhibitor, the 1-naphthyl derivative (**Compound 61**), exhibited an IC₅₀ value of 110 nM/L, and was found to be less active than trimethotrexate (IC₅₀ at 42 nM/L).

In order to produce potent new leads for anticancer drugs, Al-Rashood *et al.*, 2006, designed a new series of quinazoline analogs (**Compound 62**) to resemble methotrexate structure features and fitted with functional groups and believed to enhance inhibition of mammalian DHFR activity. The binding effects of these compounds within the active site of hDHFR were assessed by molecular modeling studies. The most active DHFR inhibitors showed IC₅₀ values in the range of 0.5 - 0.4 µM.
Thymidylate synthase (TS) is considered as an important target for the development of new drug like candidates. Meena Kumari et al., 2008, designed a series of novel antifolate inhibitors having naphthalene core, substituted quinazoline, indole, pyrrolopyrimidine, pyridopyrimidine (Compound 63), and pteridine ring using computational technique. The designed molecules showed binding affinity towards the protein compared to the other TS inhibitors.

![Compound 63](image)

Kim et al., 2009, demonstrated that, lapatinib (Compound 64) down-regulates TS through the inhibition of the nuclear translocation of Epidermal Growth Factor Receptor (EGFR) and Human Epidermal Growth Factor Receptor 2 (HER2). From the cDNA microarray experiments, it was determined that, a variety of nucleotide synthesis related genes, including TS, were down-regulated with ‘lapatinib’, and this was apparent in HER2-amplified cells. Targeted and pharmacological inhibition assays confirmed that, the dual inhibition of EGFR and HER2 was required for more effective reduction of TS as compared to what was observed with gefitinib or trasutuzumab alone.

![Compound 64](image)
DHFR plays an important role in the de novo purine synthesis. Nerkar et al., 2009, reported the in silico screening to obtain the best fit molecules as DHFR inhibitors. Quinazolinone Schiff’s bases (Compound 65) and pyridine-4-carbohydrazide Schiff’s bases (Compound 66) were synthesized and evaluated for their in vitro anticancer activity. Synthesis of these molecules was performed by using MWI and they were subjected to in vitro anticancer evaluation against five human cancer cell lines. (4-(N,N-dimethyl-amino)-phenyl) Schiff’s base of pyridine carbohydrazide (Compound 66) showed equipotent activity with the standards used in in vitro anticancer assay as per the NCI guidelines.

In 2011, Xin et al., synthesized some classical antifolates with a tricyclic benzo[4,5]thieno[2,3-d]pyrimidine scaffold and a flexible and rigid benzoylglutamate as dual TS and DHFR inhibitors. Compounds with 2-CH₃ substituents inhibited hTS (IC₅₀ = 0.26–0.8 μM), but not hDHFR. Substitution of the 2-CH₃ (Compounds 67a, b) with a 2-NH₂ (Compounds 68a, b) increased hTS inhibition by more than 10 fold and also afforded excellent hDHFR inhibition (IC₅₀ = 0.09–0.1 μM).
In 2010, Aleem et al., designed, synthesized and evaluated a novel classical antifolate $N$-{4-[(2,4-diamino-5-methyl-furo[2,3-$d$]pyrimidin-6-yl)thio]-benzoyl}-l-glutamic acid and 11 non-classical antifolates as inhibitors of DHFR and TS. The classical analogue **Compound 69** was a nanomolar inhibitor and remarkably selective inhibitor of *P. carinii* DHFR and *M. avium* DHFR at 263 fold and 2107 fold, respectively compared to mammalian DHFR. This study showed that, the furo[2,3-$d$]pyrimidine scaffold was conducive to dual human DHFR-TS inhibitory activity with high potency and selectivity for pathogen DHFR.