SYNTHESIS AND ANTICANCER PERSPECTIVE OF PYRIDOPYRIMIDINE SCAFFOLD - A REVIEW

Abstract

The core pyridopyrimidines is gaining interest in organic and heterocyclic chemistry in the recent days, as this scaffold acts as building block because of its wide range of biological and pharmacological applications like anticancer, antimicrobial, fungicidal, antiviral, CNS, antibacterial and anti-inflammatory properties. This review mainly emphases on the evolution in anticancer properties of pyridopyrimidines since 2008 especially the method of synthesis and anticancer activity of synthesized compounds with reporting of active anticancer scaffolds. Important starting materials which are widely used for the synthesis are 2-thioxopyrimidine, ethyl 2-cyanoacetate, 2-amino-3-cyano-4-trifluoromethyl-6-phenyl-pyridine, 2-amino-4,6-disubstituted nicotinonitrile, 2-chloro-3-pyridine carboxylic acid, in which 2-thioxopyrimidine is found to be mostly employed in the synthesis. Pyridopyrimidines which are synthesized from different starting materials, in which the more active compounds are, reported here which may help in further discovery/ development of novel molecules.

Keywords

Pyridopyrimidines, Anticancer, Synthesis

Introduction:

Pyridopyrimidine is a significant core with wide variety of biological activity. The heterocyclic ortho fusion of pyridine and pyrimidines led the formation of pyridopyrimidines [1]. Cancer is associated with rigorous growth of cells by uncontrolled cell division and alteration of cells by changes in their DNA [2]. Treatment for cancer remains as a major challenge to medical science in spite of intense research worldwide [3]. In the area of drug discovery, pyridopyrimidine moiety has been identified as

promising pharmacophore especially in the development of anticancer drugs [4]. Diverse mechanisms are reported for the anticancer potency of pyridopyrimidines. Inhibition of tyrosine kinases like EGFR, PDGFR[5,6] non- receptor tyrosine kinases such us PI3K, mTOR, Akt, Ras/Raf, Janus-activated kinase (JAK), signal transducers and activators of transcription (STAT) [5,7] cyclin dependent kinases**,** [6,8] DNA fragmentation and CASP3 activation leads to apoptosis [9]. This review is an attempt to document the role of pyridopyrimidines as potential anticancer drugs.

Synthetic pathways for pyridopyrimidines with their anticancer potency

Various methods of synthesis by taking different precursor molecules, for substituted pyridopyrimidines with anticancer properties which are reported in the literature are summarized.

1. 2-thioxopyrimidine (3) as precursor:

Safinaz E-S Abbas et al., [1] reported pyrido[2,3-d] pyrimidine is an important moiety with different biological properties and is present in different bioactive agents [10]. They have synthesized different derivatives of bicyclic and tricyclic pyridopyrimidines from 3. Starting precursor 3 was treated with hydrazine hydrate and phenyl hydrazine in presence of n-butanol or ethanol to get resultant compounds respectively i.e. 4 and 5. Next series of compounds i.e. 6a-e arylidene hydrazonyl derivatives were obtained with good yield by reacting compound 4 with suitable aldehyde in glacial acetic acid. **(Fig. 1)**

A new series of compounds 7a,b were obtained by S-alkylation followed by Smiles rearrangement between the dipolar nucleophile, 2-thioxopyridopyrimidinone 3 and suitable hydrazonoyl chloride by 1,3-dipolar cycloaddition in presence of dioxane and pinch of triethylamine. **(Fig. 2).**

Thiazolo[3,2-a] pyrimidine 8a–g derivatives with arylidene and arylethylidene substitution were synthesized by the reaction of 2-thioxopyridopyrimidine 3 with sodium acetate, chloroacetic acid, appropriate aldehyde or aromatic ketone in presence of mixture of an a/acetic anhydride and acetic acid. Additionally, different tricyclic pyridotriazolopyrimidines 9,10,11,12,13 are obtained with different substitution on the triazolo ring obtained by subjecting hydrazinyl derivatives into different reaction conditions **(Fig. 3).**

Tricyclic pyridotriazolopyrimidine 9, “3-thioxopyrido[2,3-d][1,2,4]triazolo[4,3- a]pyrimidine” 10, 3-methyl triazole derivatives 11, 3-phenyl triazole derivatives 12 and 3-amino triazole derivative 13 were

synthesized using N,N-dimethyl formamide, CS_2 in ethanolic KOH solution, acetyl chloride and benzoyl chloride in dry pyridine, with ammonium isothiocyanate in glacial acetic acid respectively.

From their study it was found that compounds 6b, 6e, 7b and 8d exhibited potential activity against the exposed cell lines i.e. A549, MCF-7 and PC-3 at sub micromolar level. Apoptosis was observed by 6b and 8d respectively PC-3 and MCF-7 mainly by caspase-3 activity in PC-3 cell line. Bcl2 down regulation was also observed along with CDK 4/6 inhibition. IC_{50} = 115.38 nM and IC_{50} =726.25 nM for 6b and 8d respectively for the direct inhibition of CDK6.

Nagy M. Khalifa et al., [2] described "pyrido[2,3-*d*] pyrimidin-4-ones" as inhibitors of various kinases, like., Tyrosine kinases, Phosphoinositide-3-kinase and Cyclin dependent kinases 4/6 because of the inhibition of cell growth in different cell lines [11,12,13]. In this study they reported synthesis of 5-Phenyl-7-(pyridin-3-yl)-2-thioxo substituted 2,3-dihydropyrido[2,3-d] pyrimidin-4(1H)-one 3 by refluxing sufficient quantities of "6-amino-2,3-dihydro-2-thioxopyrimidin-4(1H)-one" 1 and "α, β-unsaturated ketone" 2 for about 10h in dry DMF. Further these thioxo derivatives 3 were heated with 99% of hydrazine hydrate for about 12 hours in dry ethanolic media to get "2-Hydrazinyl-5-phenyl-7-(pyridin-3-yl) pyrido[2,3-d] pyrimidin-4(3H)-one" 4. Hydrazinyl derivatives 4 were further treated with different aromatic aldehydes like benzaldehyde, 4-nitrobenzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-methoxybenzaldehyde, 4-tolylaldehyde or 4-N,N-dimethylaminobenzaldehyde in glacial acetic acid and refluxed for 5-8 hours to obtain series of 2-arylidene derivatives of pyrido pyrimidines 5a–g. These hydrazinyl derivatives further treated with different reagents like ammonium isothiocyanate, ethyl cyanoacetate, ethyl acetoacetate or diethyl malonate after heating for 3-6 hours yields 5-substituted pyrazolones and triazolopyrimidine derivatives of pyrido pyrimidines 6-9 [2] **(Fig. 4)**.

In-vitro cytotoxicity evaluation was done against, HCT-116 and PC-3 cell Lines. All tested compounds exhibited greater than 90% inhibitory effect in comparison to standard doxorubicin (IC_{50} ~ 0.6 μM). Compounds 3–9 at 100 μM concentration shows astonishing anticancer activities in the *in-vitro* screening and compound 6 exhibited splendid activity against human liver carcinoma cell line (HepG2)

with IC_{50} approximately 0.5μM. Kinase inhibition screening by radiometric or ADP-Glo assay was performed based on the cytotoxicity assay, promising derivative 6 with concentration of 100μM was selected for the determination of *in-vitro* inhibition by the variety of proteins like AKT1, AKT2, CDK2 with cyclin A1, BRAF (V600E), EGFR, PDGFRβ, CHK1 and c-RAF kinases. Three kinases (EGFR, PDGFRβ and BRAF V600E) were highly inhibited about 90%, in comparison to all the tested kinases. EGFR was greatly and selectively inhibited (97%) by compound 6 compared to others. [2]

Mohamed Fare et al, [6] have documented pyrido[2,3-d] pyrimidines as antineoplastic agents which can be assigned to the CDK inhibition,[14] rapamycin mammalian target[15] or kinase check point [16]. Further, some of the pyridopyrimidine derivatives were also reported to induce cell apoptosis in various leukemia cell lines and solid tumors [17, 18, 19].

6-amino-2-thiouracil 2 was made to react with 3-phenyl-1-(thiophen-2-yl)prop-2-en-1-one 1 in DMF in order to get "5-phenyl-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-d]pyrimidin-4(1H)-one" 3 according to reported procedure[20]. Further 3 reacts with hydrazine hydrate in ethanolic media resulted in the formation of 2-hydrazinyl substituted pyrido[2,3-d] pyrimidin-4(3H)-one 4.

Later 4 was treated with selected aldehydes afforded 2-(2-arylidenehydrazinyl)-5-phenyl-7-(thiophen-2-yl) substituted pyrido[2,3-d] pyrimidin-4(3H)-ones 5a-e **(Fig. 5).**

In the next step key intermediate 2-Hydrazinyl substituted pyridopyrimidine derivatives 4 was used to get 3-substituted pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidines 6-10.

"6-phenyl-8-(thiophen-2-yl)pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidin-5(1H)-one" 6 was prepared from the reaction of 2-hydrazinyl derivative 4 reacts with triethyl orthoformate.

"2-oxo(thioxo)pyrido[2,3-d] [5,2,4] triazolo [4,3-a] pyrimidines" 7 and 8 were obtained by the cyclocondensation of 2-hydrazinyl derivatives 4 with dry pyridine or CS_2 in ethanolic KOH solution respectively. In the similar way, refluxing 4 with either with NH_4SCN in CH_3COOH media or acetyl chloride in dry pyridine yields 3-amino derivative 9 and 3-methyl derivative 10 **(Fig. 6).**

Few derivatives were also prepared by reacting hydrazonoyl chlorides 11a-j and 2-thioxopyridopyrimidine 3 with triethylamine in dioxane media, the reaction is further progressed by S-

alkylation resulted in the formation of S-alkylated products 12 which further undergoes smiles rearrangement yields an intermediate 13, which upon in-situ consumption by the liberation of H_2S to produce one of the products which are fused triazole derivatives 15A or 15B. Based on the spectral evaluation the isolated components 15a-j it was confirmed that form A were present rather than B **(Fig. 7).**

This finding was consistent with different recorded cyclocondensation reactions of related and condensed 2- thioxopyridopyrimidine derivatives with hydrazonoyl chloride [21, 22].

Evaluation of *in-vitro* anticancer potential of synthesized compounds were determined by taking 2 cancer cell lines like prostate cancer cell line (PC-3) and human lung adenocarcinoma cell line (A-549), where 5-FU was taken as reference standard. Some of tested compounds exhibited substantial inhibitory activity, out of which compounds 5b, 5d and 15f were found to be more potent and effective with IC_{50} of 1.54, 0.63 and 0.36µM, respectively, than the standard drug(5-FU) where IC_{50} is 12.00µM against PC-3 cell line. Additionally, compounds 6, 7 and 9 were equally potential to the 5-FU when tested on the same cell line.

Compound 15f exhibits 10 times greater potency (IC_{50} =0.41µM) than standard drug (IC_{50} =4.21µM) on A-549 cell line, while compound 5b with IC_{50} of 3.36µM showed somewhat greater cytotoxicity compared to that of standard 5-FU.

Hala B et al., [16] stated Pyrido[2,3-d] pyrimidines and fused [1,2,4] triazolo[4,3-a] pyrimidine derivatives, which were reported for anti-tumour activity [14,15,16,23,24,25]. These two groups upon combination results in the formation of tricyclic ring system containing pyridine, triazole and pyrimidines (i.e pyrido [2,3-d [1,2,4] triazolo[4,3-a] pyrimidine) and the resulting impact on the biological activities are delighted [26,27,28,29,30, 31,32].

Starting material "5-(4-chlorophenyl)-2,3-dihydro-7-phenyl-2-thioxopyrido[2,3-d]pyrimidin-4(1H)-one" 3 was synthesized by treating 6-amino-2-thiouracil 2 with "3-(4-chlorophenyl)-1-phenyl-2-propen-1-one" 1, Further compound 3 was made to react with hydrazine hydrate in ethanolic media yields "2-hydrazinopyrido[2,3-d]pyrimidin-4(3H)-one" 4. When the similar procedure was repeated in

Dimethylformamide instead of ethanol evolved in the formation of "5-oxo-1H-pyrido[2,3-d][1,2,4]triazolo[4,3-a]pyrimidine" 5. Reaction of compound 4 with triethyl orthoformate or refluxing with DMF results in the formation of same compound 5 **(Fig. 8).**

On the other hand, 3-methyl and 3-ethyl derivative 6a and 6b were obtained correspondingly, by the reaction of the acetic anhydride and propionic anhydride respectively with compound 4. In dry pyridine, compound 4 was made to react with ethyl chloroformate to get 3-oxo derivative of tricyclic pyridopyrimidines 7. Compound 4 was reacted with carbon disulphide in ethanolic KOH solution. As reaction proceeds, which resulted in the formation of 3-thioxo derivative of pyridopyrimidine 8 containing tricyclic ring systems, which upon treatment with dimethyl sulfate or alkyl halide yields 3-thioxo pyridopyrimidines with alkyl substitution on thio group 9 **(Fig. 9).**

Compound 4 was treated with different substituted benzaldehyde to get substituted hydrazones 10a,b. The 3-aryl derivatives 11a-d may be derived by two routes, 1st one was by treating compound 4 in dry pyridine with benzoyl chloride or 4-methyl benzoyl chloride and 2nd one was by the oxidative cyclization of respective hydrazones 10 a, b. 2-hydrazino derivative i.e., compound 4 in presence of acetic acid was made to react with ammonium isothiocyanate to get 3-amino substituted compound 12. Finally, compound 4 in dry pyridine media reacted with chloroacetyl chloride resulted in the formation of 3-chloromethyl derivative 13 **(Fig 10).**

The *in-vitro* cytotoxic evaluation of newly designed compounds was preformed against MCF7 (human breast cancer cell line) where, Doxorubicin was used as reference standard for this study. From the results it was revealed that, 3- substituted compounds with various substitutions like thioxo group 8, alkylthiogroup 9a,b,d or amino group 12 exhibited finest cytotoxic effects. In reference to alkylthio derivatives, methyl sulphanyl derivative 9a has shown finer result compared to ethyl sulphanyl derivative 9b or benzyl sulphanyl derivative 9c. Out of synthesized compounds, Compound 12 was emerged as most promising derivative in the *in-vitro* evaluation with IC_{50} of 3.74mg/mL. Moderate activity was observed with chloromethyl, ethyl or aryl substituted derivatives at 3rd position with IC_{50} between 9.07 and 14mg/mL.

Additionally, 3-substituted derivatives with H, CH_3 or CO groups exhibited least anticancer effects with IC_{50}>20mg/mL. It was also noted that the starting compound 3 has been shown to have good cytotoxic effects on MCF7 with IC_{50} of 5.11mg/mL.

2. Ethyl 2-cyanoacetate as precursor:

Abhay et al., [33] reported molecule with pyridopyrimidine carboxylate moiety were found to exhibit antitumor activity [34] etc. In the current study they synthesized some carboxylate derivatives of novel pyridopyrimidines by nucleophilic substitution reactions by using amidines, with 4-haloanilines and malonic acid. They prepared ethyl 3, 3 bis (methylthio)-2- cyanoacrylate in the first step using sufficient quantity of ethylcyanoacetate, DMF and carbon disulfide, by adding these reactants into ice cold KOH solution followed by stirring and cooling. Further dimethyl sulphate was added where temperature is maintained at 20°C. After 12 hrs reaction mixture was added to water **(Fig.11).**

In the second step, "2–substituted- 4 - (methylthio) – 6 - oxo-1,6-dihydro pyrimidine -5-carbonitrile" was prepared using "ethyl 2- cyano-3,3-bis(methylthio) acrylates" obtained in the first step was treated with aromatic amidines with ethanol and resultant solid was used for next step **(Fig.12).**

"2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile" is further treated with aromatic halo anilines like p-chloroaniline, p-fluroaniline, p-bromoaniline with alcohol refluxed for 1 hour which yields "4-(4-halo phenyl amino)-2-substituted-6-oxo-1,6-dihydro pyrimidine-5-carbonitrile" **(Fig.13)**, which was further treated with malonic acid in ethanol, after refluxing for one hour yields "Ethyl-5-amino-8- (4-halo phenyl) -2-substituted-4, 7-dioxo- 3,4,5,6,7,8-hexa hydro pyrido (2,3-d) pyrimidine -6- carboxylate" which were the targeted compounds **(Fig.14).**

Anticancer activity of targeted pyridopyrimidines were further evaluated for anticancer properties using 3 different human cancer cell lines like, HepG2 (liver cancer), HT29 (colon cancer), Hela (cervical cancer) and cytotoxicity was determined by MTT assay. Potential anticancer activity was observed by all the synthesized compounds.

3. 2-amino-3-cyano-4-trifluoromethyl-6-phenyl pyridine as precursor:

C. Kurumurthy et al.,[35] described the pyrido[2,3-d] pyrimidine nucleus as pharmacologically potential scaffold, including antitumor properties[36]. They also reported that, organic molecule bearing fluorine[37] or trifluoromethyl[38] group at a strategic position leads to dramatic changes in the properties and activities of the molecule.

In their study "2-amino-3-cyano-4-trifluoromethyl-6-phenyl pyridine" 1 was used as starting precursor for the synthesis, which was made to react with different aliphatic acids in presence of catalytic amount of H_2SO_4 at 120-130^{0}C for 12-20 h, results in acylation of amine and hydrolysis of nitrile to amide, which upon further cyclization yields "pyrido[2,3-d] pyrimidine" derivatives 2. In the next scheme, Compound 2 (R= alkyl) was reacted in presence of base i.e. potassium carbonate with propargyl bromide in acetone resulted in 3:1 ratio of two regioisomers 3 and 4 i.e. N-propargylated and O-propargylated pyrido [2,3-d] pyrimidines. These regioisomers were isolated depending on their polarity difference **(Fig.15)**. Further each isomer was treated with alkyl or perfluoroalkyl azide in THF separately by using copper(I) iodide as catalyst[39] undergoes 1,3-dipolar cycloaddition[40] and resulted exclusively 1,4-disubstituted-1,2,3-triazole derivatives of pyrido [2,3-d] pyrimidines 5 and 6 respectively **(Fig.16,17).**

In-vitro evaluation of the synthesized compounds, 5a-i and 6a-c were performed against 3 different cancer cell lines U937 (human leukemic monocytic lymphoma), Colo205 (human colorectal cancer), THP-1 (human acute monocytic leukemia) and using MTT assay method [41]. Decrease in cell viability was observed with compound 5b and 5e in all the three cell lines. Few compounds were active only on 2 cell lines like, compounds 5i, 5j and 6a were active against only on U-937 and THP-1, compound 5k exhibits activity against U937 and Colo205 cell lines and compound 5a exhibits its potency against THP-1 and Colo205. Further, few compounds such as 5d, 6b and 6c shows inhibition only on U937 cell lines. Toxicity was determined against U937 cell lines was found in the order of 5e > 5k > 5b > 5j > 6b>Etoposide. From the SAR, it was found that, In the second position, presence of -H or -CH_3 group and attachment of alkyltriazole ring in the 3rd position improves the activity in cells of U937 in compounds like 5b, 5e when compared to standard Etoposide [6].

Out of all the synthesised derivatives, better activity was observed with compound 5e with 2- methyl substitution and 3-alkyl group. Substitution of perfluoroalkyl functionality on triazole is considered as essential for detrimental effect. There is no added advantage on activity was observed by increasing the length of aliphatic side chain in the 2nd position. Finally, promising compounds 5b and 5e are further optimized to find out a potent derivative for further investigation.

4. 2-amino- 4, 6-disubstituted nicotinonitriles as precursor:

Few of the fused ring systems like pyridopyrimidines and quinolines have been reported by Hassan M. Faidallah [42] because of strongly observed antineoplastic [43], antiproliferative [44] and cytotoxic[45] effects.

"2-amino- 4,6-disubstituted nicotinonitriles" 1–5 are the main intermediates were prepared from Hantzsch-type synthetic method, mainly by cyclocondensation of substituted acetophenone with the suitable aromatic aldehyde (piperonal or thiophene-2-carbaldehyde) and a source of nitrogen like ammonium acetate, which is a one pot multicomponent reaction.

The reaction proceeded by the Claisen-schmidt condensation with the formation of chalcones, further, cyclocondensation was done with malononitrile and ammonium acetate.

"5, 7-disubstituted pyrido[2,3-d]pyrimidine-4(3H)-ones" 6–10 and their 2-methyl analogues 11–15 were synthesized by heating the derivatives 1-5, with formic acid or acetic anhydride respectively.

In pyridine medium, substituted "dihydropyrido[2,3-d]pyrimidine-2(1H)-thiones" 16–20 were synthesized by treating the derivatives 1–5 with the phenyl isothiocyanate while thioureido derivatives 21–23 are obtained by condensing the starting compounds 2–4 with benzoyl isothiocyanate in acetone.

The starting compounds 1–4 undergoes the direct condensation with urea at 260–300^{0}C result in the formation of target compound i.e "4-amino-5,7-disubstituted-pyrido[2,3-d]pyrimidine-2(1H)-ones" 24–27.

In the similar way, the fusion of thiourea with compounds 1–5, at temperature 260–300^{0}C, resulted in "4-amino-5,7-disubstituted-pyrido[2,3- d]pyrimidine-2(1H)-thiones" 28–32 formation.

Finally, In the presence of ethyl or methyl iodide, thioalkylation of the 2-thione derivatives 30–32, was successfully performed in 1N NaOH medium, in-order to get targeted alkylthio derivatives 33–37 **(Fig. 18)**.

In-vitro MTT assay was employed to determine the cytotoxicity of newly synthesized compounds [46] against three different human tumor cell lines, namely, hepatocellular carcinoma HePG2, colon carcinoma HT29 and Caucasian breast adenocarcinoma MCF7. Results obtained from this assay showed that the 9 compounds namely, 21, 22, 23, 30, 32, 33, 34, 36, and 37 manifested variable degree of inhibition towards three different tested cell lines, remaining compounds were either slightly active or totally inactive. Human colon carcinoma HT29 was found to be very much sensitive towards all the 9 active compounds and it shows distinguish sensitivity against derivatives 33, 34, and 37 (LC_{50} 25.2, 28.8, and 26.9μM, resp.) markedly, which was even greater than standard doxorubicin (LC_{50} =40.0μM). Meanwhile, equipotent action to doxorubicin was showed by compounds 22 and 36 (LC_{50} =46.7 and 40.4μM, resp.) and compounds 21 and 23 (LC_{50} =70.5 and 62.2μM, resp.) exhibited moderate toxicity to cells of the same tested cell line.

In case of HepG2 cell line (hepatocellular carcinoma), out of synthesized compounds, seven of them showed mild to weak activity with LC_{50} range of 64.6–111.3μM, in comparison to standard doxorubicin (LC_{50} = 3.0μM). Including this, compounds 33, 34, and 37 (LC_{50} =64.4, 70.1, and 71.2μM, resp.) exhibited the highest activity.

In this study it was revealed that, the human breast cancer cell line (MCF 7) was appeared as slightly sensitive out of all three tested cell lines since it shows sensitivity towards only 6 tested compounds. However, markable inhibition was exhibited by derivatives 33, 34, and 37 (LC_{50} =6.4, 7.9, and 8.91μM, resp.), which is about 40 to 60% of potency of standard doxorubicin (LC_{50} =4.0μM).

From the additional investigation, it was found that compounds 33, 34, 36, and 37 displayed promising broad-spectrum activity towards all three tested cell lines, particularly these derivatives exhibited special effectiveness i.e., twice the action of standard doxorubicin towards HT29 and 40-60% action of doxorubicin towards MCF 7.

Deep Structural investigation of those active molecules revealed that substituents nature (X and/or R) together with ring moiety (mono/bicyclic), exerts impact on the cytotoxic potency.

In this regard derivatives which has 4-bromo- or 4-methoxyphenyl substitution with the "benzo[d][1,3]dioxol-5-yl moiety" 22, 30, 33, and 34 showed improved cytotoxicity when compared with their 2-thienyl substituted derivatives 23, 32, 36, and 37. Additionally, the bicyclic pyrido [2,3-d] pyrimidine resulted as highly active derivative than the monocyclic nicotinonitriles.

Nicotinonitriles 1–5, were cyclized by using different reagents results in the formation of bicyclic "pyrido[2,3-d]pyrimidines" 6–10, 11–15, 16–20, and 24–27 with different substitution, where most of them were inactive against all 3 tested cell lines, Further, ketone group present at 2^{nd} position is isosterically replaced with thione group in pyrido[2,3-d]pyrimidine-2(1H)-ones 24–27, resulted in two weakly active molecules, namely, 30 (X = Br; R = 3,4-(OCH_2O)C_6H_3) and 32 (X = OCH_3 ; R=2-thienyl). But further improved cytotoxic activity was observed mainly by the thioalkylation of that 2-thione group of 28-32 mainly with methyl or ethyl group yields potentially active molecules 33, 34, 36, and 37.

5. 2-chloro-3-pyridine carboxylic acid as precursor:

Carmen Sanmartin et al., [17] explained the synthesis and prefatory evaluation of biological properties especially the anticancer potency of novel "2-(alkylsulfanyl)-N-alkylpyrido[2,3-d]pyrimidine-4-amine" derivatives. They have studied C-N-C and C-S-C linked unsymmetrically substituted pyrido[2,3-d]pyrimidines respectively at 4^{th} and 2^{nd} positions between the side-chain substituents and planar ring system.

As per the literature survey, in the recent years, class of "pyrido[2,3-d]pyrimidines" developed as inhibitors which antagonizes variety of biological targets involved in the cancer development including cyclin-dependent kinase [8], dihydrofolate reductase [47,48,49] and non-receptor tyrosine kinases [50, 51,52].

Acyl chloride of "2-chloro-3-pyridine carboxylic acid" 1 was made to react with alkyl imidothiocarbamates hydroiodides 2a–d [53] and triethylamine in a molar ratio of 1:1:2 resulted in the formation of compound 3 with 62 – 82% yield, These are good intermediates for the next class of

derivatives 4 i.e "2-(alkyl- sulfanyl)pyrido[2,3-d]pyrimidin-4-(3H)-ones" (63 – 77%) by boiling compounds 3a – d with DMF for about 15 min. Further compound 4 reacts with $POCl_3$ where DMF was added as catalyst yields compound 5. Compounds 5 was made to reflux with selected amines in ethanolic medium forms moderate yields of 6a-s **(Fig. 19)**. In the scheme 2, step for the synthesis of 6-substituted pyrido[2,3-d] pyrimidine derivatives, 14 and 15, was found to be laborious and yield was low. Preparation of compounds 7, 8, and 9 were done by following reported methods in literature [54]. 2-chloro-3-pyridine carboxylic acid was the starting material and undergoes hydrolysis which yields "2-hydroxy-3-pyridinecarboxylic acid" 7. Further Nitration of compound 7 with nitrating mixture i.e., concentrated sulfuric acid and concentrated nitric acid results in the formation of compound 8, which was modified by reacting it with $POCl_3$ with absolute ethanol into diethyl derivative 9. This diethyl derivative 9 undergoes cyclization with ethyl imidothiocarbamate 2a under reflux produced compound 12. Compound 12 was also be synthesized by treatment of compound 8 with $SOCl_2$ followed by the addition of absolute ethanol to give compound 10, which undergoes halogenation with $POCl_3$ where chlorobenzene was the solvent resulted in the formation of compound 11. Similar to derivative 9, compound 11 undergoes cyclization with 2a to give 12. Further halogenation of compound 12 at the carbonyl group of 4^{th} position with $POCl_3$ yields compound 13. This derivative was made to react with n-pentylamine to yield compound 14. Nitro group of 14 undergoes further reduction in presence of freshly prepared iron(II)hydroxide in water/DMF converted into amino group to give compound 15 **(Fig. 20)**.

Evaluation of the cytotoxicity of the compounds were performed in 3 different cancer cell lines, bladder (T-24), colon (HT-29) and breast (MD- MBA-231). According to the neutral red assay explained by Lowik and Alblas[55] evaluation of compounds for anticancer properties at concentrations of 100 and 20 microM. Camptothecin was used as a standard molecule and its IC_{50} values were 0.29 microM in MD-MB-231, 0.014 micro M in HT-29, and 0.009 micro M in T-24.

From the obtained result, synthesized derivatives can be regarded as cytotoxic and a greater potency was observed with MD-MBA-231. Compounds 6c, 6d and 6p were considered as active cytotoxic agents. These derivatives were also found to induce apoptosis in minimum two of the tested cell lines,

which displayed comparable apoptosis induction values to standard reference camptothecin (2.6 and 3.3), Compound 6p was considered as the best apoptosis inducer with value of 4.3 in T-24, 6.4 in HT-29 and 9.3 in MD-MBA-231 cell line. In case of non-tumoral cell lines, survival values were ranges between 85 and 100% for 6c, 6d, and 6p in at least one of the tested cell lines.

Few derivatives like 6c, 6d, 6j and 6p results in great increase in the caspase-3 levels in two of the tested cell lines and derivatives 6e and 6o results in markable increase in T-24. Considering these findings are of much interest since the biological function of the caspase was enhanced because of the apoptosis response.

Finally, they concluded that novel molecules 6c, 6d, 6e, 6j, 6o, and 6p are apoptotic, cytotoxic and activators of caspase-3 in at least one of the tested cell lines. Additionally, 6c, 6d, 6e, 6j, and 6p have survival values ranges from 95 – 100% in at least one of the tested nontumoral cell lines like CRL-8799 or CRL-11233.

6. 2-amino nicotinic acid as precursor:

Based on the literature, Ayman M. F. Elgohary et al., [25] reported pyrido[2,3-d]pyrimidine nucleus present in variety of active compounds since it exhibits different biological properties including antitumor activity [8,14,23,56,57]. They have synthesized new "2- propylpyrido[2,3-d]pyrimidin-4(3H)-one" derivatives possessing various substituents at 3rd position, like NH_2 and N-aryl groups mainly to examine the effect of particular substituents on antitumor activity.

2-aminonicotinic acid 1 was the staring material, which was made to react with butyroyl chloride in presence of pyridine to yield "2-propyl-4H-pyrido[2,3-d][1,3]oxazin-4-one" 2. Further, derivative 3a was prepared by the reaction between pyridooxazinone 2 with hydrazine hydrate in C_2H_5OH in water bath, "2-propylpyrido[2,3-d]pyrimidin-4(3H)-one" 3b was synthesized by reacting compound 2 with ammonium acetate in an oil bath.

3b can also be obtained by refluxing compound 2 in formamide under heat. Reaction of compound 2 with hydroxyl amine resulted in the formation of "3-hydroxy-2-propylpyrido[2,3-d]pyrimidin-4(3H)-

one" 3c. Meanwhile, compounds 3d-f were synthesized by treating compound 2 with aromatic amines like, ortho, meta and para toluidine **(Fig. 21).**

On the other hand, "3-(Arylylideneamino)-2- propylpyrido [2,3-d]pyrimidin-4(3H)-ones" 4a-k were synthesized in 3 different methods. The first method is method A (traditional method) by condensation of "3- amino-2-propylpyrido[2,3-d] pyrimidin-4(3H)-one" 3b with aryl aldehydes in ethanolic medium but the disadvantage of this method reported was low yield, long reaction time and suffering from high temperature.

So, they applied environmentally benign strategies in organic synthesis, i.e I_2/KI mediated condensation of "3-amino- 2- propylpyrido [2,3-d] pyrimidin- 4(3H) -one" 3a with different aldehydes resulted in the formation of "3-(Arylylideneamino)-2-propylpyrido [2,3-d] pyrimidin -4(3H)- ones" 4a-k in ethanol–water (method B) or boiling water (method C).

At first, saturated aqueous solution of KI was used to dissolve required quantity of molecular iodine to prepare solution of I_2 /KI. Further refluxing different aromatic aldehydes with "3-amino-2-propylpyrido [2,3-d] pyrimidin - 4(3H) -one" 3a in presence of saturated aqueous solution of I_2 /KI resulted the formation of "3-(Arylylideneamino) - 2- propylpyrido [2,3-d] pyrimidin- 4(3H)-ones" 4a-k with good yields **(Fig. 22).**

Further synthesized compounds were evaluated for anti-cancer properties by using 3-cell lines panel comprising of MCF7 (Breast), SF-268 (CNS) and NCI-H 460(Lung). In comparison to reference standard doxorubicin, some of these novel derivatives showed better anticancer activity against MCF7. Out of which compounds containing NH_2, OH or NH groups at position 3 exhibited best activity and It was found that compound 3a (IC_{50}= 3.82 mg/mL), is a 3-amino derivative was the highly promising derivative in this study.

Conclusion

This review highlights the various synthetic approach and anticancer potential of substituted pyridopyrimidines. Libraries can be developed by selecting best pathway for the synthesis. From this review, it was clearly seen the potency of pyridopyrimidines in the area of cancer research, by taking different types of cancers into consideration. Early development of useful and efficient methods and

procedures is always important in the synthesis and development of compounds with different medicinal/pharmacological values.

Out of number of starting material, 2-thioxopyrimidine is found to be mostly employed in the synthesis of pyridopyrimidines. In the different synthesisized pyridopyrimidines, arylidine hydrazonyl pyridopyrimidine with various substitution like 4-Cl, 4-CH_3, triazolo pyridopyrimidine with COOEt substitution/aryl ethylidene with F substitution/ $COCH_3$ substitution/3-thioxo group/ 3-thioxo group with alkyl substitution on thio group/ 3- amino group/ -CH_3-$(CH_2)_8$-CH_2-, -CH_3 and -CH_3-$(CH_2)_8$-CH_2-, pyrazole substituted pyridopyrimidine with -CH_3 substitution, 4-amino- 5,7- disubstituted- pyrido[2,3-d]pyrimidine-2-thiones with various substitution like Br, 2-thienyl, 2-alkylthio derivative, 3,4-$(OCH_2O)C_6H_5$, 2-thioalkyl, 5-amino alkyl substitution, 2-propyl- 3 amino-4-oxo-pyridopyrimidine were found to be effective and potent molecules, which will be helpful for the SAR studies of pyridopyrimidines by considering the contribution of different substitution on it and potent molecules can be developed further.

Consent: It is not applicable.

Ethical Approval: It is not application

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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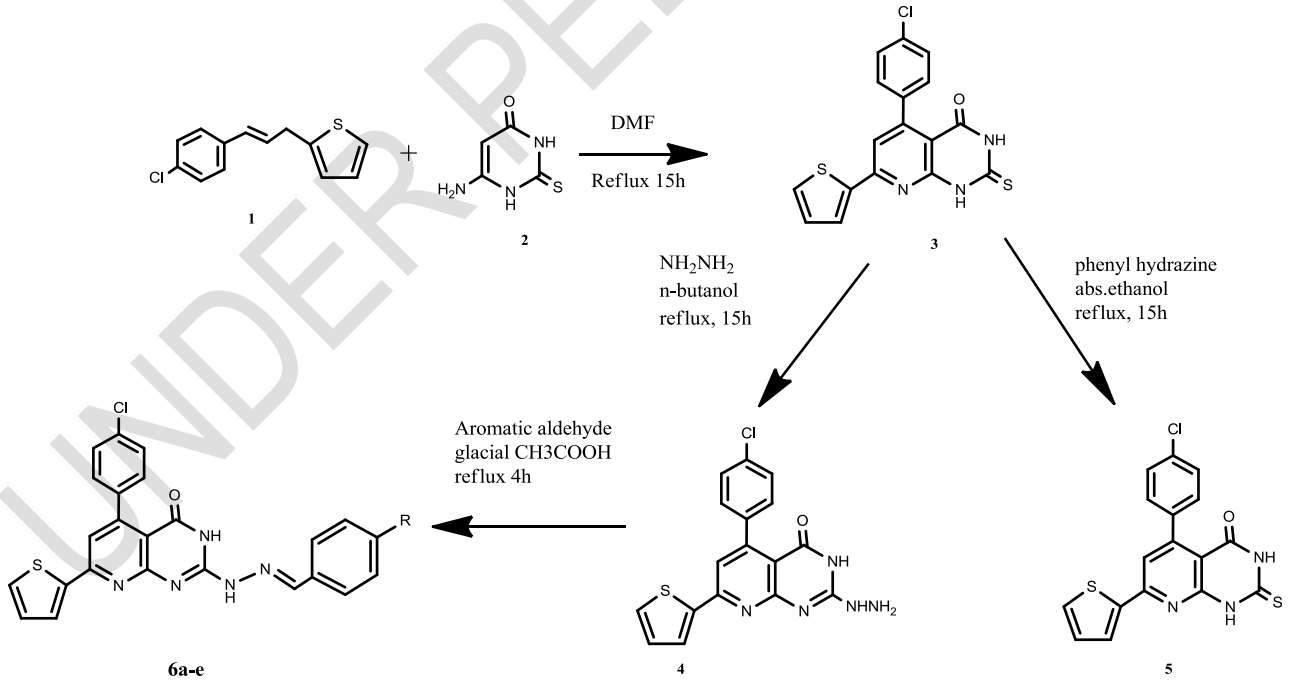
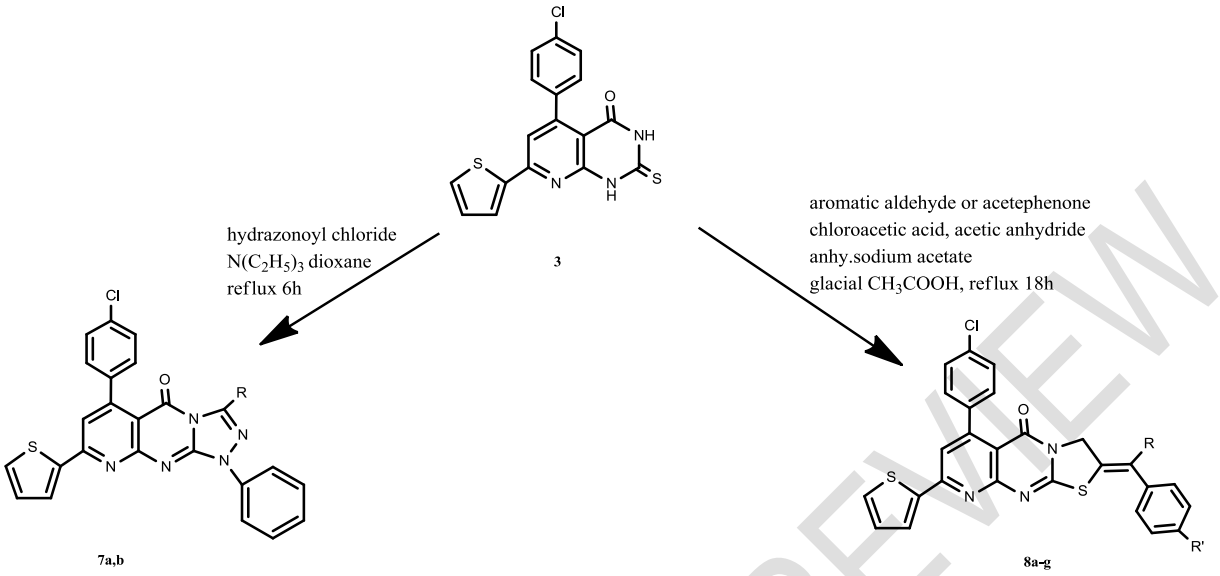


6a- R=H
6b- R=Cl
6c- R=F
6d- R=CH_3

6e- R=OCH_3

Fig. 1: Synthetic scheme for preparation of 4,5 and 6a-e



7a- R= $COCH_3$
7b- R= COOEt

8a- R=H, R'=H
8b- R=H, R'=Cl
8c- R=H, R'=F
8d- R=H, R'=F
8e- R=H, R'=OCH_3
8f- R=CH_3, R'=Cl

Fig. 2: Synthetic scheme for preparation of 7a-b and 8a-g

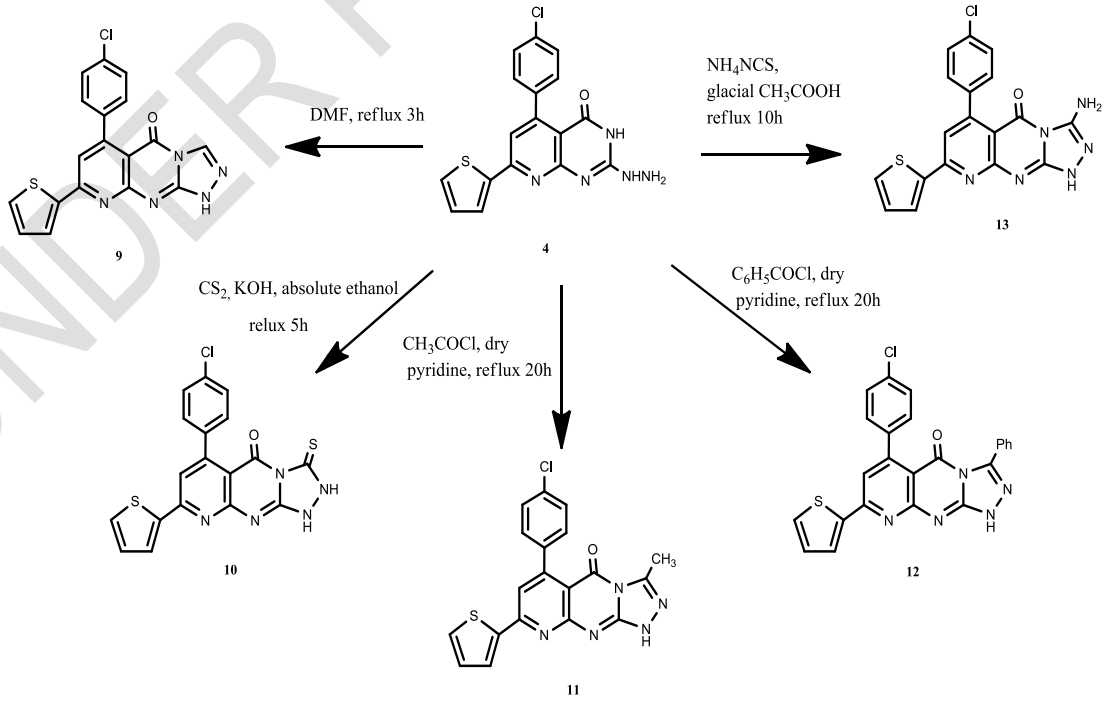


Fig. 3: Synthetic scheme for preparation of compounds 9-13

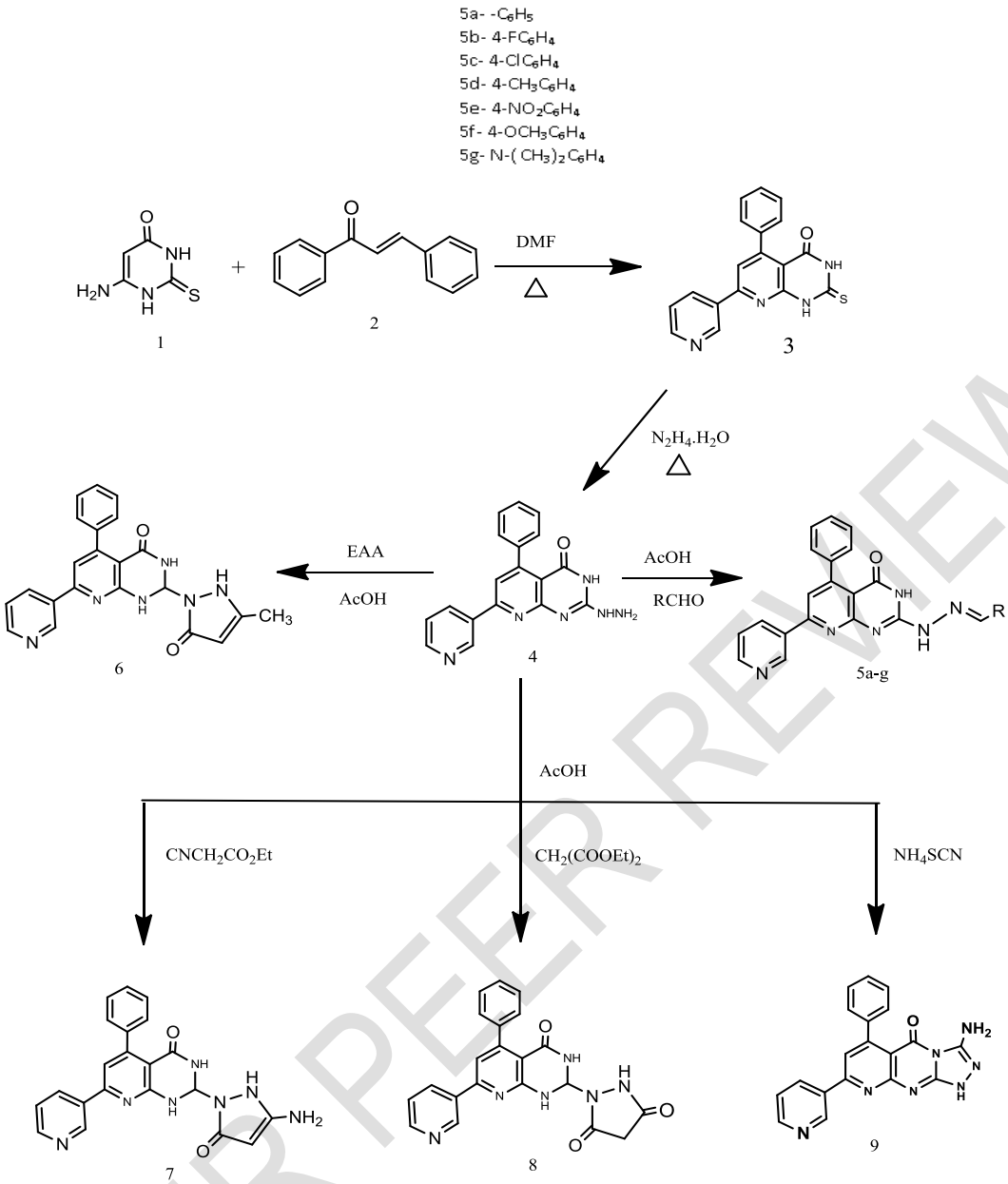
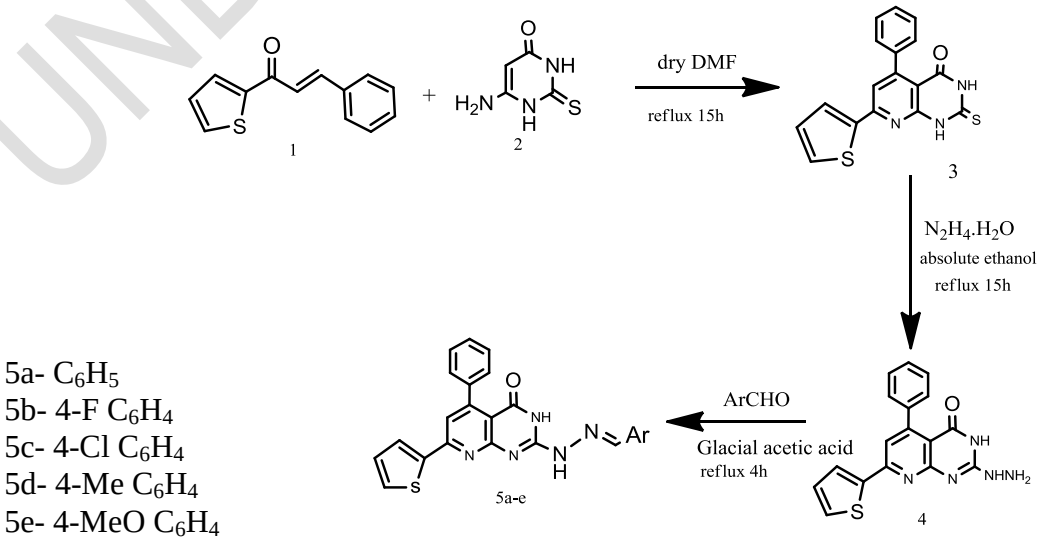


Fig. 4: Synthetic scheme for compounds 3-9



5a- C_6H_5
5b- 4-F C_6H_4
5c- 4-Cl C_6H_4
5d- 4-Me C_6H_4
5e- 4-MeO C_6H_4

Fig. 5: Pathway for the synthesis of compounds 5a-e

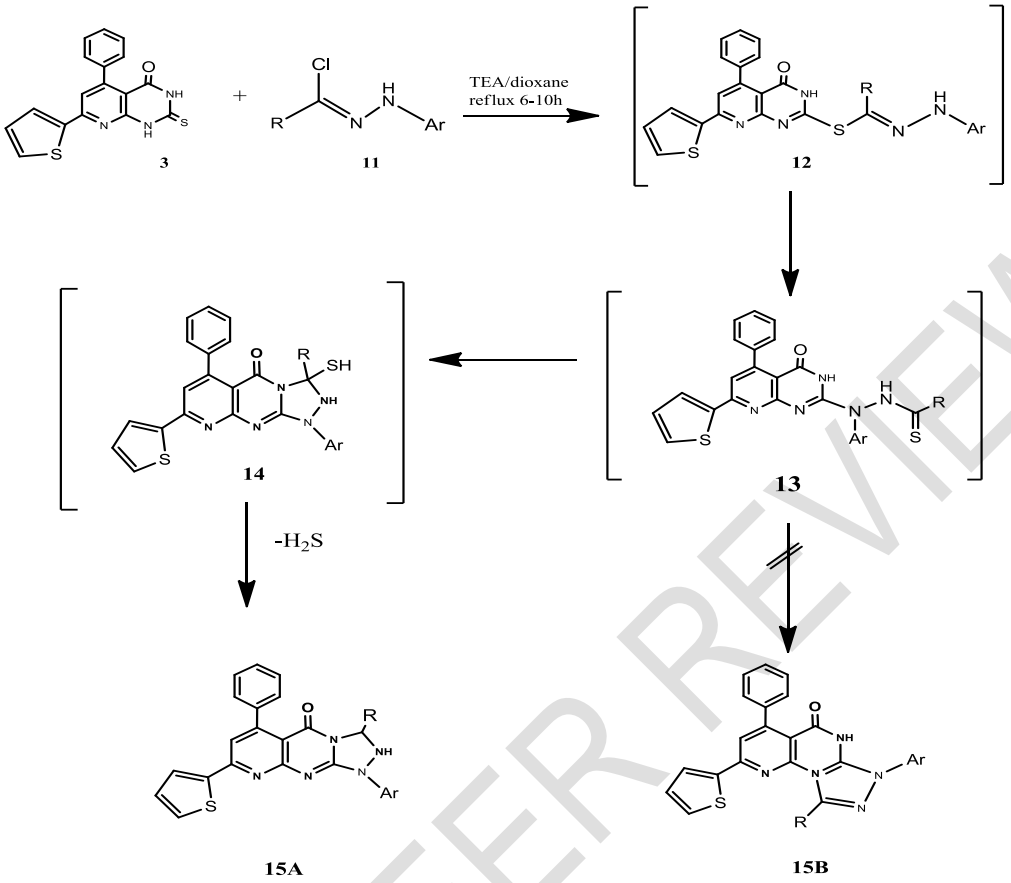


Fig. 6: Pathway for the synthesis of compounds 6-10

11,15	R	Ar
a	$COCH_3$	C_6H_5
b	$COCH_3$	4-F C_6H_4
c	$COCH_3$	4-Cl C_6H_4
d	$COCH_3$	4-Me C_6H_4
e	$COCH_3$	4-MeO C_6H_4
f	$COCH_3$	SO_2-NH_2-C_6H_4
g	COOEt	C_6H_5
h	COOEt	4-Cl C_6H_4
i	COOEt	4-Me C_6H_4
j	COOEt	SO_2-NH_2-C_6H_4

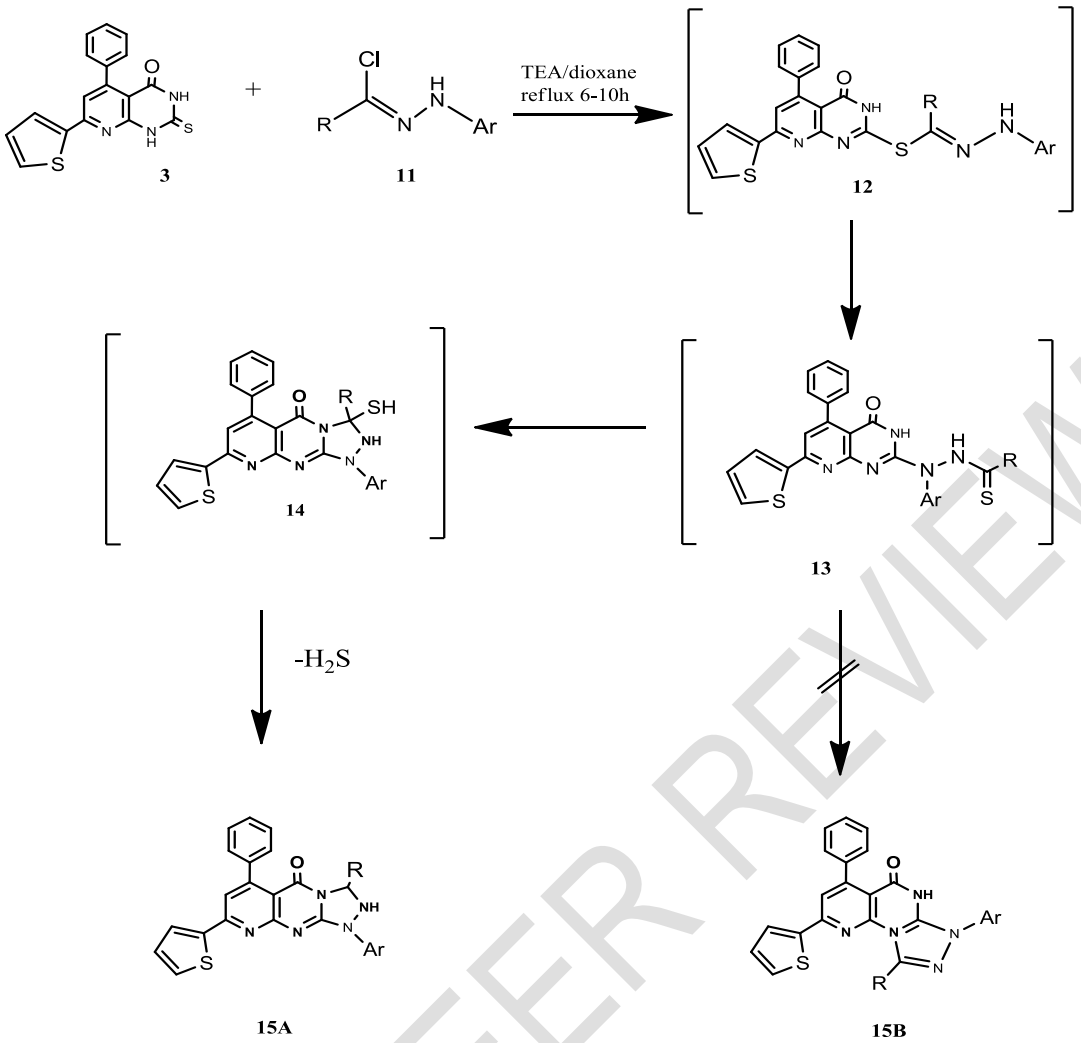


Fig.7: Pathway for the synthesis of compounds 15a-j

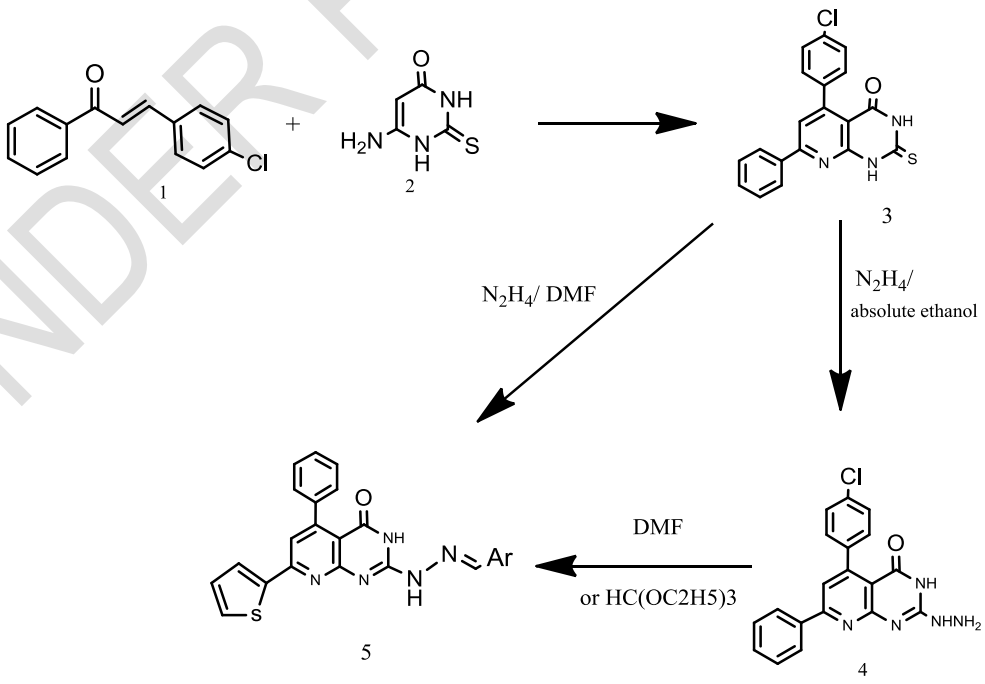


Fig. 8: Synthesis of compounds 3,4 and 5

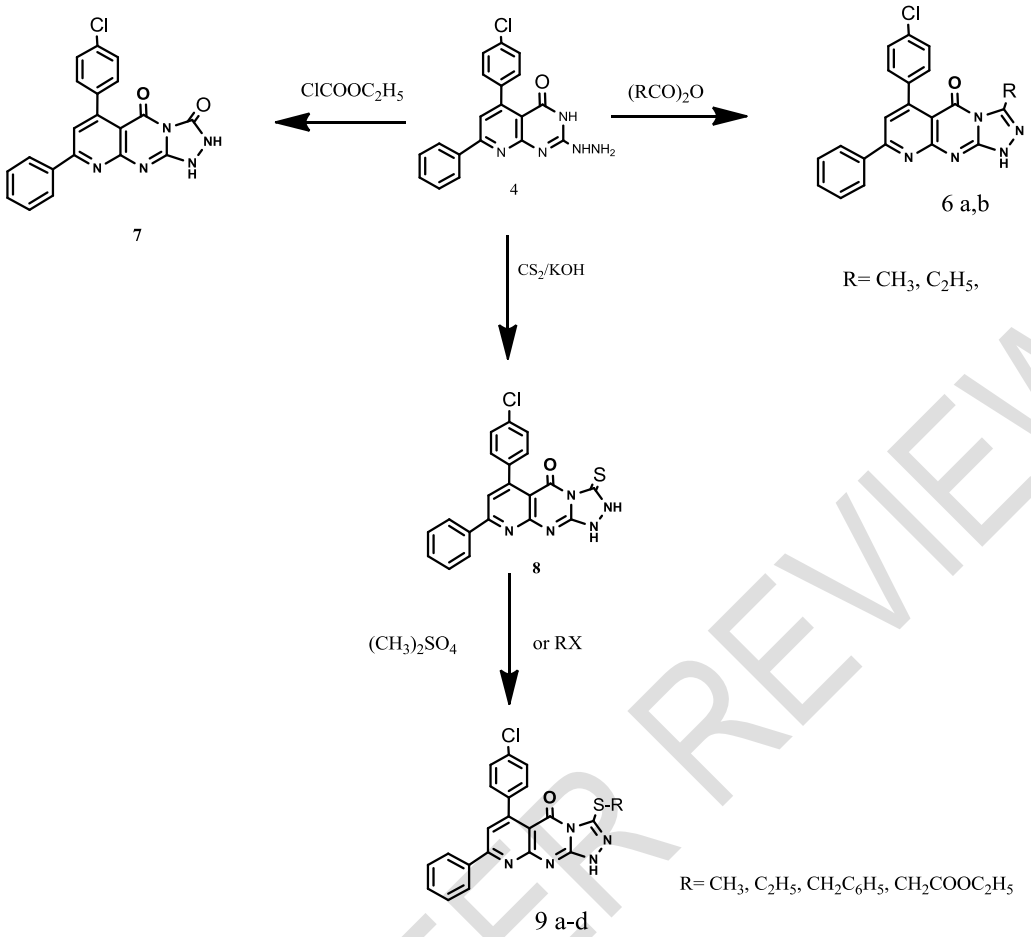


Fig. 9: Synthesis of 3- alkyl, 3-oxo, 3-thioxo and 3-alkylsulphanyl derivatives

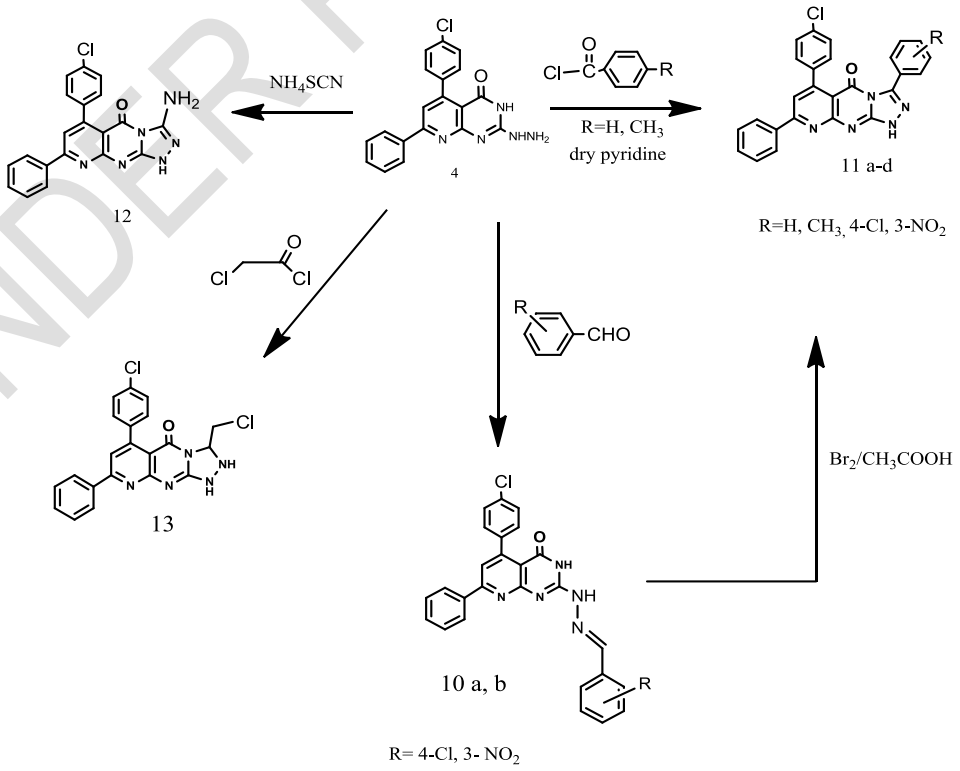


Fig. 10: Synthesis of 3-aryl, 3-amino and 3-chloromethyl derivatives

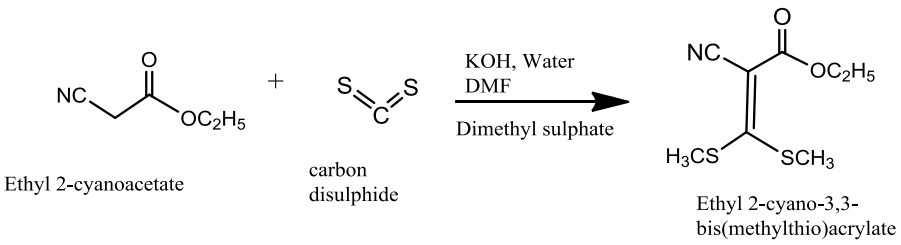


Fig. 11: Synthesis of "ethyl 2-cyano-3,3-bis(methylthio)acrylate"

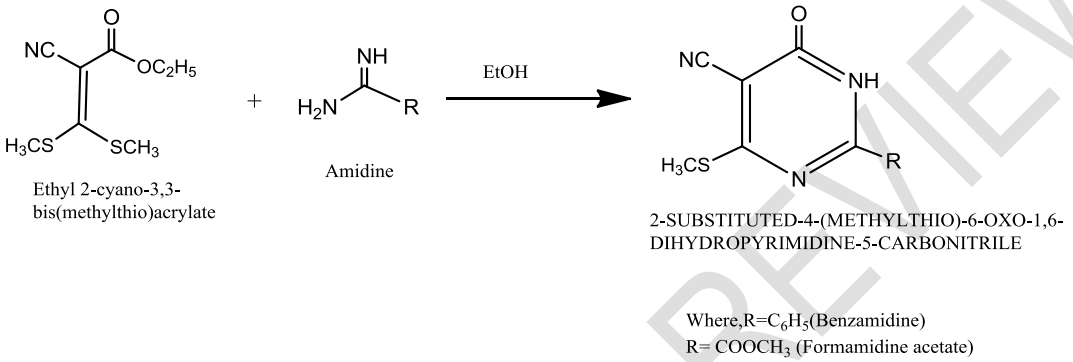


Fig no. 12, Synthesis of "2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile"

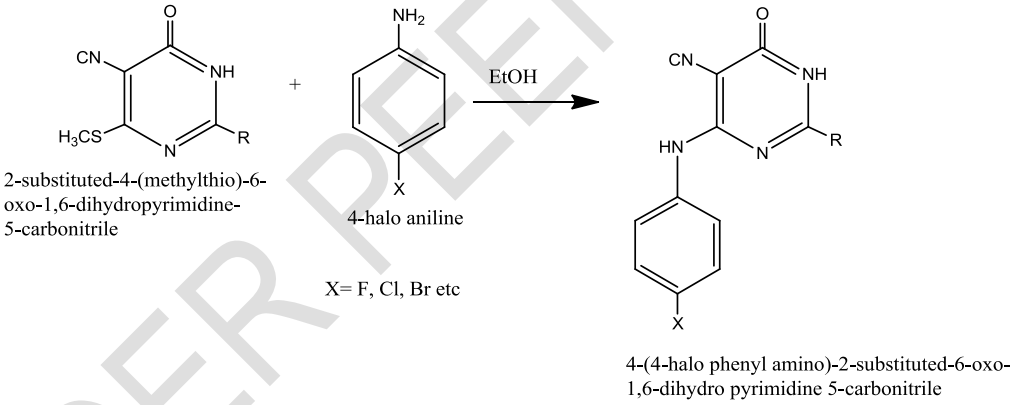


Fig. 13: Synthesis of "4-(4-halo phenyl amino)-2-substituted-6-oxo-1,6-dihydro pyrimidine 5-carbonitrile"

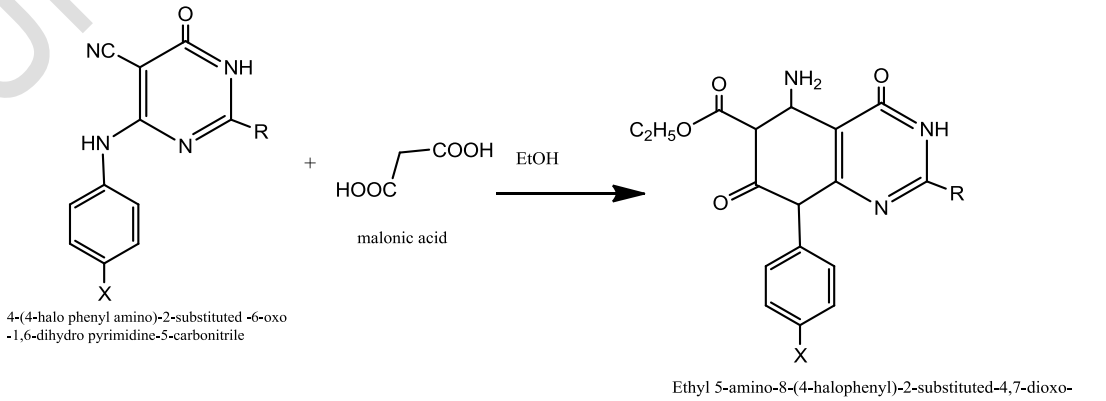
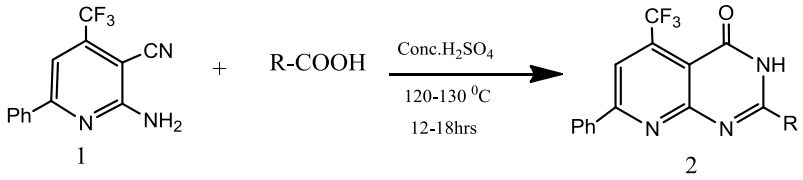
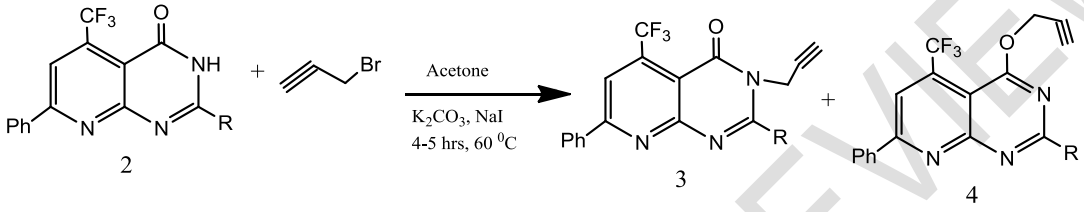


Fig. 14: Synthesis of "ethyl 5-amino-8-(4-halophenyl)-2-substituted-4,7-dioxo-3,4,5,6,7,8-hexahydro pyrido[2,3-d]pyrimidine-6-carboxylate"

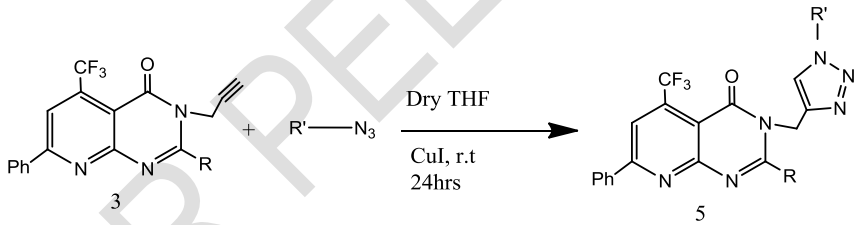


R= H, CH_3, C_2H_5, $(CH_3)_2CH$, Ph, CH_3-$(CH_2)_4$-CH_2-



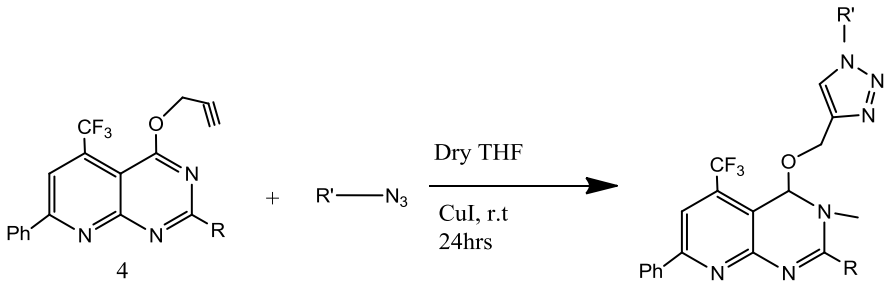
R= H, CH_3, C_2H_5, $(CH_3)_2CH$, Ph, CH_3-$(CH_2)_4$-CH_2-

Fig. 15: Synthesis of "2-substituted-5-trifluoromethyl-7-phenyl pyrido[2,3-d] pyrimidine-4(3H)-one" **(2a-f)** and "N-/O-propargyl-2-substituted-5-trifluoromethyl-7-phenyl pyrido[2,3-d] -4(3H)-one" **(3a-f)** / "pyrido[2,3-d] pyrimidines" **(4a-f)**



5a- R=H, R'=C_8H_{17}-CH_2-CH_2-
5b- R=H, R'=CH_3-$(CH_2)_8$-CH_2-
5c- R=CH_3, R'=C_6H_{13}-CH_2-CH_2-
5d- R=CH_3, R'=C_8H_{17}-CH_2-CH_2-
5e- R=CH_3, R'=CH_3-$(CH_2)_8$-CH_2-
5f- R=CH_3, R'=Ph
5g- R=C_2H_5, R'=C_6H_{13}-CH_2-CH_2-
5h- R=C_2H_5, R'=C_8H_{17}-CH_2-CH_2-
5i- R=C_2H_5, R'=CH_3-$(CH_2)_8$-CH_2-
5j- R= $(CH_3)_2$-CH-, R'=C_8H_{17}-CH_2-CH_2-
5k- R= CH_3-$(CH_2)_4$-CH_2-, R'=C_6H_{13}-CH_2-CH_2-
5l- R= CH_3-$(CH_2)_4$-CH_2-, R'=C_8H_{17}-CH_2-CH_2-

Fig. 16: Synthesis of N-alkyltriazole tagged pyrido[2,3-d]pyrimidine derivatives[5a-5l]



6a- R=CH_3, R'=C_8H_{17}-CH_2-CH_2-
6b- R=C_2H_5, R'=C_6H_{13}-CH_2-CH_2-
6c- R=Ph, R'=C_8H_{17}-CH_2-CH_2-
6d- R= $(CH_3)_2$-CH-,R'=C_6H_{13}-CH_2-CH_2-

Fig.17: Synthesis of O-alkyltriazole tagged pyrido[2,3-d]pyrimidine derivatives[6a-6d]

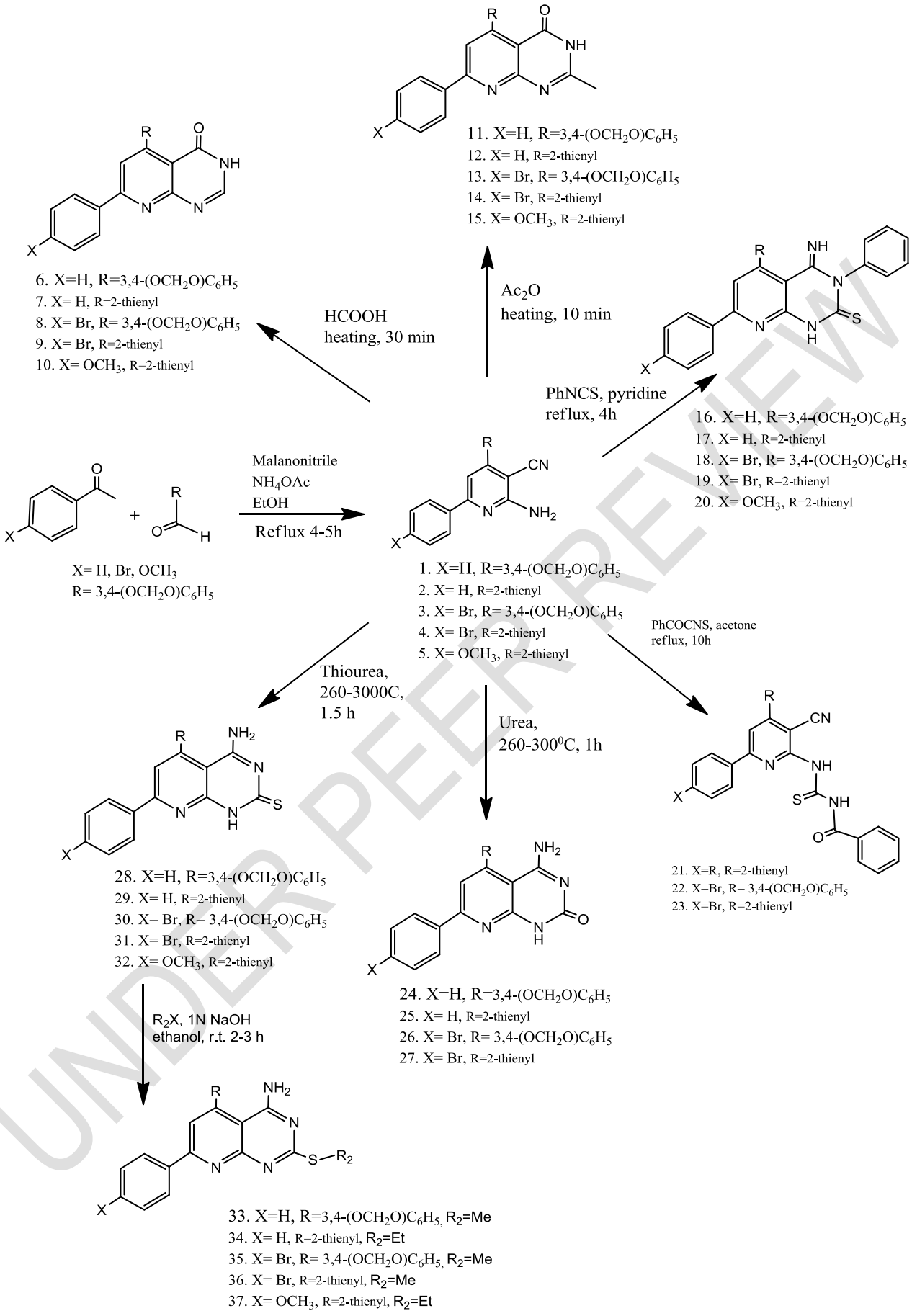


Fig.18: Synthesis of compounds 1-37

Compound	W	n	R	Z
6a	CH_3	1	H	CH_3
6b	CH_3	3	H	CH_3
6c	CH_3	4	H	CH_3
6d	CH_3	5	H	CH_3
6e	CH_3	6	H	CH_3
6f	OH	3	H	CH_3
6g	OH	4	H	CH_3
6h	OH	5	H	CH_3
6i	CH_3	3	CH_3	CH_3
6j	CH_3	4	CH_3	CH_3
6k	CH_3	5	CH_3	CH_3
6l	CH_3	6	CH_3	CH_3
6m	CH_3	3	CH_3	CH_3
6n	CH_3	3	H	$(CH_2)_3CH_3$
6o	CH_3	4	H	$(CH_2)_3CH_3$
6p	CH_3	3	H	$(CH_2)_3CH_3$
6q	CH_3	3	H	$(CH_2)_3CH_3$
6r	CH_3	4	CH_3	CH_3
6s	CH_3	5	CH_3	CH_3

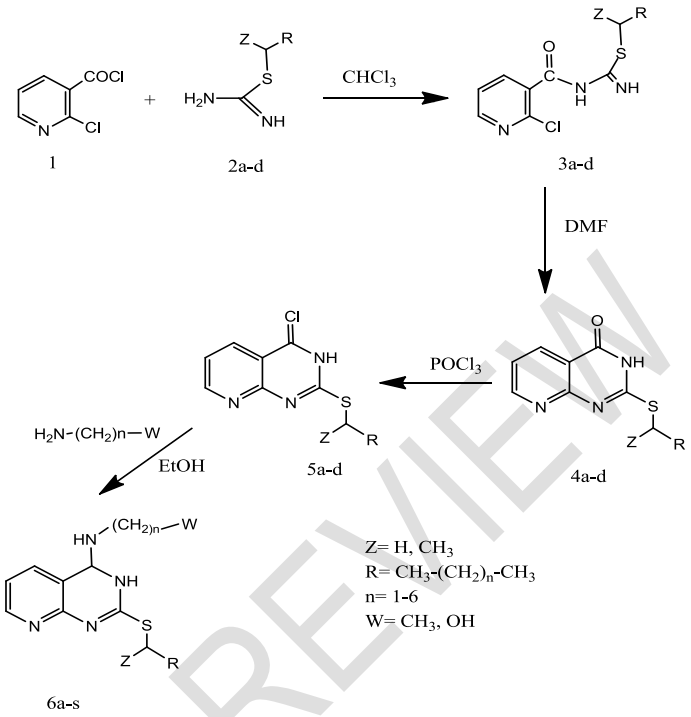


Fig. 19: Synthetic pathway for compounds 1-6

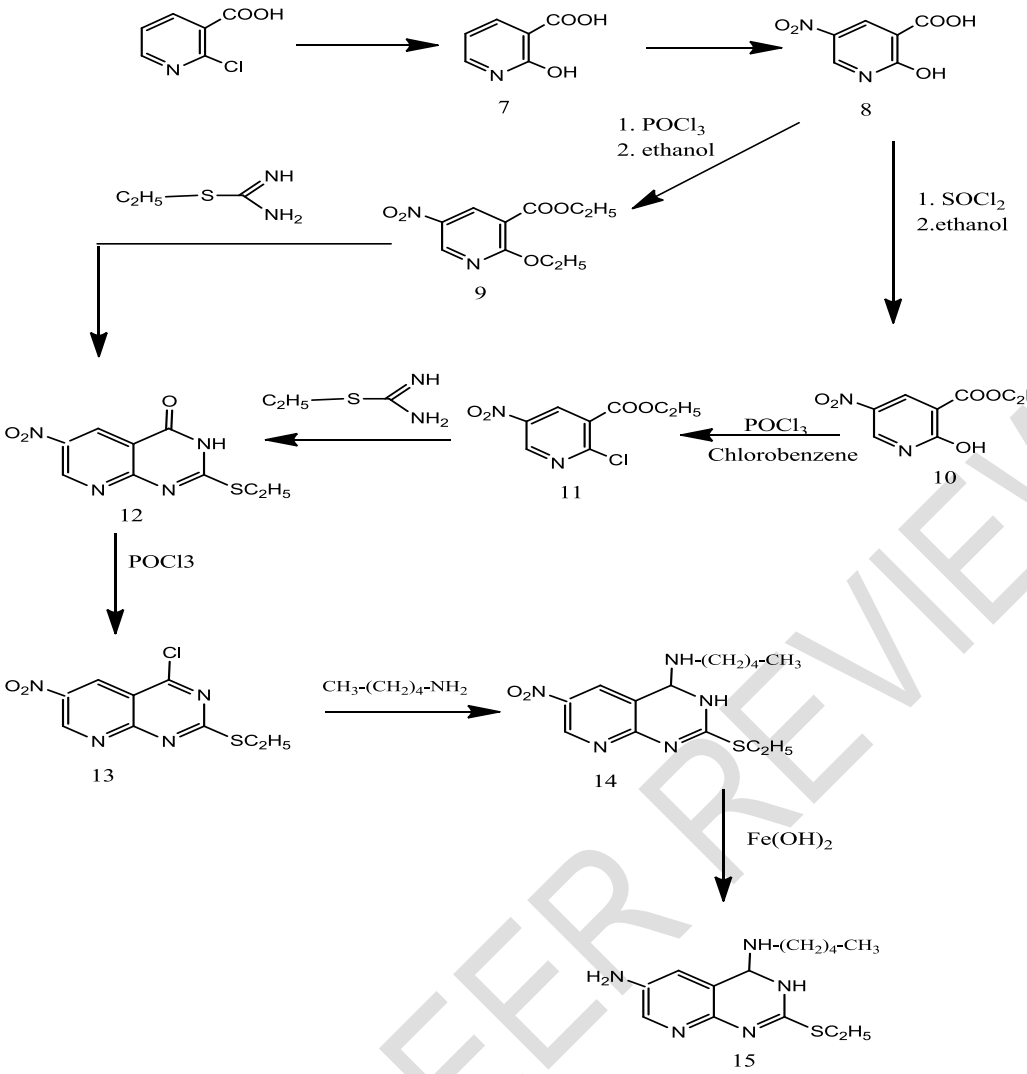
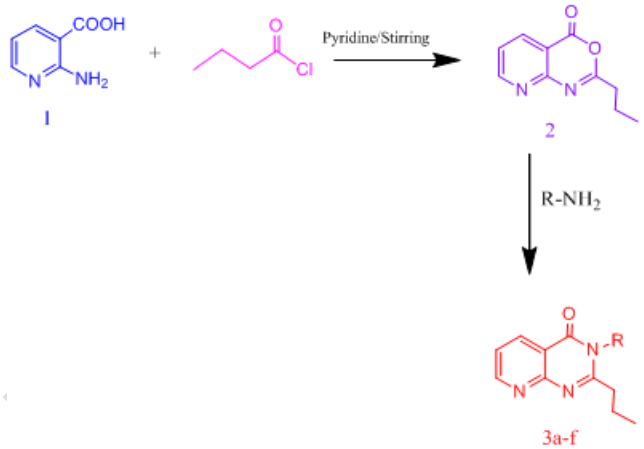


Fig.20: Synthetic scheme for compounds 7-15



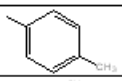
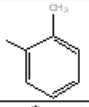
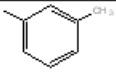
COMPOUND	R	REAGENT AND CONDITION	TIME (h)
3a	NH_2	NH_2NH_2/EtOH	0.5
3b	H	Formamide	3
	H	CH_3COONH_4, Oil bath/150^{0}C	3
3c	OH	NH_2OH/EtOH Reflux	3
3d		EtOH Reflux	4.5
3e		EtOH Reflux	5
3f		EtOH Reflux	5

Fig.21: Synthesis of 3a-f

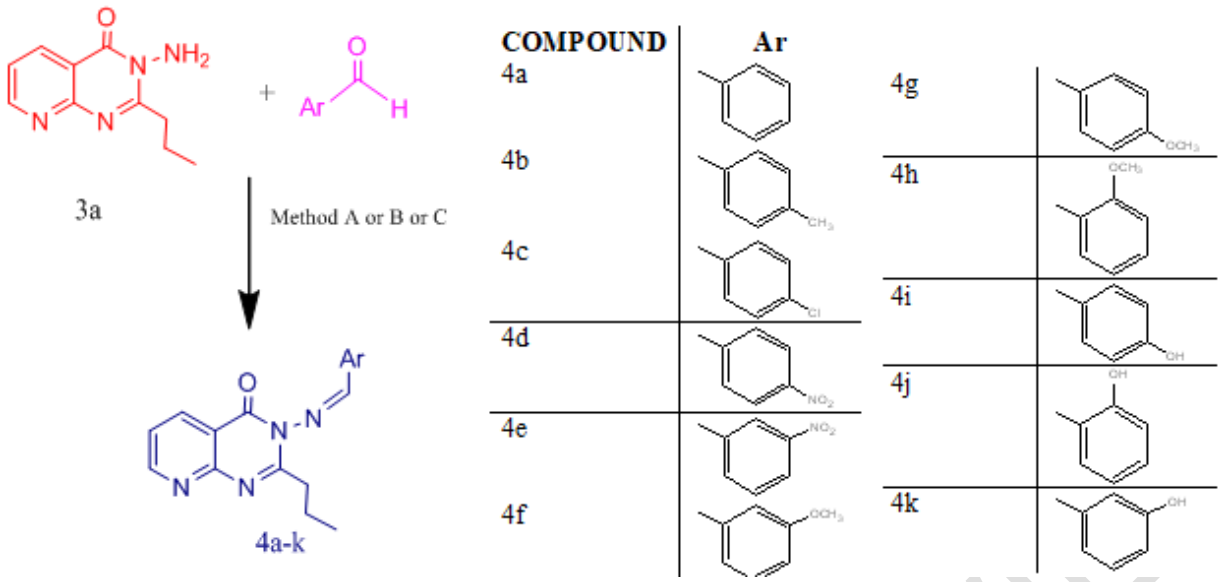


Fig. 22: Synthesis of 4a-f