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# Bicyclic 6 + 6 systems: the chemistry of pyrimido[4,5-*d*]pyrimidines and pyrimido[5,4-*d*]pyrimidines

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The present study provides an overview of the chemistry and biological significance of pyrimido[4,5-*d*]pyrimidine and pyrimido[5,4-*d*]pyrimidine analogs as types of bicyclic [6 + 6] systems. The main sections include: (1) synthesis methods; (2) the reactivities of the substituents linked to the ring carbon and nitrogen atoms; and (3) biological applications. A discussion demonstrating the proposed mechanisms of unexpected synthetic routes is intended. The aim of this study is to discuss the synthetic significance of the titled compounds and to establish the biological characteristics of this class of compounds as studied to date, where the compounds have been applied on a large scale in the medical and pharmaceutical fields. This survey will help researchers in the fields of synthetic organic and medicinal chemistry to undertake and improve new approaches for the construction of new standard biological components.

## 1. Introduction

Pyrimidopyrimidines or tetra-azanaphthalenes are two fused pyrimidine rings with four possible structural isomers. Consequently, they are bicyclic compounds with two nitrogen atoms in each ring and a possible single nitrogen atom at the ring junction. Recently, the chemistry of pyrimidopyrimidines has

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gained great importance as a result of the diverse biological applications and the synthetic importance on the large scale. Accordingly, this class of compounds inhibits the growth of cancer cells,[1] the deamination of adenosine caused by blood[2] and the formation of thrombi in rabbits.[3] Dipyridamole (Fig. 1) is a drug that prevents the formation of blood clots when given chronically but causes vasodilation in high doses over a short period of time. It is one of the most important biochemical agents, and was applied *in vivo* on a large scale, by synergistically improving synergistically the biochemical paths: (1) it acts as an inhibitor for cAMP-phosphodiesterase platelets; (2) supports adenosine inhibition of thrombocytopenia by preventing the absorption of vascular and blood cells; (3) strengthens PGI2 anti-aggregation activity and enhances the bio-synthesis of PGI2; and (4) decreases pulmonary hypertension.[4–7]

Furthermore, pyrimido[4,5-*d*]pyrimidines (Fig. 1) revealed potent activities as anticancer,[8] antioxidant,[9] dihydrofolate reductase,[10] type 2 diabetes treatment,[11] antiangiogenic,[12] resistance-modifying,[13] anti-myco-bacterium tuberculosis,[14] antibacterial,[15] antihypertensive,[16] anti-inflammatory,[17] anti-allergic,[18,19] antitumor, antiviral,[20–22] and hepatoprotective agents.[23] Moreover, pyrimidopyrimidines are conveyed as receptors for tyrosine kinase[24] and have been applied in the biosynthesis of 5-phosphoribosyl-1-pyrophosphate.[25]

Presently, the synthetic procedures of compounds that have a pyrimido[4,5-*d*]pyrimidine skeleton are variable using different routes, including: (1) the multicomponent condensation reaction of formaline with uracil analogs and amines which gives a product that has antidepressant activity;[22] and (2) several procedures including the three-component condensation of aryl aldehydes, amines such as aniline, aminouracil, thiocyanate salts or aminopyrimidine derivatives and active methylenes under catalytic conditions in a basic or acidic medium,[26–38] in addition to other reported synthetic approaches.[39,40] On the other hand, pyrimidopyrimidines were synthesized using a regiospecific one-pot reaction under solvent-free and microwave irradiation conditions.[41] The

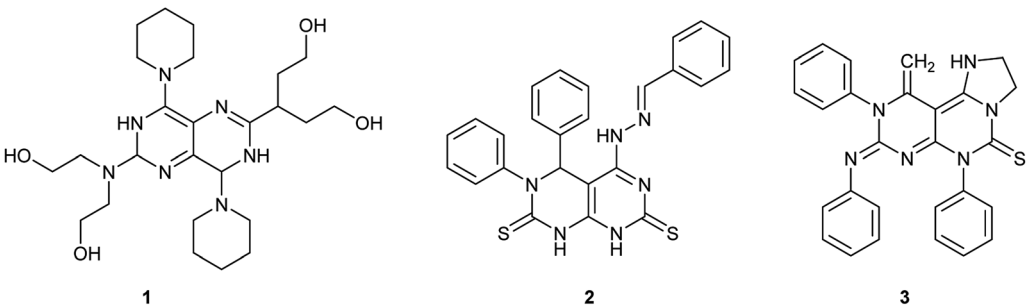


Fig. 1 Structures of the potent bioactive components.

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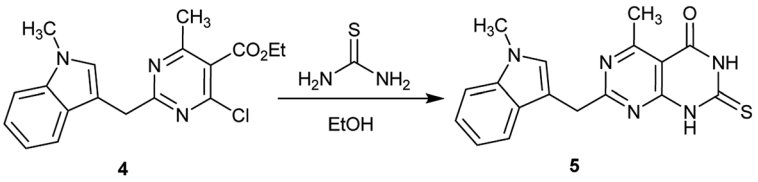
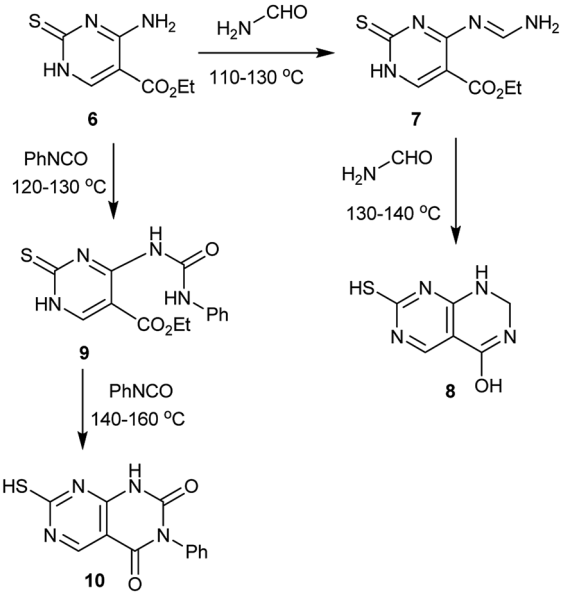



Scheme 1 Synthesis of 2-thioxo-2,3-dihydropyrimido[4,5-*d*]pyrimidin-4(1*H*)-one.

reported methods have several disadvantages, for example, the starting materials are not readily accessible or complex techniques, harsh conditions or multistep processes are required.[42,43]

The synthesis of the pyrimido[4,5-*d*]pyrimidines was first reported by Taylor *et al.*[18] who reported the reaction of the 4-aminopyrimidine-5-carboxamides with 4-amino-5-cyanopyrimidines and the application of the products as diuretic agents. Burch *et al.*[44] synthesized a series of *N*,*N*-disubstituted-2-(5-nitrofuran-2-yl)pyrimido[4,5-*d*]pyrimidin-4-amines by treating *N*-(5-cyanopyrimidin-4-yl)-5-nitrofuran-2-carboxamide with sulfuric acid followed by heating, intramolecular cyclization and subsequent chlorination with phosphorous pentasulfide/thionyl chloride and nucleophilic substitution with amines. Sharma *et al.* also reported the synthesis of 4,6-diaryl-3-(aryldiazenyl)-7-thioxo-4,6,7,8-tetrahydro-pyrimido[4,5-*d*]pyrimidine-2,5-(1*H*,3*H*)-diones as antimicrobial agents[37] or from a regioselective route by a Diels–Alder type cycloaddition from the reaction of a tetrahydropyrimidine derivative as a Biginelli type compound with *N*-arylidine-*N*′-methylformamidines and *N*-arylidine guanidine in toluene.[45] In addition, Saravanan *et al.* reported the synthesis of a series of pyrimido[4,5-*d*]pyrimidines as nucleoside transport inhibitors with improved *in vivo* pharmacokinetic properties and reduced α1-acid glycoprotein (AGP) binding relative to dipyridamole.[46] Recently, the investigated compounds were prepared from the reaction of 2-amino-4,6-dihydroxypyrimidine with 4,6-dihydroxy-pyrimidine using a multi-step synthesis process, in which the compounds have potential for use against *in vitro* HBV DNA replication inhibition and as nucleoside transport inhibitors.[47] Patil *et al.*[48a] reported the synthesis of substituted dihydro-2*H*-dipyrimido[1,2-*a*,4,5-*d*]pyrimidine-2,4-(3*H*)-diones through a convenient route using the multicomponent reaction of barbituric acid with aryl aldehydes and 2-aminopyrimidine in refluxing ethanol in the absence of a catalyst. In addition, Delia[48b] reported the hydration and ring-opening of a series of 2,4-disubstituted-pyrimido[4,5-*d*]pyrimidines with the formation of 4-amino-5-formyl-2,6-disubstituted-pyrimidines. Recently, Hao *et al.*[48c] reported the synthesis of a series of pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones as selective inhibitors for EGFR. Therefore, the multistep-synthesis was initiated by the protection of benzene-1,3-diamine followed by reaction with ethyl 2,4-dichloropyrimidine-5-carboxylate, arylamines, intramolecular cyclization, acid hydrolysis and substitution with acryloyl chloride under catalytic conditions.

The present review is an extension of our studies on the chemistry of heterocyclic compounds[49–51] with the aim of establishing the synthetic and biological importance of the pyrimido[4,5-*d*]pyrimidines and pyrimido[5,4-*d*]pyrimidines that have been widely applied in the fields of medicinal and pharmaceutical chemistry.

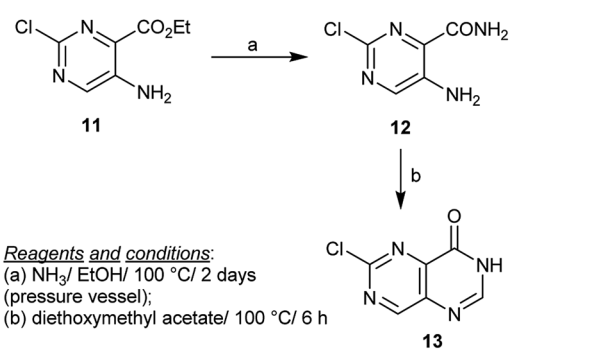



Scheme 2 Synthesis route from ethyl 4-amino-2-thioxo-1,2-dihydropyrimidine-5-carboxylate.

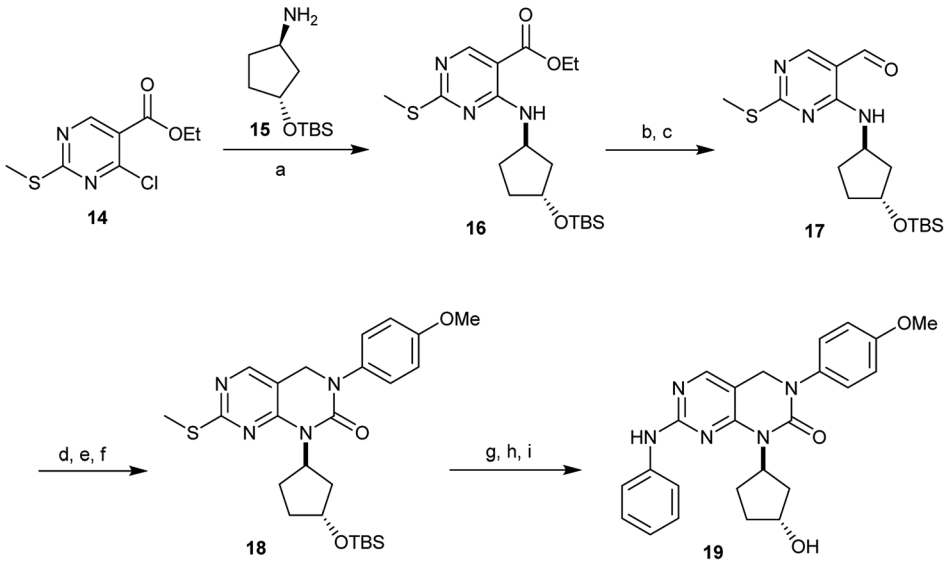
## 2. Synthetic methods

### 2.1. Synthesis of substituted pyrimidopyrimidines

**2.1.1. Synthesis from alkyl pyrimidine-carboxylates.** The reaction of ethyl 2-(1-methyl-1*H*-indol-3-yl)pyrimidine-5-




Scheme 3 Synthesis of 6-chloropyrimido[5,4-*d*]pyrimidin-4(3*H*)-one.

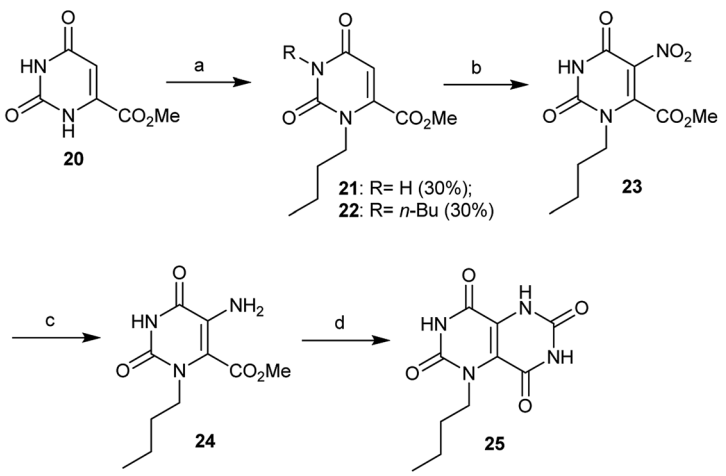



*Reagents and conditions*: (a) dioxane, 1 equiv of 2, 2 equiv $Et_3N$ reflux; (b) THF, 3.2 equiv $LiAlH_4$, rt; (c) $CH_2Cl_2$, 10 equiv $MnO_2$ (62% yield for three steps); (d) toluene, 1 equiv p-methoxy-aniline, cat. TsOH, reflux; (e) THF, 3 equiv $LiAlH_4$, rt; (f) $CH_2Cl_2$, 3 equiv $Et_3N$, 1.1 equiv $COCl_2$ 20% in toluene (57% yield for three steps); (g) THF, 2.1 equiv of mCPBA, rt; (h) aniline, 100–110 °C; (i) 25% TFA in $CH_2Cl_2$, 0 °C (73% yield for three steps).

Scheme 4 Synthesis of 1,3,7-trisubstituted-3,4-dihydro-pyrimido[4,5-*d*]pyrimidin-2(1*H*)-one (**19**).

carboxylate **4** with thiourea in refluxing ethanol containing triethylamine yielded 1*H*-indolyl-dihydropyrimido[4,5-*d*]pyrimidinone **5** (Scheme 1). The activity of compound **5** was evaluated as an analgesic and antiparkinsonian agent, and the compound had moderate results in both tests.[52]

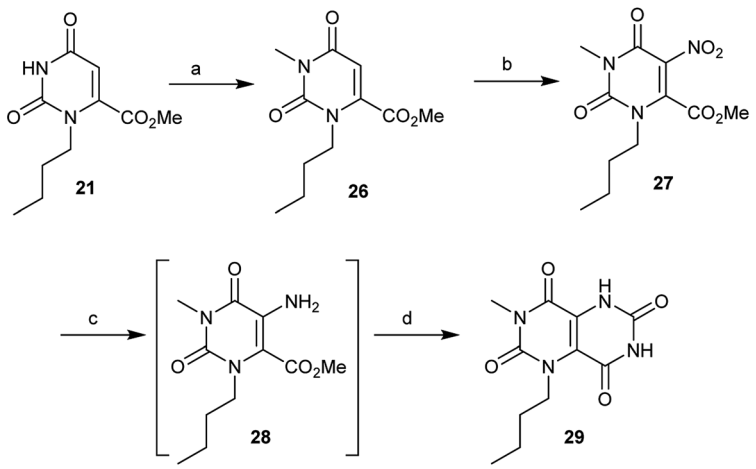
In another route, the temperature was the critical factor, below 110 °C no reaction takes place and above 140 °C, the products decompose with the formation of aminomercaptopyrimidine. Thus, heating ethyl 4-amino-2-thioxo-1,2-dihydropyrimidine-5-carboxylate (**6**) with formamide at 110–130 °C leads to the formation of the Schiff base intermediate **7**, which reacted with another mole of formamide 130–140 °C to give 7-mercapto-1,2-dihydropyrimido[4,5-*d*]pyrimidin-4-ol (**8**). On the other hand, heating of **6** with phenyl isocyanate at 120–130 °C generates the amide intermediate **9**, which reacted with another mole of phenyl isocyanate at 140–160 °C to give the desired compound 7-mercapto-3-phenylpyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione (**10**) (Scheme 2).[53]




*Reagents and conditions*: (a) NaH, *n*-BuI (1.0 equiv), DMF, rt; (b) $HNO_3/H_2SO_4$ (v/v = 1/4), 0 °C- rt, 90%; (c) Zn/AcOH; (d) urea, 210 °C, 30% over 2 steps.

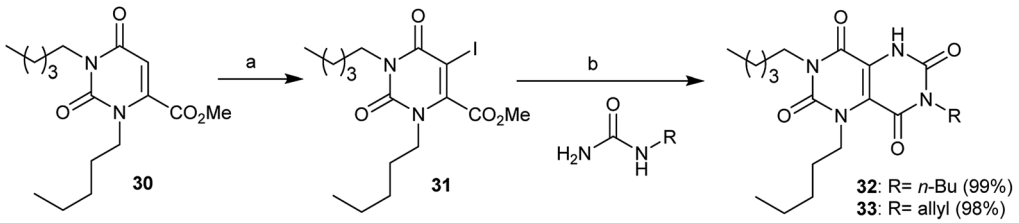
Scheme 5 Synthesis of 1-butyl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraone.




Reagents and conditions: (a) MeI (1.5 equiv), NaH (1.2 equiv), DMF, rt, 95%; (b) $HNO_3/H_2SO_4$ (v/v = 1/4), 0 °C-rt, 84%; (c) Zn/AcOH; (d) urea, 210 °C, 20% over two steps.

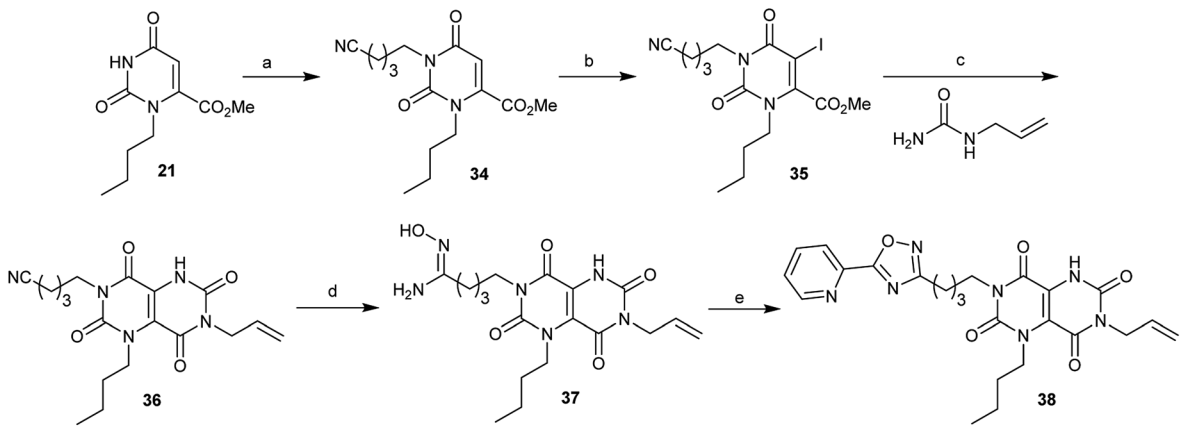
Scheme 6 Synthesis of 1,3-dialkyl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraone.




Reagents and conditions: (a) NIS, TFAA, TFA, 80 °C, 68%; (b) $Pd_2(dba)_3$, $Cs_2CO_3$, xantphos, dioxane, 100 °C.

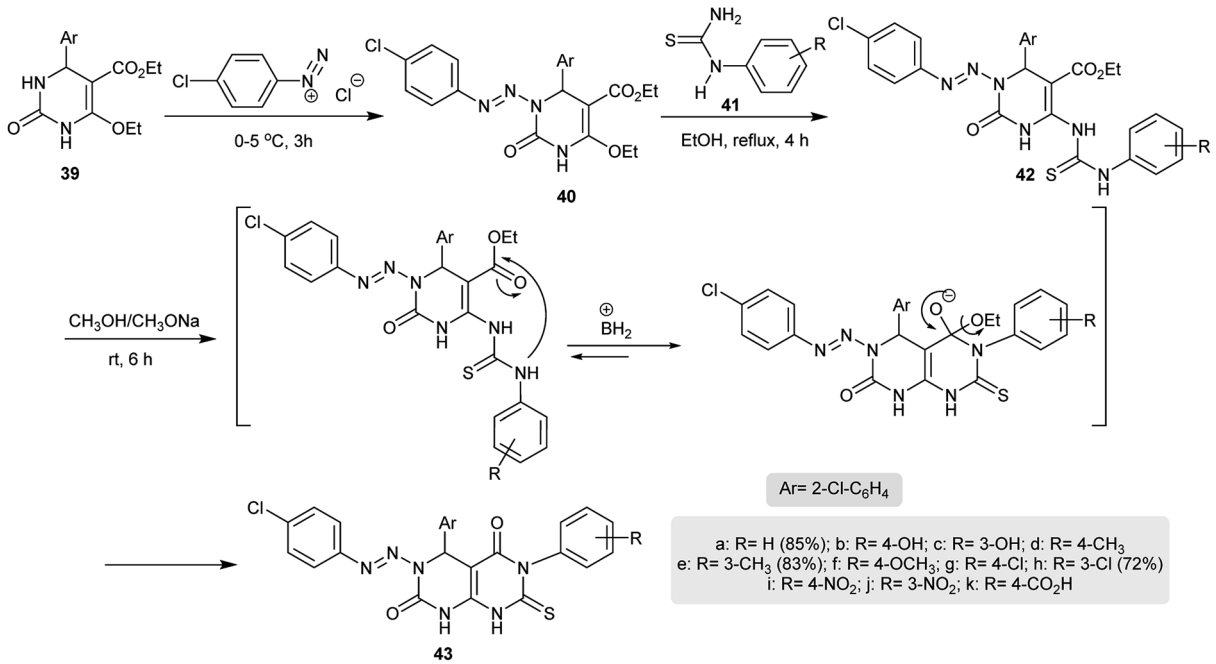
Scheme 7 Synthesis of 7-alkyl-1,3-dipentyl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetra-ones.

Heating of ethyl 5-amino-2-chloropyrimidine-4-carboxylate (**11**) with ammonia in ethanol yielded the respective amide **12**. The amide **12** reacted with triethyl orthoformate or with diethoxymethyl acetate to give the cyclization product **13** in a much higher yield, in the case of diethoxymethyl acetate two C=N bonds and one C–N bond were formed (Scheme 3).[24]



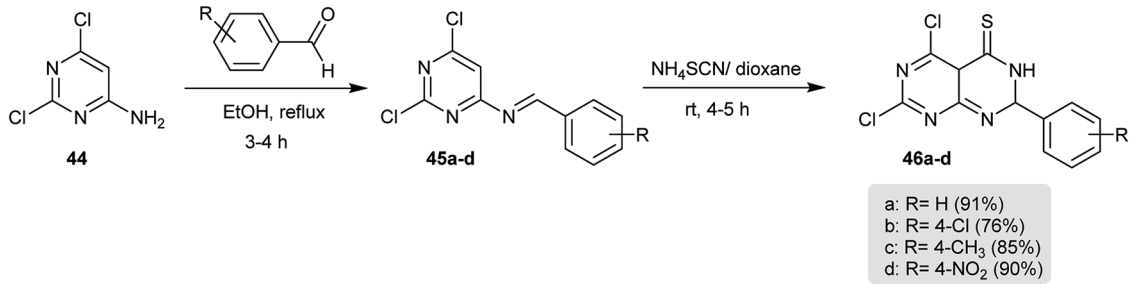

Reagents and conditions: (a) $Br(CH_2)_4CN$ (1.5 equiv), NaH (1.2 equiv), DMF, 90%; (b) NIS, TFAA, TFA, 80 °C, 68%; (c) $Pd_2(dba)_3$, $Cs_2CO_3$, Xantphos, dioxane, 100 °C, 63%; (d) $HONH_2HCl$, $K_2CO_3$, $EtOH/H_2O$, 66%; (e) 2-py$CO_2H$, CDI, DMF, 100 °C, 50%.

Scheme 8 Synthesis of $N_7$-alkyl-heteryl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraone.

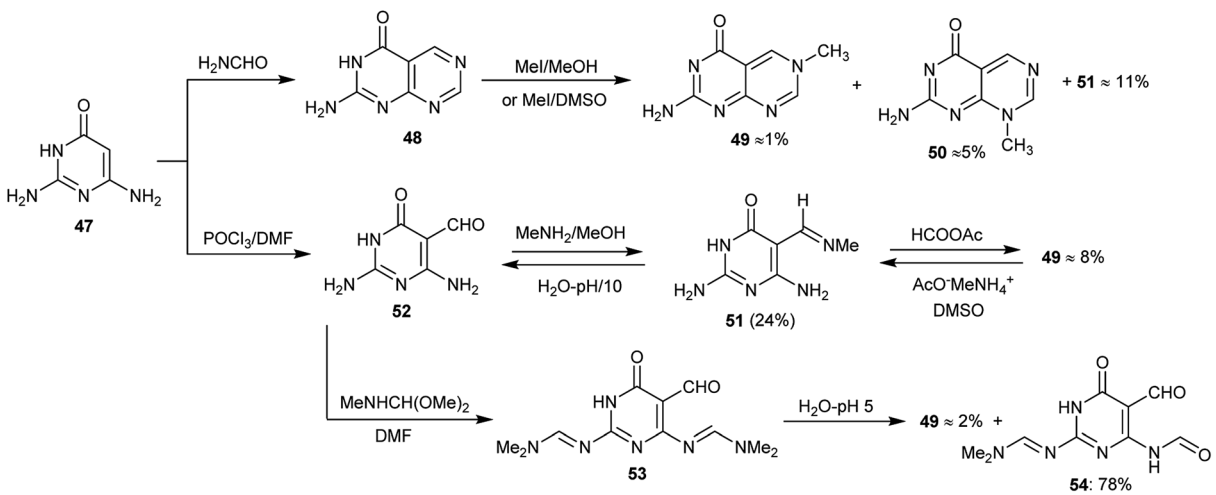



Scheme 9 Synthesis of 7-thioxo-4,6,7,8-tetrahydropyrimido[4,5-*d*]-pyrimidine-2,5(1*H*,3*H*)-diones.

Refluxing of ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate (**14**) with the racemic protected-(1*R*,3*R*)-3-((*tert*-butyl-dimethyl-silyl)oxy)cyclopentan-1-amine (**15**) in dioxane containing triethylamine gave the imine **16.** Reduction of **16** with lithium aluminum hydride followed by subsequent oxidation with manganese(IV) oxide furnished the respective

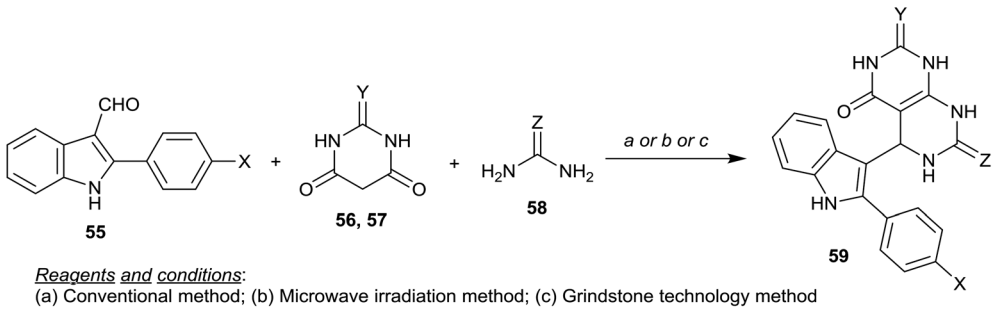



Scheme 10 Synthesis of 5,7-dichloro-2-aryl-2,4*a*-dihydropyrimido[4,5-*d*]pyrimidine-4(3*H*)-thiones.




Scheme 11 Synthesis of 2-aminopyrimidopyrimidinones.




*Reagents and conditions*:
(a) Conventional method; (b) Microwave irradiation method; (c) Grindstone technology method

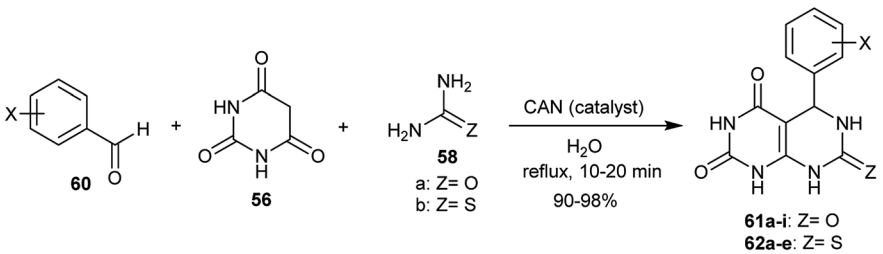
| Product 59 | X | Y | Z | Conventional yield % | Microwave irradiation yield % | Grindstone technology yield % |
|---|---|---|---|---|---|---|
| **a** | H | O | O | 62 | 86 | 92 |
| **b** | H | O | S | 60 | 90 | 94 |
| **c** | H | S | O | 65 | 84 | 92 |
| **d** | H | S | S | 64 | 88 | 90 |
| **e** | $CH_3$ | O | O | 58 | 90 | 92 |
| **f** | $CH_3$ | O | S | 65 | 85 | 92 |
| **g** | $CH_3$ | S | O | 62 | 88 | 94 |
| **h** | $CH_3$ | S | S | 55 | 90 | 92 |

Scheme 12 Synthesis of 5-(1*H*-indol-3-yl)-5,8-dihydropyrimido[4,5-*d*]pyrimidines.

aldehyde **17**. Condensation of the aldehyde **17** with *p*-anisidine yielded the imine intermediate which was reduced with lithium aluminum hydride and cyclized upon reaction with phosgene to give the respective pyrimidopyrimidinone **18**. Oxidation of **18** followed by reaction with aniline and hydrolysis of the protected group with trifluoroacetic acid gave the desired dihydro-pyrimido[4,5-*d*]pyrimidin-2(1*H*)-one **19** (Scheme 4). Compound **19** was evaluated as a tyrosine kinase inhibitor against KDR, FGFR, and PDGFR and found to be effective with a high bioavailability and plasma exposure at 25 mg $kg^{-1}$.[54]

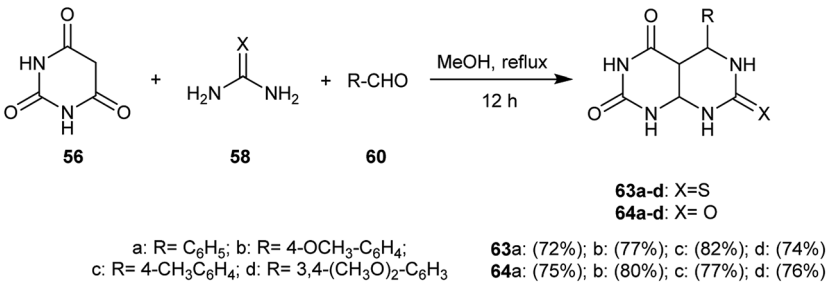
A regioselective synthetic route to prepare pyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraones **9** was reported by Huang *et al.*[55] In this route, methyl 2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (**20**) was alkylated with *n*-butyl iodide in the presence of sodium hydride to give the mono- and dialkylated products **21** and **22**. The alkylation with an alkyl halide took place at the N1 and N3 atoms. The nitration of **21** with $HNO_3/H_2SO_4$ (v/v = 1/4) yielded the nitro derivative **23** in a 90% yield. Reduction of **23** with Zn/AcOH gave the corresponding amine **24**, which was cyclized by reacting with urea to give the desired 1-butyl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraone (**25**) through the nucleophilic attack of the amino group of urea on the carbonyl ester of **24** and a subsequent cyclocondensation process (Scheme 5).

Similarly, methylation of methyl carboxylate **21** with methyl iodide in the presence of sodium hydride yielded the alkylation product **26**, which underwent nitration with $HNO_3/H_2SO_4$ to give the corresponding nitro derivative **27**. Reduction of **27** by treatment of zinc in acetic acid, followed by cyclization by heating with urea afforded the respective 1-butyl-3-methyl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraone (**29**) through the formation of the intermediate **28** (Scheme 6).[55]

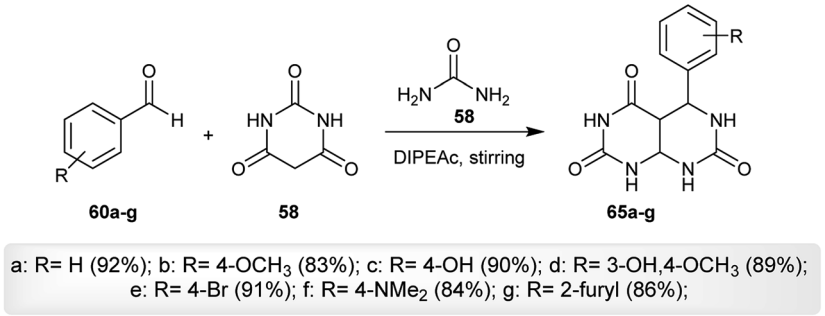



**61a**: X= H (92%); b: X= 4-Cl; (98%); c: X= 4-F (95%); d: X= 4-OH (95%); e: X= 2-OH (92%); f: X= 4-$CH_3$ (90%); g: X= 4-$OCH_3$ (98%); h: X= 2-$OCH_3$ (96%); i: X= 4-$NMe_2$ (95%)
**62a**: X= H (94%); b: X= 4-Cl; (98%); c: X= 4-OH (98%); d: X= 2-OH (96%); e: X= 4-$OCH_3$ (98%)

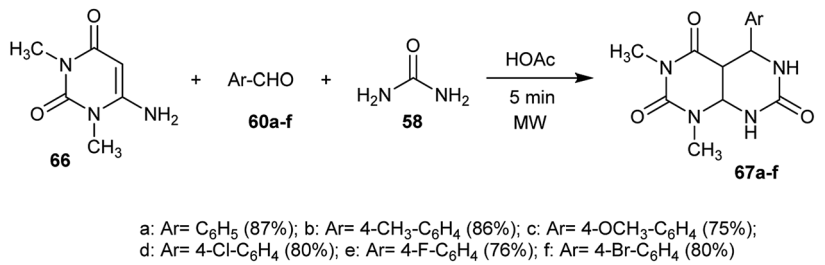
Scheme 13 Synthesis of 5-aryl-(7-thioxo and 7-oxo)-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine-diones.




Scheme 14 Synthesis of 5-aryl-7-(thioxo and oxo)-hexahydropyrimido[4,5-*d*]pyrimidine-2,4-diones.



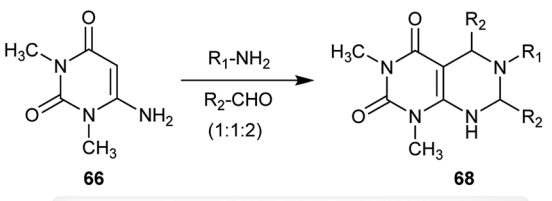

Scheme 15 Synthesis of 5-aryl-tetrahydro-pyrimido[4,5-*d*]pyrimidine-2,4,7(1*H*,3*H*,4a*H*)-triones.




Scheme 16 Synthesis of 1,3-dimethyl-5-aryl-tetrahydropyrimido[4,5-*d*]pyrimidine-2,4,7(1*H*,3*H*,4a*H*)-triones.

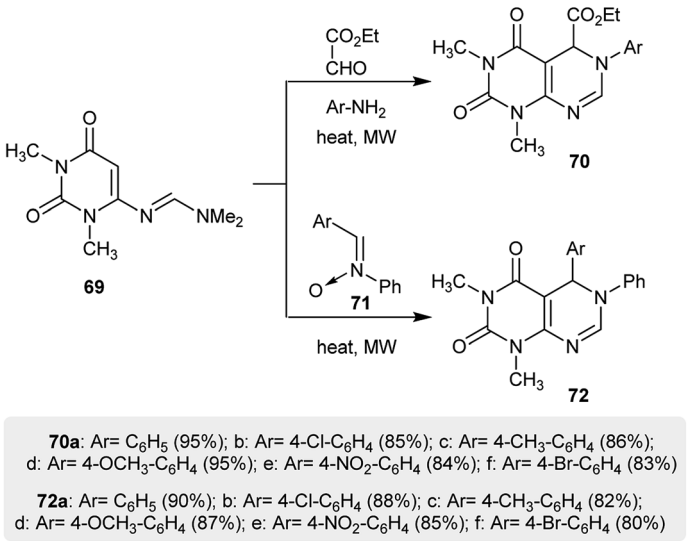
Furthermore, the iodination of 1-alkyl-methyl 2,6-dioxo-3-pentyl-1,2,3,6-tetrahydropyrimidine-4-carboxylate (**30**) was achieved by heating **30** in trifluoroacetic acid with NIS and TFAA to yield the iodo derivative **31**. The halogenation took place at C5. The palladium catalyzed cyclization reaction of **31** with alkyl urea derivatives in dioxane in the presence of cesium carbonate yielded 3,7-dialkyl-1-pentyl-1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraones **32** and **33** (Scheme 7).[55]

Additionally, alkylation of methyl 3-butyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidine-4-carboxylate (**21**) with 5-bromopentanenitrile in DMF containing sodium hydride gave the cyanoalkylation product **34**. The alkylation process took place at N1. Similar to the iodination of previous products (**30** → **31**), iodination of **34** with NIS in the presence of TFA and TFAA yielded the iodo derivative **35**. The cyclization step was proceeded by the reaction of **35** with allyl urea under palladium catalyzed conditions to give the cyclization product **36**. Nucleophilic addition of hydroxyl amine to the cyano function of tetraoxo-1,4,5,6,7,8-hexahydro-pyrimido[5,4-*d*]pyrimidine **36** in a mixture of ethanol/water containing potassium carbonate yielded the intermediate **37**, which was cyclized by the reaction with picolinic acid by heating in DMF containing catalytic CDI




Scheme 17 Multicomponent synthesis of 1,3-dimethyl-5,6,7-triaryl-5,6,7,8-tetrahydropyrimido[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-diones.



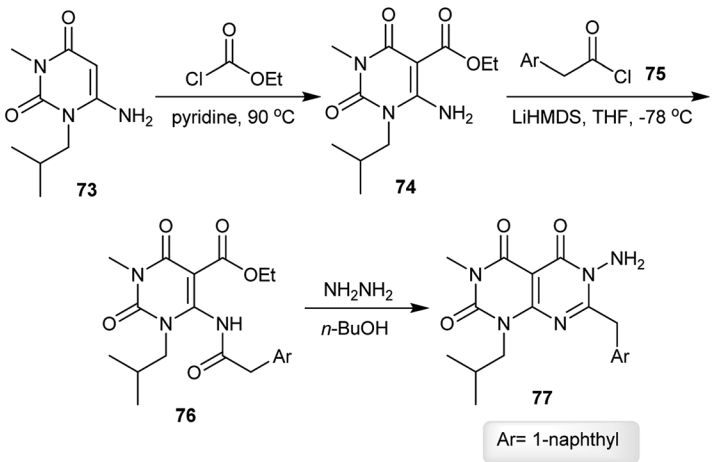

Scheme 18 Synthesis of hexahydro- and 5,6-dihydro-pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones.

to furnish the respective 1,2,4-oxadiazole **38** in 50% yield (Scheme 8).[55]

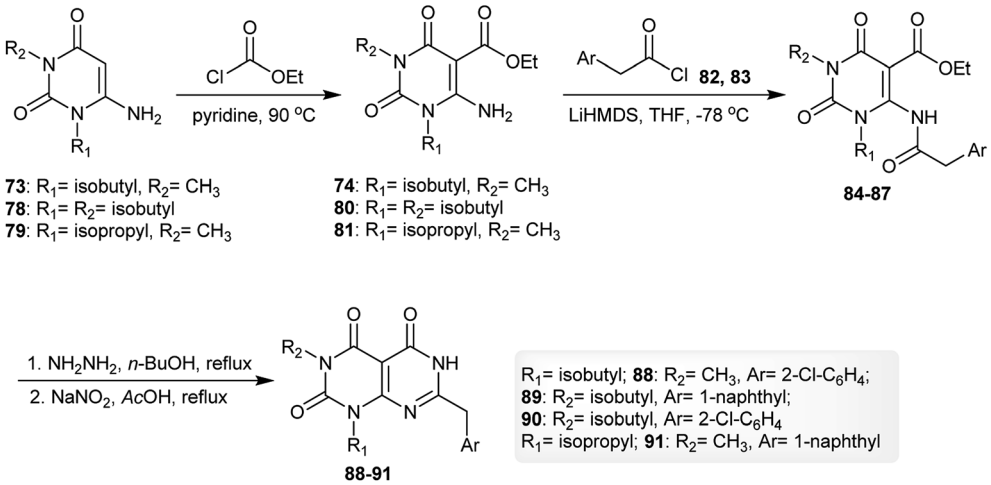
In another route, excellent yields of the desired 4,6-diaryl-3-(aryldiazenyl)-7-thioxo-4,6,7,8-tetrahydro-pyrimido[4,5-*d*]pyrimidine-2,5(1*H*,3*H*)-diones **43** were obtained through a multistep synthesis. Thus, diazocoupling of pyrimidinone **39** with the diazonium salt of *p*-chloroaniline under diazotization conditions gave the arylazo derivative **40**. Refluxing of **40** with *N*-arylthiourea derivatives in ethanol yielded compound **42**. Cyclization of **42** took place by treatment in sodium methoxide solution at room temperature by nucleophilic attack of the aryl-NH group on the carbonyl ester to afford the corresponding tetrahydropyrimido[4,5-*d*]pyrimidine-2,5(1*H*,3*H*)-diones **43** (Scheme 9). Compounds **43** were assessed *in vitro* as antimicrobial agents against *E. coli*, *P. diminuta*, *S. aureus*, *B. subtilis*, *A. niger*, and *C. albicans* species. The best results were noted for the compounds having hydroxy, chloro, nitro or methyl substituents in the meta position.[37]

**2.1.2. Synthesis from 4-amino-2,6-dichloropyrimidine.** The Schiff bases **45a–d** were prepared by condensation of 2,6-dichloropyrimidin-4-amine (**44**) with aromatic aldehydes in refluxing ethanol. Successive treatment of **45a–d** with ammonium thiocyanate in 1,4-dioxane yielded the cyclization products **46a–d**, respectively (Scheme 10).[56]

**2.1.3. Synthesis from 2,6-diaminopyrimidin-4(3*H*)-one.** Three methods for the preparation of 2-amino-pyrimidopyrimidinones have been reported by Gebauer *et al.*[10] although the products **48**, **49** and **50** were obtained with very low yields. The 2-aminopyrimido-pyrimidinones are analogs of 8-alkylpterins (N8-alkyl-2-aminopteridin-4(8*H*)-ones), which are well-known inhibitors for dihydrofolate reductase. The low yield of the products is due to the deficiency of the π-electrons of the heterocycles **49** and **50** which make the quaternized pyrimidine ring susceptible to the addition of a nucleophile with the promotion of the pyrimidopyrimidine ring-opening (Scheme




Scheme 19 Synthesis of 1,3-dialkyl-6-amino-(naphthalen-1-ylmethyl)pyrimido[4,5-*d*]pyrimidine-2,4,5-(1*H*,3*H*,6*H*)-trione.




Scheme 20 Synthesis of 7-alkylaryl-1,3-dialkyl-pyrimido[4,5-*d*]pyrimidine-2,4,5(1*H*,3*H*,6*H*)-triones.

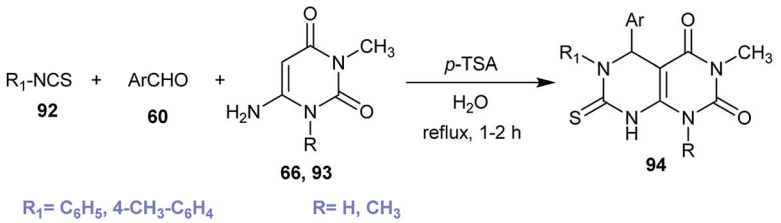
11). Compounds **49** and **50** showed remarkable activities as inhibitors of dihydrofolate reductase.

**2.1.4. Synthesis from barbituric and thiobarbituric acids.** Gupta *et al.*[57] reported proficient procedures for the synthesis of 5-(1*H*-indol-3-yl)-5,8-dihydropyrimido[4,5-*d*]pyrimidines **59** in dry media through multicomponent reactions of 2,3-disubstituted indoles **55** with barbituric or thiobarbituric acids **56, 57** and urea or thiourea **58** under microwave irradiation conditions. Compounds **59** were also prepared following conventional and grinding methods in good yields (Scheme 12). In addition, compounds **59a–h** were evaluated as antimicrobial agents against well-known microbial species, the results prove that compound **59d** has the ability to protect against microbial growth and that compound **59e** has a good activity against *F. oxysporum* at concentrations of 600, 800 and 1000 ppm. On the other hand, compounds **59a** and **59g** are potent agents against *P. aeruginosa* at 800 and 1000 ppm.[57]

An efficient procedure for the synthesis of two series of tetrahydropyrimido[4,5-*d*]pyrimidine-diones **61** and **62** has been reported through a Biginelli-type reaction of the aryl aldehydes **60** with barbituric acid **56** and urea or thiourea **58** in the presence of ceric ammonium nitrate (CAN) as a catalyst. The reactions proceeded in a one-pot multicomponent step in refluxing water and the products **61** and **62** were obtained in excellent yields (Scheme 13).[58]

Multicomponent condensation reactions of barbituric acid (**56**) with urea or thiourea (**58**) and aromatic aldehydes **60** in equivalent amounts in refluxing methanol yielded a series of 5-aryl-7-(thioxo and oxo-)-hexa-hydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **63a–d** and **64a–d** (Scheme 14).[59]

Three component reactions were employed for the preparation of a series of 5-aryl-tetrahydro-pyrimido[4,5-*d*]pyrimidine-2,4,7(1*H*,3*H*,4*aH*)-triones (**65a–g**) by reacting aryl aldehydes (**60a–g**) with barbituric acid (**56**) and urea (**58**) at room temperature *via* a one-pot procedure using diisopropyl-ethyl-ammonium acetate (DIPEAc) as an alternative solvent and a catalyst, which promotes the cyclocondensation step-reaction providing high yields from the reactions and reusability of the



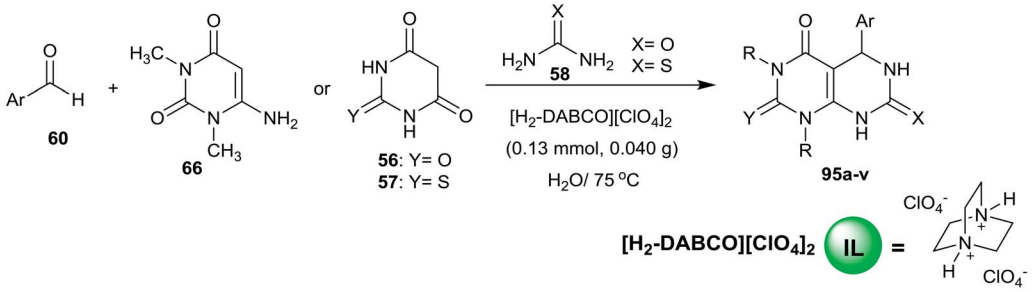

a: Ar= 4-Cl-$C_6H_4$, R= $CH_3$, $R_1$= $C_6H_5$ (70%); b: Ar= 4-$CH_3$-$C_6H_4$, R= $CH_3$, $R_1$= $C_6H_5$ (80%); c: Ar= 4-$OCH_3$-$C_6H_4$, R= $CH_3$, $R_1$= $C_6H_5$ (82%); d: Ar= 4-Br-$C_6H_4$, R= $CH_3$, $R_1$= $C_6H_5$ (71%); e: Ar= 4-$NO_2$-$C_6H_4$, R= $CH_3$, $R_1$= $C_6H_5$ (67%); f: Ar= 2-Br-$C_6H_4$, R= $CH_3$, $R_1$= $C_6H_5$ (63%); g: Ar= $C_6H_5$, R= H, $R_1$= $C_6H_5$ (73%); h: Ar= 4-Cl-$C_6H_4$, R= H, $R_1$= $C_6H_5$ (68%); i: Ar= 4-$CH_3$-$C_6H_4$, R= H, $R_1$= $C_6H_5$ (79%); j: Ar= 4-$OCH_3$-$C_6H_4$, R= H, $R_1$= $C_6H_5$ (80%); k: Ar= 4-$NO_2$-$C_6H_4$, R= H, $R_1$= $C_6H_5$ (65%); l: Ar= 4-Br-$C_6H_4$, R= H, $R_1$= $C_6H_5$ (66%); m: Ar= 2-Br-$C_6H_4$, R= H, $R_1$= $C_6H_5$ (60%); n: Ar= $C_6H_5$, R= $CH_3$, $R_1$= $C_7H_8$ (77%); o: Ar= 4-Cl$C_6H_4$, R= $CH_3$, $R_1$= $C_7H_8$ (72%); p: Ar= 4-$CH_3$-$C_6H_4$, R= $CH_3$, $R_1$= $C_7H_8$ (78%); q: Ar= 4-$OCH_3$-$C_6H_4$, R= $CH_3$, $R_1$= $C_7H_8$ (84%); r: Ar= $C_6H_5$, R= $CH_3$, $R_1$= $C_6H_5$ (75%);

Scheme 21 Synthesis of 5,6-diaryl-1-alkyl-3-methyl-7-thioxo-5,6,7,8-tetrahydropyrimido[4,5-*d*]-pyrimidine-2,4(1*H*,3*H*)-diones.

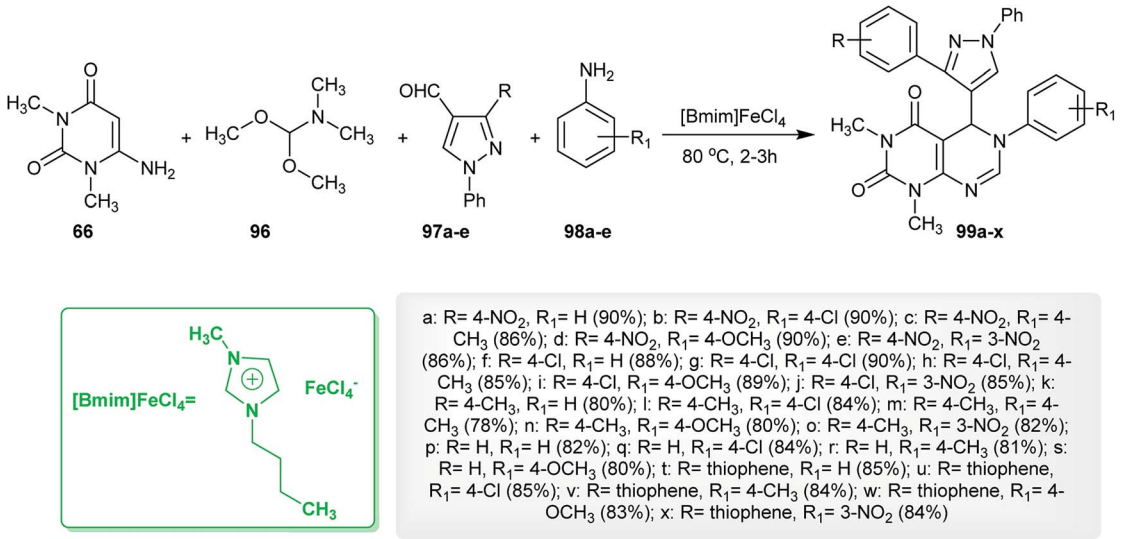
catalyst. The procedure provided high yields in a short time (Scheme 15). The desired tetrahydro-pyrimido[4,5-*d*] pyrimidine-triones (**65a–g**) were assessed *in vitro* as anticancer agents against MCF-7, HeLa, A-549, and MCF-10A cancer cell lines relative to the standard Adriamycin and tyrosinase inhibitors. The compounds showed moderate anticancer activities against all tested cell lines and compound **65f** was found to be the most potent agent relative to the results of the other compounds against MCF-7, HeLa, and A-549 cell lines. The compounds are inactive against the MCF-10A cancer cell line. The presence of nitrogen substitution at position 4 of the aryl ring is essential to achieving potency. On the other hand, compounds **65c**, **65d** and **65f** are tyrosinase inhibitors, while the other compounds are inactive agents. The presence of phenyl-substituted groups with stable radicals enhances the biological results.[60]

**2.1.5. Synthesis from 6-amino-1,3-dialkyl-pyrimidine-2,4(1*H*,3*H*)-dione.** The reaction of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**66**) with aryl aldehydes **60** and urea (**58**) in the presence of a catalytic amount of acetic acid under microwave irradiation conditions yielded the respective tetrahydropyrimido[4,5-*d*]pyrimidine-triones **67**. The best yield was obtained using acetic acid as a catalyst and the use of the electron-withdrawing substituents of the aldehydes. The reaction mechanism was proceeded by the initial condensation of one mole of the aryl aldehydes with two moles of pyrimidindione **66** followed by nucleophilic attack of the amino group of urea on the formed intermediate and subsequent intramolecular cyclization after the evolution of ammonia to give the tetrahydropyrimido[4,5-*d*]pyrimidine-2,4,7(1*H*,3*H*,4*aH*)-triones **67** (Scheme 16).[26]



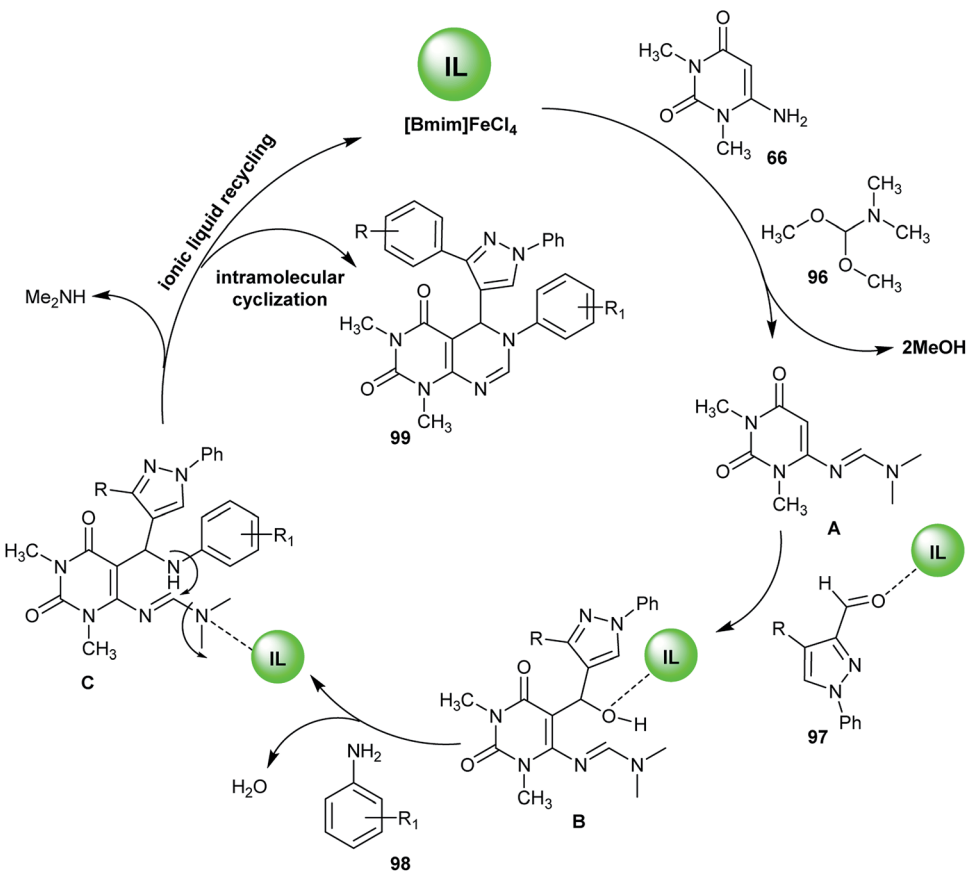

| Product | R | X | Y | Ar | Yield (%) | Reference |
|---|---|---|---|---|---|---|
| **a** | H | O | O | $C_6H_5$ | 90 | [64] |
| **b** | H | O | O | 4-Cl-$C_6H_4$ | 92 | [64] |
| **c** | H | O | O | 4-Br-$C_6H_4$ | 95 | [65] |
| **d** | H | O | O | 4-$NO_2$-$C_6H_4$ | 90 | [65] |
| **e** | H | O | O | 3-$NO_2$-$C_6H_4$ | 90 | [66] |
| **f** | H | O | O | 4-$OCH_3$-$C_6H_4$ | 87 | [65] |
| **g** | H | O | O | 4-$CH_3$-$C_6H_4$ | 90 | [65] |
| **h** | H | O | O | 4-OH-$C_6H_4$ | 85 | [58] |
| **i** | H | O | O | 2-OH-$C_6H_4$ | 85 | [58] |
| **j** | H | O | O | 4-$NMe_2$-$C_6H_4$ | 80 | [65] |
| **k** | H | S | O | $C_6H_5$ | 90 | [64] |
| **l** | H | S | O | 4-Cl-$C_6H_4$ | 90 | [64] |
| **m** | H | S | O | 3-$NO_2$-$C_6H_4$ | 88 | [66] |
| **n** | H | S | O | 4-$OCH_3$-$C_6H_4$ | 90 | [65] |
| **o** | H | S | O | 4-$CH_3$-$C_6H_4$ | 95 | [64] |
| **p** | H | S | O | 4-OH-$C_6H_4$ | 85 | [58] |
| **q** | H | S | O | 2-OH-$C_6H_4$ | 87 | [58] |
| **r** | H | S | S | $C_6H_5$ | 90 | [38] |
| **s** | H | S | S | 2-OH-$C_6H_4$ | 88 | [38] |
| **t** | $CH_3$ | O | O | 4-Cl-$C_6H_4$ | 80 | [26] |
| **u** | $CH_3$ | O | O | 4-Br-$C_6H_4$ | 82 | [26] |
| **v** | $CH_3$ | O | O | 4-$CH_3$-$C_6H_4$ | 78 | [26] |

Scheme 22 Catalytic multicomponent synthesis of 2,3,5,6,7,8-hexahydropyrimido[4,5-*d*]pyrimidinones.

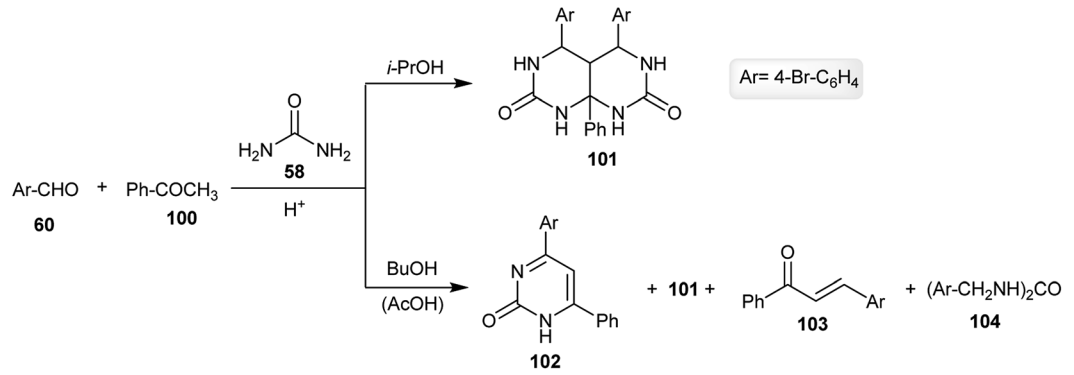



Scheme 23 Four-component synthesis of 5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones.

The multicomponent reaction of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**66**) with arylamines and aldehydes in a ratio of 1 : 1 : 2 yielded the respective 5,6,7-trisubstituted-1,3-dimethyl-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **68** in good to moderate yields. The best yield was noticed for the reaction of one equivalent of **66** with *p*-anisidine and 4-methoxybenzaldehyde (2 equivalents) for the preparation of compound **68c** (93%) and the other reactions gave the products with moderate to low yields. The use of this technique provided the best product yields in each case




Scheme 24 The proposed mechanistic route for the synthesis of 5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones.



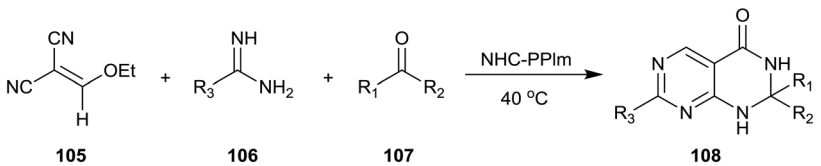

Scheme 25 Synthesis of hexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-dione.

depending on the substituents attached to the amines and aldehydes (Scheme 17).[61]

A procedure reported by Prajapati *et al.*[41] provided a proficient synthetic route for the synthesis of pyrimidopyrimidines **70** in a one-pot step with exceptional yields. Thus, [4 + 2] cycloaddition reactions of uracil **69** with various glyoxylate imines (which were *in situ* generated by the reaction of ethyl 2-oxoacetate with aryl amines) and imine oxides **71** were used to afford ethyl hexahydro-pyrimido-[4,5-*d*]pyrimidine-carboxylates **70** and 5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **72** under microwave irradiation conditions (Scheme 18).

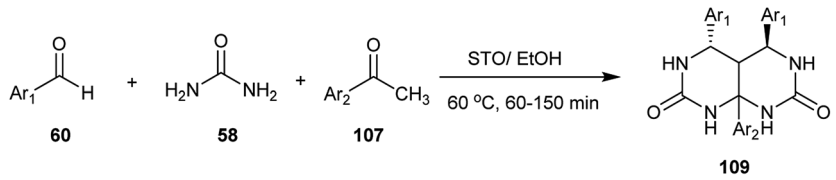
The reaction of 6-amino-1-isobutyl-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (**73**) with ethyl carbono-chloridate by heating in pyridine gave the respective ester **74**, which reacted with 2-(naphthalen-1-yl)acetyl chloride (**75**) in THF in the presence of LiHMDS to give the desired amide **76.** Cyclization of the amide **76** was achieved by reaction with hydrazine hydrate in *n*-butanol to afford pyrimido[4,5-*d*]pyrimidine-2,4,5-(1*H*,3*H*,6*H*)-trione **77** (Scheme 19).[62]

Similarly, using three aminouracils, pyrimido[4,5-*d*]pyrimidine-2,4,5(1*H*,3*H*,6*H*)-triones **88–91** were prepared in four step-reactions under the same conditions described for the synthesis




| Product 108 | $R_1$ | $R_2$ | $R_3$ | Yield (%) |
|---|---|---|---|---|
| **A** | $R_1 + R_2 = (CH_2)_5$ | -- | $NH_2$ | 85 |
| **B** | $R_1 + R_2 = (CH_2)_6$ | -- | $NH_2$ | 81 |
| **C** | $CH_3$ | $CH_3$ | $NH_2$ | 88 |
| **D** | $CH_3$ | $CH_3CH_2$ | $NH_2$ | 87 |
| **E** | $CH_3$ | $CH_3CH_2CH_2$ | $NH_2$ | 92 |
| **F** | $CH_3CH_2$ | $CH_3CH_2$ | $NH_2$ | 90 |
| **G** | $CH_3$ | $(CH_3)_2CH$ | $NH_2$ | 82 |
| **H** | $CH_3$ | Ph | $NH_2$ | 78 |
| **I** | H | Ph | $NH_2$ | 75 |
| **J** | $R_1 + R_2 = (CH_2)_5$ | -- | $(CH_3)_2N$ | 82 |
| **K** | $CH_3$ | $CH_3$ | $(CH_3)_2N$ | 86 |
| **L** | $CH_3$ | $CH_3CH_2$ | $(CH_3)_2N$ | 85 |
| **M** | $CH_3$ | $CH_3CH_2CH_2$ | $(CH_3)_2N$ | 86 |
| **N** | $CH_3$ | $(CH_3)_2CH$ | $(CH_3)_2N$ | 79 |
| **O** | $R_1 + R_2 = (CH_2)_5$ | -- | PhNH | 91 |
| **P** | $R_1 + R_2 = (CH_2)_6$ | -- | $CH_3NH$ | 81 |
| **Q** | $R_1 + R_2 = (CH_2)_5$ | -- | $C_2H_5NH$ | 86 |
| **R** | $CH_3$ | $CH_3$ | Ph | 81 |
| **S** | $CH_3$ | $CH_3$ | $CH_3$ | 75 |

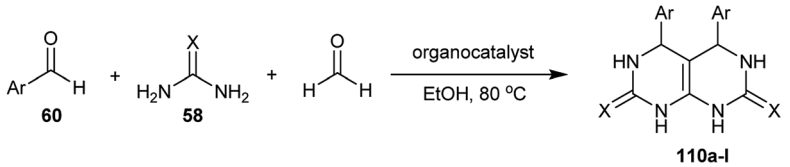
Scheme 26 Synthesis of 2,2,7-trisubstituted-2,3-dihydropyrimido[4,5-*d*]pyrimidin-4(1*H*)-ones.




60 58 107 109

**109**a: $Ar_1$= 4-Cl, $Ar_2$= H (91%); b: $Ar_1$= 3-$NO_2$, $Ar_2$= H (88%); c: $Ar_1$= 4-Cl, $Ar_2$= 4-OH (92%); d: $Ar_1$= 3-$NO_2$, $Ar_2$= 4-OH (85%); e: $Ar_1$= 4-Br, $Ar_2$= 4-Me (94%); f: $Ar_1$= 3-$NO_2$, $Ar_2$= 4-Me (89%); g: $Ar_1$= 4-$NO_2$, $Ar_2$= 4-OMe (90%); h: $Ar_1$= 4-Br, $Ar_2$= 4-OMe (93%); i: $Ar_1$= 4-Cl, $Ar_2$= 4-OMe (89%); j: $Ar_1$= 4-F, $Ar_2$= 4-OMe (87%); k: $Ar_1$= 4-Br, $Ar_2$= 4-F (91%); l: $Ar_1$= 4-Cl, $Ar_2$= 4-F (86%); m: $Ar_1$= H, $Ar_2$= 4-F (87%); n: $Ar_1$= 4-$NO_2$, $Ar_2$= 4-F (84%); o: $Ar_1$= 4-Cl, $Ar_2$= 2-OH (29%); p: $Ar_1$= 4-Cl, $Ar_2$= 2-Br (0%)

Scheme 27 Synthesis of 4,5,8*a*-trisubstituted-hexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-diones.




60 58 110a-l

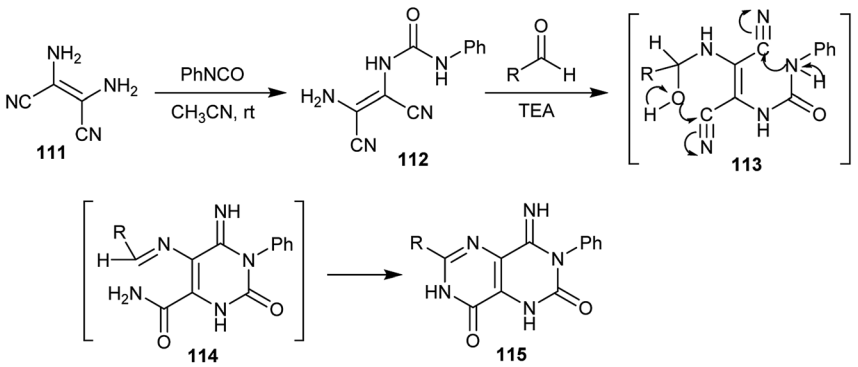
a: X= O, Ar= 4-OH-$C_6H_4$ (89%); b: X= O, Ar= 2-OH-$C_6H_4$ (88%); c: X= O, Ar= 4-$OCH_3$-$C_6H_4$ (90%); d: X= O, Ar= $C_6H_5$ (80%); e: X= O, Ar= 4-$NO_2$-$C_6H_4$ (78%); f: X= O, Ar= 3-$NO_2$-$C_6H_4$ (79%); g: X= S, Ar= 4-OH-$C_6H_4$ (85%); h: X= S, Ar= 2-OH-$C_6H_4$ (87%); i: X= S, Ar= 4-$OCH_3$-$C_6H_4$ (88%); j: X= S, Ar= $C_6H_5$ (79%); k: X= S, Ar= 4-$NO_2$-$C_6H_4$ (77%); l: X= S, Ar= 3-$NO_2$-$C_6H_4$ (75%)

Scheme 28 Multicomponent synthesis of 4,5-diaryl-4,5,6,8-tetrahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-diones and thiones.

of pyrimido[4,5-*d*]pyrimidine-2,4,5-(1*H*,3*H*,6*H*)-trione **77**. Compounds **74**, **80** and **81** were obtained with yields of 60–70%, compounds **84–87** were obtained with yields of 47–52% and compounds **88–91** were obtained with yields of 31–54% (Scheme 20).[62]

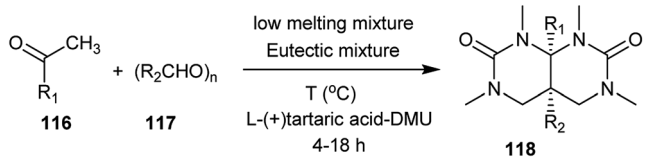
An efficient multicomponent synthetic route for the synthesis of 5,6-diaryl-1-alkyl-3-methyl-7-thioxo-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **94** was reported by Majumder and coworkers.[36] A regioselective one-pot multicomponent reaction of isothiocyanates **92** with aryl aldehydes **60** and 1-alkyl-6-amino-3-methylpyrimidine-2,4(1*H*,3*H*)-diones **66** and **93** yielded the desired products **94** in good yields. The reactions proceeded in water under catalytic conditions using *p*-TSA as a Lewis acid catalyst (Scheme 21). The use of 20 mol% of *p*-TSA was found to give the best yield of **93** (75%). The mechanism of the reactions progressed through the initial nucleophilic attack of the amino group of **93** into the C=N of the isothiocyanates with the formation of the NH–C bond. Next, the C=C bond of the formed intermediate attack the carbonyl group of the aldehyde, in which the presence of the catalyst supports this step, and consecutive intramolecular cyclization and condensation furnished the anticipated products **94**.[36]

Shirini *et al.*[63] reported the synthesis of 1,4-diazabicyclo[2.2.2]octane-1,4-diium perchlorate ($[H_2\text{-DABCO}][ClO_4]_2$) as an efficient base ionic liquid in 95% yield by stirring a mixture of 1,4-diazabicyclo[2.2.2]octane (DABCO) in dry dichloromethane in an ice-bath followed by the addition of perchloric acid (70%, 2 equiv.). The catalyst ($[H_2\text{-DABCO}][ClO_4]_2$) was used in the multicomponent synthesis of 1,3-disubstituted-5-aryl-2,7-dithioxo/dioxo-2,3,5,6,7,8-hexahydropyrimido[4,5-*d*]pyrimidin-



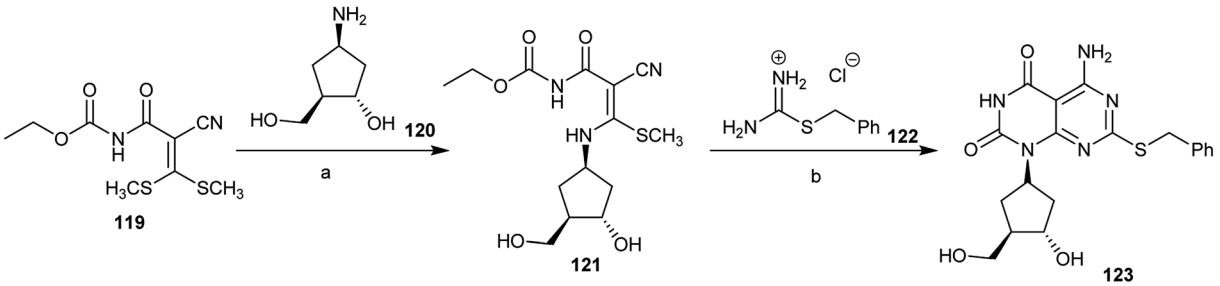

111 112 113 114 115

Scheme 29 Synthesis of 1,3,4,7-tetrahydropyrimido[5,4-*d*]pyrimidine-2,8-diones.




a: $R_1$= Ph, $R_2$= H (95%); b: $R_1$= Ph, $R_2$= $CH_3$ (82%); c: $R_1$= $(OCH_3)_3C_6H_2$, $R_2$= H (93%); d: $R_1$= $(OCH_3)_2C_6H_3$, $R_2$= H (91%); e: $R_1$= 2-thienyl, $R_2$= H (94%); f: $R_1$= Boc-NH-$C_6H_4$, $R_2$= $CH_3$ (84%); g: $R_1$= 2-naphthyl, $R_2$= H (90%); h: $R_1$= 3-$NO_2C_6H_4$, $R_2$= H (90%); i: $R_1$= 4-$BrC_6H_4$, $R_2$= H (85%); j: $R_1$= 4-$OBnC_6H_4$, $R_2$= H (88%); k: $R_1$= 2-furyl, $R_2$= H (87%); l: $R_1$= 4-$CH_3C_6H_4$, $R_2$= H (95%); m: $R_1$= 4-$FC_6H_4$, $R_2$= H (90%); n: $R_1$= 4-I-$C_6H_4$, $R_2$= H (93%); o: $R_1$= 2-OH-$C_6H_4$, $R_2$= H (84%); p: $R_1$= 3-$OCH_3C_6H_4$, $R_2$= H (96%)

Scheme 30 Synthesis of hexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-diones.

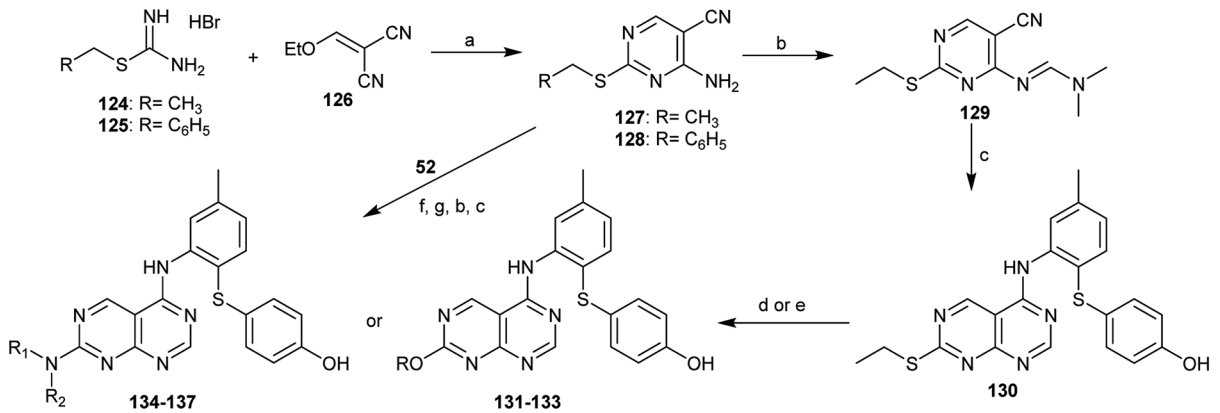



*Reagents and conditions*: a) dry $NEt_3$ (2 equiv), ethanol, reflux, 55 %; b) $NEt_3$ (2 equiv), *i*-PrOH, reflux, 61%

Scheme 31 Synthesis of cyclopentyl-pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione.

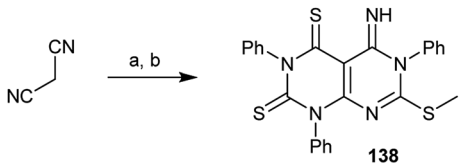
4(1*H*)-ones **95a–v** for the reaction of aryl aldehydes with 6-amino-1,3-dimethyluracil **66**, barbituric acid **56** or thiobarbituric acid **57** with urea or thiourea **58** (Scheme 22).[26,38,58,64–66] The procedure provides short reactions times, saves costs, allows catalyst reusability, is moisture resistant and gives high yields.

Similarly, an efficient method for the synthesis of 1,3-dimethyl-6-aryl-5-heteroaryl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **99** using 1-butyl-3-methyl-1*H*-imidazol-3-ium iron(III) chloride ([Bmim]$FeCl_4$) as an ionic liquid was reported by Suresh *et al.*[67] by applying four-component reactions of 6-amino-1,3-dimethyluracil (**66**), DMF-DMA (**96**), 3-substituted-1-phenyl-1*H*-pyrazole-4-carbaldehydes **97** and aryl-




*Reagents and conditions*: (a) Hunig's base, ethanol, 4 h, 39-45%; (b) N,N-dimethylforamide dimethyl acetal, toluene, reflux, 3 h, 100%; (c) 4-((2-amino-4-methylphenyl)thio)phenol, acetic acid solvent, reflux, 1.5 h, 15-45%; (d) excess sodium alkoxide, EtOH, heating, 32%; (e) excess morpholine or piperidine, microwave irradiation, 180 °C, 2 h, 38-65%; (f) mCPBA, $CH_2Cl_2$, 95%; (g) excess ethyl amine or methyl amine, EtOH, heating, 37-44%.

Scheme 32 Synthesis of 2,5-disubstituted-pyrimido[4,5-*d*]pyrimidines.




Reagents and conditions:
(a) $C_6H_5NCS$ (1 equiv), NaH (1.5 equiv), dry DMF, 5 °C, then $C_6H_5NCS$ (2 equiv), rt, 15 h;
(b) $CH_3I$, $NaHCO_3$, rt, 12 h

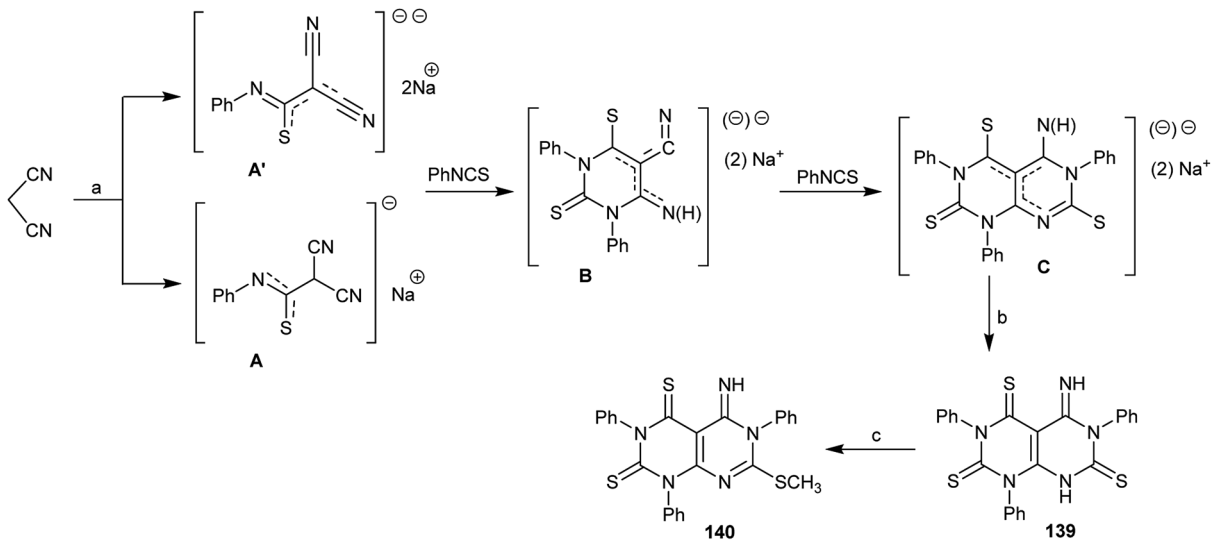
Scheme 33 Synthesis of 5-imino-7-(methylthio)-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dithione.

amines under reflux conditions. The synthesis of compound **99f** was established using X-ray crystallography (Scheme 23). The investigated analogs were estimated as antibacterial agents, in which compounds **99c**, **99i**, **99l**, and **99m** have potent results with minimum inhibitory concentration (MIC) values ranging between 3.9–15.6 μg $mL^{-1}$ and the minimum bactericidal concentration (MBC) was found to be 2-fold greater than the antibacterial results. In addition, the compounds of the series **99** were assessed as biofilm inhibitors. Compounds **99l** and **99m** revealed potent results with half maximal inhibitory concentration ($IC_{50}$) values ranging between 1.8–8.2 μg $mL^{-1}$. Compound **99l** increases the level of intracellular reactive oxygen species (ROS) when treating biofilms of *Micrococcus luteus* (MTCC 2470) cultured at a concentration of 0.5 μg $mL^{-1}$ and increases the membrane permeability allowing protein leakage and the successive death of the bacterial cell.[67]

The ionic liquid catalyst ($[Bmim]FeCl_4$) was recycled from the previous reaction using a method similar to that reported by Shirini *et al.*[63] using 1,4-diazabicyclo[2.2.2]octane-1,4-diium perchlorate ($[H_2\text{-DABCO}][ClO_4]_2$) as an ionic liquid catalyst. The reaction mechanism proceeded through the initial formation of the amidine intermediate **A** by reaction of aminopyrimidindione **66** with DMF-DMA (**96**). The addition of formyl pyrazole **97** led to reaction with intermediate **A** and the formation of intermediate **B**. Dehydration of intermediate **B** with arylamines **98** resulted in the formation of intermediate **C**. Subsequent intramolecular cyclization on the intermediate **C** yielded the respective 1,3-dimethyl-6-aryl-5-heteroaryl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones **99** with recycling of the ionic liquid catalyst and elimination of the dimethylamine molecule (Scheme 24).[67]

## 2.2. Ring synthesis from acyclic compounds

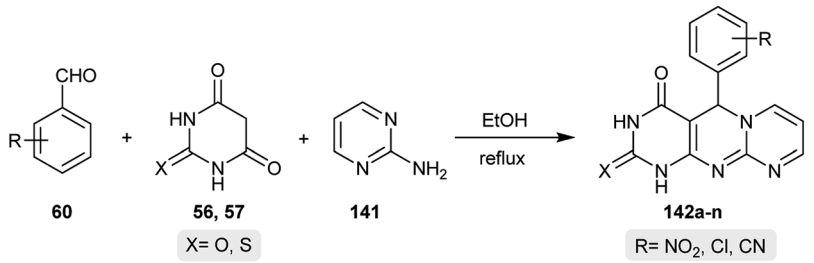
The multicomponent one-pot reaction of the aromatic aldehyde **60** with acetophenone (**100**) and urea (**58**) in an excess amount (1 : 1 : 4) in isopropanol yielded the respective hexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-dione **101** (63% yield) instead of the expected products **102** or its tautomeric form. The product **101** was obtained in a good yield through a cyclocondensation step reaction. The ratio of the reactants (1.5 : 1 : 3) allowed the formation of compound **102** with the formation of hexahydro-pyrimidopyrimidine-dione **101**. On the




Reagents and conditions: (a) $C_6H_5NCS$ (1 equiv), NaH (1.5 equiv), anhydrous DMF, 5 °C, then $C_6H_5NCS$ (2 equiv), rt, 15 h;
(b) 1M $CH_3COOH$; (c) $CH_3I$, $NaHCO_3$, DMF, heat.

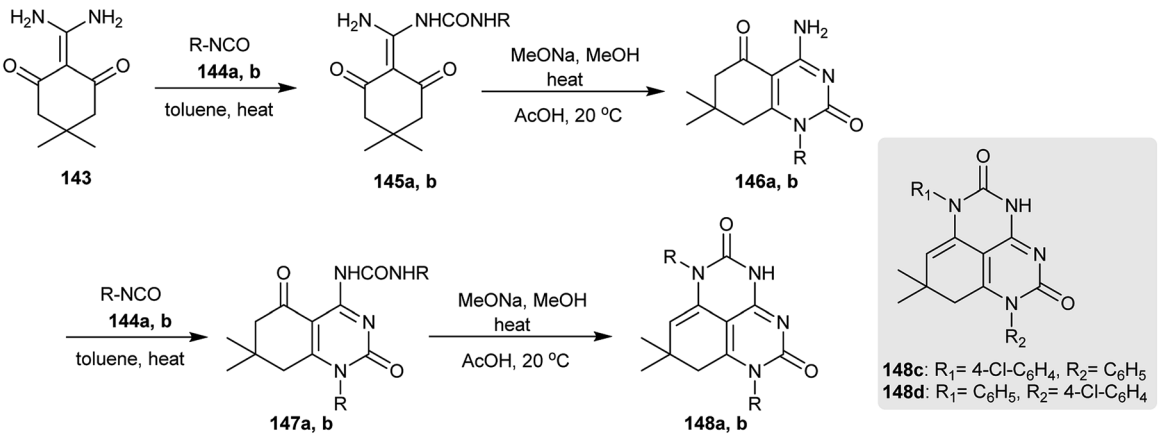
Scheme 34 Synthesis of 5-imino-7-(methylthio)-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4-(1*H*,3*H*)-dithione.




a: X=O, R= 4-Cl (90%); b: X=O, R= 4-$NO_2$ (92%); c: X=O, R= 4-CN (88%); d: X=O, R= 4-Br (85%); e: X=O, R= 3-Cl (89%); f: X=O, R= 3-$NO_2$ (90%); g: X=O, R= 2-Cl (88%); h: X=O, R= 2-$NO_2$ (92%); i: X=S, R= 4-Cl (91%); j: X=S, R= 4-$NO_2$ (90%); k: X=S, R= 4-CN (85%); l: X=S, R= 3-$NO_2$ (88%); m: X= O, R= H (84%); n: X= O, R= 3-OH (80%)

Scheme 35 Synthesis of tetrahydro-4*H*-dipyrimido[1,2-*a*:4′,5′-d]pyrimidin-4-ones.

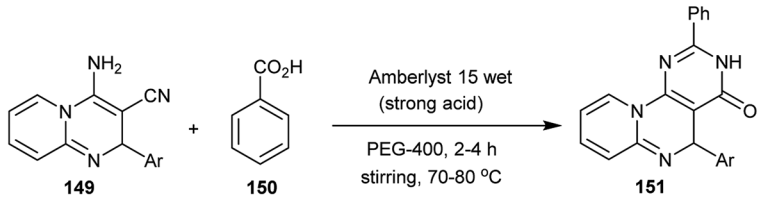



a: R= $C_6H_5$; b: R= 4-Cl-$C_6H_4$

Scheme 36 Synthesis of 7,8-dihydro-1*H*-pyrimidoquinazoline-2,5(3*H*,6*H*)-diones.

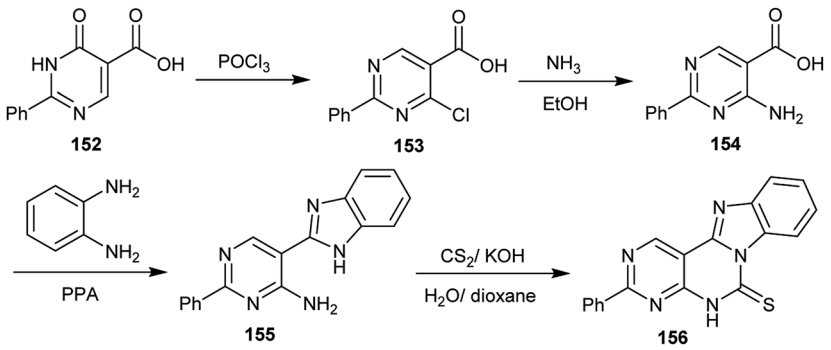
other hand, changing the solvent in the previous reaction to butanol gave rise to the possibility of the formation of by-products **102**, **103** and **104** in addition to the formation of the main product **101** (Scheme 25).[68]

The multicomponent one-pot reactions of 2-(ethoxymethylene)malononitrile (**105**) with guanidine or amidines derivatives **106** and ketones or aldehydes **107** in the presence of catalytic N-heterocyclic carbenes gave the respective 2,2,7-trisubstituted-2,3-dihydropyrimido[4,5-*d*]pyrimidin-4(1*H*)-ones **108**. The reaction mechanism involved the Michael addition of compound **105** with guanidine followed by subsequent cyclization, isomerization and aromatization to form the intermediate 4-amino-2-substituted-pyrimidine-5-carbonitrile. Next, nucleophilic attack of different nucleophiles (formed from the attack of the catalytic NHC-PPIm on the ketones or aldehydes) on the nitrile function of the recently formed intermediates took place, followed by release of the catalyst and then the Dimroth rearrangement to afford **108**. The best yield of **108a** were obtained by heating the reactants in ethanol containing N-




a: Ar= 4-Cl-$C_6H_4$; b: Ar= 4-$NO_2$-$C_6H_4$; c: Ar= 4-F-$C_6H_4$; d: Ar= 3-$NO_2$-$C_6H_4$; e: Ar= 2-Cl-$C_6H_4$; f: Ar= 2-OH-$C_6H_4$; g: Ar= $C_6H_5$; h: Ar= 1-Ph-3-$CH_3$-5-Cl-pyrazole; i: Ar= 2-furyl; j: Ar= 4-$OCH_3$-$C_6H_4$

Scheme 37 Synthesis of 5-aryl-2-phenyl-3,5-dihydro-4*H*-pyrido[1,2-*a*]pyrimido[5,4-*e*]pyrimidin-4-ones.




Scheme 38 Synthesis of 3-phenylbenzo[4,5]imidazo[1,2-*c*]pyrimido[5,4-*e*]pyrimidine-6(5*H*)-thione.

heterocyclic carbenes (NHCs)-assisted "NHC-PPlm" (0.4 equiv.) at 40 °C (Scheme 26).[27]

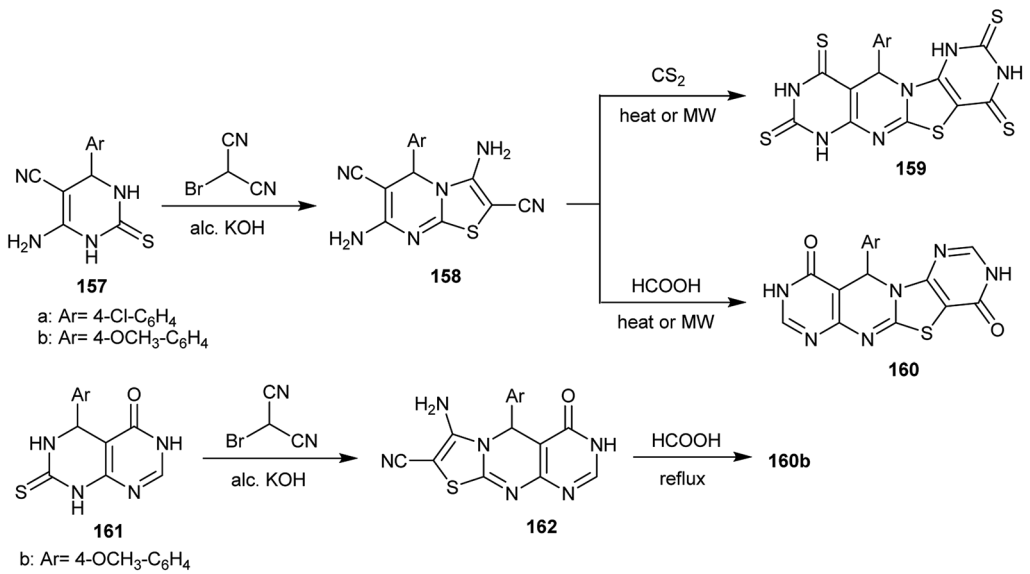
The procedure involved the synthesis of the Biginelli-type, 4,5,8*a*-trisubstituted-hexahydro-pyrimido-[4,5-*d*]pyrimidine-diones **109** was described by Magar and coworkers.[40] The process specified a multicomponent one-pot synthesis from the reaction of aromatic aldehydes **60** with urea (**58**) and acetophenones **107** under optimum conditions in the presence of a recyclable catalyst, sulfated tin oxide (STO), to give the hexahydro-pyrimido-pyrimidine-diones **109**. The catalyst was prepared by heating a solution of stannous chloride with a few drops of nitric acid and the subsequent addition of ammonia solution at pH = 8. The procedure allows the preparation of pyrimidopyrimidines with high yields after a short reaction time (Scheme 27).

Multicomponent one-pot reactions of aromatic aldehydes **60** with urea or thiourea **58** and formaldehyde in boiling ethanol containing the organocatalyst gave the respective tetrahydropyrimido[4,5-*d*]pyrimidine-diones and thiones **110a–l**. The best yield of compound **110a** was achieved by treating the reactants with ethanol as a solvent and (*S*)-*N*-(4-fluorophenyl)-1-tosylpyrrolidine-2-carboxamide as a catalyst after 15 h (Scheme 28).[69]

A series of 4-imino-6-substituted-3-phenyl-1,3,4,7-tetrahydropyrimido[5,4-*d*]pyrimidine-2,8-diones (**115**) was first synthesized by Ohtsuka in 1978,[70] through the reaction of phenyl isocyanate with 2,3-diaminomaleonitrile (**111**) *via* a condensation process to give the respective 1-(2-amino-1,2-dicyanovinyl)-3-phenylurea (**112**) followed by reaction with diverse aldehydes in the presence of triethylamine as a basic medium. The reaction involved the formation of two intermediates, **113** and **114** (Scheme 29).

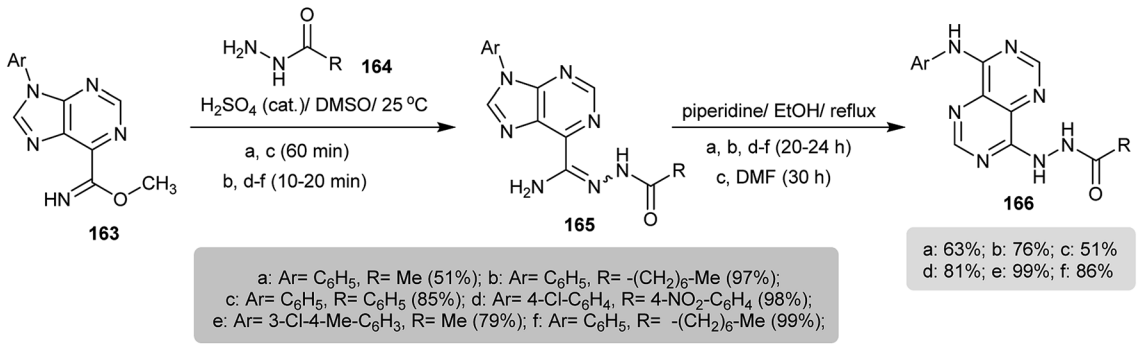
Under mild conditions, a proficient method for the synthesis of a series of 4a,8a-disubstituted-1,3,6,8-tetramethylhexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-diones **118** from the reactions of ketones **116** with para-formaldehydes **117** following the use of l-(+)-tartaric acid-dimethylurea as a catalyst, reaction medium, and solvent at the same time. The catalyst l-(+)-tartaric acid-DMU (70 : 30) was used to achieve the best yields from the reactions by reacting 1 mole of the ketone with 4 moles of paraformaldehyde **117**. The method is an eco-friendly technique for the preparation of the products **118** in excellent yields (Scheme 30).[71]

Condensation of ethyl (2-cyano-3,3-bis(methylthio)acryloyl) carbamate (**119**) with racemic 4-amino-2-(hydroxymethyl)

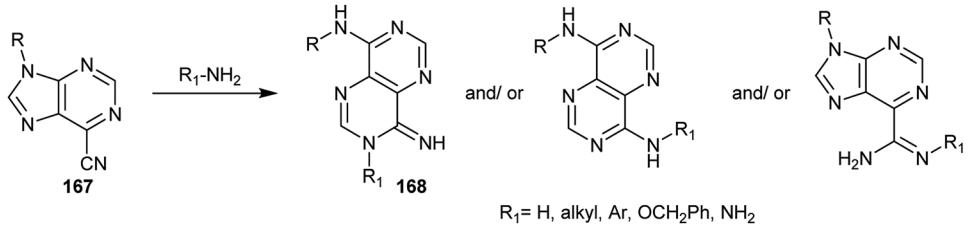



Scheme 39 Synthesis of polycyclic pyrimido[4,5-*d*]pyrimidines.



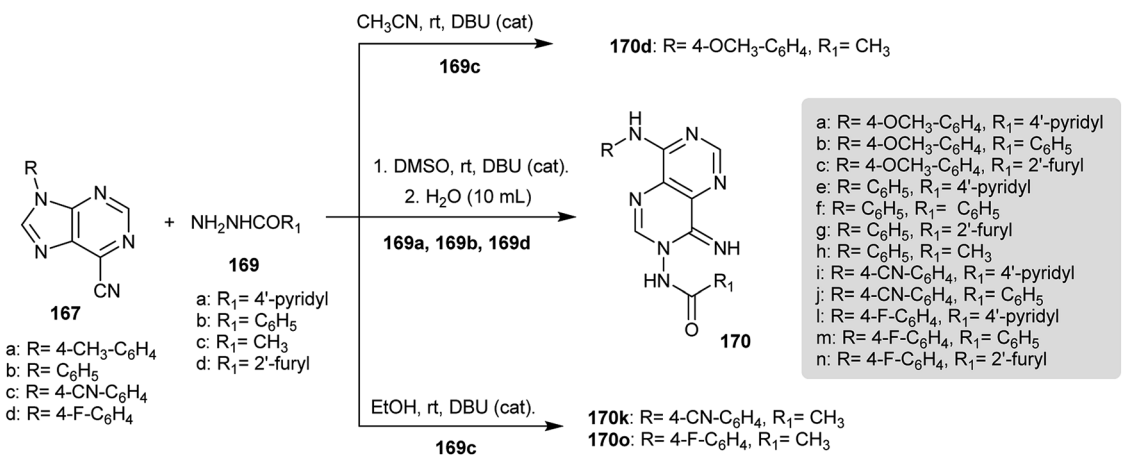

Scheme 40 Synthesis of *N*′-(8-(aryl-amino)pyrimido[5,4-*d*]pyrimidin-4-yl)substituted-hydrazides.



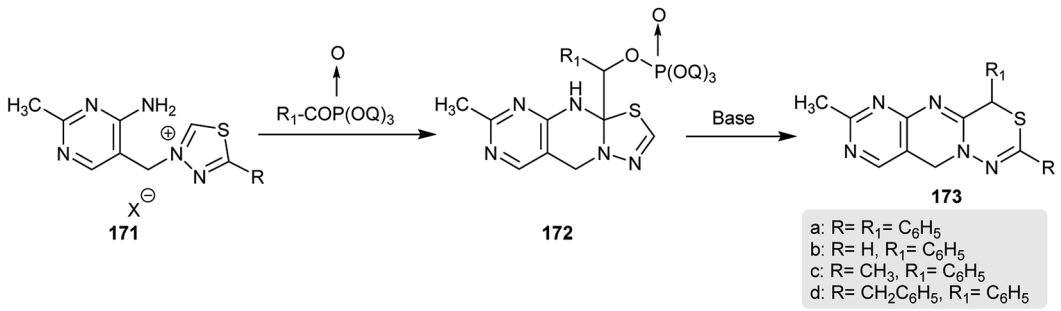

Scheme 41 Reactions of 9-substituted-9*H*-purine-6-carbonitriles with different amines.

cyclopentan-1-ol (**120**) in refluxing ethanol containing triethylamine yielded the condensation intermediate **121.** The cyclization of the respective ethyl carbamate intermediate **121** did not occur using different bases and catalysts. The cyclization of **121** through reaction with 2-benzyl-isothiouronium chloride (**122**) was achieved by refluxing isopropanol in the presence of triethylamine to give the desired cyclopentyl-pyrimido[4,5-*d*] pyrimidine-dione **123** (Scheme 31).[72]

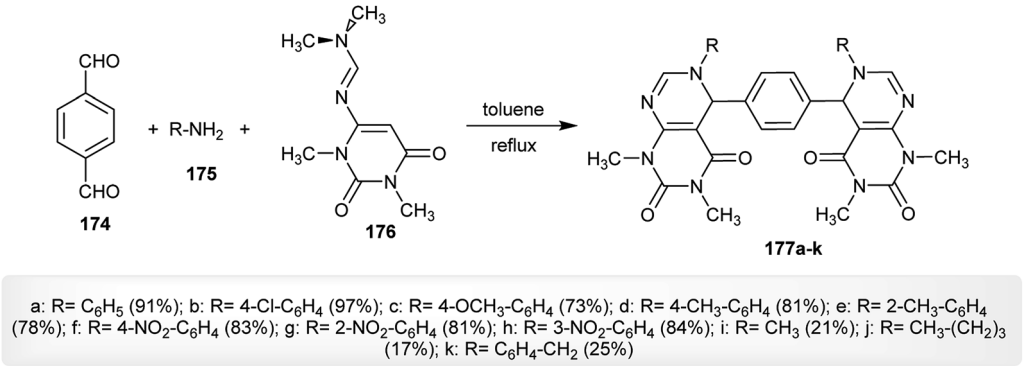
In another synthetic route, substituted-methyl carbamimidothioate hydrobromides **124** and **125** both reacted with 2-(ethoxymethylene)malononitrile (**126**) in ethanol containing Hunig's base to give the analogous aminopyrimidines **127** and **128.** In refluxing toluene, the amine **127** was transformed into the respective amidine **129** by reaction with DMF-DMA. Heating of **129** with 4-((2-amino-4-methylphenyl)-thio)phenol in acetic acid gave the cyclization product **130.** In a strong basic medium, the ethylsulfanyl moiety of **130** was displaced by nucleophiles such as morpholine or piperidine under microwave irradiation conditions to give the respective ether **131–133** and amine **134, 135** derivatives. Consecutively, the oxidation of the mercapto moiety of **128** into the respective sulfone followed by nucleophilic displacement with amines yielded the amines **136** and **137** (Scheme 32). The compounds were evaluated as inhibitors for hepatitis C virus replication using two assays (genotype 1a and 1b cell culture HCV replicon), in which compound **130** demonstrated the highest potency against 1b replication (half maximal effective concentration ($EC_{50}$) = 10 μM) and the other compounds exhibited activities with an $EC_{50}$ greater than 2 μM.[73]




Scheme 42 Synthesis of 3,8-disubstituted-amino-4-iminopyrimido[5,4-*d*]pyrimidines.




Scheme 43 Synthesis of 7-methyl-2,4-disubstituted-4,10-dihydropyrimido[4′,5′:4,5]pyrimido[1,2-*d*]-[1,3,4]-thiadiazines.



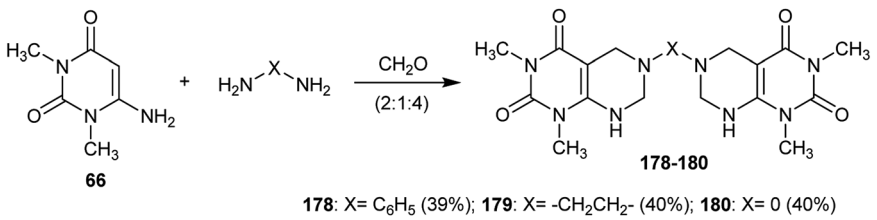

Scheme 44 Synthesis of bis-pyrimidopyrimidines.

Condensation of malononitrile with three equivalents of phenyl isothiocyanate in DMF/sodium hydride and subsequent methylation of the SH group of the formed intermediate at C7 gave the respective products incorporating a dihydropyrimido[4,5-*d*]pyrimidine core **138** in good yield (Scheme 33).[74]

The intermediate, 5-imino-1,3,6-triphenyl-5,8-dihydropyrimido[4,5-*d*]pyrimidine-2,4,7-(1*H*,3*H*,6*H*)-trithione (**139**) was synthesized through the reaction of malononitrile with one equivalent of phenyl isothiocyanate in DMF containing sodium hydride followed by the addition of two equivalents of phenyl isothiocyanate to the generated mono and dianion intermediates **A** and **A′** and subsequent treatment of the intermediate **C** with acetic acid. The presence of sodium hydride in the first step facilitates the acidic proton abstraction from malononitrile to form the anion which attacks the C=S of phenyl isothiocyanate. Methylation of **139** was accessed by reaction with methyl iodide in DMF containing sodium bicarbonate to give the methylation product **140** (Scheme 34).[75]

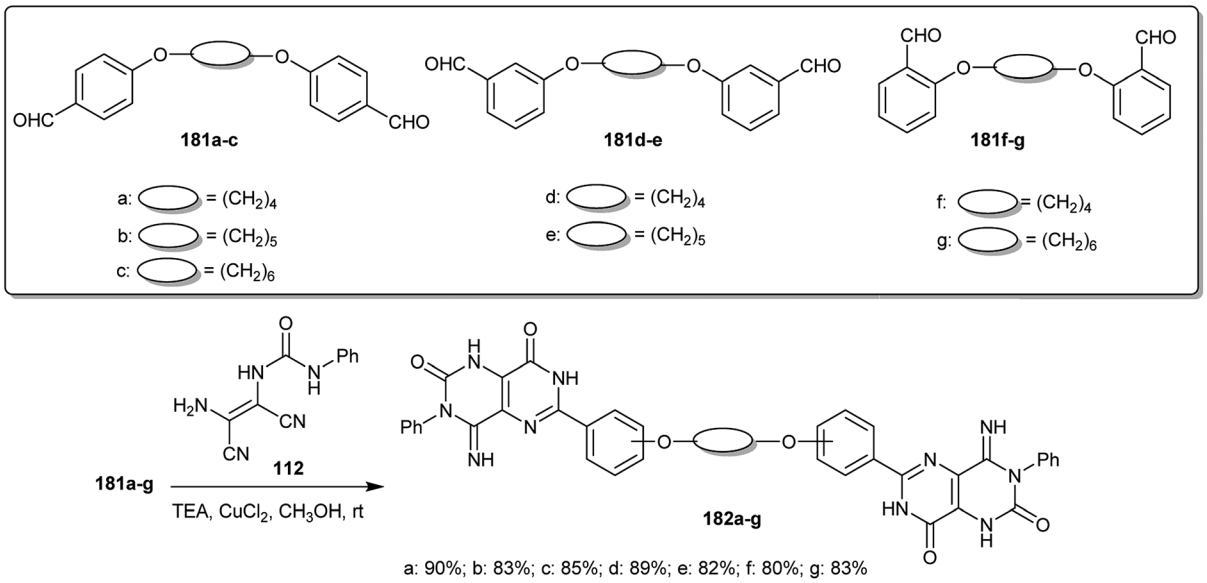
## 2.3. Synthesis of tricyclic systems

**2.3.1. Multicomponent synthesis.** Patil and coworkers[48] developed a new method for the synthesis of tetrahydro-4*H*-dipyrimido[1,2-*a*:4′,5′-*d*]pyrimidin-4-ones. The reactions involved one-pot multicomponent reactions of aryl aldehydes **60**, barbituric or thiobarbituric acids **56** and **57** and 2-aminopyrimidine (**141**). The respective tetrahydro-4*H*-dipyrimido[1,2-*a*:4′,5′-d]pyrimidin-4-ones **142a–n** were obtained by refluxing the reactants in ethanol under non-supported catalytic conditions (Scheme 35). The mechanism for the formation of products **142a–n** was proposed to be an initial Knoevenagel condensation of the aryl aldehydes **60** with barbituric or thiobarbituric acids **56** and **57** to form a conjugate enone intermediate. 2-Aminopyrimidine (**141**) followed an amine-imine

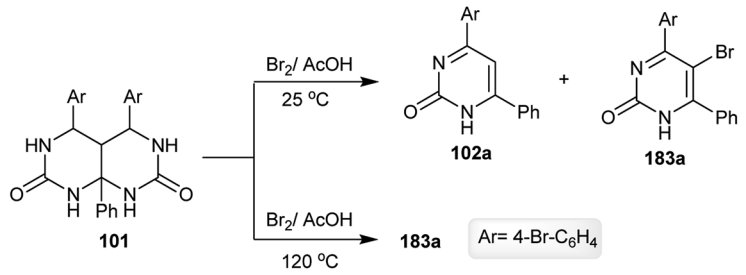



Scheme 45 Synthesis of bis(1,3-dimethyl-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones).



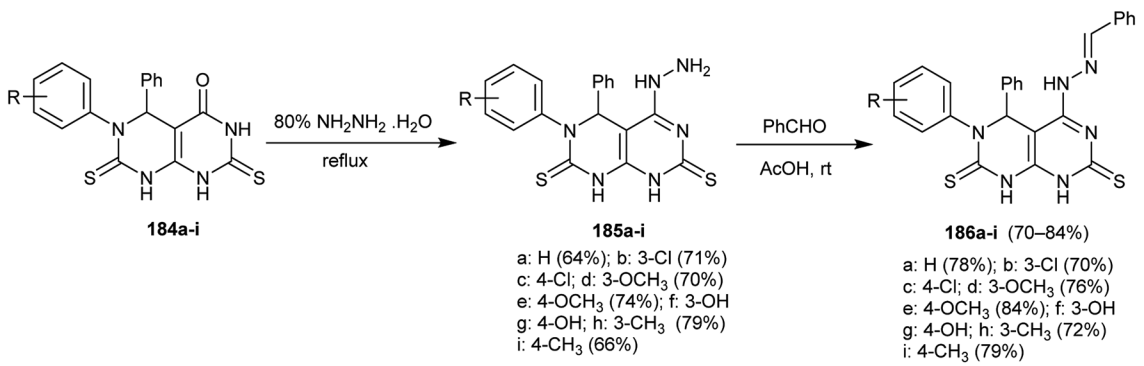

Scheme 46 Synthesis of bis-1,3,4,7-tetrahydropyrimido[5,4-*d*]pyrimidine-2,8-diones.



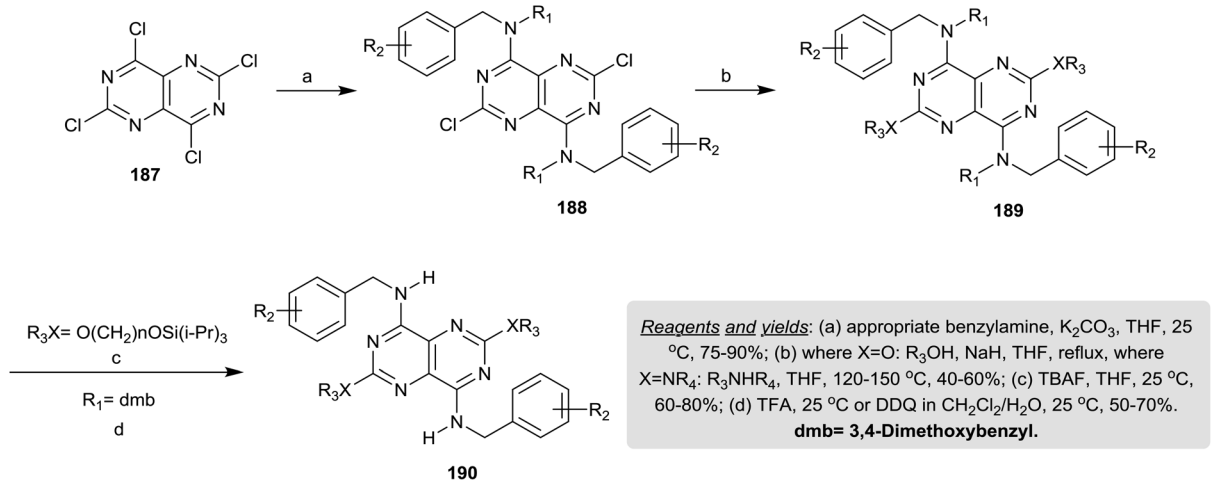

Scheme 47 Bromination of hexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-dione.

rearrangement resulting in the nucleophilic attack of the pyrimidine nitrogen at the enone intermediate double bond and the subsequent Michael addition led to the formation of the pyrimidinyl-iminopyrimidine intermediate. Cyclization and oxidation of the recently formed intermediate gave the anticipated products **142a–n**.

**2.3.2. Synthesis of pyrimidoquinazoline-diones.** The reaction of 2-(diaminomethylene)-5,5-dimethylcyclohexane-1,3-dione (**143**) with excess aryl isocyanates **144** in refluxing toluene gave the urea derivatives **145**. Cyclization of **134** by heating in sodium methoxide solution yielded the respective aminopyrimidinones **146**, in which the enamine moiety was involved in the heterocyclization. In addition, heating the aryl isocyanates **144** with aminopyrimidinones **146** gave urea derivatives **147a** and **147b** in 87–89% yields. Heterocyclization of **147** by heating in a sodium methoxide solution afforded the




Scheme 48 Synthesis of 4,8-dihydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-dithiones.

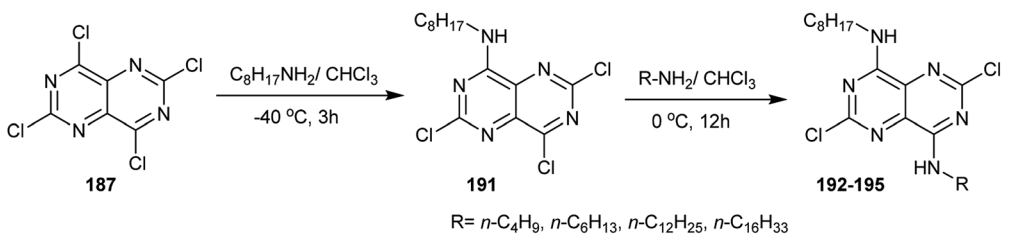



| Product 189 | $R_1$ | $R_2$ | $XR_3$ |
|---|---|---|---|
| **a** | H | H | $N(CH_2CH_2OH)_2$ |
| **b** | H | 4-OMe | $N(CH_2CH_2OH)_2$ |
| **c** | H | 3,4-$(OMe)_2$ | $N(CH_2CH_2OH)_2$ |
| **d** | Me | 4-OMe | $N(CH_2CH_2OH)_2$ |
| **e** | H | 4-OMe | $NHCH_2CH(OH)CH_3$ |
| **f** | H | 3,4-$(OMe)_2$ | $NHCH_2CH(OH)CH_3$ |
| **g** | Me | 4-OMe | $NHCH_2CH(OH)CH_3$ |
| **h** | Me | 3,4-$(OMe)_2$ | $NHCH_2CH(OH)CH_3$ |
| **i** | H | 4-OMe | $O(CH_2)_2OH$ |
| **j** | H | 3,4-$(OMe)_2$ | $O(CH_2)_2OH$ |
| **k** | H | 4-OMe | $O(CH_2)_2OH$ |
| **l** | Me | 4-OMe | $O(CH_2)_2OH$ |
| **m** | Me | 4-OMe | $O(CH_2)_2OH$ |

Scheme 49 Nucleophilic substitution reactions.

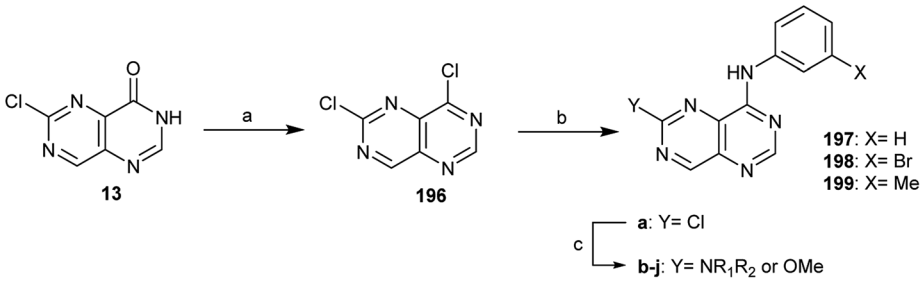
desired 7,8-dihydro-1*H*-pyrimidoquinazoline-2,5(3*H*,6*H*)-diones **148a** and **148b** with yields of 80 and 65%, respectively. Changing the aryl isocyanates allowed the preparation of **148c** and **148d** with yields of 67 and 80% following similar preceding synthetic approaches (Scheme 36).[76]

**2.3.3. Synthesis of pyridopyrimidopyrimidinones.** A series of 5-aryl-2-phenyl-3,5-dihydro-4*H*-pyrido[1,2-*a*]pyrimido[5,4-*e*] pyrimidin-4-ones **151** were prepared by the reaction of benzoic acid (**150**) with 2-aryl-3-cyano-4-amino-2*H*-pyrido[1,2-*a*]pyrimidines **149** through a green technique in PEG-400 (polyethylene glycol) in the presence of a catalytic amount of Amberlyst 15-wet. The use of PEG-400 enabled the availability and low toxicity and the catalyst Amberlyst 15-wet provided a strong acid medium (Scheme 37). The mechanism of the reaction proceeded through condensation between the carboxylic group of the benzoic acid and the amino group of **149** and the subsequent intramolecular cyclization. The compounds **151** were assessed as good antifungal agents against *A. niger*, *C. albicans*, and *A. flavus* species and the results were compared to the standard antibiotic Nystatin at 50 μg $mL^{-1}$.[77]




Scheme 50 Synthesis of diamines.

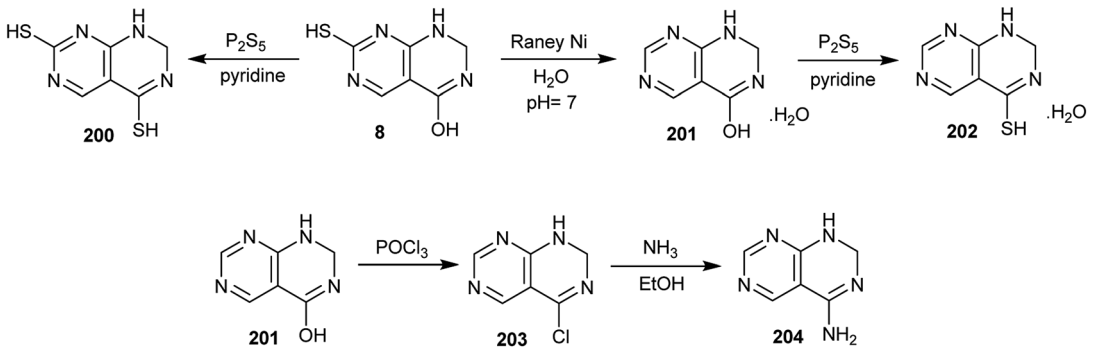
 



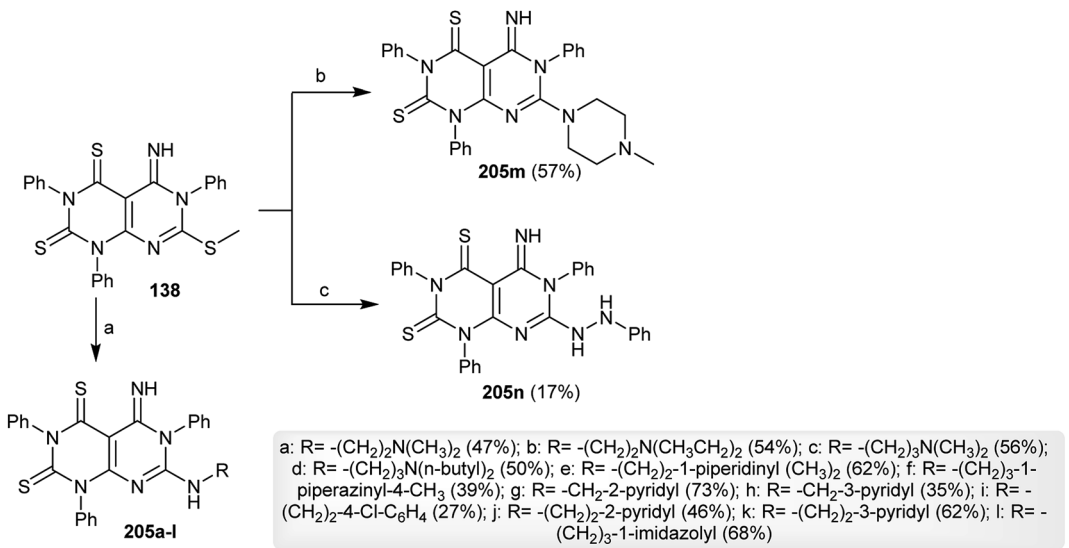

*Reagents and conditions*: (a) $SOCl_2$/DMF (trace)/ reflux/ 30 min; (b) $ArNH_2$/ 2-propanol/ reflux / 10 min; (c) $R_1R_2NH$/ DMSO/ 100 °C or MeOH/ $Et_3N$/ reflux.

**197a**: Y= Cl (79%); b: Y= $NHCH_3$ (82%); **198a**: Y= Cl (80%); b: Y= $NH_2$ (72%); c: Y= $NHCH_3$ (68%); d: Y= $N(CH_3)_2$ (70%); e: Y= $OCH_3$ (100%); f: Y= $NH(CH_2)_2N(CH_3)_2$ (64%); g: Y= $NH(CH_2)_2$-N-morpholino (51%); h: Y= $NH(CH_2)_3$-N-morpholino (92%); i: Y= $NH(CH_2)_2$-imidazol-4-yl (59%); j: Y= $NH(CH_2)_3$-imidazol-1-yl (93%); **199a**: Y= Cl (71%); b: Y= $NH_2$ (54%); c: Y= $NHCH_3$ (61%); d: Y= $N(CH_3)_2$ (92%); e: Y= $NH(CH_2)_2$-N-morpholino (78%); f: Y= $NH(CH_2)_2$-imidazol-4-yl (75%);

Scheme 51 Synthesis of mono- and disubstituted-amines.



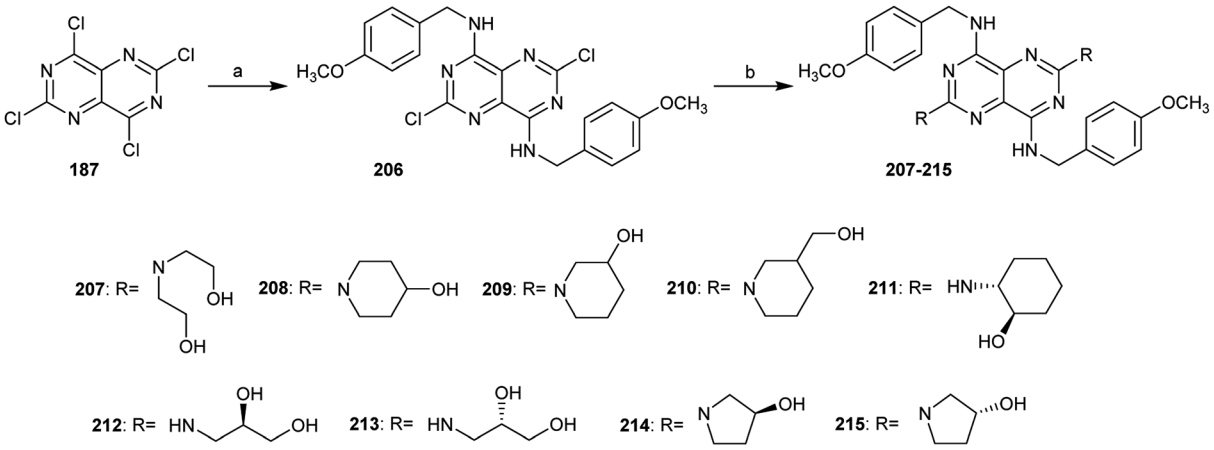

Scheme 52 Reactivity of 7-mercapto-1,2-dihydropyrimido[4,5-*d*]pyrimidin-4-ol.



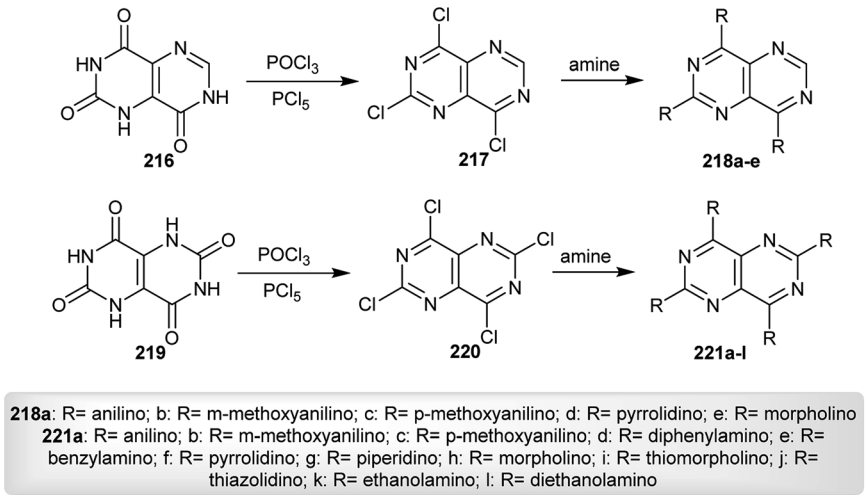

a: R= $-(CH_2)_2N(CH_3)_2$ (47%); b: R= $-(CH_2)_2N(CH_3CH_2)_2$ (54%); c: R= $-(CH_2)_3N(CH_3)_2$ (56%); d: R= $-(CH_2)_3N(n\text{-butyl})_2$ (50%); e: R= $-(CH_2)_2$-1-piperidinyl $(CH_3)_2$ (62%); f: R= $-(CH_2)_3$-1-piperazinyl-4-$CH_3$ (39%); g: R= $-CH_2$-2-pyridyl (73%); h: R= $-CH_2$-3-pyridyl (35%); i: R= $-(CH_2)_2$-4-Cl-$C_6H_4$ (27%); j: R= $-(CH_2)_2$-2-pyridyl (46%); k: R= $-(CH_2)_2$-3-pyridyl (62%); l: R= $-(CH_2)_3$-1-imidazolyl (68%)

*Reagents and conditions*: (a) R-$NH_2$ (1.2 equiv), dry DMF, (DIPEA), rt 12 h; (b) N-methylpiperazine, 120 °C, 5 h; (c) $C_6H_5NHNH_2$ ACN/EtOH, reflux, 10 h.

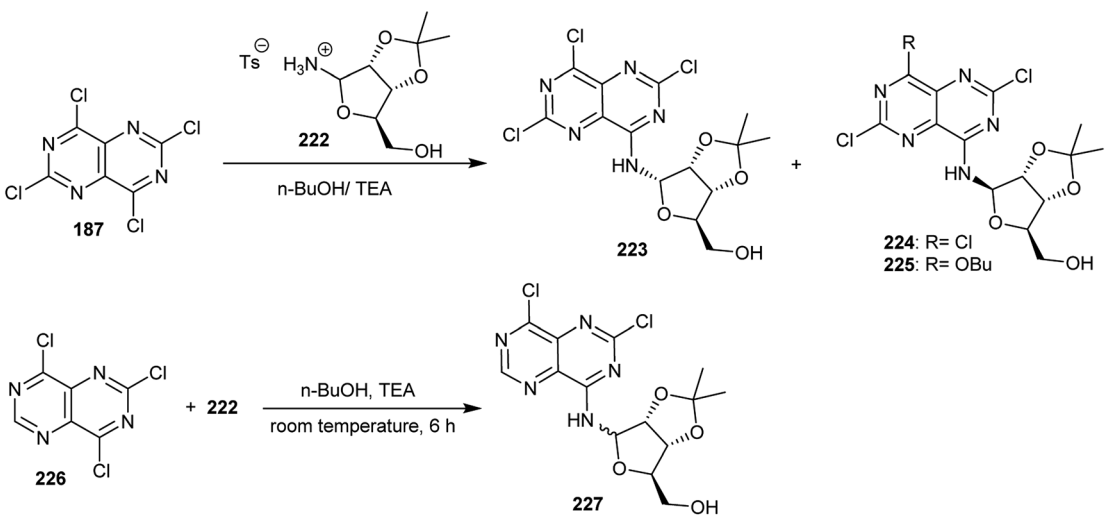
Scheme 53 Synthesis of 7-substituted-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4-(1*H*,3*H*)-dithiones.




Scheme 54 Synthesis of tetra-substituted-amines.




Scheme 55 Synthesis of tri- and tetra-substituted-amines of pyrimido[5,4-*d*]pyrimidines.




Scheme 56 Synthesis of nucleosides incorporated with a pyrimido[5,4-*d*]pyrimidine core.

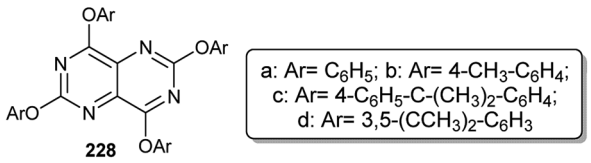
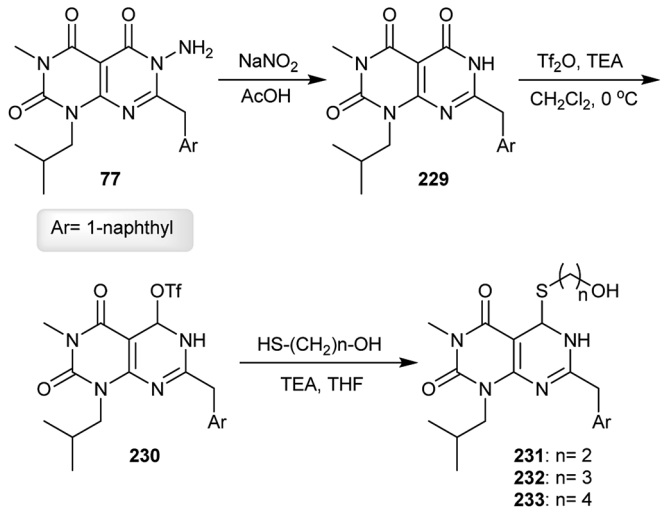



Fig. 2 The structure of 2,4,6,8-tetrakis(substituted-phenoxy)pyrimido[5,4-*d*]pyrimidines.




Scheme 57 Reactivity of the ring substituents.

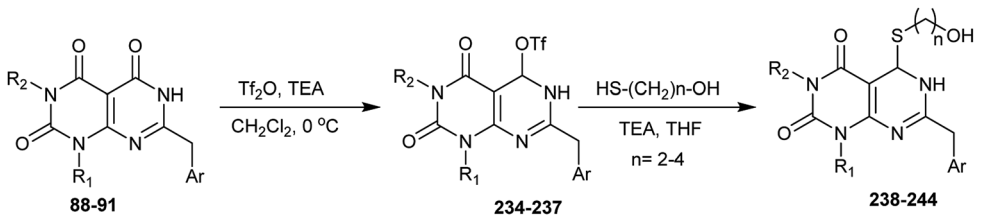
### 2.4. Synthesis of tetracyclic systems

Chlorination of 6-oxo-2-phenyl-1,6-dihydropyrimidine-5-carboxylic acid (**152**) with phosphorus oxychloride yielded the respective 4-chloro-2-phenylpyrimidine-5-carboxylic acid (**153**). Treatment of **153** with ammonia in ethanol gave the amine **154**, which was cyclized to the desired 5-(1*H*-benzo[*d*]imidazol-2-yl)-2-phenylpyrimidin-4-amine (**155**) upon heating with *o*-phenylenediamine in polyphosphoric acid. Compound **155** reacted with carbon disulfide in a mixture of water and dioxane containing potassium hydroxide to afford benzimidazo-pyrimido-pyrimidine-thione **156** in a 34% yield (Scheme 38).[78]

The reaction of 6-amino-4-aryl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitriles **157** with 2-bromo-malononitrile in an alcoholic potassium hydroxide solution yielded the respective 3,7-diamino-5-aryl-5*H*-thiazolo[3,2-*a*]pyrimidine-2,6-dicarbonitriles **158**. The thiazolopyrimidines **158** reacted with both carbon disulfide and formic acid under microwave-assisted conditions to afford the corresponding polycyclic pyrimido[4,5-*d*]pyrimidines **159** and **160**, respectively. The mechanism of this reaction involved the initial condensation of the two amino groups of **158** with two moles of formic acid, the partial hydrolysis and the cyclocondensation step. Compound **160b** could be obtained by another route, the reaction of 5-aryl-7-thioxo-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidin-4(3*H*)-one **161** with 2-bromo-malononitrile in alcoholic potassium hydroxide solution and subsequent cyclization by reaction of the formed dihydro-4*H*-pyrimidothiazolo[3,2-*a*]pyrimidine **162** with formic acid (Scheme 39).[79]

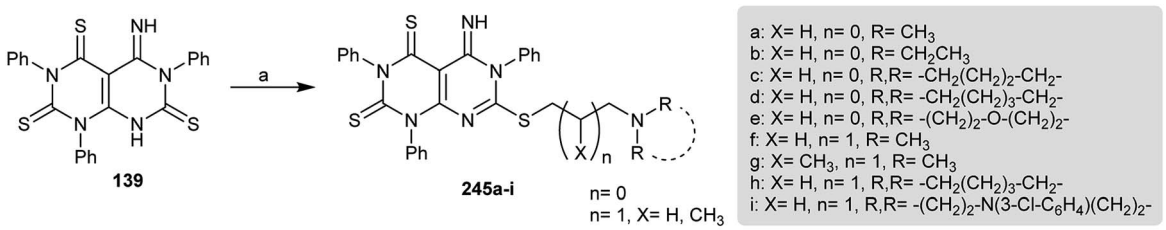
### 2.5. Ring synthesis by transformation of another ring

The reaction of methyl 9-aryl-9*H*-purine-6-carbimidates **163** with hydrazides **164** in DMSO containing a catalytic amount of sulfuric acid yielded *N*′-acetyl-9-aryl-9*H*-purine-6-carbohydrazonamides **165** in good to excellent yields. Subsequent refluxing of **165** in ethanol containing catalytic drops of piperidine resulted in imidazole ring



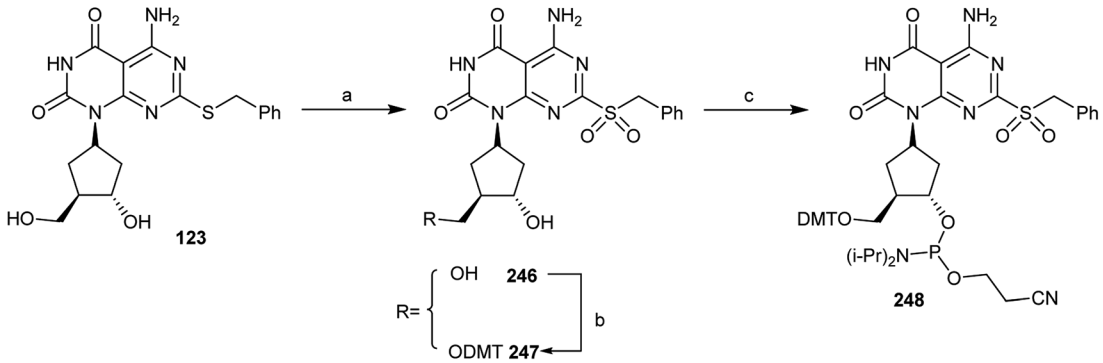

R1= isobutyl; **238**: R2= CH3, n= 3, Ar= 2-Cl-C6H4; **239**: R2= CH3, n= 4, Ar= 2-Cl-C6H4; **240**: R2= isobutyl, n= 3, Ar= 1-naphthyl; **241**: R2= isobutyl, n= 4, Ar= 1-naphthyl; **242**: R2= isobutyl, n= 3, Ar= 2-Cl-C6H4
R1= isopropyl; **243**: R2= CH3, n= 3, Ar= 1-naphthyl; **244**: R2= CH3, n= 4, Ar= 1-naphthyl

Scheme 58 Synthesis of 1,3,5,7-tetra-substituted-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones.




Reagents and conditions: (a) ω-chloroalkyldialkyl/cyclo-amines, various bases and solvents (methods A-F), heat.

Scheme 59 Synthesis of 7-(alkylthio)-5-imino-1,3,6-triphenyl-5,6-dihydropyrimido[4,5-*d*]pyrimidine-2,4-(1*H*,3*H*)-dithiones.




*Reagents and conditions*: a) *m*-CPBA (3 equiv), MeOH, $CHCl_3$, 72%; b) dimethyltryptamine (DMT)-Cl (1.1 equiv), DIPEA (1.1 equiv), 1,4-dioxane, 51%; c) 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.1 equiv), DIPEA (1.1 equiv), dry $CH_2Cl_2$, molecular sieves (4 Å), 60%.

Scheme 60 Reactivity of cyclopentyl-pyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dione.

cleavage followed by intramolecular cyclization of the formed intermediate to afford the respective *N*′-(8-(aryl-amino)pyrimido[5,4-*d*]pyrimidin-4-yl)substituted-hydrazides **166** (Scheme 40).[80]

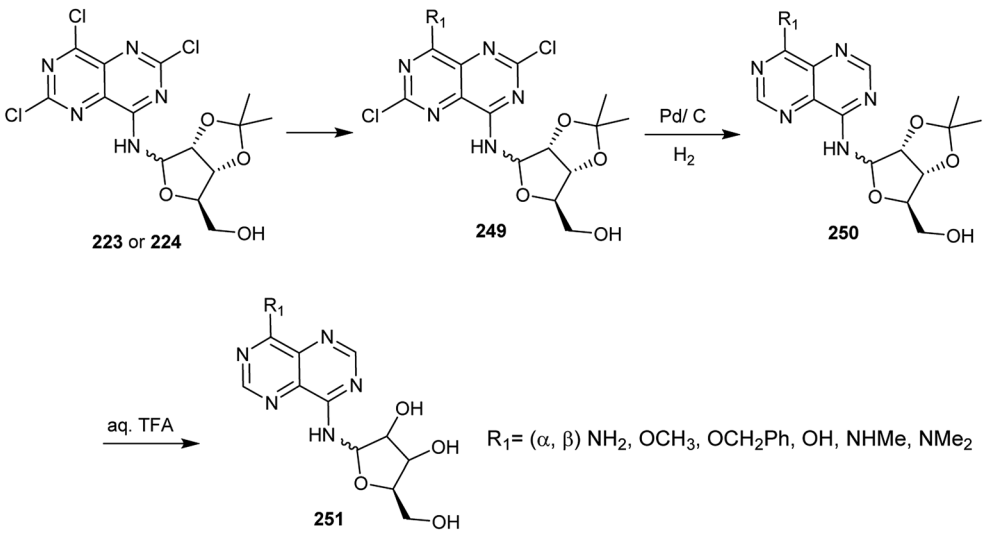
Furthermore, 9-substituted-9*H*-purine-6-carbonitriles **167** were used as synthetic intermediates for the synthesis of pyrimido[5,4-*d*]pyrimidine analogs **168** by reaction with various amines through ring transformations (Scheme 41).[14,24,81–89]

A series of pyrimido[5,4-*d*]pyrimidines **170** were prepared by reaction of 9-substituted-9*H*-purine-6-carbonitriles (**167**) with various hydrazides **169** by treatment in DMSO and water using catalytic DBU. Compound **170d** was obtained by the reaction of 9-aryl-9*H*-purine-6-carbonitriles **167** with acetohydrazide (**169c**) in acetonitrile instead of DMSO and compounds **170k** and **170o** were prepared in ethanol (Scheme 42). The compounds were tested as antibacterial agents against *Mycobacterium tuberculosis* strains in which the biological impact is based on the substituents at the C8 and N3 atoms of the pyrimidopyrimidine skeleton. The incorporation of 4-F-aryl and 4-$OCH_3$-aryl substituents into C8 and the heteroaryl substituents into N3 enhance the activity. Compound **170l** has a high antitubercular activity at $IC_{90} = 3.58\ \mu g\ mL^{-1}$.[14]

In 1974, Takamizawa and Sato[90] patented a different technique for the production of dihydro-pyrimido-pyrimido-thiadiazines **173**. Therefore, treatment of 3-((4-amino-2-methylpyrimidin-5-yl)-methyl)-5-substituted-1,3,4-thiadiazol-3-ium salts **171** with diethyl benzoyl phosphonate followed by treating the formed intermediates **172** with a base afforded the respective dihydro-pyrimido-[4′,5′:4,5]-pyrimido[1,2-*d*][1,3,4]-thiadiazines **173** (Scheme 43).

### 2.6. Synthesis of bis-pyrimidopyrimidines

In refluxing toluene and the absence of a catalyst, multicomponent one-pot aza-Diels–Alder reactions of terephthalaldehyde (**174**) with amines **175** and *N*′-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-*N*,*N*-dimethylformimidamide (**176**)




Scheme 61 Reactivity of chlorine atoms attached to the ring carbon atoms.

gave the anticipated bis-pyrimidopyrimidines **177a–k** in a procedure developed by Das *et al.*[30] in another route, a catalytic amount of indium(III)trifluoro-methane-sulfonate [$In(OTf)_3$] was used in chloroform for the successive multicomponent one-pot synthesis of bis-pyrimido-pyrimidines **177a–k** through three reaction steps (Scheme 44).

In addition, the respective bis(1,3-dimethyl-5,6,7,8-tetrahydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-diones) **178–180** were synthesized in moderate yields by the reaction of 6-amino-1,3-dimethylpyrimidine-2,4(1*H*,3*H*)-dione (**66**) with diamines and formaldehyde in a ratio of (2 : 1 : 4) through multicomponent reactions (Scheme 45).[61]

The copper(II) catalyzed reactions of bis-aldehydes (**181a–g**) "prepared from $S_{N^2}$ reactions of the alkyl dibromides with the respective aryl aldehydes in DMF containing potassium carbonate" with 1-(2-amino-1,2-dicyanovinyl)-3-phenylurea (**112**) in methanol containing triethylamine at room temperature yielded the desired 4-imino-6-(phenoxy)-3-phenyl-1,3,4,7-tetrahydropyrimido[5,4-*d*]pyrimidine-2,8-diones **182a–g**, respectively, in excellent yields (Scheme 46). The formation of the bis-1,3,4,7-tetrahydropyrimido[5,4-*d*]pyrimidine-2,8-diones **182** was illustrated in a reported mechanism by the nucleophilic addition of the copper complex formed with phenyl urea **2** to the bis-aldehydes (**181**) (in a molar ratio of 2 : 1) and subsequent cyclocondensation of the formed intermediates, a H-shift and an oxidative cyclization step.[91]

# 3. Reactivity

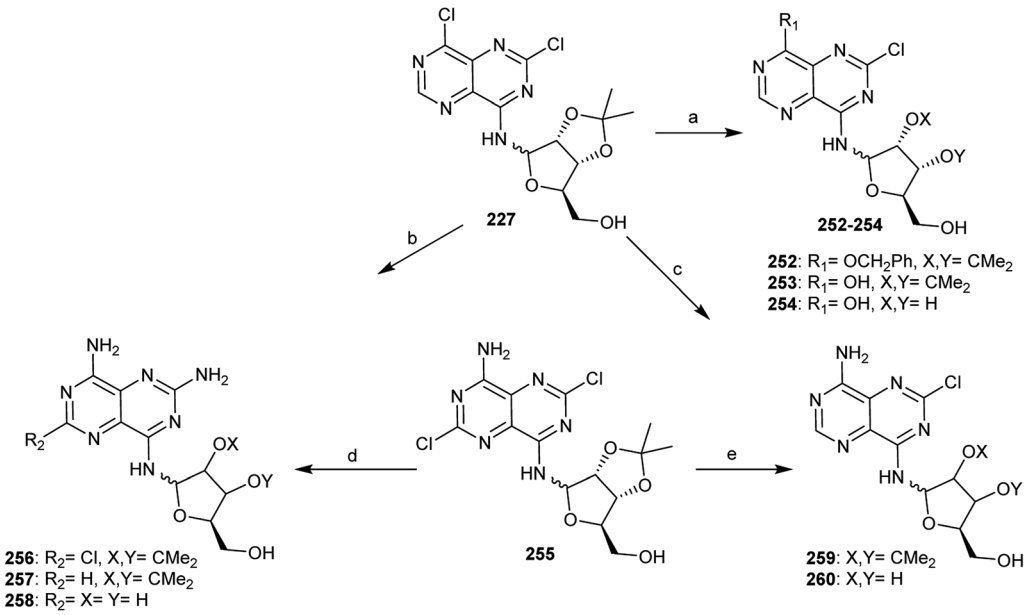
## 3.1. Ring cleavage

Hexahydropyrimido[4,5-*d*]pyrimidine-2,7(1*H*,3*H*)-dione **101** is unstable against bromination in acetic acid at room temperature, and gave two products of pyrimidinones **102a** and **183a**. The formation of the product **183a** was established by the bromination of **102a**. The bromination in acetic acid at 120 °C yielded the desired bromopyrimidinone **183a** (24% yield) (Scheme 47).[68]

## 3.2. Reactivity of substituents attached to ring carbon and heteroatoms

**3.2.1. Condensation reactions.** Condensation of 5,6-diaryl-2,7-dithioxo-2,3,5,6,7,8-hexahydropyrimido[4,5-*d*]pyrimidin-4(1*H*)-ones **184a–i**[37] with hydrazine hydrate yielded the corresponding hydrazine analogs **185a–i**. In addition, the Schiff bases **186a–i** were obtained by condensation of **185a–i** with benzaldehyde in acetic acid at room temperature (Scheme 48). The compounds **186a–i** were evaluated as antibacterial agents against different types of bacterial strains with potent results against Gram-positive bacterial species mainly, the substituted *N*-benzylidinehydrazine compounds conveyed an intense effect relative to the results of Ampicillin.[92]

**3.2.2. Nucleophilic substitution reactions.** Treatment of perchloropyrimido[5,4-*d*]pyrimidine (**187**) with various benzyl amines in THF containing potassium carbonate at room temperature yielded a series of diamines **188** in 75–90% yields through nucleophilic displacement at C4 and C8. Refluxing of




*Reagents and conditions:*

a) (1): BnOH, TEA, room temperature; (2): Pd/C, $H_2$, atmospheric pressure; (3): aqueous TFA; (b): liquid $NH_3$, room temperature; (c): Pd/C, $H_2$, atmospheric pressure; $MeOH/NH_3$, 0 °C, 15 min; (d): liquid $NH_3$, room temperature; Pd/C, $H_2$, atmospheric pressure; (e): (1): Pd/C, $H_2$, atmospheric pressure; (2): aqueous TFA

Scheme 62 Synthesis of the pyrimido[5,4-*d*]pyrimidine skeleton incorporating nucleosides.

**188** with alcohols in THF/sodium hydride or with amines in THF gave the pyrimidopyrimidines **189**, in which the substitution took place at C2 and C6. The removal of the triisopropylsilyl groups was achieved by treatment with TBAF and the protecting dmb groups were cleaved by treatment with trifluoroacetic acid or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) at room temperature to give the anticipated pyrimidopyrimidines **190** in a good yield (Scheme 49). The tetra-substituted-pyrimidopyrimidines **190** were assessed as resistance-modifying agents by inhibiting the transportation of the nucleoside in the presence of the α1-acid glycoprotein.[13]

Treatment of perchloropyrimido[5,4-*d*]pyrimidine (**187**) with long chain alkyl amines in chloroform afforded the mono-alkylamines **191** through nucleophilic displacement at C4. Subsequent treatment of **191** with alkyl amines, such as *n*-butylamine, *n*-hexylamine, *n*-dodecylamine and *n*-hexadecyl-amine in chloroform, afforded the diamines **192–195**, in which the substitution took place at C8 (Scheme 50).[93]

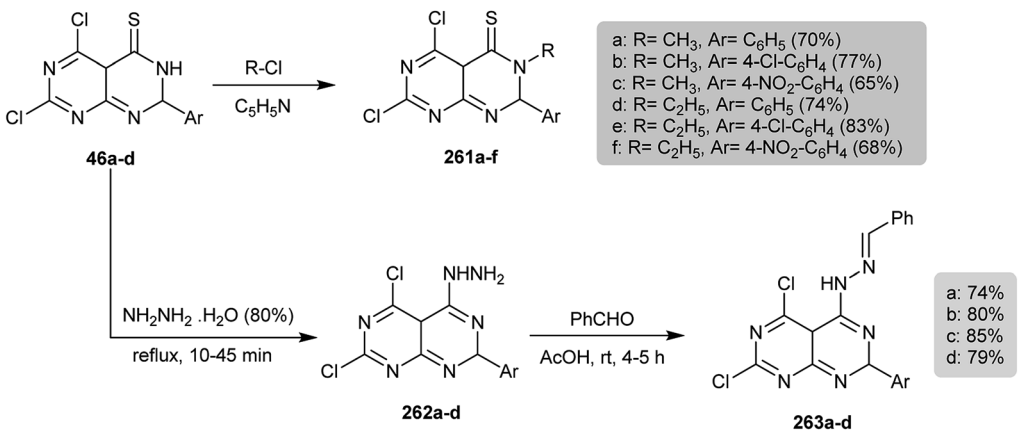
The chlorination of 6-chloropyrimido[5,4-*d*]pyrimidin-4(3*H*)-one (**13**) with thionyl chloride in refluxing DMF provides the dechlorinated product **196**, which cannot be isolated and reacted directly with the respective aryl amines in refluxing isopropanol to yield the amination products **197a–199a** in exceptional yields. Heating of **198a** in methanol containing triethylamine is a simple route to prepare **198e**, which has the possibility of reacting with nucleophilic amines. Reactions of secondary amines with **197a–199a** by heating in DMSO yielded the respective diamines **197a**, **197b**, **198a–j** and **199a–f** by nucleophilic displacement of the chlorine atom at C6 (Scheme 51). The anticipated pyrimidopyrimidines **197–199** were investigated as inhibitors for EGFR autophosphorylation, in which the presence of amine substituents provides high solubility and weakly basic characteristics. Compound **198c** has a higher potency for the inhibition of the autophosphorylation of EGFR in intact A431 cells.[24]

In addition, treatment of 7-mercapto-1,2-dihydropyrimido[4,5-*d*]pyrimidin-4-ol (**8**) with $P_2S_5$ in pyridine afforded the dimercapto analog **200**. In addition, the reduction of 7-mercapto-1,2-dihydropyrimido[4,5-*d*]pyrimidin-4-ol (**8**) with RANEY® Ni in neutral medium gave 1,2-dihydropyrimido[4,5-*d*]pyrimidin-4-ol (**201**), which was treated with $P_2S_5$ in pyridine to afford the respective mercapto analog (**202**). Chlorination of compound **201** with phosphorus oxychloride yielded the desired 4-chloro-1,2-dihydropyrimido[4,5-*d*]pyrimidine (**203**), which was aminated by the reaction with ammonia in ethanol to afford 1,2-dihydropyrimido[4,5-*d*]pyrimidin-4-amine (**204**) (Scheme 52).[53]

Nucleophilic substitution reactions of dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dithiones **138** with various alkyl-/cycloalkyl-/arylamines in DMF containing a catalytic amount of DIPEA yielded the respective amines **205a–l** in moderate to good yields. The same reaction of **138** with *N*-methylpiperazine was accomplished by heating at 120 °C and the reaction of **138** with phenylhydrazine was processed in a refluxing mixture of acetonitrile and ethanol to afford the respective dihydropyrimido[4,5-*d*]pyrimidine-2,4(1*H*,3*H*)-dithiones **205m** and **205n**, respectively, through amination step reactions by nucleophilic attack of the amines to the methylmercaptan moiety (Scheme 53).[74]

Treatment of perchloropyrimido[5,4-*d*]pyrimidine (**187**) with 4-methoxybenzylamine in THF containing potassium carbonate provided the diamines **206** through the nucleophilic displacement of chlorine atoms at C4 and C8. Heating the diamine **206** with the appropriate primary and secondary amines yielded the analogs pyrimidopyrimidines **207–215** (Scheme 54). The previous synthetic method is similar to those reported by Curtin *et al.*[94] The compounds were assessed *in vitro* as inhibitors for cellular proliferation and motility prompted by h-prune in breast cancer, and high potency was found for compounds **214** and **215**.[8]

Nakashima and Akiyama[95] prepared a series of tri- and tetra-substituted-amines of pyrimido[5,4-*d*]pyrimidines **218** and **221** by chlorination of 1,7-dihydropyrimido[5,4-*d*]pyrimidine-2,4,8(3*H*)-trione (**216**) and 1,5-dihydropyrimido[5,4-*d*]pyrimidine-2,4,6,8(3*H*,7*H*)-tetraone (**219**), each with phosphorus oxychloride or phosphorus pentasulfide followed by a nucleophilic substitution reaction of the respective halogenated products **217** and **220** with various amines (Scheme 55). The investigated




Scheme 63 Synthesis of *N*-alkyl- and hydrazine-substituents.

pyrimidopyrimidines **218** and **221** were applied as fluorescence reagents in dioxane in a peroxyoxalate–chemiluminescence reaction, wherein the compounds with 3-methoxy-aniline substituents have the highest intensity.

Treatment of perchloropyrimido[5,4-*d*]pyrimidine (**187**) with free 2,3-*O*-isopropylidine-D-ribofuranosyl-amine, which was formed *in situ* from its stable salt **222** by the addition of triethylamine in *n*-butanol at room temperature, yielded a mixture of two nucleosides **224** and its α-anomer **223** in a ratio of 1 : 0.77. At ambient temperature, the product **225** was also obtained after 16 h from the same reaction, providing the possibility of a chlorine atom at the C4 position of compound **224** to be displaced by the nucleophile. Similarly, an anomeric mixture of pyrimidopyrimidines **227** was obtained by treatment of **226** with **222** in *n*-butanol containing triethylamine at room temperature after 6 h (Scheme 56).[20]

Gall and coworkers[96] reported a synthetic technique for the preparation of 2,4,6,8-tetrakis(3,5-di-*tert*-butyl-phenoxy)pyrimido[5,4-*d*]pyrimidine (**228d**) in a yield of 28.5% from the reaction of 2,4,6,8-tetrachloropyrimido[5,4-*d*]pyrimidine (**187**) with four equivalents of 3,5-di-*t*-butylphenol in THF containing sodium hydride. The structure of **228d** was established using X-ray crystallography. The relative pyrimidopyrimidines **228a–c** were obtained in 67, 33, and 72% yields, respectively (Fig. 2).

The respective pyrimido[4,5-*d*]pyrimidine **229** was obtained in a 38% yield under diazotization conditions of the analogs 77 with sodium nitrite in acetic acid through the cleavage of the N–N bond. Treatment of **229** with triflic anhydride in the presence of triethylamine gave the corresponding triflate **230** in a 64% yield. The nucleophilic substitution reactions of **230** with mercaptoalcohols yielded the thioethers **231–233** in 67–77% yields (Scheme 57).[62]

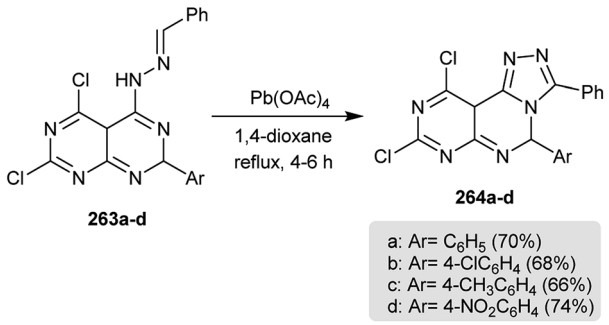
As stated in the previous reaction sequence, dihydropyrimido[4,5-*d*]pyrimidine-triones **88–91** reacted with triflic anhydride in dichloromethane containing triethylamine to give the anticipated triflates **234–237** in 52–83% yields. The reactions of **234–237** with mercaptoalcohols in THF/TEA afforded the dihydropyrimido[4,5-*d*]pyrimidine-diones **238–244** in 45–82% yields through a nucleophilic substitution step (Scheme 58).[62]

**3.2.3. Alkylation.** Regio-alkylation of dihydropyrimido[4,5-*d*]pyrimidine-trithione **139** was accomplished by treatment with a stirred solution of 40% Triton (dried from methanol) in DMF followed by subsequent addition of *o*-chloroalkyl- and cycloalkyl amines to give the anticipated *S*-alkyl derivatives **245a–i**. The alkylation process occurred at the sulfur atom attached to the C7 of the pyrimidopyrimidine ring (Scheme 59). The compounds of the series **245** were assessed as antiproliferative agents, in which compounds **245b**, **245d**, **245f**, **245h**, **245i**, and **245j** revealed potent activities against the tested leukemia and prostate cell lines.[75]

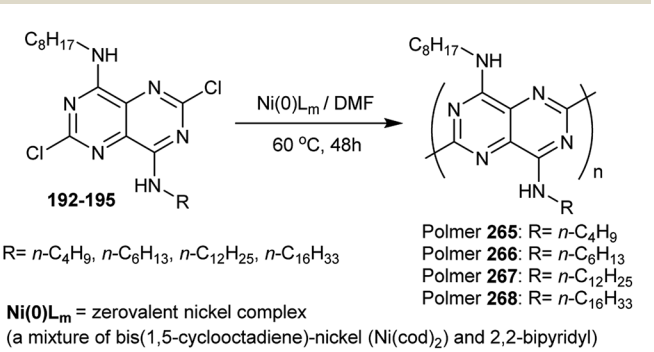
**3.2.4. Oxidation and substitution reactions.** Oxidation of cyclopentyl-pyrimido[4,5-*d*]pyrimidine-dione **123** with *m*-chloroperoxybenzoic acid (*m*-CPBA) yielded the sulfone **246** in a 72% yield. Protection of the hydroxymethyl group was achieved by reaction of **246** with dimethyltryptamine (DMT)-Cl in a 1,4-dioxane/DIPEA system to yield the protected nucleotide **247**, which was converted into the respective phosphoramidite **248** at a yield of 60% by reaction with 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite in dichloromethane containing DIPEA through the nucleophilic displacement of the chlorine atom of the desired chlorophosphoramidite (Scheme 60).[72]

**3.2.5. Miscellaneous reactions.** Treatment of compounds **223** and **224**, each with ammonia in ethanol at 0 °C, sodium methoxide solution in methanol, benzyl alcohol in the presence of TEA at rt (>60% yield), methyl amine or dimethyl amine gave the respective amines and their α-anomer **249** ($R_1$ = (α, β) $NH_2$, $OCH_3$, $OCH_2Ph$, OH, NHMe, $NMe_2$). Catalytic hydrogenation of **249** using Pd/C yielded the desired dehalogenation pyrimidopyrimidines and their α-anomer **250**. Subsequent treatment of **250** with an aqueous solution of trifluoroacetic acid (90%) at room temperature afforded pyrimidopyrimidines **251** through isopropylidene ring cleavage in high yields (Scheme 61).[20]

Treatment of **227** with benzyl alcohol in the presence of triethylamine gave **252**. In addition, the catalytic reduction of **30** with Pd/C yielded **253**, which after hydrolysis with trifluoroacetic acid afforded **254** in a yield of 58%. Treatment of **255** with liquid ammonia for six days gave a mixture of isomers identified as β-anomer **256** (59% yield) and α-anomer **256** (15% yield). Catalytic hydrogenation of **256** afforded the respective **34**. Amonolysis of **29** with liquid ammonia after six days furnished another synthetic method for **257**. Deisopropylidenation of **257** yielded the corresponding pyrimidopyrimidine **258**, which was isolated as a salt of TFA. Pyrimidopyrimidine **259** was obtained in a yield of 65% by hydrogenation of **235** with the removal of




Scheme 64 Synthesis of 3-aryl-5,10*a*-dihydropyrimido[5,4-*e*][1,2,4]triazolo[4,3-*c*]pyrimidines.




Scheme 65 Synthesis of polymers bearing alkylamino groups.

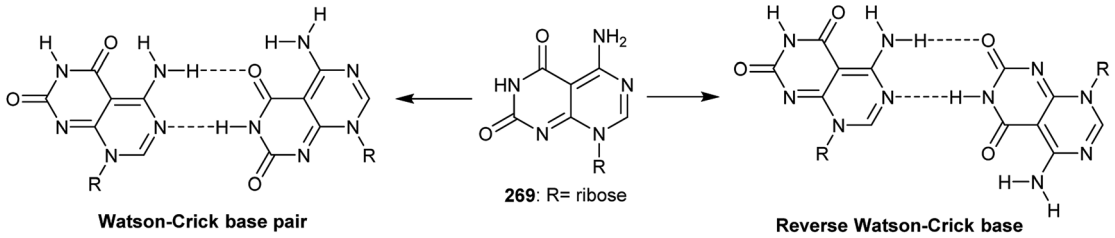



Fig. 3 The structures of the two probable monomeric conformers as base pair motifs.

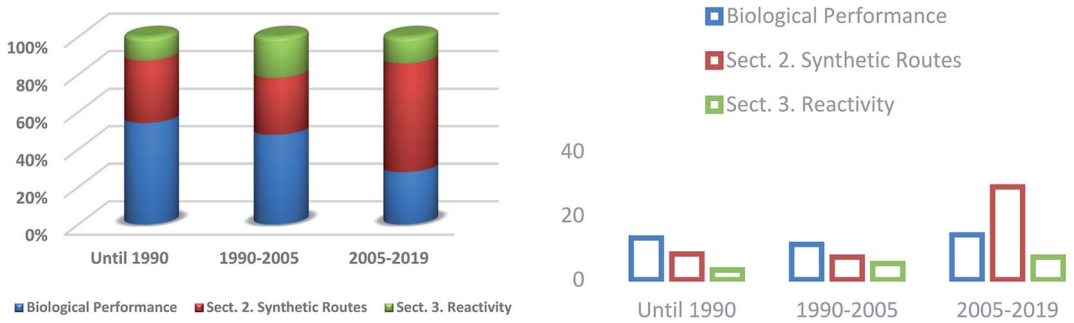



Fig. 4 A comparison of the different areas of research in the last decade.

the chlorine atom at C2. Successive deisopropylidenation of **259** yielded the respective pyrimidopyrimidine **260** in a yield of 67% (Scheme 62).[20]

Under reflux conditions, the electrophilic substitution of the proton at N3 of **46a–d** in the reaction with alkyl halides in pyridine yielded the corresponding *N*-alkyl derivatives **261a–f** in good yields. On the other hand, refluxing of thiones **46a–d** with hydrazine hydrate yielded the respective hydrazines **262a–d**, which were condensed with benzaldehydes in acetic acid to give Schiff bases **263a–d**, respectively (Scheme 63).[56]

Intramolecular cyclization of **263a–d** in refluxing 1,4-dioxane containing catalytic palladium acetate gave the desired dihydropyrimido[1,2,4]triazolo-pyrimidines **264a–d** (Scheme 64). The transformation mechanism to products **6** was reported through proton, 1,5-hydrogen abstraction and subsequent cyclization.[56]

**3.2.6. Synthesis of polymers bearing alkylamino groups.** A series of conjugated polymers of pyrimidopyrimidines **265–268** were prepared in high yields by heating the corresponding pyrimidopyrimidines with a nickel(0) complex in DMF. The procedure involved dehalogenation polycondensation of the diamines **192–195** at C2 and C6 of the monomers. The polymers displayed photo-luminescence in the solution and in the film. In the solid-state, the polymers have an inclination to form a stacked structure (Scheme 65).[93]

**3.2.7. Synthesis of ribose–supramolecular complex.** Ribose bearing 5-amino-pyrimido[4,5-*d*]pyrimidine-2,4(3*H*,8*H*)-dione (**269**) is considered as a structurally biological active molecule and has received attention from different industrial fields. Zhao *et al.*[97] studied the mechanism of the formation of a flower-shaped supramolecular complex structure of a nucleoside derived pyrimido[4,5-*d*]pyrimidine using X-ray crystallography, NMR spectroscopy, dynamic light scattering, and electron microscopy scanning. The structure has two possible conformers, a Watson–Crick base pair and a reverse Watson–Crick base (Fig. 3).

## 4. Conclusions

The present study reviews and summarizes the research into the chemistry of pyrimido[4,5-*d*]pyrimidine and pyrimido[5,4-*d*]pyrimidine analogs published to date. The diverse biological efficacy of pyrimidopyrimidine compounds has been extensively tested on a large scale. This type of compound can be obtained *via* several synthetic routes using different techniques, for example: (a) the reactions of β-haloester with thiourea, or heating β-aminoester with formamide or phenyl isocyanate, or a multistep-synthesis starting from β-aminoester or α,β-unsaturated esters; (b) preparation from 4-amino-2,6-dichloropyrimidine and 2,6-diaminopyrimidin-4(3*H*)-one; (c) multicomponent reactions of barbituric and thiobarbituric acids with aryl aldehydes and urea, aryl isothiocyanates, thiourea or arylamines; (d) *via* a definite route, in which pyrimidopyrimidines were also synthesized from the reaction of 1,5-diketones with hydrazine hydrate; (e) preparation from multicomponent reactions of acyclic reagents such as ureas with ketones (or aldehydes) and aryl aldehydes or 2-(ethoxymethylene)malononitrile; and (f) other techniques that have been applied for the synthesis of tricyclic and tetracyclic systems or the transformation of another ring.

On the other hand, the substituted compounds are reactive with respect to nucleophilic, electrophilic, substitution and

condensation reactions and provide the possibility of obtaining a number of compounds incorporating a pyrimidopyrimidine nucleus with the aim of obtaining novel antibiotics. Therefore, improvement of the method used for the synthesis of pyrimido[4,5-*d*]pyrimidines and pyrimido[5,4-*d*]pyrimidines would be particularly useful for the preparation of bicyclic pyrimidines, which could be useful in several areas of chemistry, for example, chelating derivatives, bases, and drug design.

## 5. Literature overview

Numerous investigations have been performed and the frequency with which published articles from different fields have been published has shown continual growth(Fig. 4). Synthetic strategies (Section 2) used to access heterocycles with a pyrimido[4,5-*d*]pyrimidine and pyrimido[5,4-*d*]pyrimidine skeleton have been considerably extended and there has been a significant increase in interest from researchers for the synthesis of this class of compounds in recent years (2005–2019). Modified methods of preparation have reached a remarkable level of versatility and efficiency. In view of the synthetic methods, it has been found that they have increased considerably in recent years owing to the high biological diversity of this class of compounds. During the period before 1990, the biological importance of the compounds and the synthetic procedures produced great interest, as well as the reactivity of substituents, which has increased over time.

## 6. Future perspectives

The synthesis of pyrimido[4,5-*d*]pyrimidines was first reported by Taylor *et al.*[18] from the reaction of 4-aminopyrimidine-5-carboxamides with 4-amino-5-cyanopyrimidines. Since then, many types of research have been published for the synthesis of compounds incorporating a pyrimidopyrimidine core and their subsequent application in the medicinal field, as these compounds have a diverse and remarkable biological significance. The interest of researchers should be focused on the evaluation of these compounds in different areas of medicinal chemistry, for example, ATPase activity enzymes that catalyze the decomposition of ATP", human CaR antagonism, hepatic metabolism, antitumor activity, fungicidal activity, antimycobacterial activity, and tyrosine kinase inhibitors, which are applications of the analogs of pyrido[4,3-*d*]pyrimidines.[49a]

## Conflicts of interest

The authors declare no conflicts of interest.

## Abbreviations

| | |
|---|---|
| HBV-DNA | Hepatitis B virus DNA |
| KDR | Kinase insert domain receptor (a type III receptor tyrosine kinase) |
| EGFR | Epidermal growth factor receptor |
| FGFRs | Fibroblast growth factor receptors |
| PDGFR | Platelet-derived growth factor receptors |
| TFAA | Trifluoroacetic anhydride |
| DMF | *N*,*N*-Dimethylformamide |
| TFA | Trifluoroacetic acid |
| THF | Tetrahydrofuran |
| DMU | L-(+)-Tartaric acid-dimethyl urea |
| CDI | 1,1′-Carbonyldiimidazole |
| NIS | *N*-Iodosuccinimide |
| *B. subtilis* | *Bacillus subtilis* (bacteria) |
| *S. aureus* | *Staphylococcus aureus* |
| *E. coli* | *Escherichia coli* |
| *A. niger* | *Aspergillus niger* |
| *C. albicans* | *Candida albicans* |
| *P. diminuta* | *Pseudomonas diminuta* |
| ppm | Parts per million |
| *P. aeruginosa* | *Pseudomonas aeruginosa* |
| LiHMDS | Lithium bis(trimethylsilyl)amide |
| *p*-TSA | *p*-Toluenesulfonic acid |
| NHC-PPIm | N-heterocyclic carbene 1,3-dipropylimidazole-2-ylidene |
| DMF-DMA | *N*,*N*-Dimethylformamide dimethyl acetal |
| $EC_{50}$ | Half maximal effective concentration |
| DMSO | Dimethyl sulfoxide |
| IC | Inhibitory concentration |
| A431 cells | Human epidermoid carcinoma cell line |
| $P_2S_5$ | Phosphorus pentasulfide |
| DIPEA | *N*,*N*-Diisopropylethylamine |

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