REVIEW ARTICLE

SYNTHESIS AND ANTICANCER PERSPECTIVE OF PYRIDOPYRIMIDINE SCAFFOLD - AN OVERVIEW

ABSTRACT

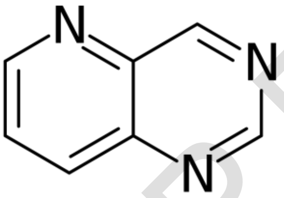
The core pyridopyrimidines are gaining interest in organic and heterocyclic chemistry in recent days, as this scaffold acts as a building block because of its wide range of biological and pharmacological applications like anticancer, antimicrobial, fungicidal, antiviral, CNS, antibacterial, and anti-inflammatory properties. This review mainly emphasizes 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, pharmacological applications

1. INTRODUCTION:

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



Basic ring structure of pyridopyrimidines

2. 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.

2.1 2-thioxopyrimidine (2) 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 2-thioxopyrimidine(2). Thiophene incorporated α,β-unsaturated compound (1) was made to react with precursor (2) to get substituted 2-thioxo-pyridopyrimidinone (3) was treated with hydrazine hydrate and phenylhydrazine in presence of n-butanol or ethanol to get resultant compounds respectively i.e. (4) and (5).

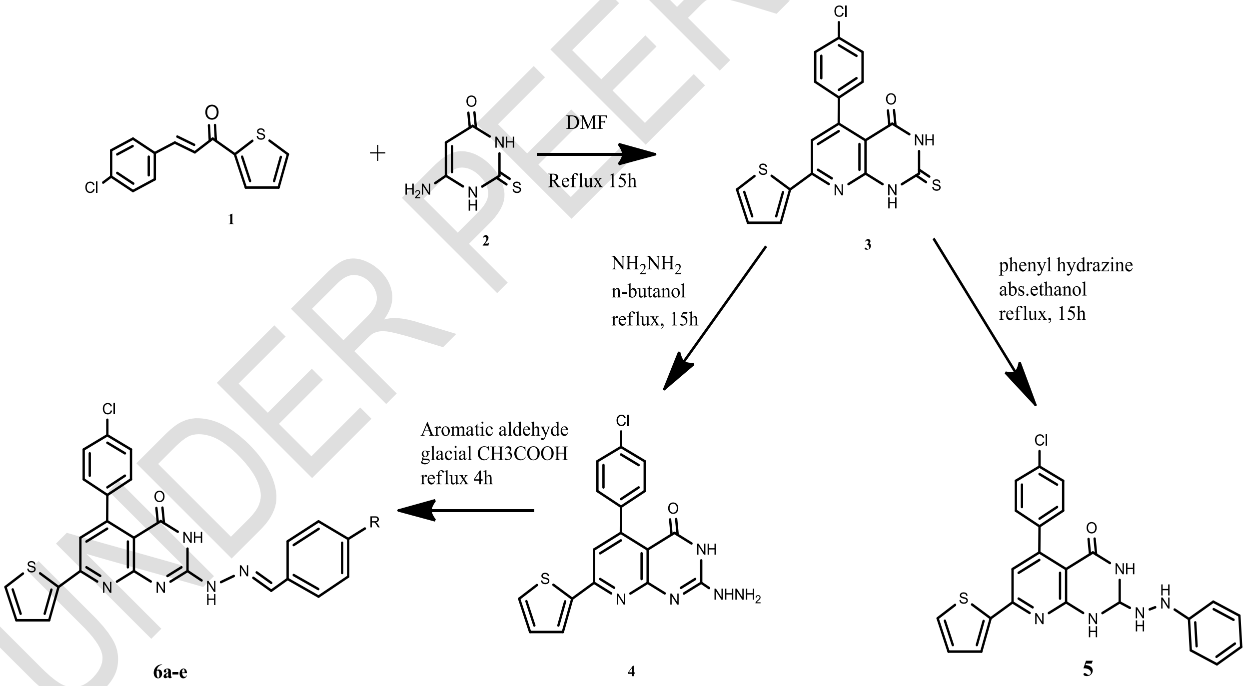
The next series of compounds i.e. arylidene hydrazonyl derivatives (6a-e) were obtained with good yield by reacting compound (4) with a suitable aldehyde in glacial acetic acid. **(Fig. 1)**

A new series of compounds (7a,b) were obtained by S-alkylation followed by Smiles rearrangement between the dipolar nucleophile, 2-thioxo-pyridopyrimidinone (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-thioxo-pyridopyrimidine (3) with sodium acetate, chloroacetic acid, appropriate aldehyde, **or** aromatic ketone in presence of a mixture of an a/acetic anhydride and acetic acid. Additionally, different tricyclic pyridotriazolopyrimidines **(9-13)** are obtained with different substitutions on the triazole ring obtained by subjecting hydrazine derivatives (4) into different reaction conditions **(Fig. 3).**

Tricyclic pyridotriazolopyrimidine (9), 3-thioxopyrido[2,3-d][1,2,4]triazolo[4,3- a]pyrimidin (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 (lung cancer cell line), MCF-7 (breast cancer cell line), and PC-3 (prostate cancer cell line) at submicromolar level. Apoptosis was observed by 6b and 8d respectively in PC-3 and MCF-7 mainly by caspase-3 activity in the PC-3 cell line. Bcl2 downregulation 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.



6a- R=H

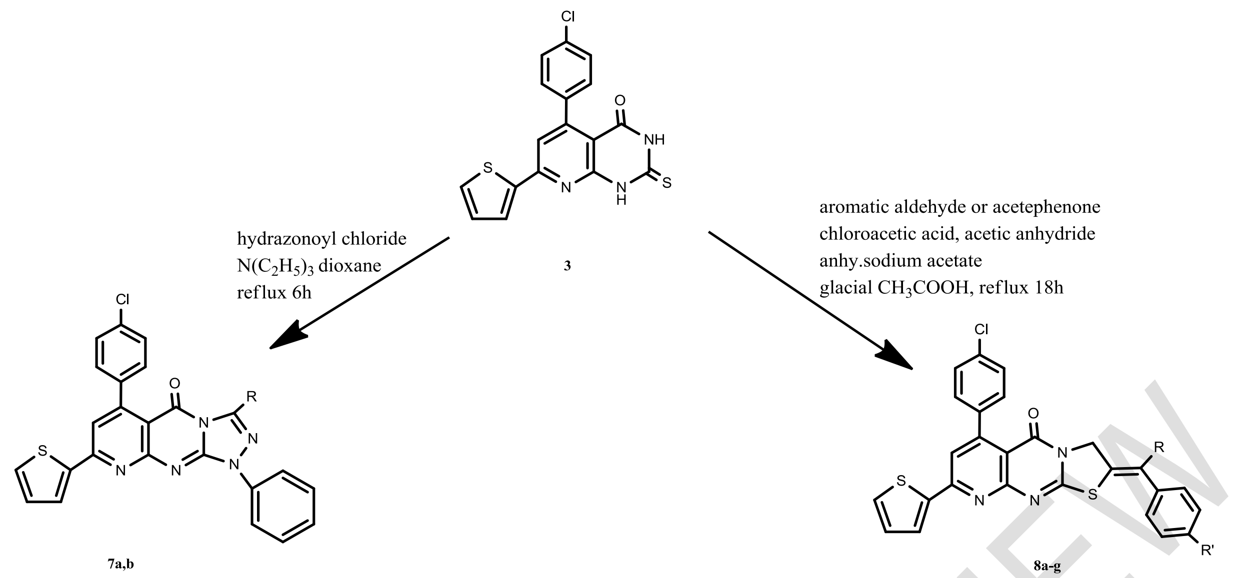
6b- R=Cl

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
8g- R=CH_3, R’= CH_3

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

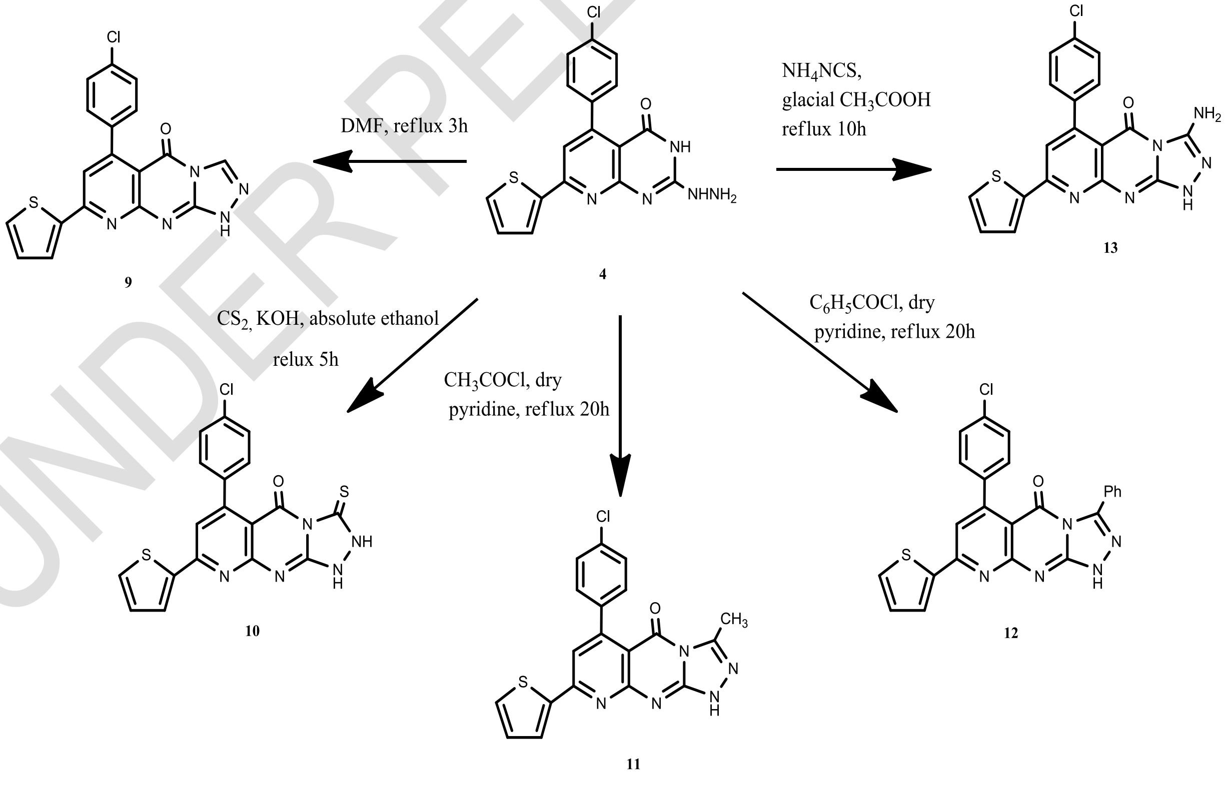


Fig 3. Synthetic scheme for preparation of compounds 9-13

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 [11-13]. In this study they reported the 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 12h 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 5-8h to obtain a 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 3-6h yields 5-substituted pyrazolones and triazolopyrimidine derivatives of pyridopyrimidines (6-9) [2]. **(Fig. 4)**.

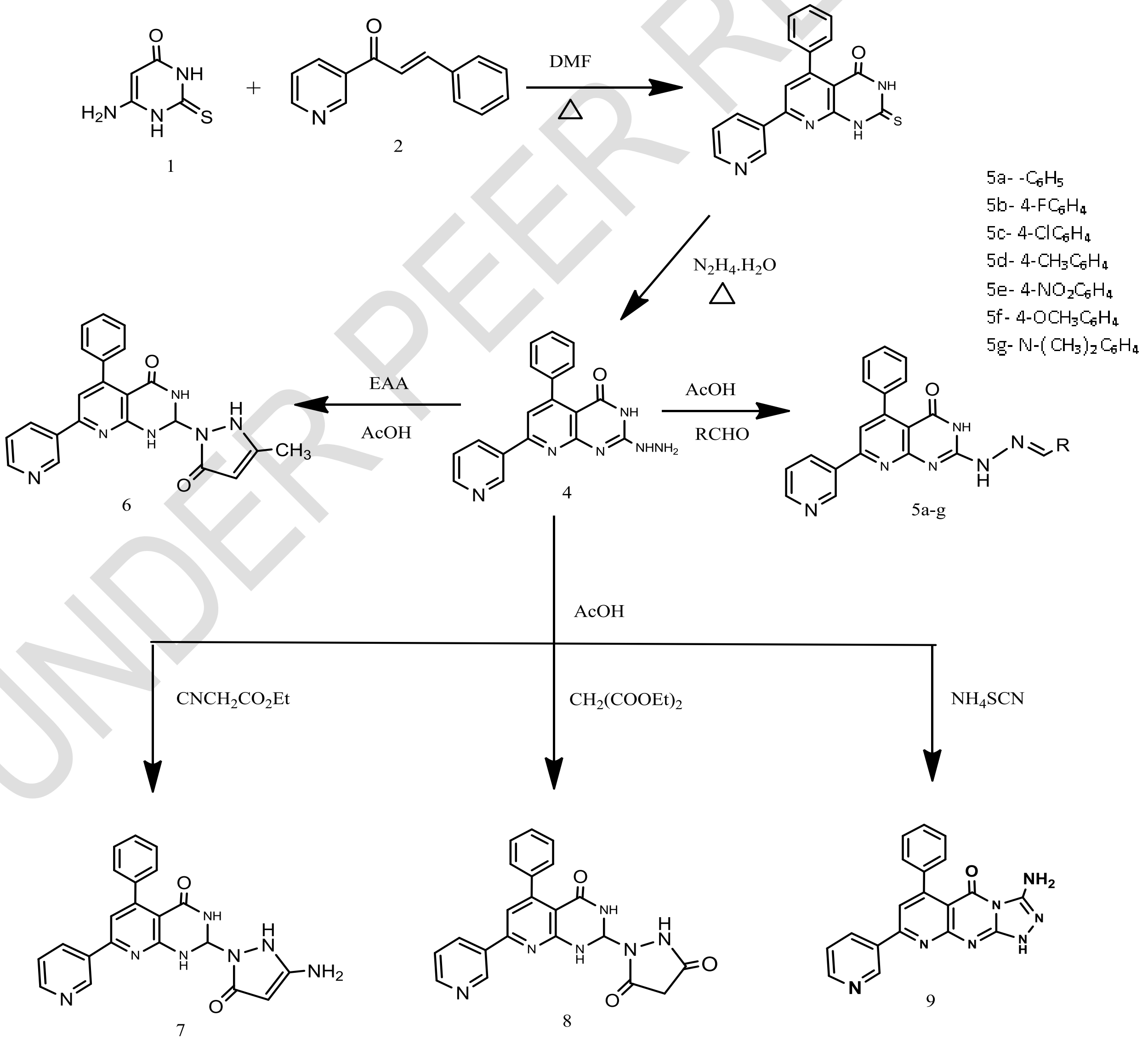


Fig no.4 Synthetic scheme for compounds 3-9

In-vitro cytotoxicity evaluation was done against, HCT-116(colorectal carcinoma cell line) and PC-3 (prostate cancer cell line). 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 IC50 approximately 0.5μM. Kinase inhibition screening by radiometric or ADP-Glo assay was performed based on the cytotoxicity assay, promising derivative (6) with a 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 by 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 checkpoint [16]. Further, some of the pyridopyrimidine derivatives were also reported to induce cell apoptosis in various leukemia cell lines and solid tumors [17-19].

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 cyclo condensation of 2-hydrazinyl derivatives (4) with dry pyridine or CS_2 in ethanolic KOH solution respectively.

Similarly, 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 of the isolated components (15a-j) it was confirmed that form A was present rather than B **(Fig. 7).**

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

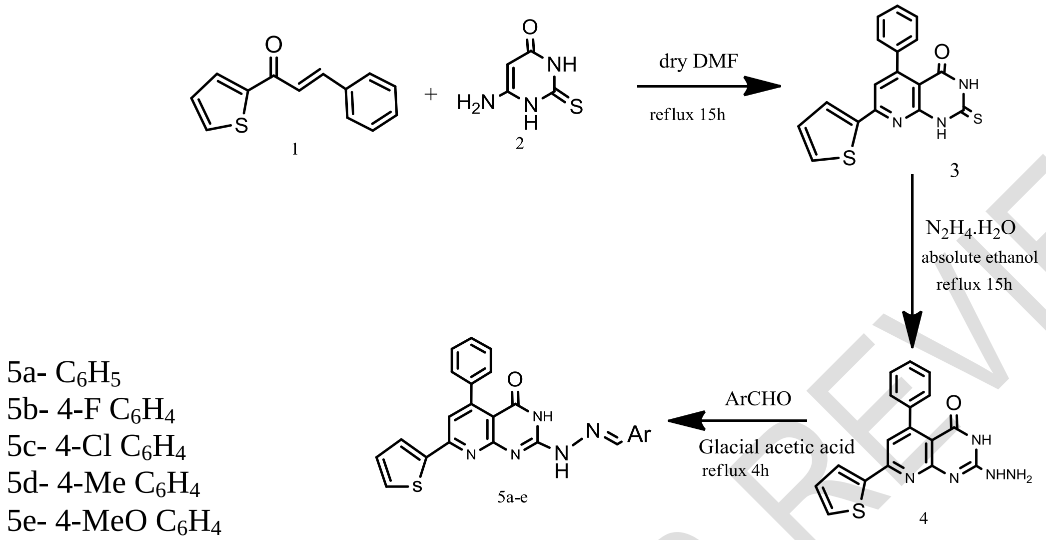


Fig No. 5, Pathway for the synthesis of compounds 5a-e

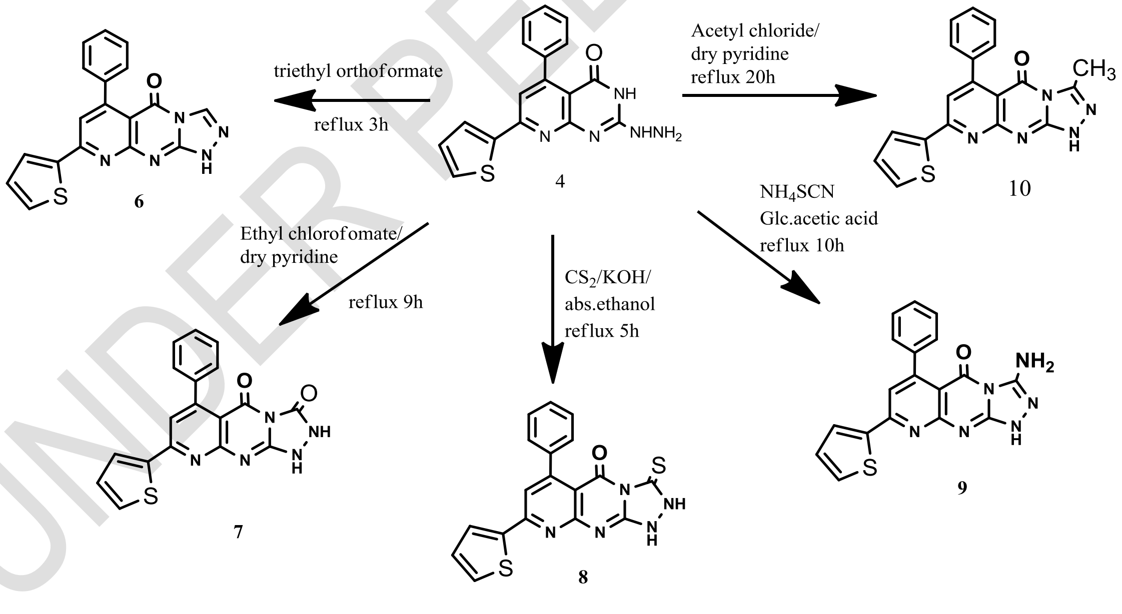
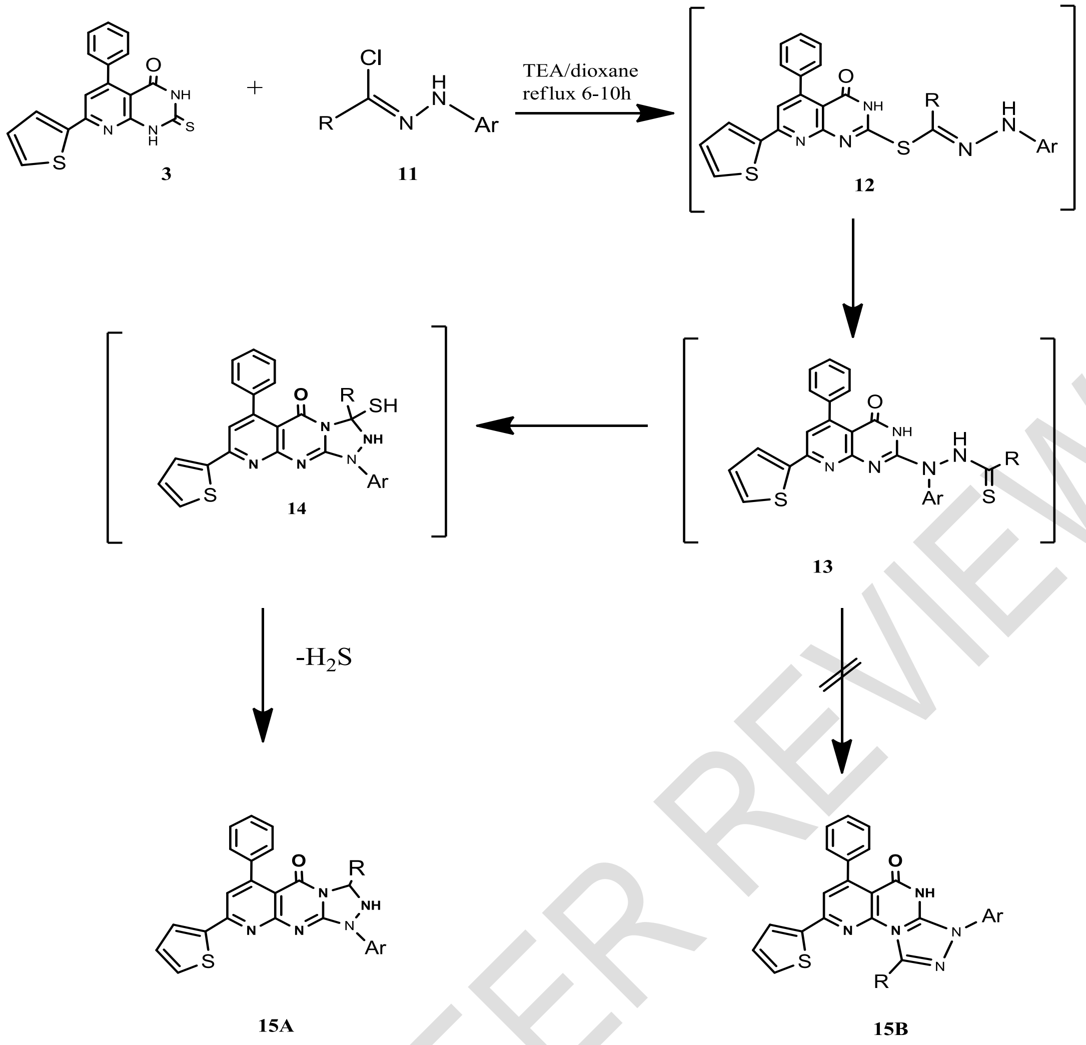


Fig no. 6, Pathway for the synthesis of compounds 6-10



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 no.7, Pathway for the synthesis of compounds 15a-j

Evaluation of *the in-vitro* anticancer potential of synthesized compounds was 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 the reference standard. Some of the 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 [14-25]. 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 [26-32].

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

On the other hand, 3-methyl and 3-ethyl derivatives (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 the 3-oxo derivative of tricyclic pyridopyrimidines (7). Compound (4) was reacted with carbon disulfide in ethanolic KOH solution. As reaction proceeds, which resulted in the formation of the 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 the 2nd one was by the oxidative cyclization of respective hydrazones (10a,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 performed against MCF7 (human breast cancer cell line) where Doxorubicin was used as a 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 the finest cytotoxic effects. About 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 emerged as the 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 the 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.

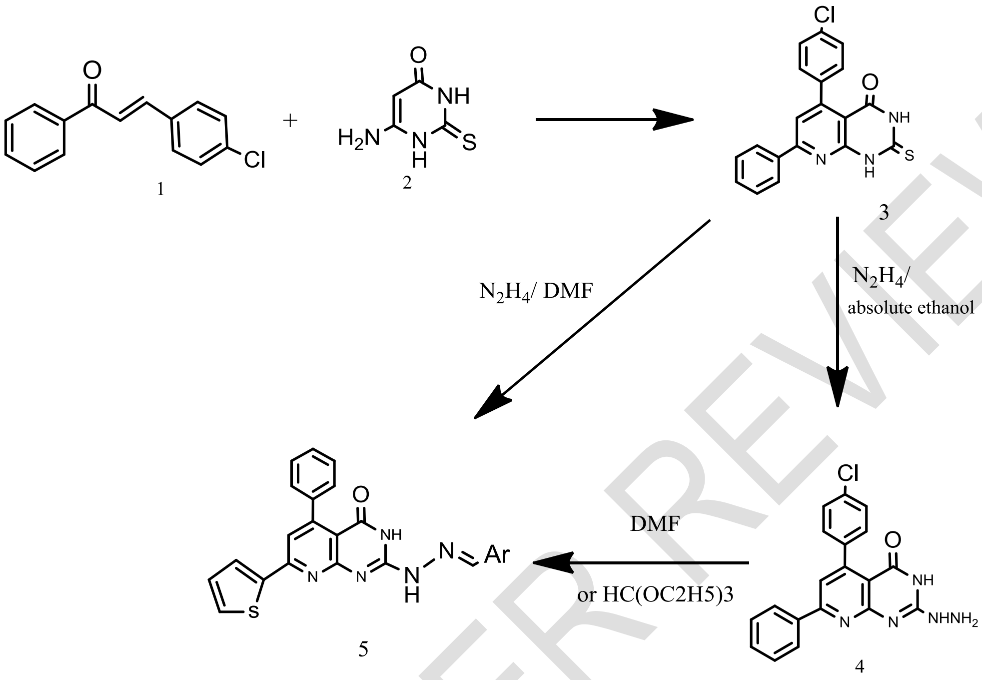


Fig no. 8, Synthesis of compounds 3,4 and 5

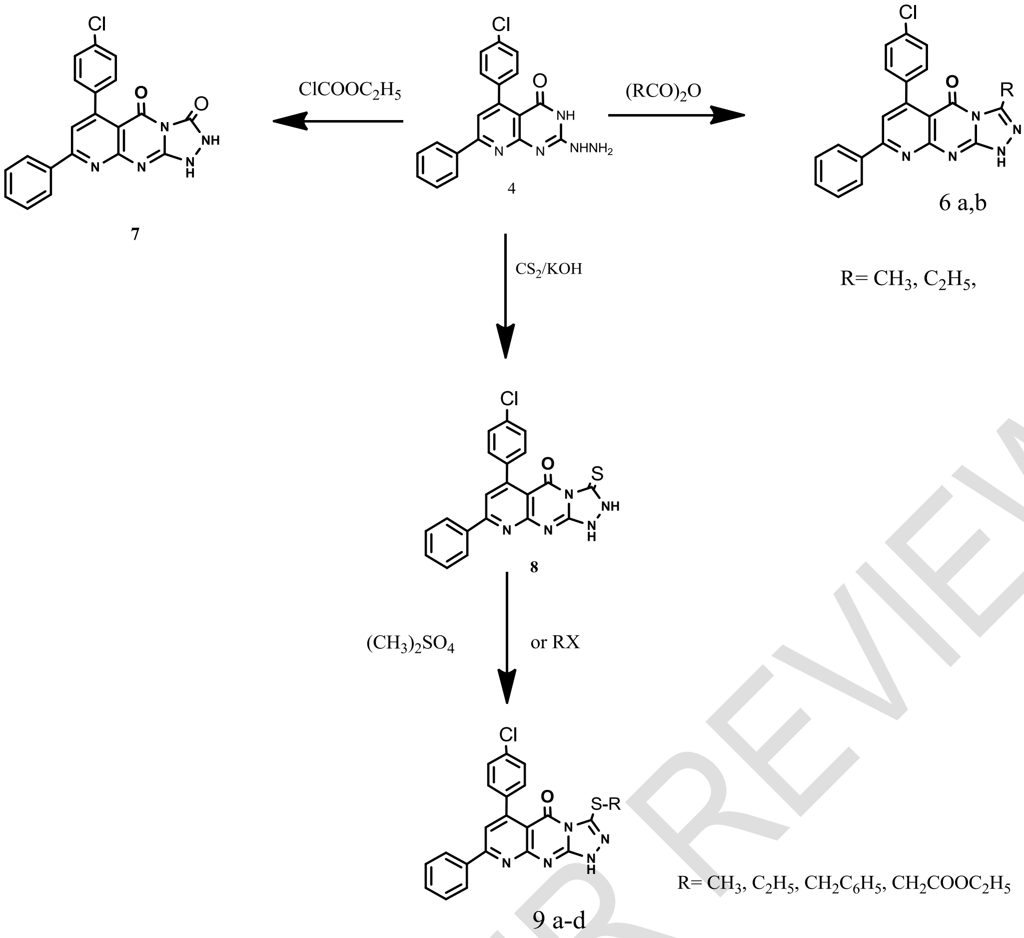


Fig no. 9, Synthesis of 3- alkyl, 3-oxo, 3-thioxo and 3-alkylsulphanyl derivatives

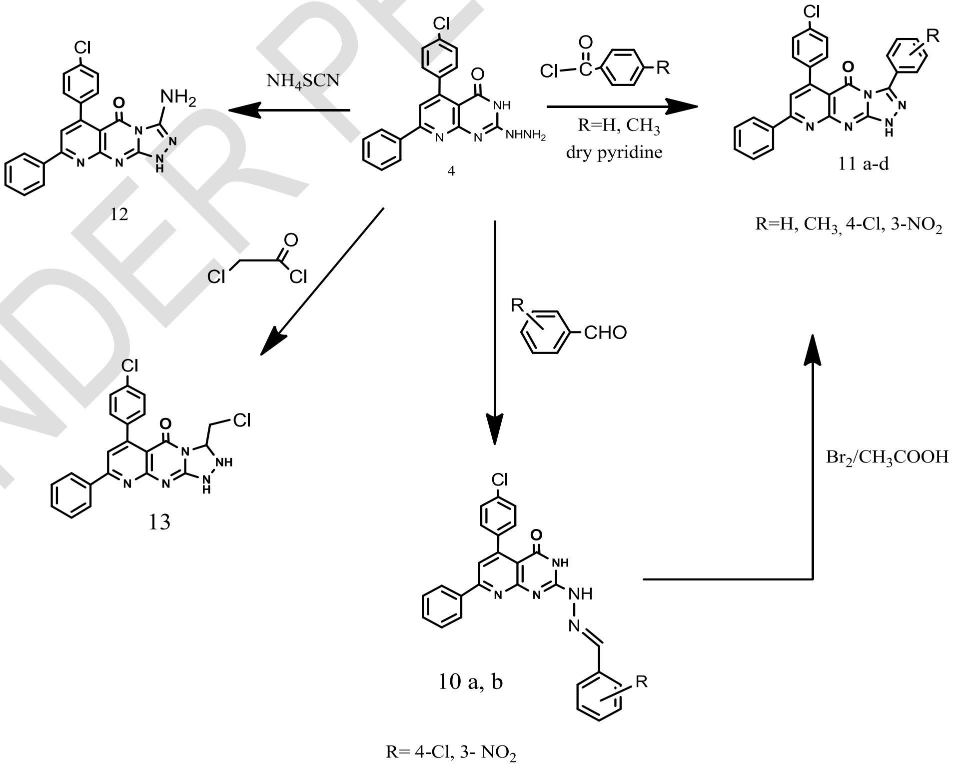


Fig no. 10, Synthesis of 3-aryl, 3-amino and 3-chloromethyl derivatives

2.2 Ethyl 2-cyanoacetate as precursor:

Abhay et al., [33] reported molecules with pyridopyrimidine carboxylate moiety were found to exhibit antitumor activity [34]. 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 a sufficient quantity of ethylcyanoacetate (1), carbon disulfide (2) and DMF, by adding these reactants into ice cold KOH solution followed by stirring and cooling. Further dimethyl sulfate was added where the temperature was maintained at 20°C. After 12h reaction mixture was added to water to get ethyl 2-cyano-3,3-bis(methylthio) acrylates (3) **(Fig.11).**

In the second step, 2–substituted-4-(methylthio)–6-oxo-1,6-dihydropyrimidine-5-carbonitrile (4) was prepared using ethyl 2-cyano-3,3-bis(methylthio) acrylates (3) 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 (4) is further treated with aromatic halo anilines like p-chloroaniline, p-fluroaniline, p-bromoaniline with alcohol refluxed for 1h which yields 4-(4-halophenylamino)-2-substituted-6-oxo-1,6-dihydro-pyrimidine-5-carbonitrile(5) **(Fig.13)**, which was further treated with malonic acid in ethanol, after refluxing for 1h yields "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 (6) which were the targeted compounds **(Fig.14).**

Anticancer activity of targeted pyridopyrimidines was 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.

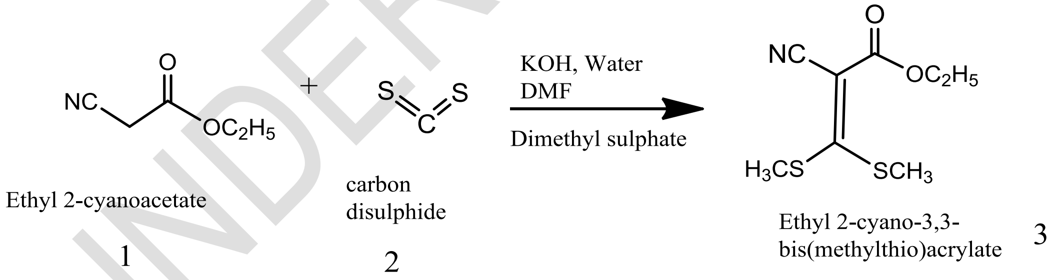


Fig no. 11, Synthesis of "ethyl 2-cyano-3,3-bis(methylthio)acrylate"

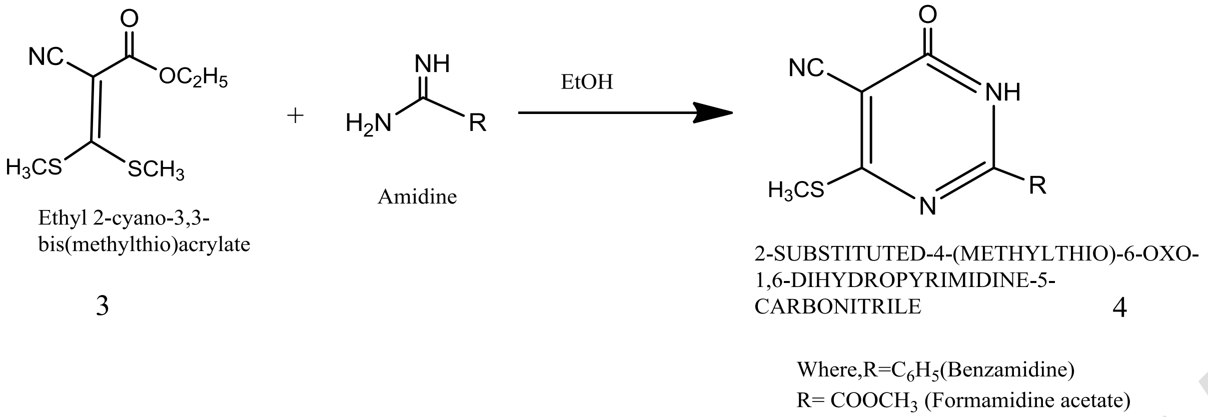


Fig no. 12, Synthesis of "2-substituted-4-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile"

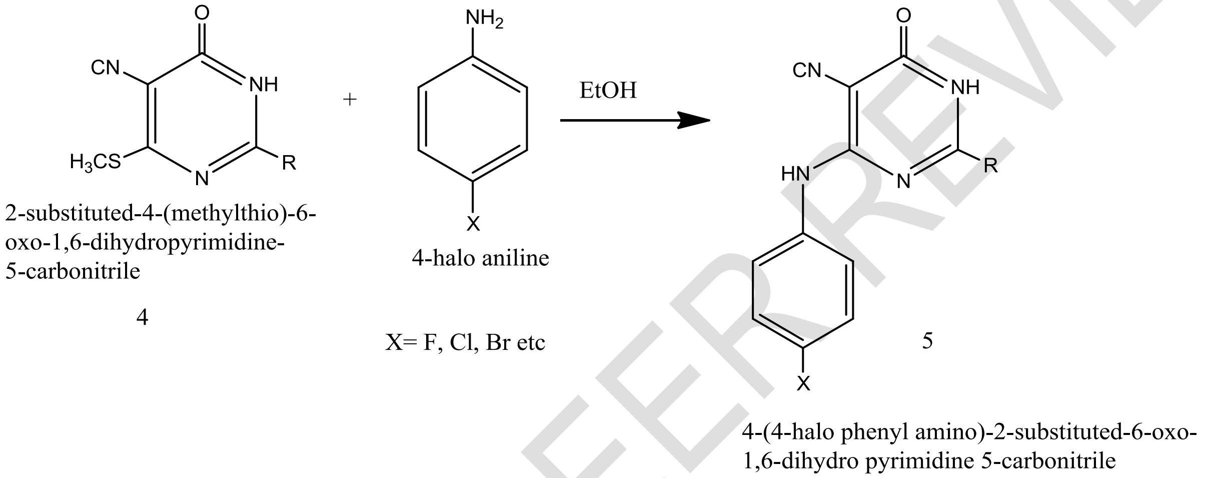


Fig no. 13, Synthesis of "4-(4-halo phenyl amino)-2-substituted-6-oxo-1,6-dihydro pyrimidine 5-carbonitrile"

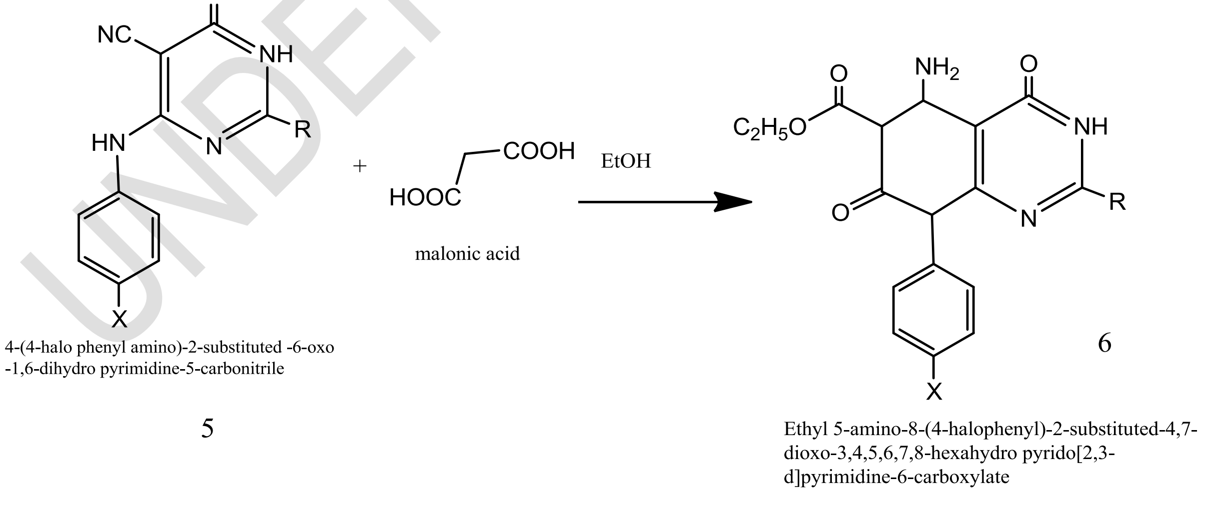
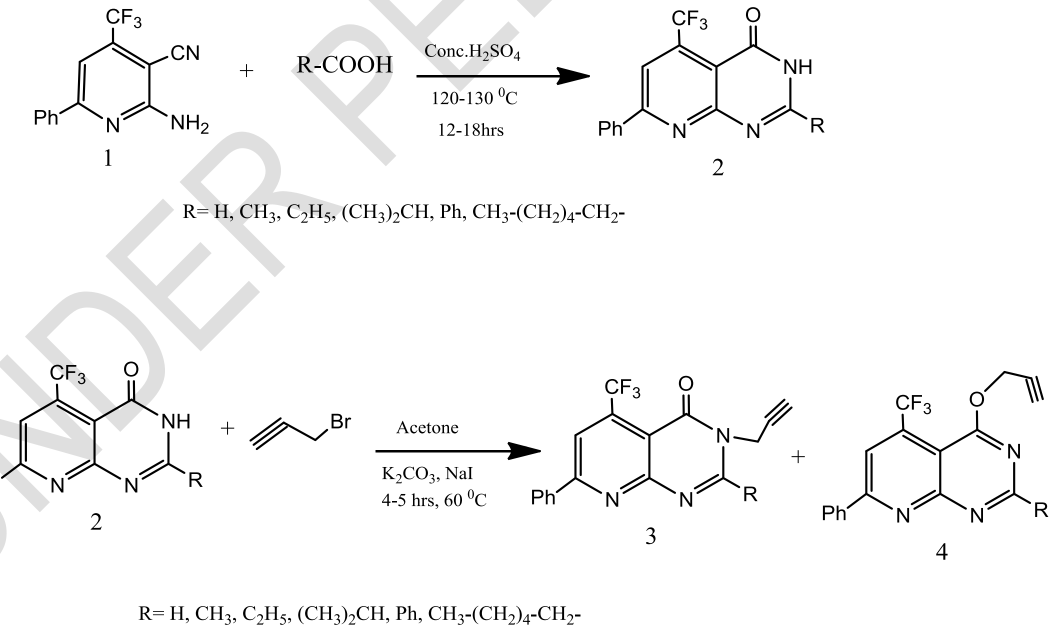


Fig no. 14. Synthesis of "ethyl 5-amino-8-(4-halophenyl)-2-substituted-4,7-dioxo-3,4,5,6,7,8-hexahydro pyrido[2,3-d]pyrimidine-6-carboxylate"

2.3 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 a 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.

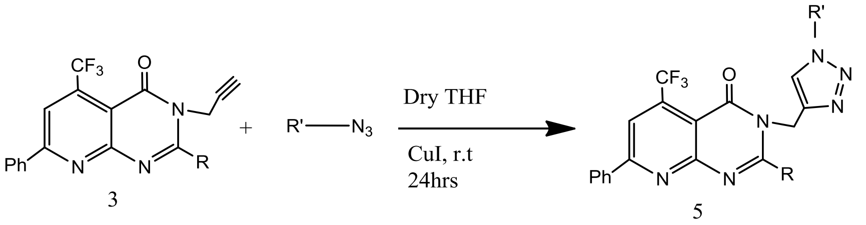
In their study "2-amino-3-cyano-4-trifluoromethyl-6-phenyl pyridine"(1) was used as starting precursor for the synthesis, which was made to react with different aliphatic acids in presence of a catalytic amount of H_2SO_4 at 120-130^{0}C for 12-20h, resulting in the acylation of amine and hydrolysis 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).**



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

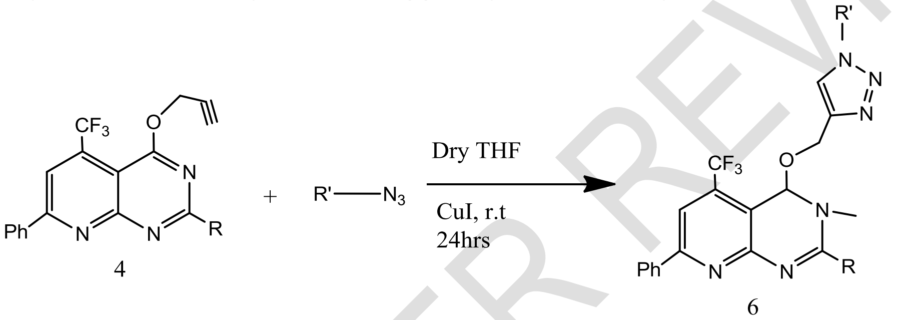
R= H, CH_3, C_2H_5, $(CH_3)_2CH$, Ph, CH_3-$(CH_2)_4$-CH_2-

Fig no. 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 no. 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. no.17. Synthesis of O-alkyltriazole tagged pyrido[2,3-d]pyrimidine derivatives[6a-6d]

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 the MTT assay method [41]. A decrease in cell viability was observed with compounds (5b) and (5e) in all 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, a 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, the 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 synthesized derivatives, better activity was observed with compound (5e) with 2- methyl substitution and 3-alkyl group. Substitution of perfluoroalkyl functionality on triazole is considered essential for detrimental effects. There is no added advantage on activity was observed by increasing the length of the 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.

2.4 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 the 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 multi-component reaction.

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

“5, 7-disubstituted pyrido[2,3-d]pyrimidine-4(3H)-ones” (6–10) and their 2-methyl analogs **(**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) undergo the direct condensation with urea at 260–300^{0}C result in the formation of the target compound i.e “4-amino-5,7-disubstituted-pyrido[2,3-d]pyrimidine-2(1H)-ones” (24–27).

Similarly, 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, 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, 21-23, 30, 32-34, 36, and 37 manifested a variable degree of inhibition towards three different tested cell lines, remaining compounds were either slightly active or 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 shown 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 the 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 the 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 substituent's nature (X and/or R) together with ring moiety (mono/bicyclic), exerts impact on the cytotoxic potency.

In this regard derivatives which have 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 a 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 2nd position is isostatically replaced with thione group in pyrido[2,3-d]pyrimidine-2(1H)-ones (24–27), resulted in two weakly active molecules, namely, 30 ($X = Br$; $R = 3,4\text{-}(OCH_2O)C_6H_3$) and 32 ($X = OCH_3$; R=2-thienyl). But further improved cytotoxic activity was observed 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.

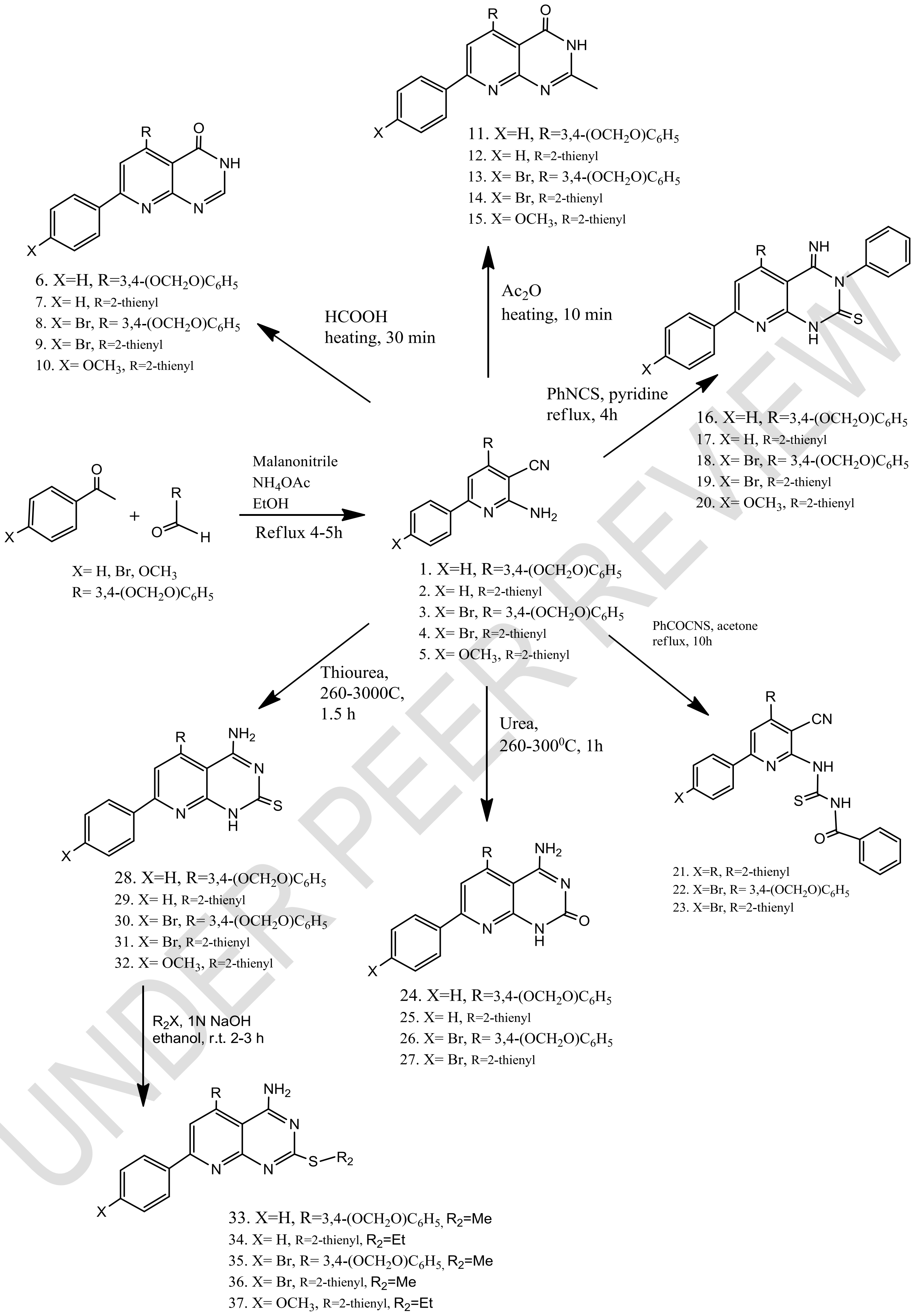


Fig no.18. Synthesis of compounds 1-37

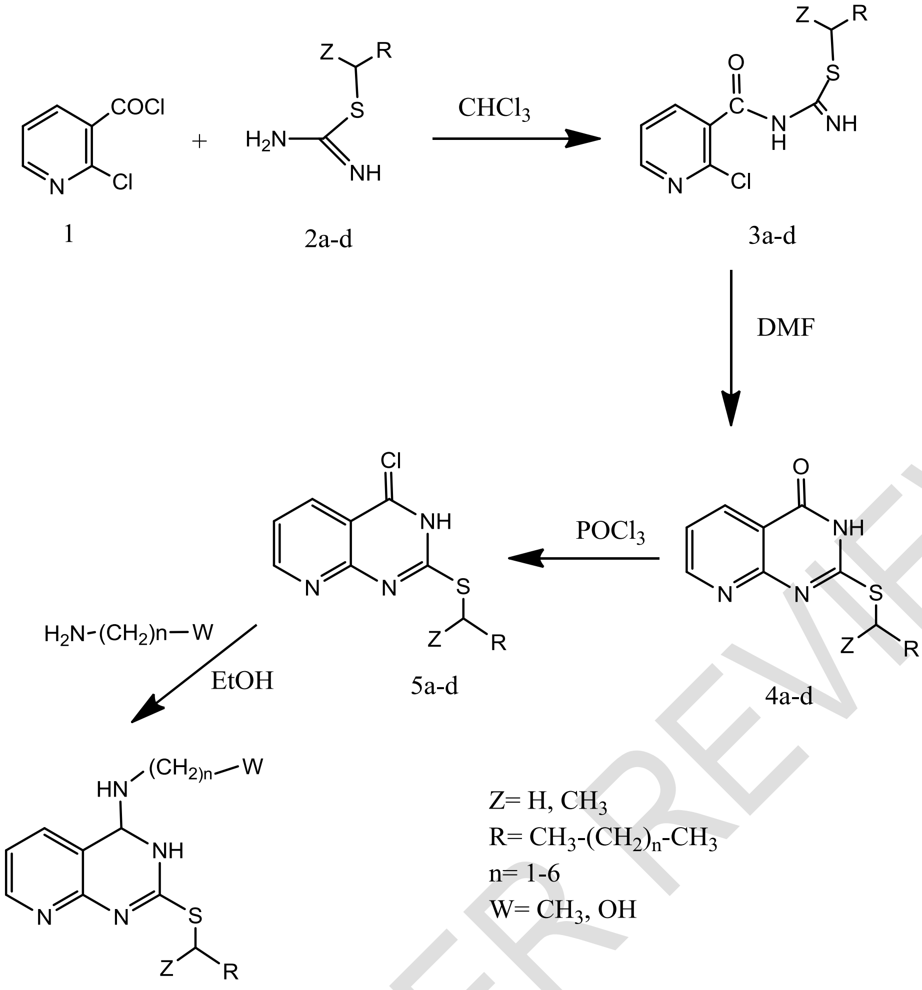
2.5. 2-chloro-3-pyridine carboxylic acid as precursor:

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 4th and 2nd positions between the side-chain substituents and planar ring system.

As per the literature survey, in recent years, the class of "pyrido[2,3-d]pyrimidines" developed as inhibitors that antagonize a variety of biological targets involved in 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 imidothiocarbamate hydroiodides (2a–d) [53] and triethylamine in a molar ratio of 1:1:2 resulted in the formation of compounds (3a-d) with 62 – 82% yield, These are good intermediates for the next class of derivatives (4a-d) 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 these compounds (4a-d) reacts with $POCl_3$ where DMF was added as catalyst yield compounds (5a-d). Compounds (5a-d) were made to reflux with selected amines in ethanolic medium forms moderate yields of (6a-s) **(Fig. 19)**. In scheme 2, the 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) was done by following reported methods in literature [54].

2-chloro-3-pyridine carboxylic acid (7) was the starting material and undergoes hydrolysis which yields "2-hydroxy-3-pyridine-carboxylic acid" (8). Further Nitration of compound (8) with nitrating mixture i.e., concentrated sulfuric acid and concentrated nitric acid results in the formation of compound (9), which was modified by reacting it with $POCl_3$ with absolute ethanol into diethyl derivative (10). This diethyl derivative (10) undergoes cyclization with ethyl imidothiocarbamate under reflux produced compound (13). Compound (13) was also be synthesized by treatment of compound (9) with $SOCl_2$ followed by the addition of absolute ethanol to give compound (11), which undergoes halogenation with $POCl_3$ where chlorobenzene as the solvent resulted in the formation of compound (12). Similar to derivative 9, compound (12) undergoes cyclization with ethyl imidothiocarbamate to give (13). Further halogenation of compound (13) at the carbonyl group of 4th position with $POCl_3$ yields compound (14). This derivative was made to react with n-pentylamine to yield compound (15). Nitro group of (15) undergoes a further reduction in presence of freshly prepared iron(II)hydroxide in water/DMF converted into an amino group to give compound 16 **(Fig. 20)**.



1

2a-d

3a-d

4a-d

5a-d

6a-s

Z= H, CH_3

R= CH_3-$(CH_2)_n$-CH_3

n= 1-6

W= CH_3, OH

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. no. 19. Synthetic pathway for compounds 1-6

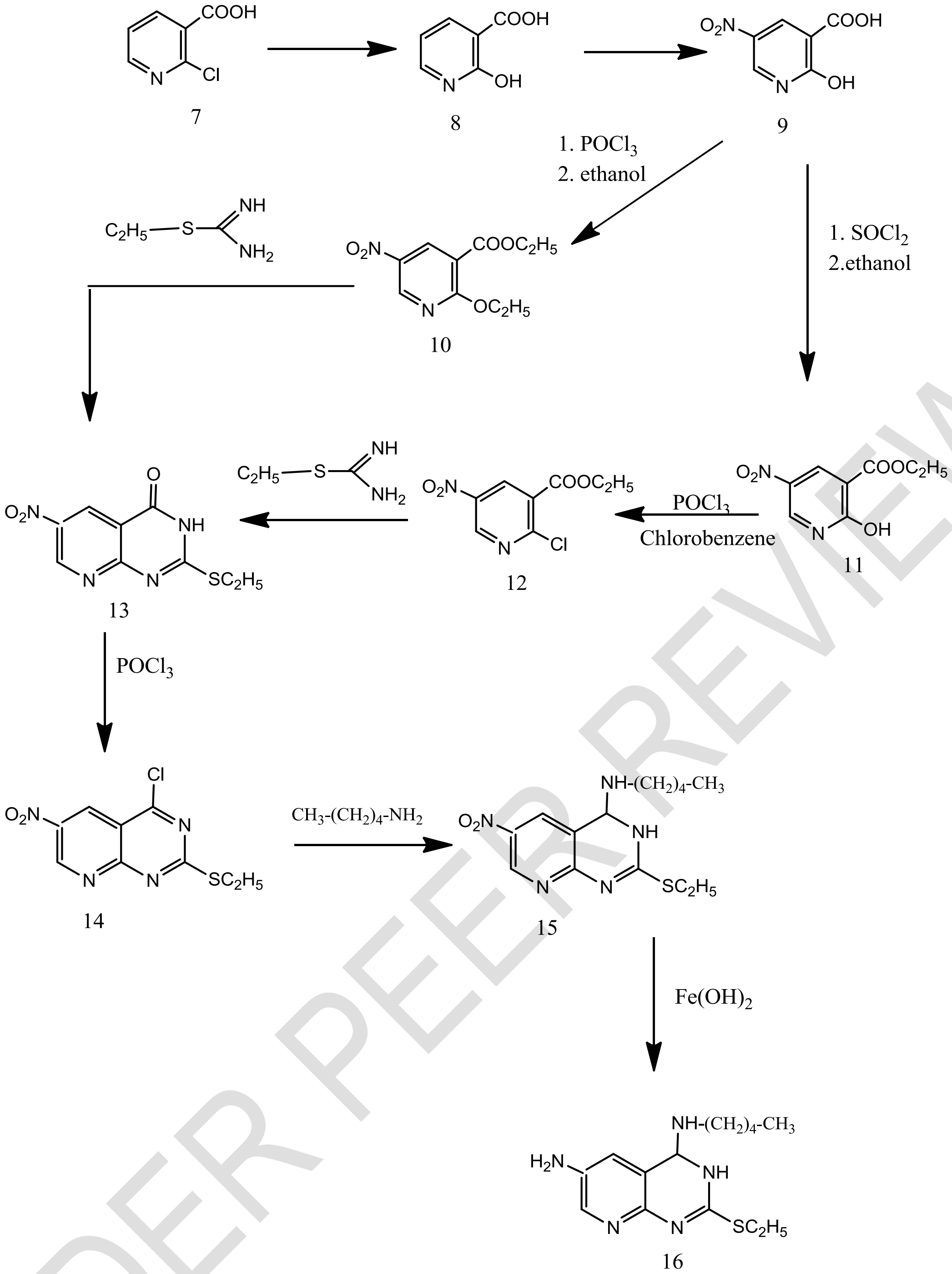


Fig.no. 20. Synthetic scheme for compounds 7-16

Evaluation of the cytotoxicity of the compounds **was** performed in 3 different cancer cell lines, bladder T-24 (bladder cancer call line), HT-29 (colon cancer cell line), and MD- MBA-231 (breast cancer cell line). According to the neutral red assay explained by Lowik and Alblas [55] evaluation of compounds for anticancer properties at concentrations of 100 and 20µM. Camptothecin was used as a standard molecule and its IC_{50} values were 0.29µM in MD-MB-231, 0.014µM in HT-29, and 0.009µM 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 a minimum of 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 the value of 4.3 in T-24, 6.4 in HT-29 and 9.3 in the MD-MBA-231 cell line. In the 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) result in a great increase in the caspase-3 levels in two of the tested cell lines, and derivatives (6e) and (6o) result in a 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 ranging from 95–100% in at least one of the tested nontumoral cell lines like CRL-8799 or CRL-11233.

2.6. 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 a 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-amino-nicotinic acid (1) was the starting material, which was made to react with butyryl 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 (2) with hydrazine hydrate in C_2H_5OH in a 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.

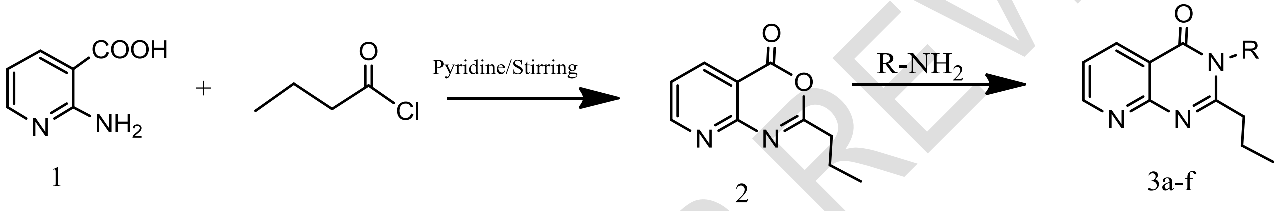
3b can also be obtained by refluxing compound (2) in formamide under heat. The reaction of compound (2) with hydroxylamine resulted in the formation of “3-hydroxy-2-propylpyrido[2,3-d]pyrimidin-4(3H)-one” (3c). Meanwhile, compounds (3d-f) were synthesized by treating compound (2) with aromatic amines like ortho, meta, and para toluidine **(Fig. 21).**

On the other hand, “3-(Arylylideneamino)-2- propyl-pyrido [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-propyl-pyrido [2,3-d]pyrimidin- 4(3H) -one” (3a) with different aldehydes resulted in the formation of “3-(Arylylideneamino)-2-propyl-pyrido [2,3-d]pyrimidin -4(3H)- ones” (4a-k) in ethanol-water (method B) or boiling water (method C).

At first, a saturated aqueous solution of KI was used to dissolve the required quantity of molecular iodine to prepare a solution of I_2/KI. Further refluxing different aromatic aldehydes with “3-amino-2-propyl-pyrido[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-propyl-pyrido [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 different cell lines panel comprising of MCF7 (breast cancer cell line), SF-268 (glioblastoma cell line), and NCI-H 460(lung cancer cell line). 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 the 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.



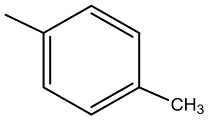
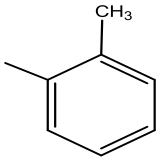
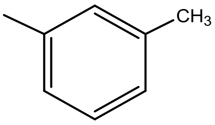
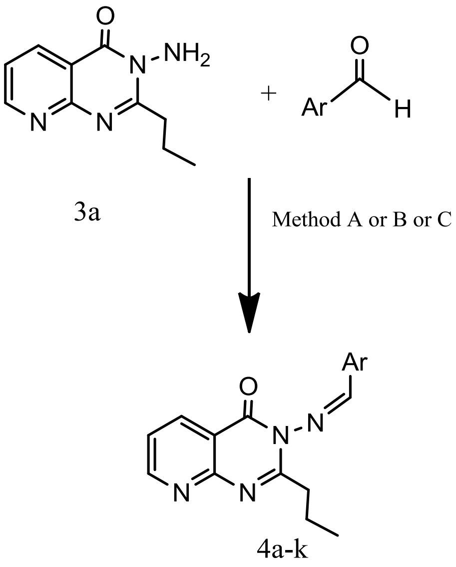
COMPOUND	R	REAGENT AND CONDITION	TIME(h)
3a	NH_2	NH_2NH_2/EtOH	0.5
3b	H H	Formamide CH_3COONH_4, Oil bath/150^{0}C	3 3
3c	OH	NH_2OH/EtOH Reflux	3
3d		EtOH Reflux	4.5
3e		EtOH Reflux	5
3f		EtOH Reflux	5

Fig.no.21. Synthesis of 3a-f



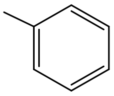
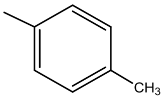
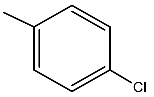
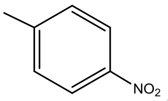
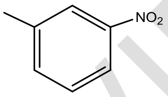
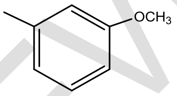
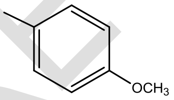
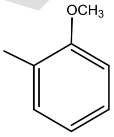
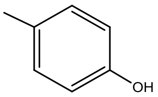
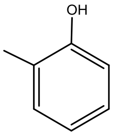
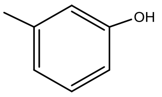
COMPOUND	Ar
4a	
4b	
4c	
4d	
4e	
4f	
4g	
4h	
4i	
4j	
4k	

Fig no. 22. Synthesis of 4a-f

3. CONCLUSION

This review highlights the various synthetic approach and anticancer potential of substituted pyridopyrimidines. Libraries can be developed by selecting the best pathway for synthesis. From this review, it was seen the potency of pyridopyrimidines in the area of cancer research, by considering different types of cancers. 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 the number of starting materials, 2-thioxopyrimidine is found to be mostly employed in the synthesis of pyridopyrimidines. From the SAR point of view, in the reported pyridopyrimidines important substitution for the better anticancer activities on pyridopyrimidine core moiety are discussed i.e., 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 with important substitution, which will be helpful for the further SAR studies of pyridopyrimidines by considering the contribution of different substitution on it and potent molecules can be developed further.

4. CONSENT: It is not applicable.

5. ETHICAL APPROVAL: It is not applicable.

6. 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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