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Review

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Advanced updates in the synthetic strategies and reaction pathways of pyrazolo[3,4-*d*]pyrimidine derivatives

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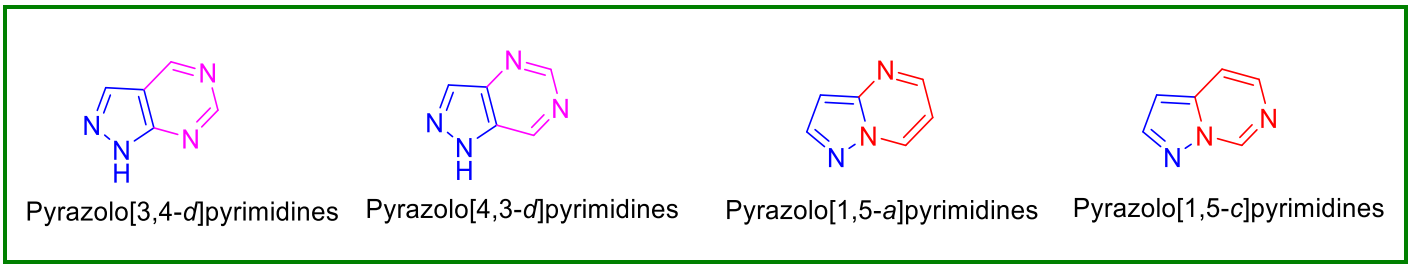
Abstract

This review presents a concise overview of recent advances in the synthesis of pyrazolo[3,4-*d*]pyrimidines, an important class of fused heterocycles of considerable synthetic and medicinal interest. Emphasis is placed on efficient and versatile methodologies affording structurally diverse derivatives, including routes from pyrazole, pyrimidine, and acyclic precursors, as well as one-pot, solid-phase, and Diels–Alder-type approaches. The reported biological activities of this scaffold, including antimicrobial, anticancer, anti-inflammatory, antiviral, antihypertensive, and xanthine oxidase inhibitory properties, are also briefly highlighted. Thus, the review provides a useful reference for the design of new synthetic strategies and biologically relevant pyrazolo[3,4-*d*] pyrimidine derivatives.



Keywords: Pyrazolo[3,4-*d*]pyrimidin-4-one, 4-chloro pyrazolo[3,4-*d*]pyrimidine, 4-amino pyrazolo[3,4-*d*] pyrimidine, 4-imino-5-aminopyrazolo[3,4-*d*]pyrimidine, 4-hydrazino pyrazolo[3,4-*d*]pyrimidine

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1. Introduction

The pyrazolo[3,4-*d*]pyrimidine scaffold is a privileged heterocyclic framework that has attracted considerable attention due to its broad spectrum of biological activities, including antiviral,[1] antibacterial,[2,3] antioxidant,[4,5] anticonvulsant,[6] and anticancer[7-10] effects. Recent studies have highlighted of diverse pyrazolo[3,4-*d*] pyrimidine derivatives as anticancer agents through the inhibition of the CDK2 enzyme pathway interactions with multiple molecular targets,[11] particularly via inhibition of tubulin polymerization,[12] epidermal growth factor receptor (EGFR),[13] vascular endothelial growth factor receptor-2 (VEGFR-2),[14, 15] and cyclin-dependent kinase 2 (CDK2).[15-17]

The pyrazolo[3,4-*d*]pyrimidine core is widely recognized in medicinal chemistry as a valuable pharmacophore for drug design,[18] owing to its ability to mimic hinge-region interactions within kinase active sites and its role as a bioisostere of the adenine moiety of ATP.[19, 20] Consequently, this nucleus has been incorporated into compounds exhibiting diverse pharmacological profiles, including antimicrobial, antitubercular, antimalarial, and antidiabetic activities.[21, 22] Moreover, previous studies have demonstrated that the pyrazolo[3,4-*d*]pyrimidine nucleus displays significant anti-inflammatory activity.[22-29] Notably, when fused with or conjugated to other heterocyclic ring systems, pyrazolo[3,4-*d*]pyrimidines constitute a valuable and versatile scaffold that has been extensively employed in the design of numerous biologically and pharmacologically active compounds.[30-32] Moreover, structural hybridization of the pyrazolo[3,4-*d*]pyrimidine nucleus via ring fusion or conjugation with other heterocyclic motifs has generated diversified candidates of considerable biological relevance, notably those demonstrating significant preferential inhibition of COX-2 compared to COX-1.[28, 33, 34]

It was mentioned that[28] the pyrazolo[3,4-*d*]pyrimidine derivatives incorporating thiazolidine and pyrazole moieties (compounds **2** and **3**) were identified as selective COX-2 inhibitors, exhibiting selectivity

indices (SI) of 12.45 and 8.52, respectively, exceeding that of the reference drug celecoxib (SI = 7.23). Moreover, compound pyrazolo[3,4-*d*]pyrimidine **4** demonstrated marked inhibition of 5-lipoxygenase (5-LOX) activity (~72%),[35] while compound **5** was identified as a dual COX/iNOS inhibitor, suggesting potential anti-inflammatory and analgesic efficacy (Fig. 1).[36]

Furthermore, structures featuring an acetamide linker or related derivatives have gained attention as key motifs in the development of anti-inflammatory agents.[37, 38] In this context, compounds **I** and **II** displayed notable COX-2 inhibitory activity, with IC_{50} values of 78.9 µM and 23.8 µM, respectively (Fig. 1), demonstrating comparable or superior potency relative to NS398 (IC_{50} = 54.0 µM) and celecoxib (IC_{50} = 54.0 µM) as reference inhibitors.[39]

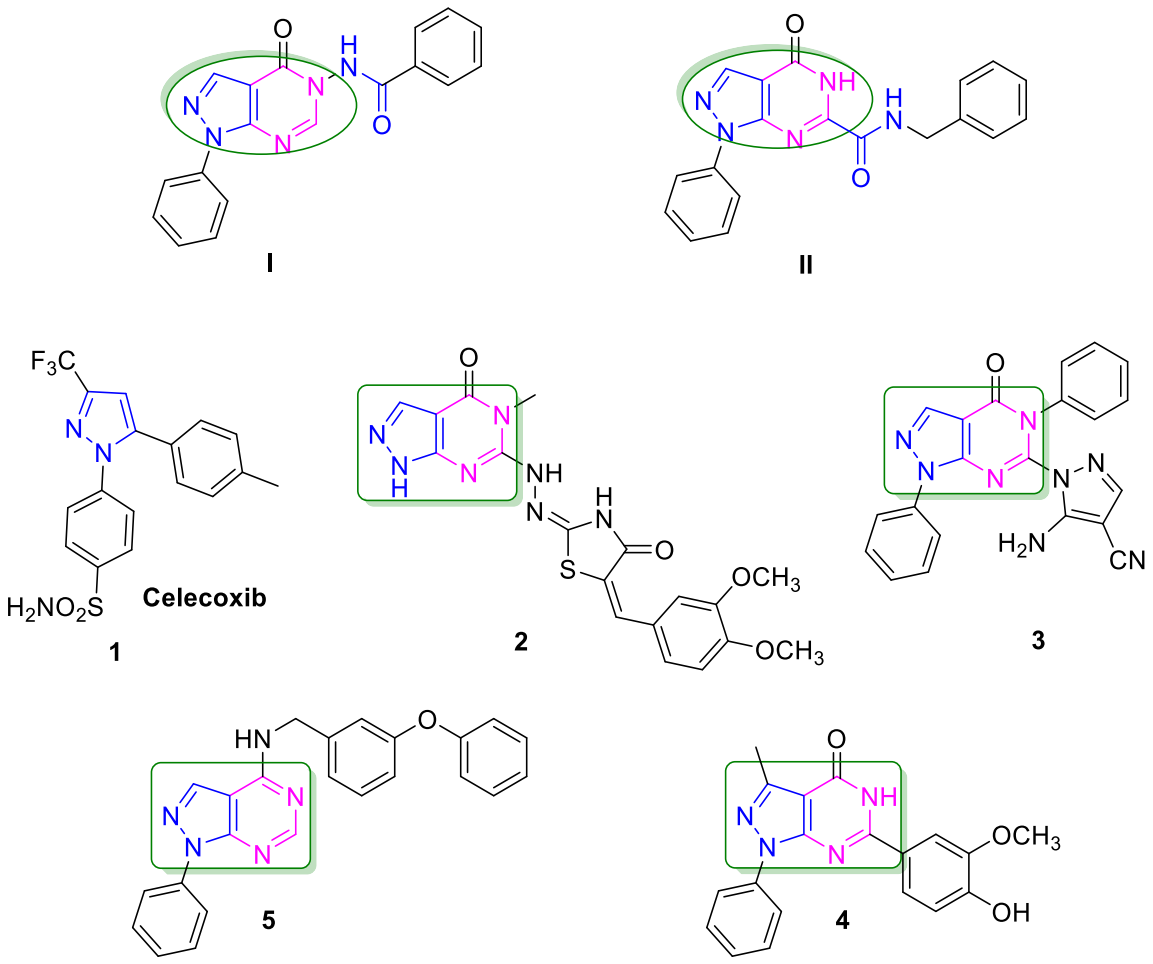


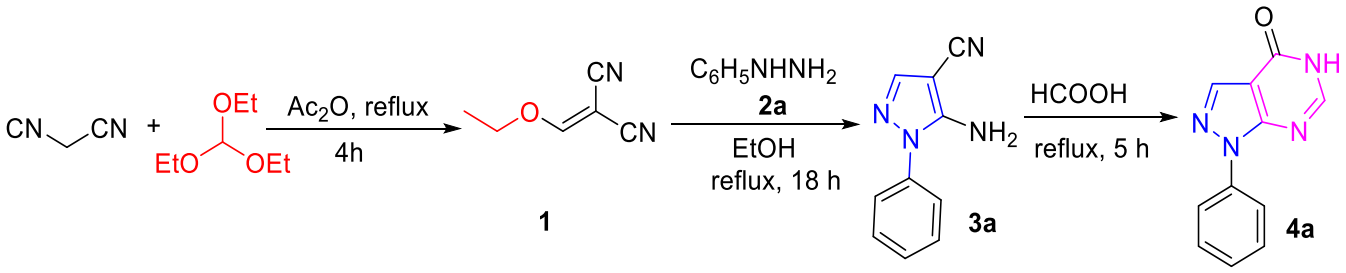
Figure 1. Chemical structures of celecoxib 1 and the pyrazolo[3,4-*d*]pyrimidine reported as: selective COX-2 inhibitors 2 and 3, specific anti-LOX agent 4, and the COXs/iNOS dual inhibitor.

2. Synthesis of Pyrazolo[3,4-*d*]pyrimidine derivatives

2.1 Synthesis of pyrazolo[3,4-*d*]pyrimidin-4-one

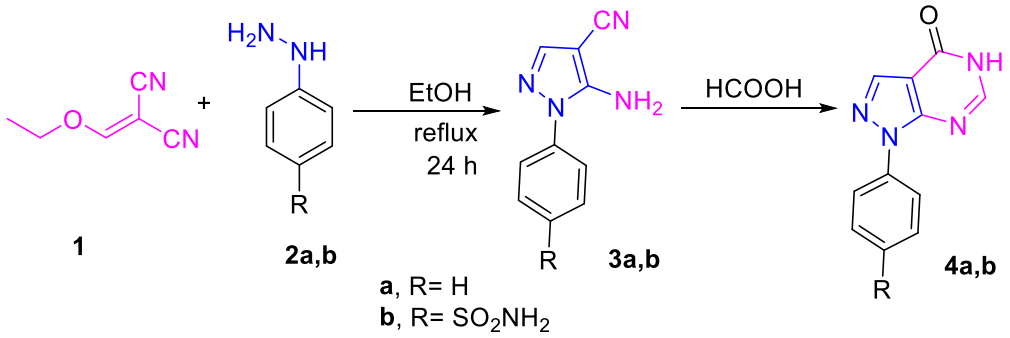
In 2025, Roshdi *et al*. reported that the intermediate **1** was synthesized according to the reported procedure[36,40] through the reaction of malonitrile with triethyl orthoformate in the presence of acetic anhydride under reflux conditions. Subsequent treatment of compound **1** with phenylhydrazine in refluxing ethanol furnished 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles **2**.[41] The key bicyclic intermediate **3** was then obtained by cyclization of

compound **2** in refluxing formic acid, affording the desired pyrazolo[3,4-d]pyrimidin-4(5*H*)-one scaffold **3** in good yield (Scheme 1).[41]



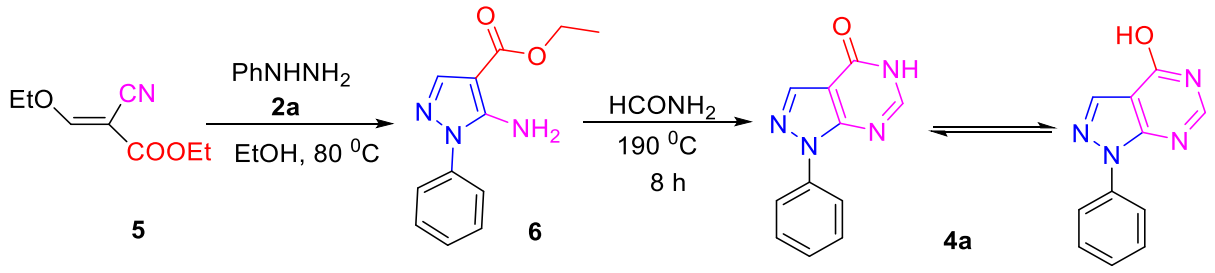
Scheme 1. Synthesis of pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one derivative **3.**

Compound **1**, 2-(ethoxymethylene)malononitrile,[27] was previously prepared in a one-pot procedure by refluxing malononitrile with ethyl orthoformate and acetic anhydride for 4–5 h.[42] Reaction of **1** with phenylhydrazine derivatives **2a,b**[43] in refluxing ethanol for 24 h furnished the corresponding 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles **3a**. Subsequent cyclocondensation of compounds **3a** with formic acid under reflux for 6 h afforded the pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one derivatives **4a** in good yields, in accordance with the reported procedure (Scheme 2). [44, 45]



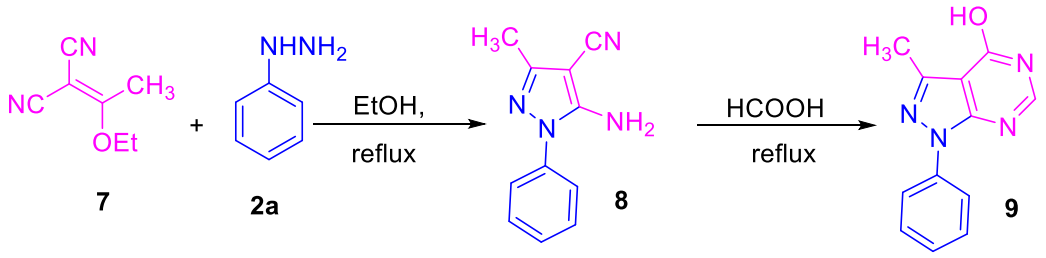
Scheme 2. Synthesis of the pyrazolo[3,4-*d*]pyrimidin-4-one **4a,b**.

In 2023,[46] it was mentioned that the cyclocondensation of ethyl (ethoxymethylene)cyanoacetate **5** with phenylhydrazine provided ethyl 5-amino-1-phenyl-1*H*-pyrazole-4-carboxylate **6**.[47, 48] Treatment of compound **6** with formamide promoted further ring closure, affording the corresponding pyrazolo[3,4-*d*] pyrimidinone derivative **4a**[49, 50] as illustrated in Scheme 3.



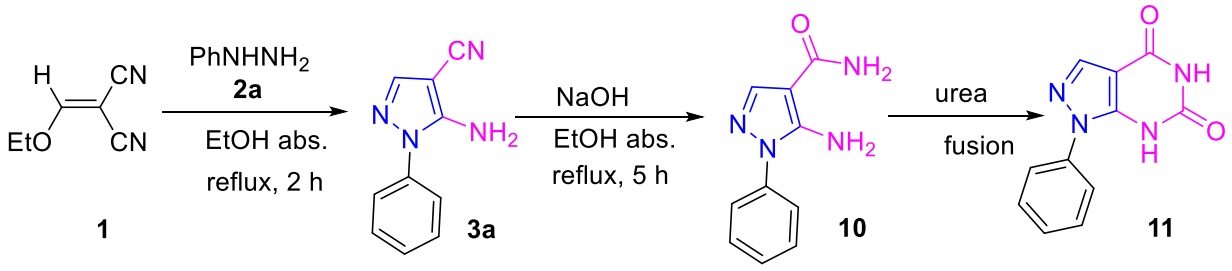
Scheme 3. Synthetic pathway of pyrazolo[3,4-*d*]pyrimidinone derivative **4a**.

El Hafi M. *et al.* reported that[51] reaction of 2-(1-ethoxyethylidene)malononitrile **7** with phenylhydrazine **2a** in ethanol under reflux for 2 h afforded 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile **8**.[41] compound **8** was subsequently subjected to cyclization by refluxing with formic acid for 7 h to yield the key intermediate pyrazolo[3,4-d]pyrimidine derivative **9** in good yield (Scheme 4).



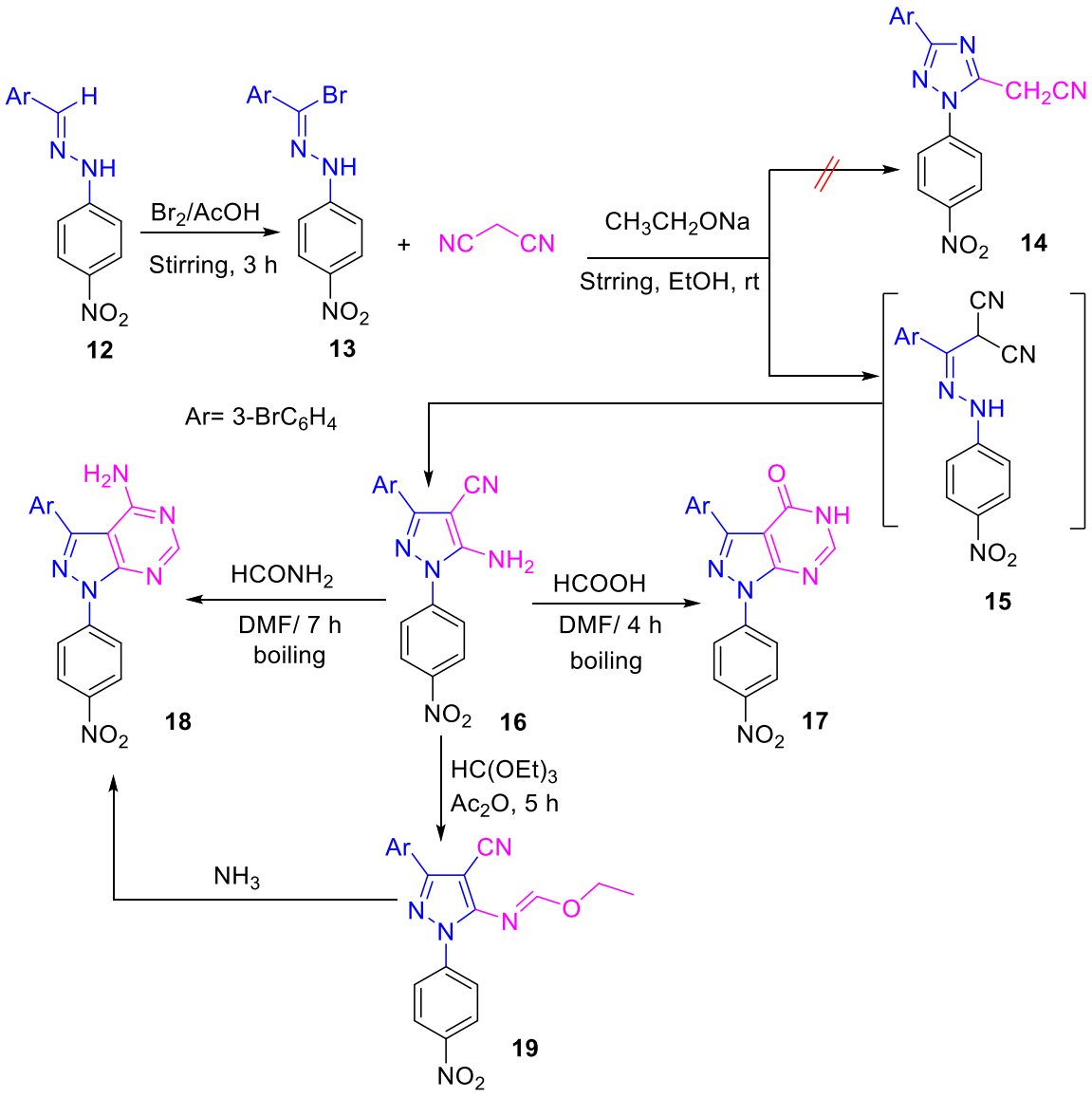
Scheme 4. Synthesis of 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ol **9.**

In 2022 it was reported[52] that treatment of ethoxymethylenemalononitrile **1**[53] with phenylhydrazine afforded the corresponding 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **3a**.[54] Subsequent partial hydrolysis of **3a** with alcoholic sodium hydroxide afforded the corresponding carboxamide derivative **10**.[55] Fusion of compound **10** with urea then furnished 1-phenyl-1,7-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*)-dione **11** (Scheme 5).



Scheme 5. Synthetic protocol of 1-phenyl-1,7-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*)-dione **11.**

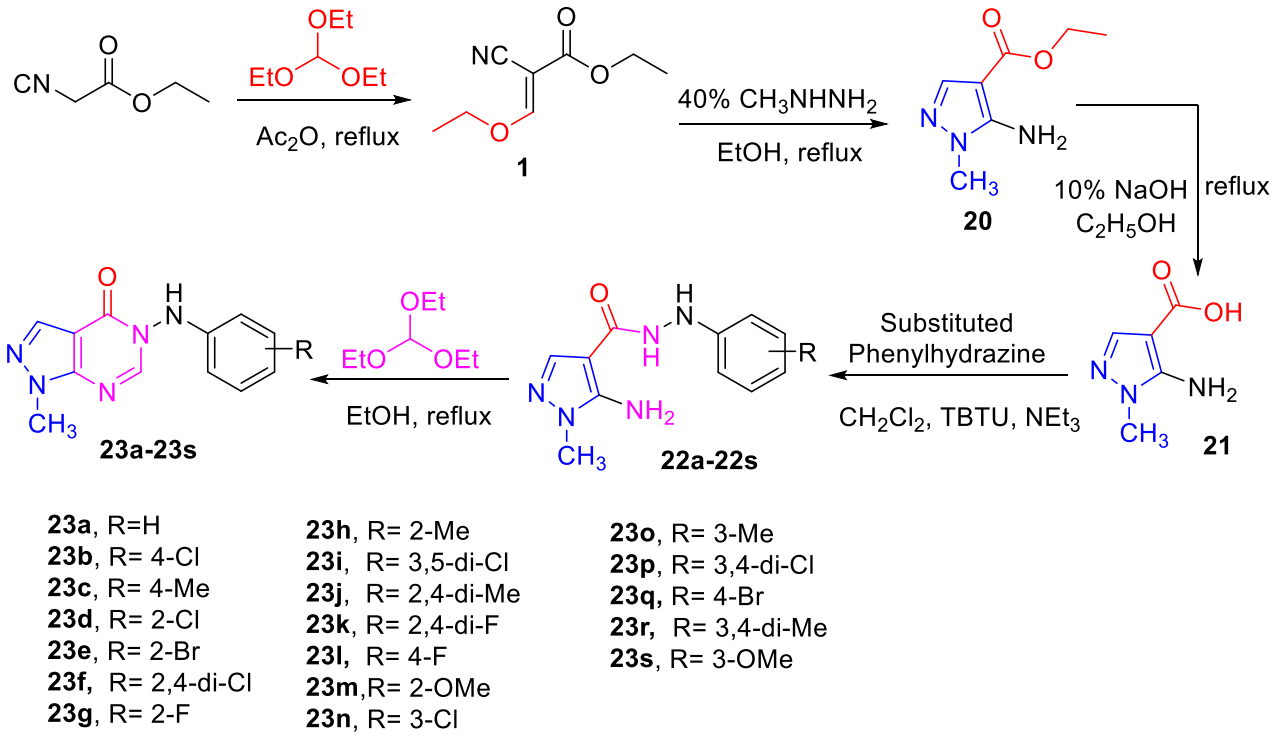
It was reported that hydrazonoyl bromide **13** was synthesized via bromination of compound **12** in acetic acid at room temperature.[56] Subsequent reaction of **13** with malononitrile in ethanolic sodium ethoxide did not afford 2-(3-aryl-1-(4-nitrophenyl)-1*H*-1,2,4-triazol-5-yl) acetonitrile **14** but afforded the corresponding intermediate (*E*)-2-(aryl(2-(4-nitrophenyl)hydrazineylidene)methyl)malononitrile **15** followed by the formation the corresponding pyrazole derivative **16**. Treatment of **16** with formic acid, formamide, or triethyl orthoformate yielded the pyrazolo[3,4-*d*]pyrimidine derivatives **17** and **18**, and the ethoxymethylene derivative **19**, respectively. Furthermore, compound **19** underwent ammonolysis in ethanol to give compound **18** (Scheme 6).



Scheme 6. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **17**, **18**.

In 2025, a series of 1-methyl-5-(phenylamino)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one **23a–23s**[57] was synthesized through the sequence outlined in Scheme 7. Ethyl 2-cyanoacetate was first treated with triethyl orthoformate to afford Ethyl 2-cyano-3-ethoxyacrylate (**1**). Condensation of compound **1** with methylhydrazine in refluxing ethanol furnished ethyl 5-amino-1-methyl-1*H*-pyrazole-4-carboxylate (**20**). Subsequent alkaline hydrolysis with sodium hydroxide provided 5-amino-1-methyl-1*H*-pyrazole-4-carboxylic acid (**21**). Coupling of compounds **21** with appropriately substituted phenylhydrazines in dichloromethane, using triethylamine and TBTU as the base and coupling reagent, respectively, afforded the corresponding 5-amino-1-methyl-N'-phenyl-1*H*-pyrazole-4-carbohydrazides **22a–22s**. Cyclocondensation of intermediates **22a–22s** with triethyl orthoformate in refluxing ethanol gave the desired target compounds **23a–23s** (Scheme 7).

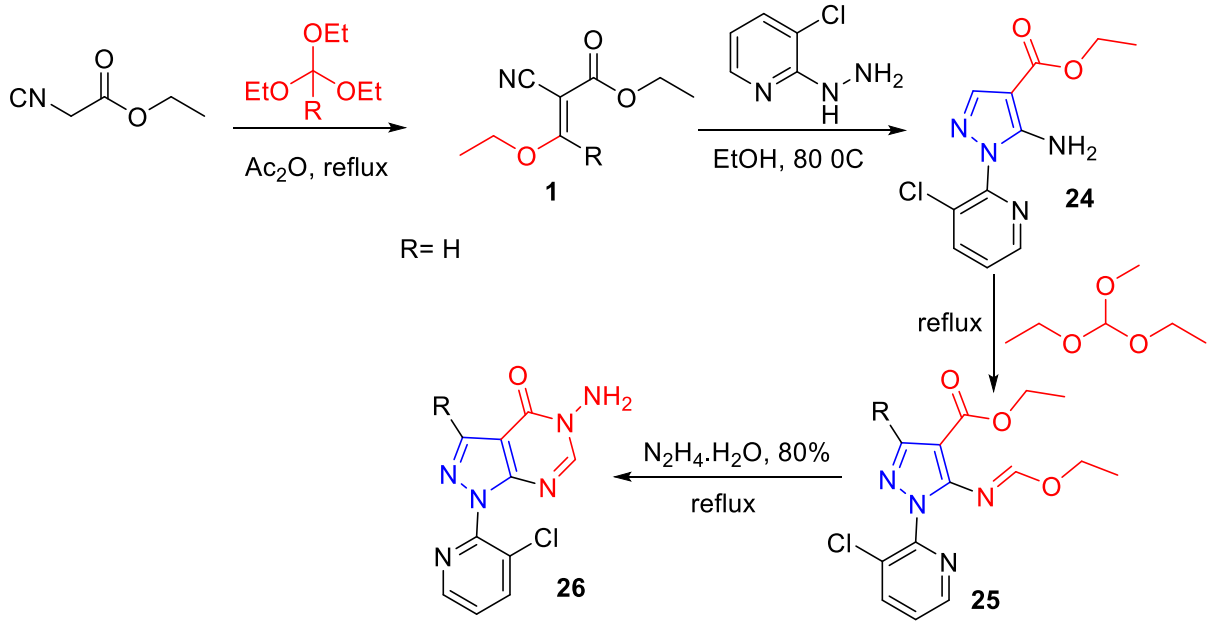
In 2024, the synthesis of substituted pyrazolo[3,4-*d*]pyrimidine derivatives **26** was reported.[58] The synthetic pathway commenced from ethyl cyanoacetate, which was treated with triethyl orthoformate in the presence of acetic anhydride under reflux to afford the corresponding ethoxymethylene derivative **1**.



23a, R=H
23b, R= 4-Cl
23c, R= 4-Me
23d, R= 2-Cl
23e, R= 2-Br
23f, R= 2,4-di-Cl
23g, R= 2-F
23h, R= 2-Me
23i, R= 3,5-di-Cl
23j, R= 2,4-di-Me
23k, R= 2,4-di-F
23l, R= 4-F
23m,R= 2-OMe
23n, R= 3-Cl
23o, R= 3-Me
23p, R= 3,4-di-Cl
23q, R= 4-Br
23r, R= 3,4-di-Me
23s, R= 3-OMe

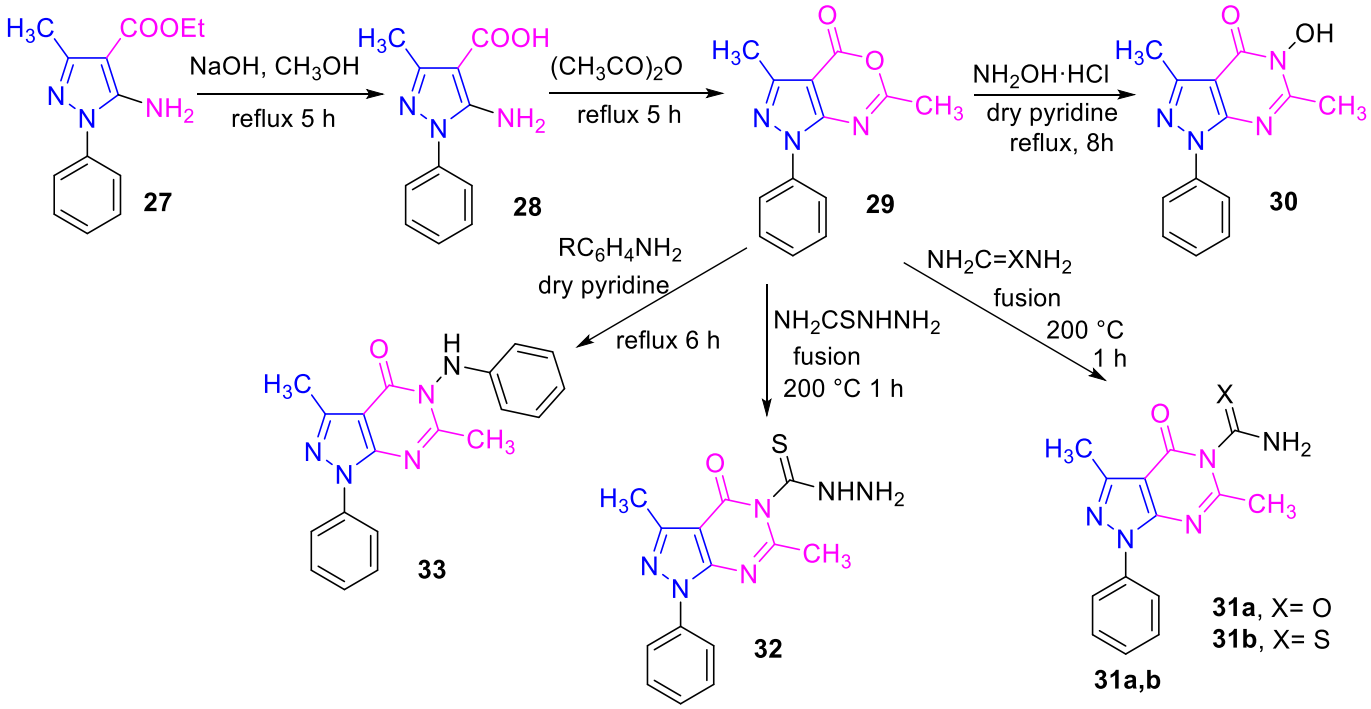
Scheme 7. Synthetic routes of 1-methyl-5-(phenylamino)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one **23a-23s.**

Cyclocondensation of intermediate **1** with an aminopyridine derivative in ethanol at 80 °C furnished the pyrazole intermediate **24**. Subsequent reaction of compound **24** with triethyl orthoformate under reflux provided the formimidate intermediate **25**. Treatment of **25** with 80% hydrazine hydrate induced intramolecular cyclization to construct the fused pyrazolo[3,4-*d*]pyrimidine derivative **26** (Scheme 8).



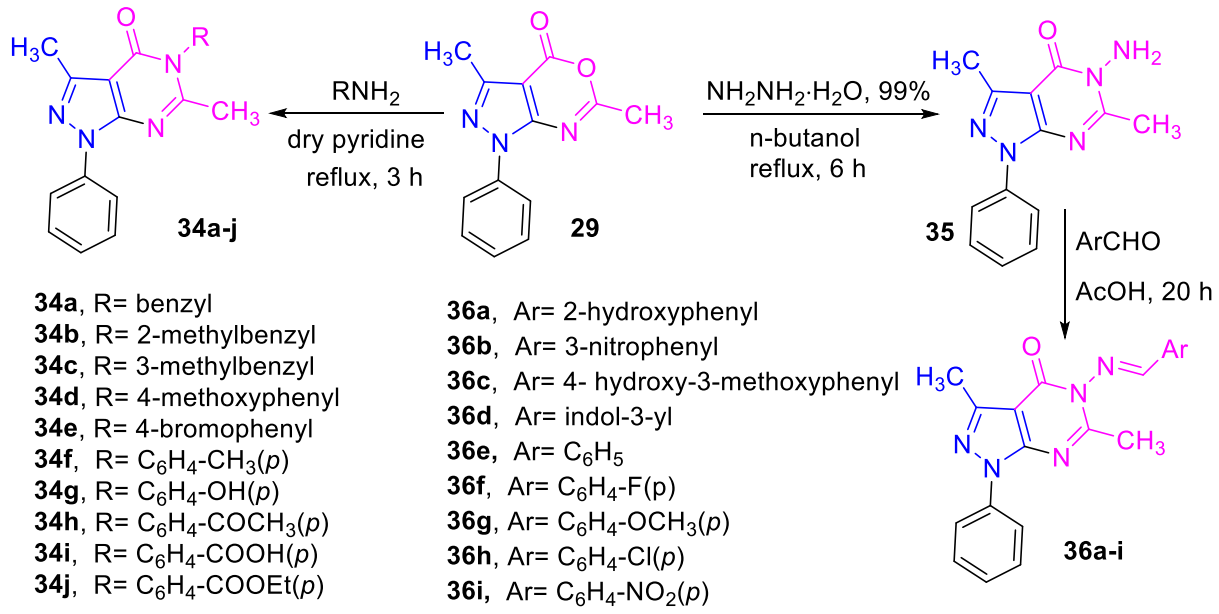
Scheme 8. Synthesis of 4*H*-Pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **26.**

Abdellatif *et al*.[59] mentioned that the ethyl 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylate (**27**), was prepared according to the reported procedure.[60] Alkaline hydrolysis of ester 1 afforded the corresponding carboxylic acid, 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**28**).[59] Heating compound **28** in acetic anhydride effected cyclodehydration to give 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**29**). This transformation is consistent with intramolecular ring closure of the initially acetylated intermediate accompanied by elimination of acetic acid. The synthetic utility of the oxazinone intermediate **29** was subsequently explored through its reactions with a variety of nitrogen nucleophiles. Thus, treatment of compound **29** with hydroxylamine hydrochloride in dry pyridine resulted in ring transformation to furnish 5-hydroxy-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**30**). Likewise, fusion of compound **29** with urea, thiourea, and thiosemicarbazide at 200 °C afforded the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives **31a, 31b**, and **32**, respectively. Under similar conditions, reaction of compound **29** with phenylhydrazine provided 3,6-dimethyl-1-phenyl-5-phenylamino-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**33**). These transformations proceed through nucleophilic attack at the electrophilic C-4 carbonyl carbon of the oxazinone ring, followed by ring opening and subsequent cyclization to generate the fused pyrazolo[3,4-*d*]pyrimidine system (Scheme 9).



Scheme 9. Synthetic pathway for pyrazolo[3,4-*d*]pyrimidine derivatives **30-33.**

Recently,[11] the reactivity of oxazine intermediate **29** toward various nitrogen nucleophiles was explored. Fusion of **29** with a range of aromatic amines at 180 °C in a sand bath promoted ring transformation, affording the corresponding 5-substituted-3,6-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **(34a–j)**. Similarly, treatment of **29** with hydrazine hydrate in n-butanol gave 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one **(35)**.[59] The latter amino derivative proved to be a useful precursor for further derivatization. Thus, condensation of **35** with different aromatic aldehydes under suitable reaction conditions furnished the corresponding 5-(arylideneamino)-3,6-dimethyl-1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **(36a–i)** (Scheme 10).

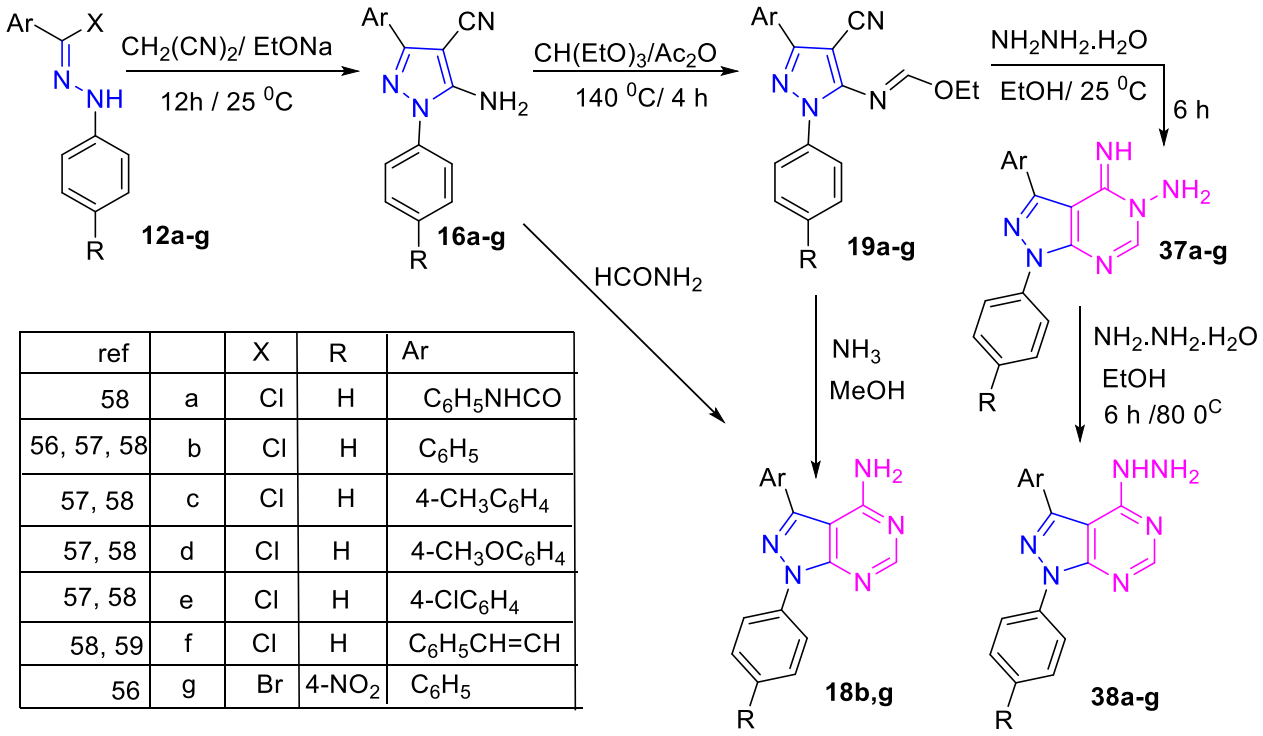


Scheme 10. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **34-36.**

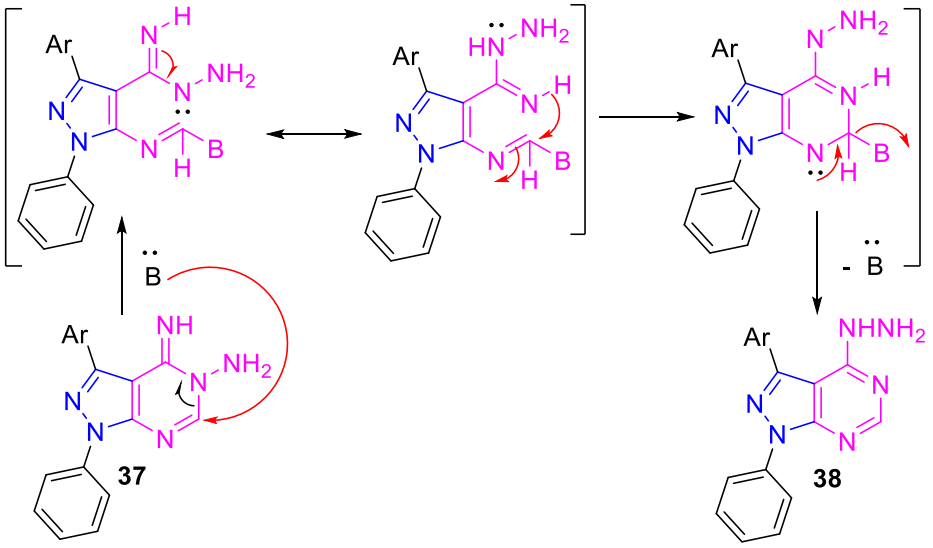
2.2. Synthesis of 4-imino-5-aminopyrazolo[3,4-*d*]pyrimidine

Recently, Helmy *et al*. and others reported that, [61-64] treatment of acetohydrazonoyl derivative **12a-g** with malononitrile in ethanolic sodium ethoxide afforded 5-aminopyrazole-4-carbonitrile derivative **16a-g**. [61] Compound **16a-g** was then refluxed with triethyl orthoformate in acetic anhydride to give the corresponding formimidate derivative **19a-g**. Subsequent treatment of **19a-g** with hydrazine hydrate in ethanol at room temperature promoted intramolecular cyclization, furnishing the aminoimino derivatives **37a-g**. [61] On the other hand, ammonolysis of compounds **19b,g** in methanolic ammonia afforded the corresponding 4-amino-1,3-diphenyl-1*H*-pyrazolo[3,4-*d*] pyrimidines **(18b,g)** in 70% yield.[65] Further reaction of the aminoimino derivative **37a-g** with hydrazine hydrate in refluxing ethanol gave 4-hydrazino compound **38a-g** (Scheme 11).[38, 64, 66]

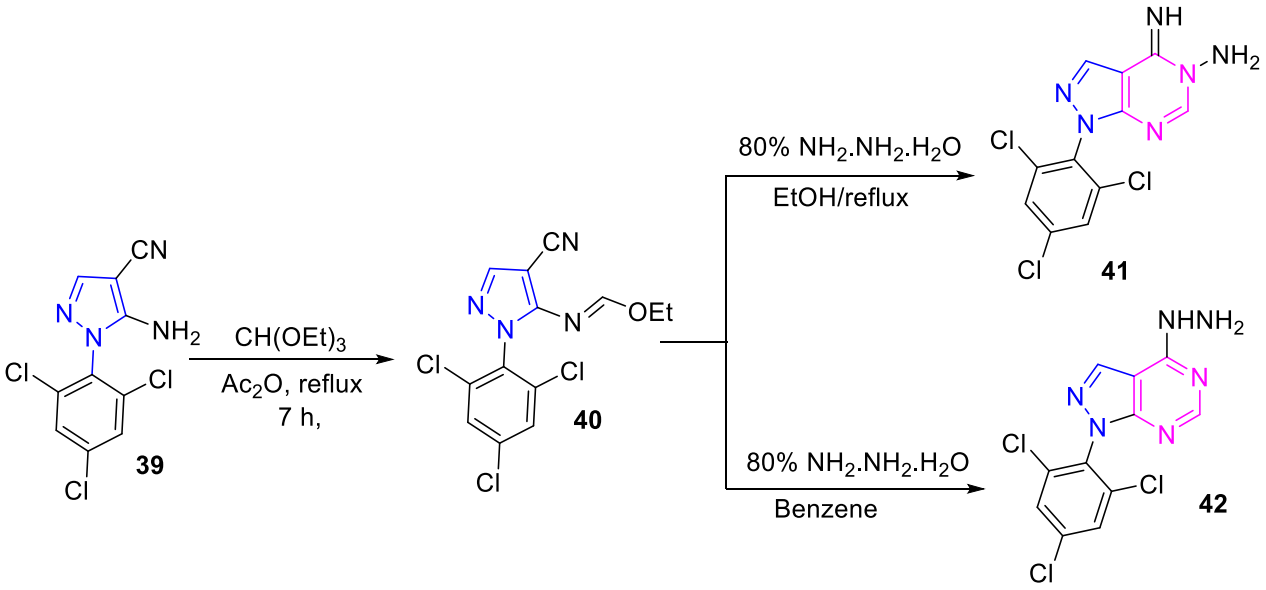
Nemr *et al*. reported that[67] the key intermediate 5-amino-1-(2,4,6-trichlorophenyl)-1*H*-pyrazole-4-carbonitrile (**39**) underwent condensation with triethyl orthoformate in acetic anhydride to furnish the corresponding ethoxymethyleneamino derivative **40**. [68-70] Subsequent treatment of compound **40** with 80% hydrazine hydrate afforded different products depending on the reaction conditions. When the reaction was performed under reflux in ethanol, cyclization readily occurred to yield the fused pyrazolo[3,4-*d*]pyrimidine derivative **41**.[71-75] In contrast, stirring the same intermediate with hydrazine hydrate in benzene at room temperature led to the isolation of the hydrazino derivative **42**. [68, 76] These findings demonstrate that both solvent polarity and reaction temperature exert a decisive influence on the course of the transformation: elevated temperature in a polar medium promotes intramolecular cyclization,[75] whereas milder conditions in a non-polar solvent favor formation of the corresponding hydrazine intermediate **4** (Scheme 12).[70, 76, 77]



ref		X	R	Ar
58	a	Cl	H	C_6H_5NHCO
56, 57, 58	b	Cl	H	C_6H_5
57, 58	c	Cl	H	4-$CH_3C_6H_4$
57, 58	d	Cl	H	4-$CH_3OC_6H_4$
57, 58	e	Cl	H	4-ClC_6H_4
58, 59	f	Cl	H	C_6H_5CH=CH
56	g	Br	4-NO_2	C_6H_5

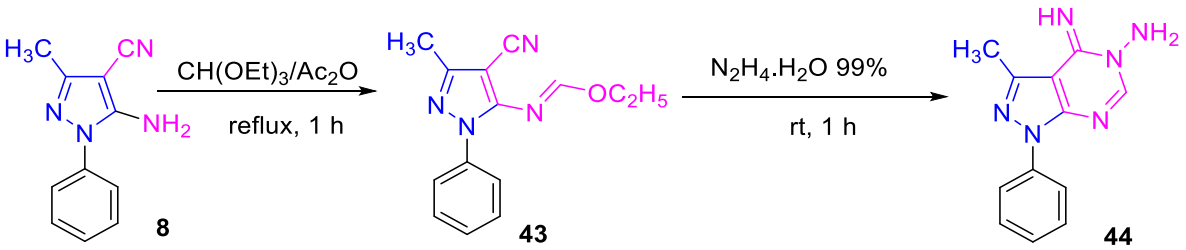


Scheme 11. Synthesis of pyrazolo[3,4-*d*] pyrimidines **38b-f**. Proposed Mechanism of Dimroth rearrangement.



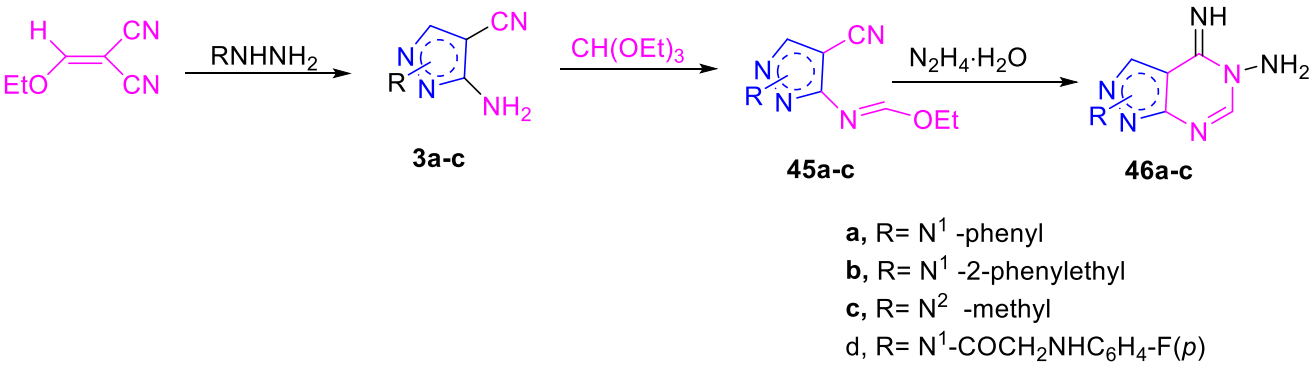
Scheme 12. Reaction of ethoxymethyleneamino derivative **40** with hydrazine hydrate.

Recently it was described that,[11] according to the reported procedure, 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**8**) [78] was treated with triethyl orthoformate to afford the corresponding formimidate derivative ethyl *N*-(4-cyano-3-methyl-1-phenyl-1*H*-pyrazol-5-yl)formimidate (**43**). Compound **44**, namely 4-imino-3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-5(4*H*)-amine, has previously been obtained by the reaction of compound **43** with hydrazine hydrate in absolute ethanol, [75] or in absolute ethanol in the presence of metallic sodium as a catalyst. [79] In the present study, however, compound **44** was prepared in excellent yield (95%) by stirring compound **43** with hydrazine hydrate (99%) in dry benzene at room temperature, following the method previously described (Scheme 13).[80]



Scheme 13. Synthetic pathway for the pyrazolo[3,4-*d*]pyrimidine derivatives **44.**

Baraldi *et al*.[75, 81] reported that the N^1- and N^2-substituted amino-cyanopyrazoles **3a–c**[69, 70] underwent formylation upon treatment with triethyl orthoformate to give the corresponding ethoxymethyleneamino derivatives **45a–c**. Subsequent reaction of these intermediates with hydrazine hydrate resulted in intramolecular cyclization to afford the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives **46a–c**, which served as key intermediates in the synthetic sequence (Scheme 14).

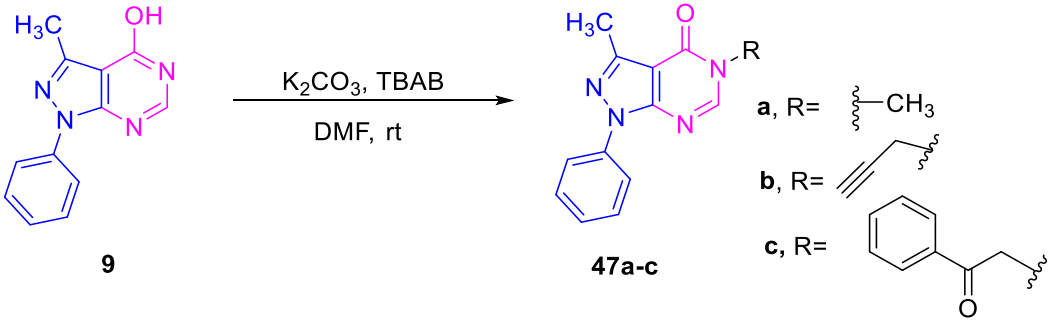


Scheme 14. Synthesis of 4-imino-1-substitued-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-amine **46a-c.**

3. Reactions of Pyrazolo[3,4-*d*]pyrimidine

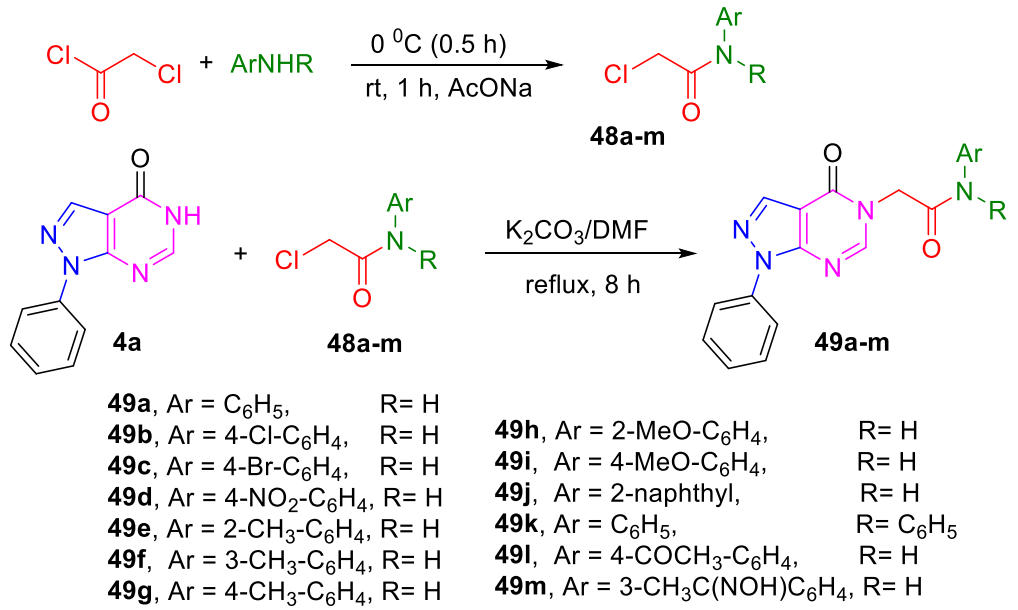
3.1 Alkylation of pyrazolo[3,4-*d*]pyrimidin-4-one

El Hafi *et al* reported that,[51] pyrazolo[3,4-*d*]pyrimidine derivatives **47a-c** were synthesized via the synthetic pathway illustrated in Scheme 15. Functionalization of **9** was achieved through N-alkylation using different alkylating agents, namely methyl iodide, propargyl bromide, and phenacyl bromide, leading to the formation of the corresponding derivatives **47a-c**, respectively, in good yields.



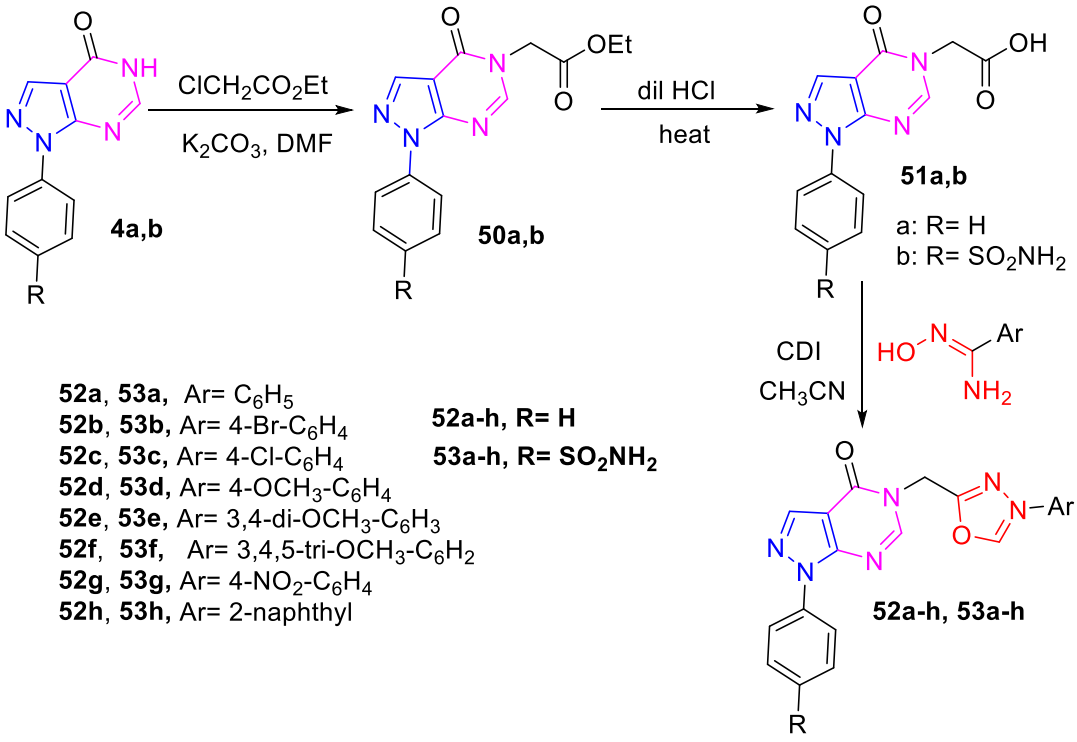
Scheme 15. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **47a-c**.

The 2-chloro-*N*-phenylacetamide derivatives **48a–m** were prepared by acylation of the appropriate substituted anilines with chloroacetyl chloride in glacial acetic acid in the presence of sodium acetate as a base, according to reported procedures.[82-84] Subsequent N-alkylation of the NH group of 1-phenyl-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **4a** was accomplished by refluxing the latter with the corresponding chloroacetamides **48a–m** in *N,N*-dimethylformamide (DMF) using anhydrous potassium carbonate as the base, furnishing the corresponding compounds **49a–m** (Scheme16).[40]



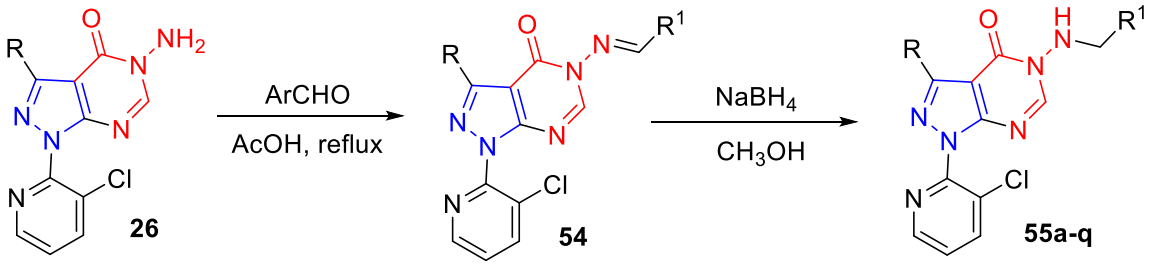
Scheme 16. Alkylation of the NH group of pyrazolo[3,4-*d*]pyrimidine derivative (**4a**).

N-Alkylation[27] of compounds **4a** and **4b** was accomplished by refluxing the corresponding substrates with ethyl chloroacetate in dry *N,N*-dimethylformamide (DMF) in the presence of anhydrous potassium carbonate as a base, as outlined in Scheme 17.[85] This reaction furnished the corresponding ethyl ester intermediates **50a** and **50b**. Subsequent hydrolysis of these esters with dilute hydrochloric acid afforded the target carboxylic acids **51a** and **51b**. The final compounds **52a–h** and **53a–h** were then synthesized through coupling of acids **51a** and **51b** with (*Z*)-4-substituted-*N'*-hydroxybenzimidamides using *N,N'*-carbonyl-diimidazole (CDI) as the activating reagent in DMF under reflux conditions. This strategy provided the desired products **52a–h** and **53a–h** in moderate to good yields (Scheme 17). [86]



Scheme 17: Alkylation of pyrazolo[3,4-*d*]pyrimidine **4** with ethyl chloroacetate.

As reported by Wang Ya and co-workers,[58] the amino intermediate **26** was condensed with a series of substituted aromatic aldehydes (ArCHO) in glacial acetic acid under reflux to furnish the corresponding Schiff base intermediates **54**. Subsequent reduction of the azomethine (imine) group with sodium borohydride in methanol afforded the desired secondary amine derivatives **55a–q** (Scheme 18).

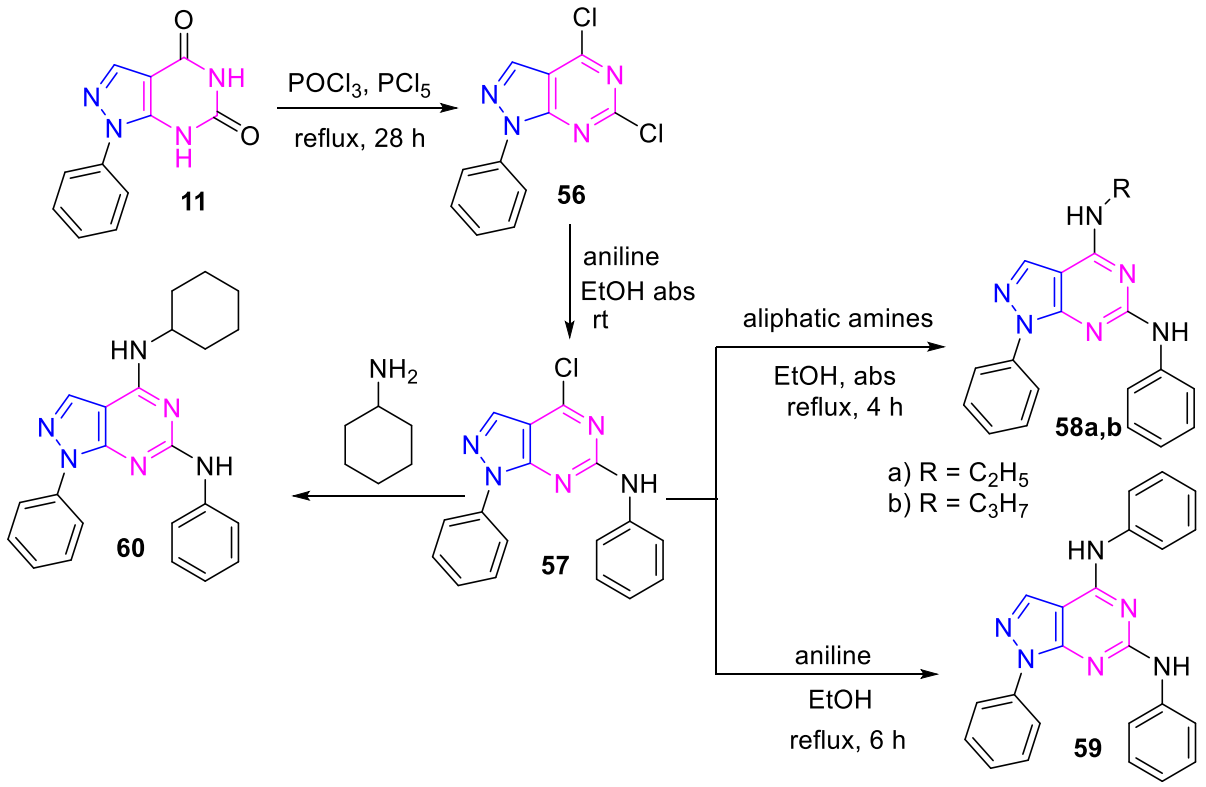


55a, R= Me, R^1= 6-bromopyridin-3-yl
55b, R= Me, R^1= 5-methylthiophen-2-yl
55c, R= Me, R^1= pyrrol-2-yl
55d, R= Me, R^1= 5-bromopyridin-2-yl
55e, R= Me, R^1= 2-fluoro-4-cayno-ph
55f, R= Me, R^1= thiophen-2-yl
55g, R= Me, R^1= 4-bromothiophen-2-yl
55h, R= Me, R^1= 2,6-dichloropyridin-3-yl
55i, R= Me, R^1= 2-CF_3-Ph
55j, R= Me, R^1= pyridin-3-yl
55k, R= H, R^1= 4-bromothiophen-2-yl
55l, R= H, R^1=5-bromofuran-2-yl
55m, R= H, R^1= pyrrol-2-yl
55n, R= H, R^1= 5-chloro-2-hydroxy-ph
55o, R= H, R^1= 3-chloro-5-(triflurомethyl)pyridin-2-yl
55p, R= H, R^1= 2-fluoro-ph
55q, R= H, R^1= 5-bromofuran-2-yl

Scheme 18. Synthesis of Hydrazino derivatives **55a-q** of pyrazolo[3,4-*d*]pyrimidine.

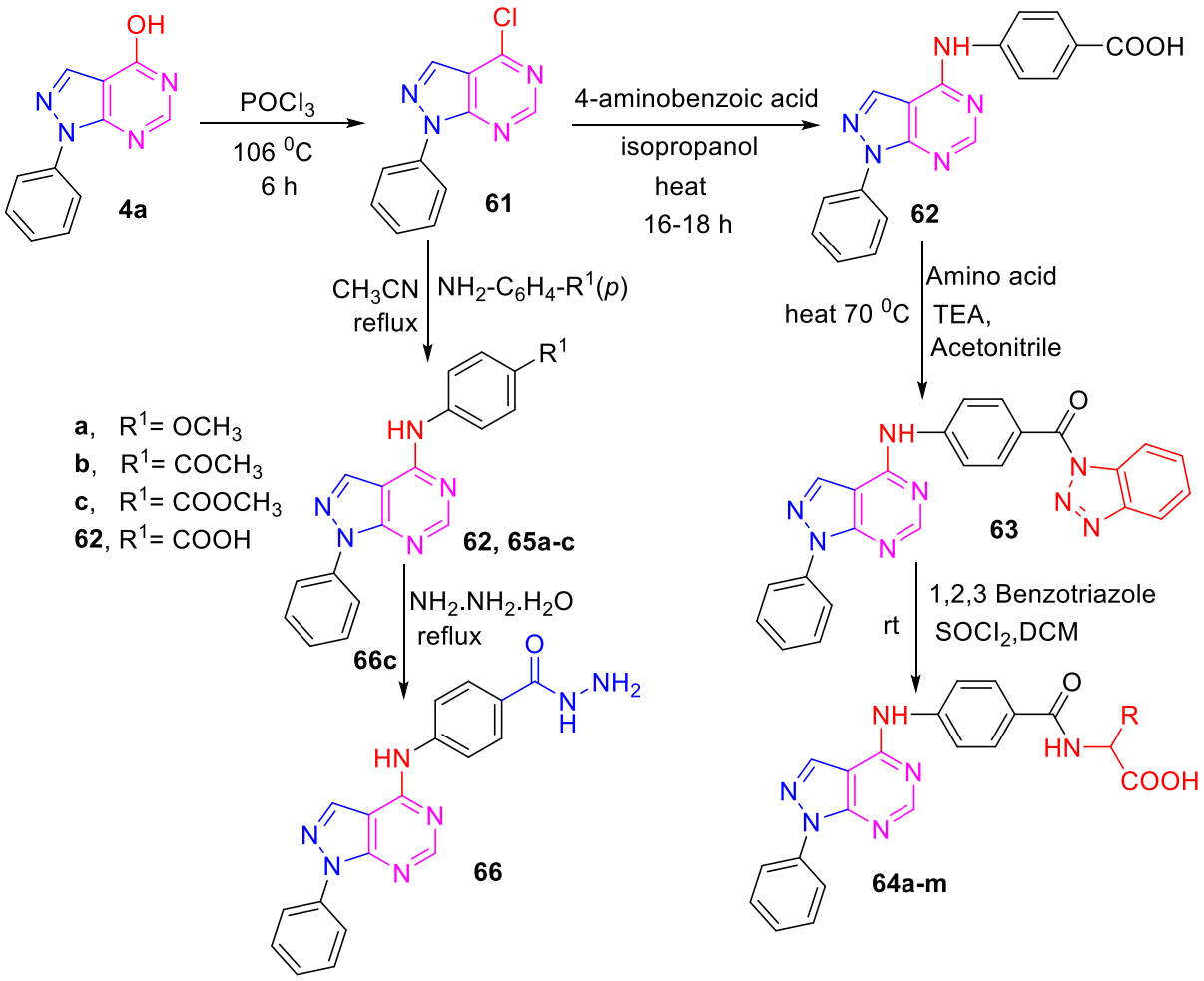
3.2. Chlorination of pyrazolo[3,4-*d*]pyrimidin-4-one and its nucleophilic substitution reactions

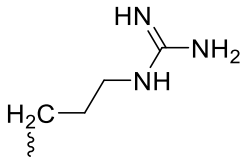
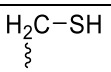
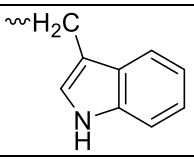
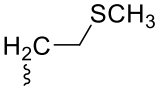
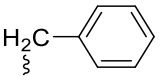
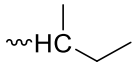
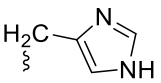
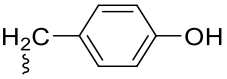
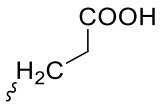
Treatment[52] of compound **11** with phosphorus oxychloride and phosphorus pentachloride resulted in deoxychlorination at the 4- and 6-positions, yielding 4,6-dichloro-1-phenyl-1*H*-pyrazolo[3,4-d]pyrimidine (**56**).[87] Subsequent stirring of compound **56** with aniline at room temperature led to selective nucleophilic substitution of one chlorine atom to afford 4-chloro-*N*,1-diphenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**57**).[88] Treatment of **57**[52] with commercially available different amines, namely ethylamine, propylamine, aniline, and cyclohexylamine in the presence of triethylamine afforded the corresponding compounds **58a,b, 59,** and **60,** respectively (Scheme 19).



Scheme 19. Nucleophilic substitution reactions of 4-chloro-*N*,1-diphenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*] pyrimidin-6-amine (**57**).

Chlorination[46] of compound **4a** using phosphoryl chloride produced 4-chloro-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **61**.[89] This activated intermediate **61** underwent nucleophilic aromatic substitution with 4-aminobenzoic acid to yield the key intermediate 4-[(1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)amino]benzoic acid **62**.[41] Activation of compound **62** with 1*H*-benzotriazole afforded the corresponding *N*-acyl benzotriazole derivative **63**, which was subsequently condensed with a series of amino acids to furnish the desired *N*-acyl amino acid derivatives **64a–m**. Reaction[90] of **61** with the relevant amine produced the matching derivatives **62** and **65a-c**. Refluxing of the methyl ester derivative **65c** with hydrazine hydrate resulted in the corresponding hydrazide **66** (Scheme 20).

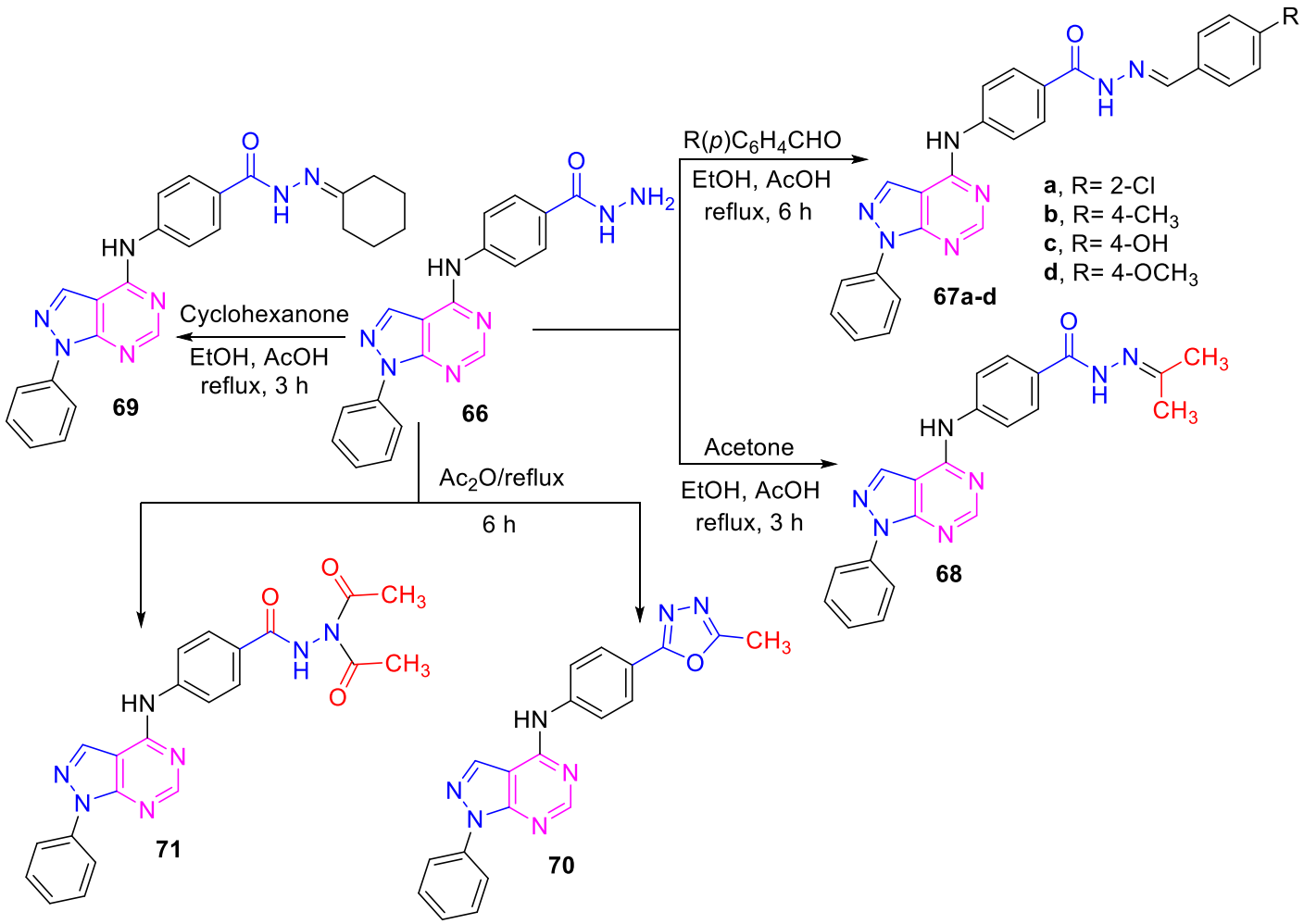


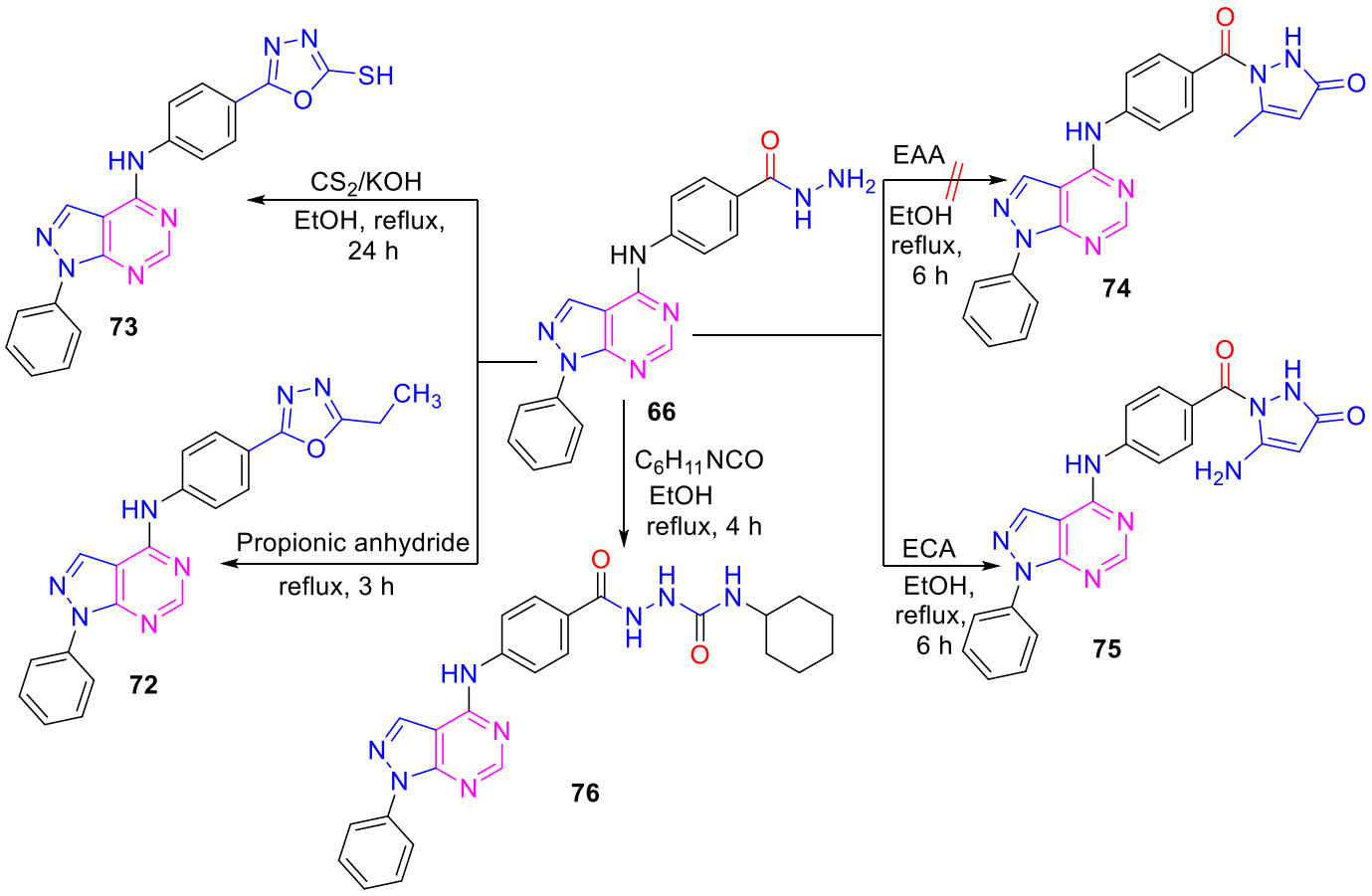
No	R	No	R	No	R
64a	H	**64f**		**64j**	
64b	CH_3	**64g**		**64k**	
64c		**64h**		**64l**	
64d		**64i**		**64m**	
64e					

Scheme 20. Synthetic pathway for preparation of pyrazolo[3,4-*d*]pyrimidine derivatives **62-66.**

Furthermore,[90] treatment of hydrazide **66** with a variety of aromatic aldehydes, acetone, and cyclohexanone furnished the corresponding Schiff bases **67a–d**, **68**, and **69**, respectively (Scheme 21). Attempts to synthesize the expected 5-methyl-1,3,4-oxadiazole derivative **70** by refluxing hydrazide **66** with acetic anhydride were unsuccessful, the diacetylated product **71** was obtained.

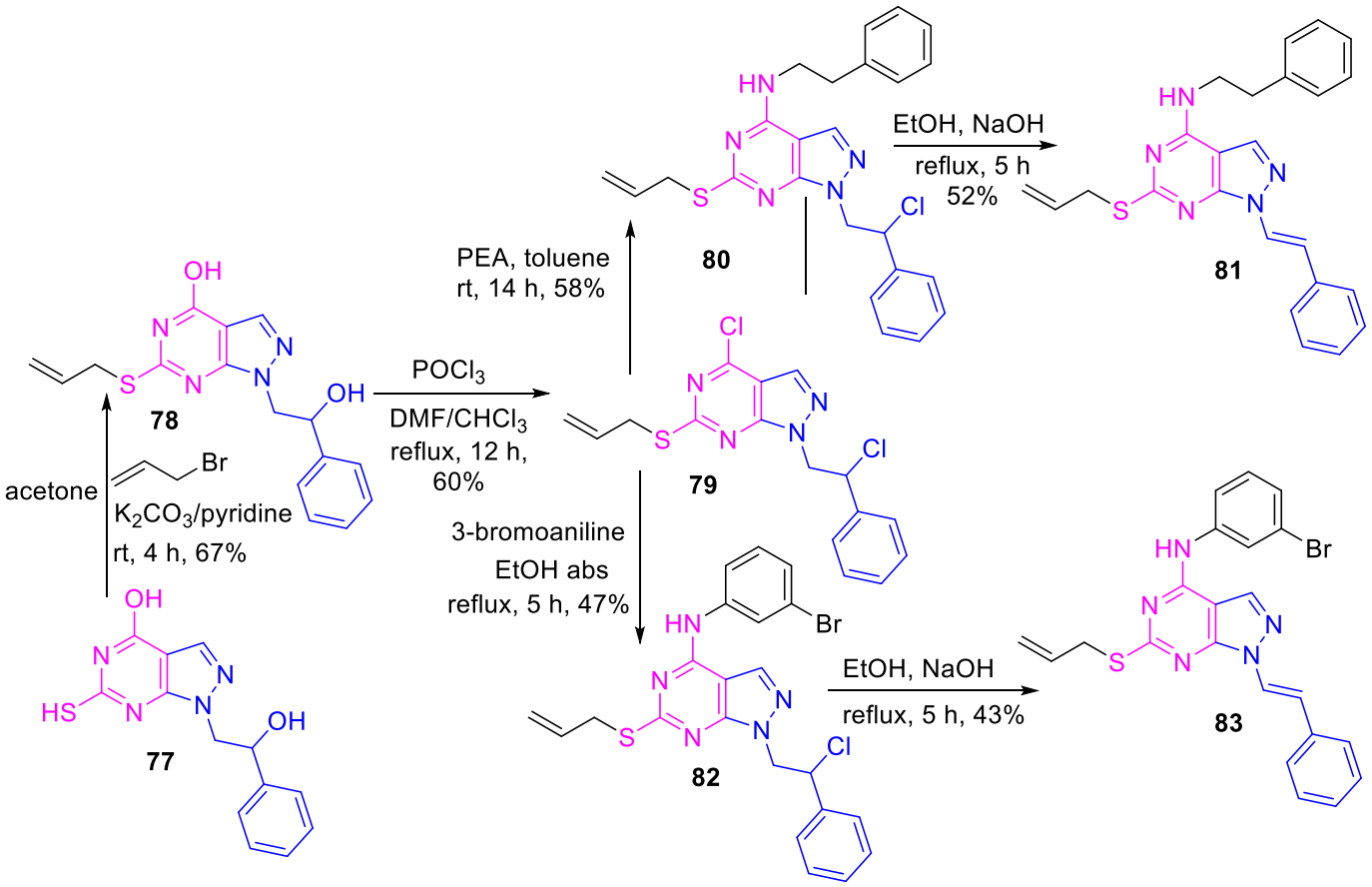
On the other hand, heating of **66** with propionic anhydride and carbon disulfide afforded the 5-ethyl-1,3,4-oxadiazole **72** and the 1,3,4-oxadiazole-5-thiol derivative **73**, respectively. Moreover, cyclocondensation of hydrazide **66** with ethyl acetoacetate or ethyl cyanoacetate gave the corresponding pyrazol-3-one derivatives **74** and **75**. Reaction of **66** with cyclohexyl isocyanate afforded the semicarbazide derivative **76** (Scheme 21).





Scheme 21. Synthesis of varieties of pyrazolo[3,4-*d*]pyrimidine derivatives **67-76**.

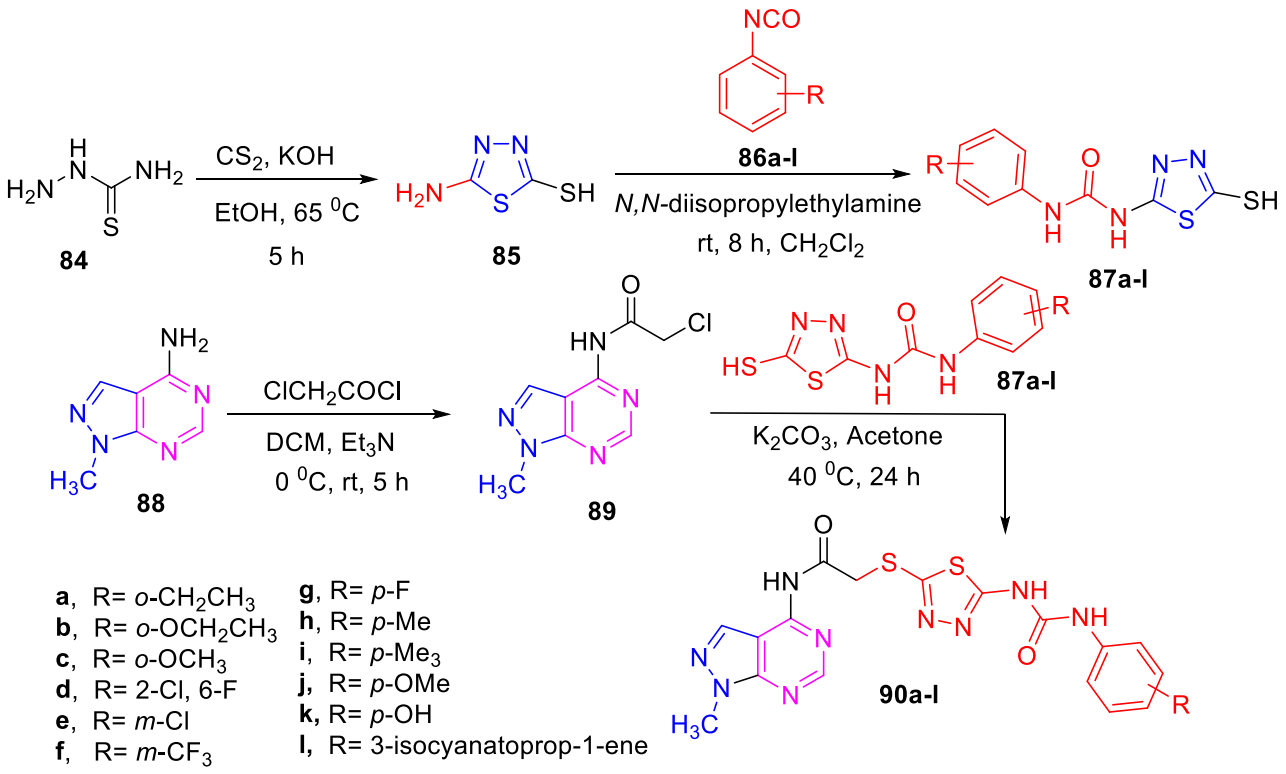
In 2024 it was mentioned that[91] the verities of pyrazolo[3,4-*d*]pyrimidine **78-83** derivatives described in this study were synthesized starting from pyrazolo[3,4-*d*]pyrimidin-4-ol **77**, following a previously reported procedure.[92] Initially, compound **77** was reacted with allyl bromide in acetone to afford the corresponding C6 thioallyl derivative (**78**) in 67% yield. Subsequent treatment of compound **78** with the Vilsmeier reagent system ($POCl_3$/DMF) produced the key dichloro intermediate **79** in good yield (60%). This intermediate was then reacted with phenethylamine to yield the 4-phenethylamino derivative **80** in 58% yield, which upon reflux with sodium hydroxide in ethanol furnished the final compound **81** in a satisfactory yield of 52% (Scheme 22). In a parallel pathway, compound **79** was treated with 3-bromoaniline to give compound **82** in 47% yield; further reaction under reflux conditions with sodium hydroxide in ethanol afforded the corresponding unsaturated analogue **83** in 43% yield (Scheme 22).



Scheme 22. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **78**–**83.**

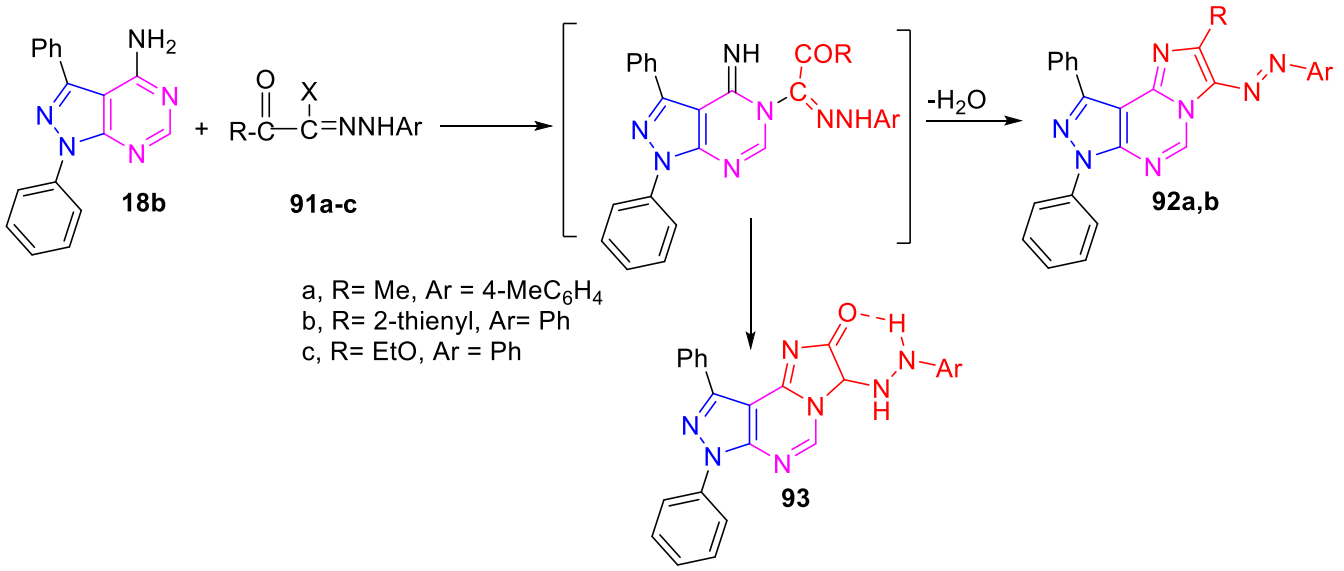
3.3. Reactions of 4-aminopyrazolo[3,4-*d*]pyrimidine

Peduri *et al*. reported that[93] the key intermediate, 1,3,4-thiadiazole derivative **85**, was synthesized through the reaction of hydrazine carbothioamide **84** with carbon disulfide in the presence of potassium hydroxide, according to literature procedures[94]. Subsequently, compound **85** was condensed with a series of substituted phenyl isocyanates **86a–86l** in dry dichloromethane at ambient temperature, using *N,N*-diisopropylethylamine (DIPEA) as a base, to furnish 1-(5-mercapto-1,3,4-thiadiazol-2-yl)-3-phenylurea intermediates **87a–87l** in high yields.[94] In a parallel step, commercially available 1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**88**) was treated with 2-chloroacetyl chloride in dichloromethane to yield 2-chloro-*N*-(1-methyl-1*H*-pyrazolo[3,4-*d*] pyrimidin-4-yl)acetamide **89**. Nucleophilic substitution of the chlorine atom in compound **89** with intermediates **87a–87l** was carried out in acetone using K_2CO_3 at 40 °C overnight, affording the corresponding compounds **90a–90l** in 73–89% yields (Scheme 23).



Scheme 23. Reaction of 4-amino pyrazolo[3,4-*d*]pyrimidines **90a-l**.

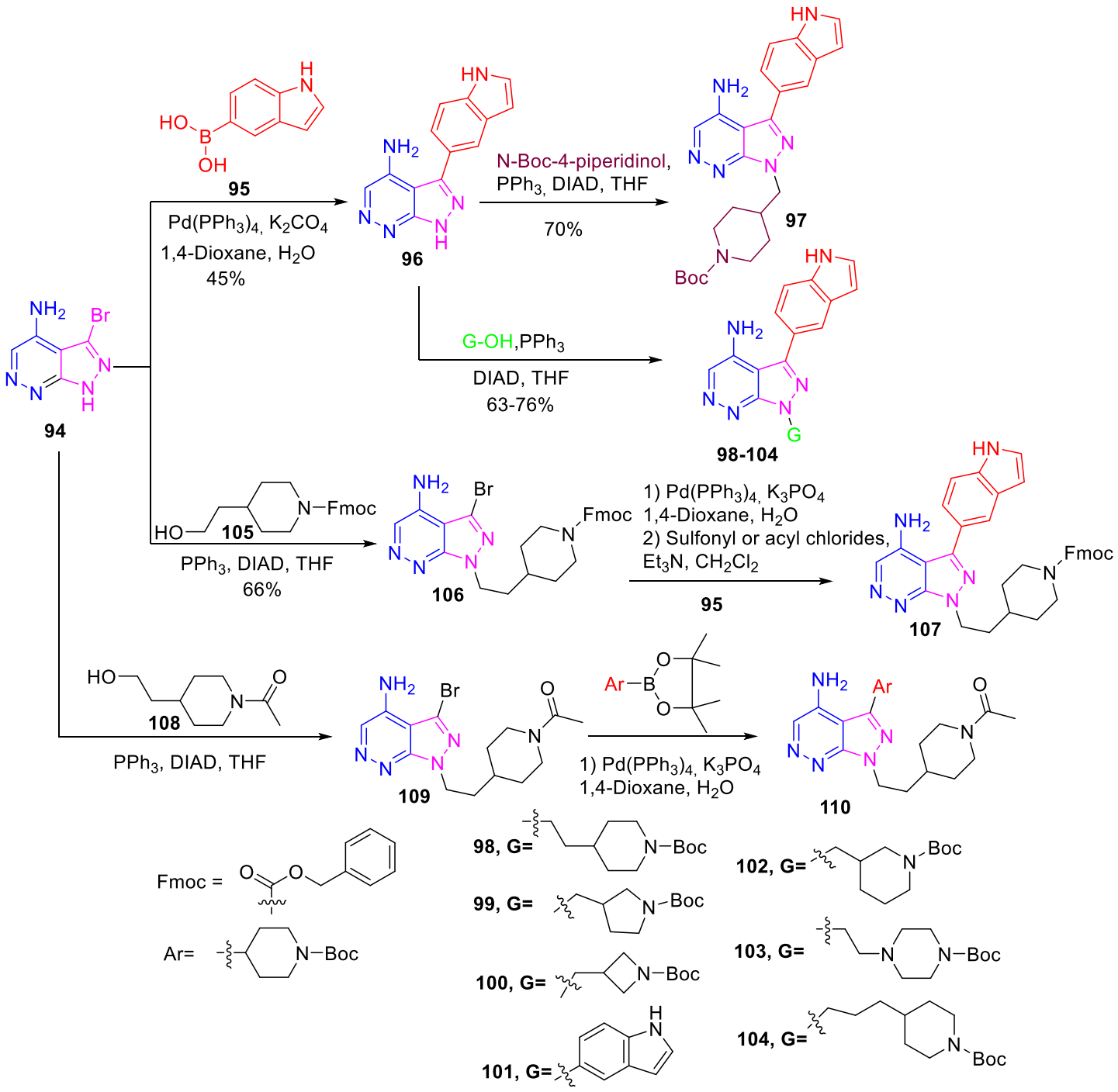
Reactions of **18b** with the hydrazonoyl halides **91a-c** benzene in the presence of triethylamine furnished the corresponding derivatives **92a, 92b** and **93** respectively In summary the present investigation offers rapid and effective new procedures for the synthesis of the title ring systems (Scheme 24).[61]



Scheme 24. Synthesis of imidazopyrazolo[3,4-*d*]pyrimidine derivative **92a,b**.

In 2024, it was reported[95] that Suzuki–Miyaura cross-coupling reaction of brominated pyrazolo[3,4-*d*] pyrimidine derivative **94** with the corresponding aryl boronic acid **95** in the presence of $Pd(PPh_3)_4$ as a catalyst

and K_3PO_4 as a base in a 1,4-dioxane/H_2O solvent system, affording the aryl-substituted intermediate **96** in 45% yield. Subsequently, compound **96** is subjected to a Mitsunobu reaction with *N*-Boc-4-piperidinol using triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD) in THF, resulting in *N*-alkylation of the heterocyclic scaffold to produce the tert-butyl 4-((4-amino-3-(1*H*-indol-5-yl)-1*H*-pyrazolo[3,4-*c*]pyridazin-1-yl)methyl)piperidine-1-carboxylate (**97**) in 70% yield. Also,[95] compound **96** was allowed to react with the appropriate alcohol (G–OH) in the presence of triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD) in THF to afford the corresponding *N*-alkylated derivatives **98-104** in moderate to good yields (63–76%). Similarly, compound **94** reacts with an Fmoc-protected piperidine alcohol **105** under Mitsunobu conditions to yield intermediate **105** in 66% yield. Subsequent Suzuki–Miyaura cross-coupling of **106** with **95** using $Pd(PPh_3)_4$ and K_3PO_4 in a 1,4-dioxane/H_2O mixture introduces aryl substituents at the brominated position, followed by deprotection and further functionalization with sulfonyl or acyl chlorides in the presence of triethylamine to furnish derivatives **107** in 89% yields. In a parallel pathway, compound **110** was treated with another piperidine-based alcohol **108** under Mitsunobu conditions gave the intermediate **109** in (66%), which then undergoes Suzuki coupling with aryl boronic esters (Ar–B) under similar palladium-catalyzed conditions to produce the final arylated products **110** in 45–88% yields (Scheme 25).

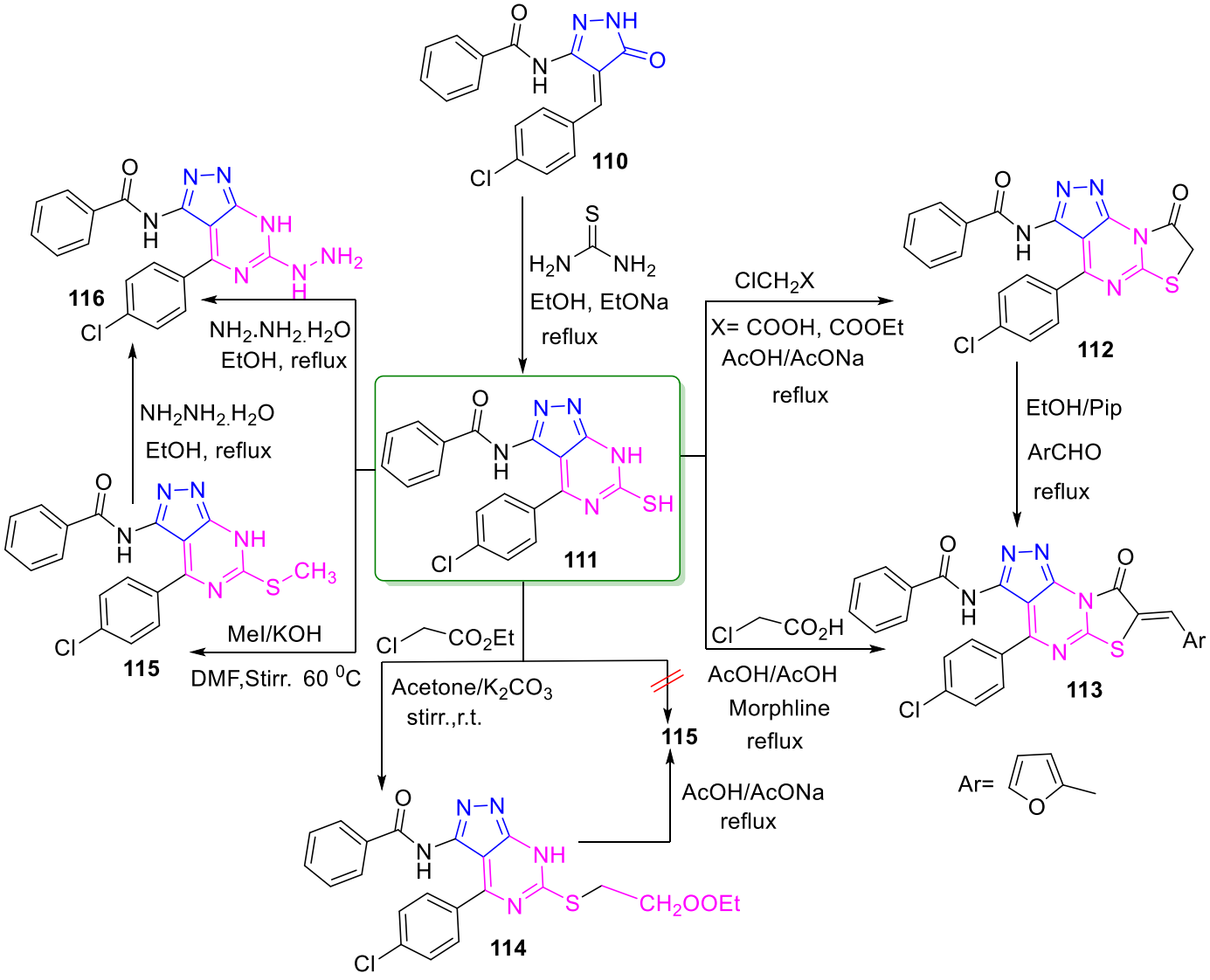


Scheme 25. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **96-110**.

3.4. Reactions of pyrazolo[3,4-*d*]pyrimidin-6-thione

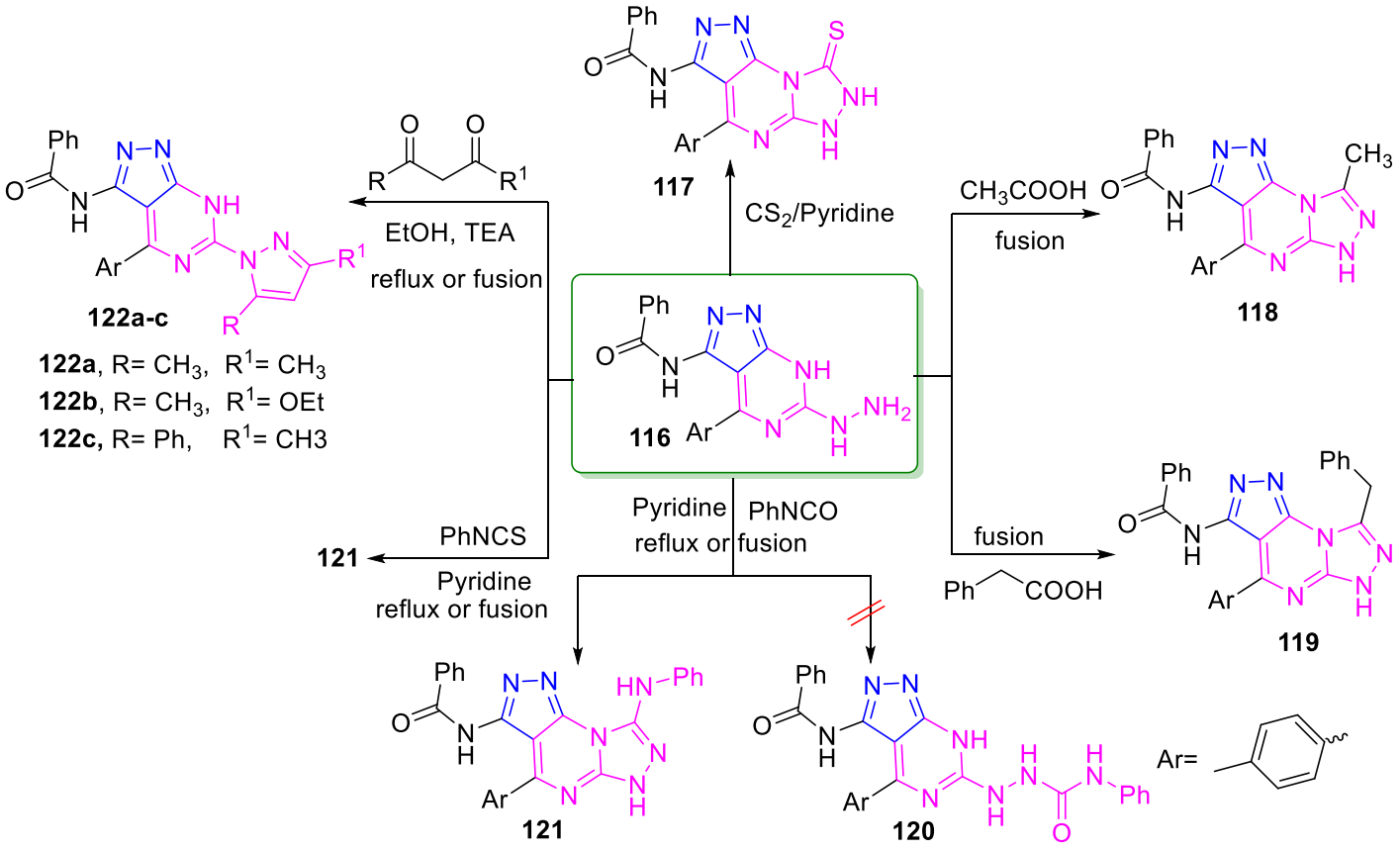
It was reported[10] that treatment of *N*-(4-(4-chlorobenzylidene)-5-oxo-4,5-dihydro-1*H*-pyrazol-3-yl)benzamide compound (**110**)[96] with thiourea in ethanol in the presence of a catalytic amount of sodium ethoxide, or alternatively under solvent-free fusion conditions, afforded *N*-(4-(4-chlorophenyl)-6-thioxo-6,7-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)benzamide (**111**) in good yield. Reaction of the thiol derivative **111** with chloroacetic acid and/or ethyl chloroacetate in glacial acetic acid, in the presence of anhydrous sodium acetate, afforded the corresponding thiazolo[3,2-*a*]pyrimidine derivative **112**. Knoevenagel condensation of compound **112** with 2-furancarboxaldehyde in the presence of piperidine as a base catalyst furnished the corresponding

product *N*-(4-(4-chlorophenyl)-7-(furan-2-ylmethylene)-8-oxo-7,8-dihydropyrazolo[4,3-*e*] thiazolo[3,2-*a*]pyrimidin-3-yl)benzamide **113**. In addition, compound **113** was efficiently synthesized via a one-pot multicomponent reaction of compound **111**, chloroacetic acid, and furfural in the presence of morpholine as a catalytic base. Alternatively, stirring the thiol derivative **111** with ethyl chloroacetate in dry acetone at room temperature in the presence of a catalytic amount of anhydrous potassium carbonate afforded the uncyclized S-alkylated product, ethyl 2-((3-benzamido-4-(4-chlorophenyl)-7*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl) thio)acetate (**114**). No evidence for the formation of the cyclized thiazolo[3,2-*a*] pyrimidine derivative **112** was observed under these conditions. Subsequent cyclization of compound **114** was achieved via treatment with acetic acid in the presence of sodium acetate, leading to the formation of the corresponding thiazolo[3,2-*a*] pyrimidine derivative **112** through a one-pot condensation process. In a related transformation, alkylation of thiol derivative **111** with methyl iodide in dry *N,N*-dimethylformamide in the presence of potassium hydroxide at 60 °C for 2.5 h afforded the methylthio derivative **115** in 89% yield. Furthermore, hydrazinolysis of the thiol derivative **111** or the methylthio **115** with hydrazine hydrate resulted in the formation of the derivative **116** (Scheme 26).



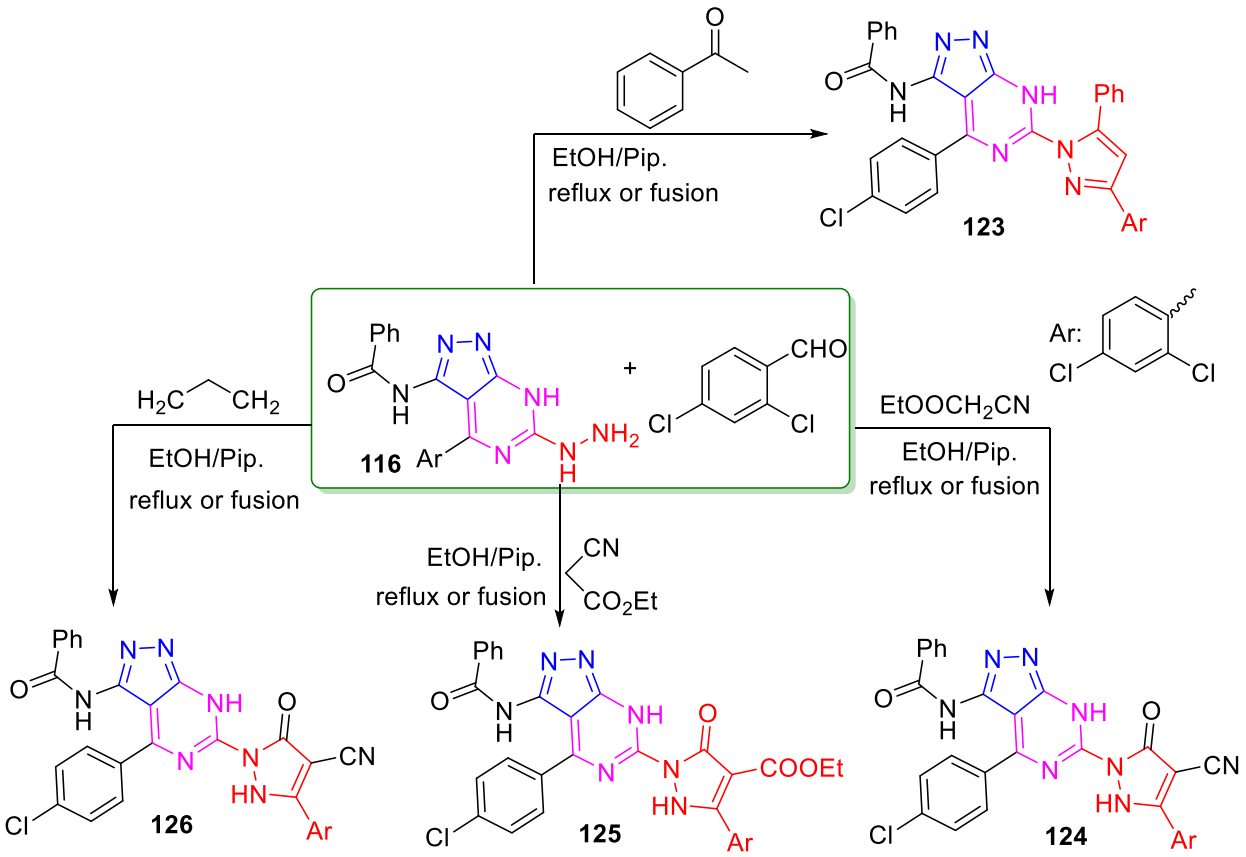
Scheme 26. Synthesis of some pyrazolo[3,4-*d*]pyrimidine derivatives **112-116**.

Furthermore,[10] treatment of compound **116** with carbon disulfide in dry pyridine under water-bath heating afforded the corresponding thioxo derivative **117**. When compound **116** was refluxed in glacial acetic acid for 3 h, intramolecular cyclization occurred smoothly to furnish *N*-(4-(4-chlorophenyl)-8-methyl-6*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl) benzamide (**118**). Likewise, fusion of compound **116** with phenylacetic acid in the absence of solvent led selectively to the formation of the condensed derivative **119**. The reactivity of compound **116** toward heterocumulenes was also investigated. Thus, refluxing compound **113** with phenyl isocyanate in dry pyridine for 15 h gave the cyclized pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*a*] pyrimidine derivative **121**, whereas the anticipated open-chain N-phenylhydrazine-1-carboxamide intermediate **120** was not detected. In contrast, reaction of compound **116** with phenyl isothiocyanate, either in refluxing pyridine or under solvent-free fusion conditions, afforded a desulfurized product identified as N-(4-(4-chlorophenyl)-8-(phenylamino)-6*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*a*]pyrimidin-3-yl)benzamide (**121**). In addition, reaction of compound **116** with representative β-dicarbonyl compounds, namely acetylacetone, ethyl acetoacetate, and benzoylacetone, under either conventional reflux or solvent-free fusion conditions, afforded the corresponding pyrazol-1-yl-substituted pyrazolo[3,4-*d*]pyrimidine derivatives **122a–c** (Scheme 27).



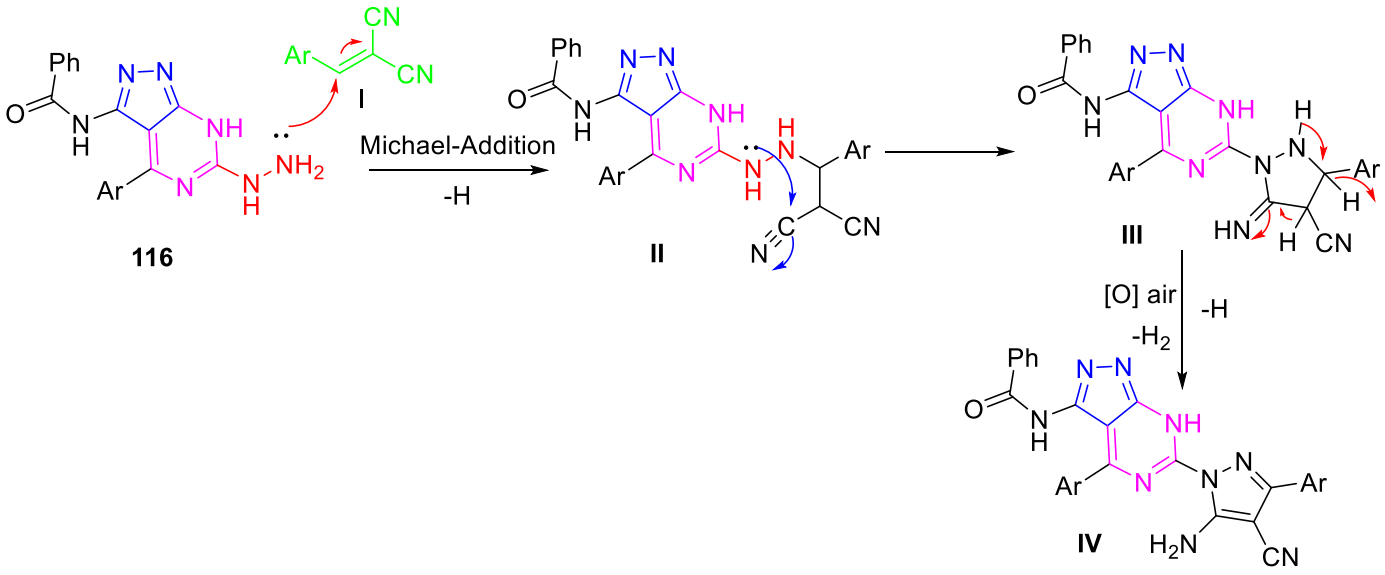
Scheme 27. Synthesis of fused heterocyclic systems incorporating the pyrazolo[3,4-*d*]pyrimidine scaffold **117-122**.

The one-pot multicomponent reactions of compound 116[10] with 2,4-dichlorobenzaldehyde and active methylene compounds, namely acetophenone, ethyl cyanoacetate, diethyl malonate, and malononitrile were carried out under both conventional and green conditions yielded a series of (1*H*-pyrazol-1-yl)-4-(4-chlorophenyl)-7*H*-pyrazolo[3,4-*d*]pyrimidine derivatives (**123–126**) (Scheme 28).



Scheme 28. Synthesis of series of (1*H*-pyrazol-1-yl)-4-(4-chlorophenyl)-7*H*-pyrazolo[3,4-*d*]pyrimidine derivatives (**123–126**).

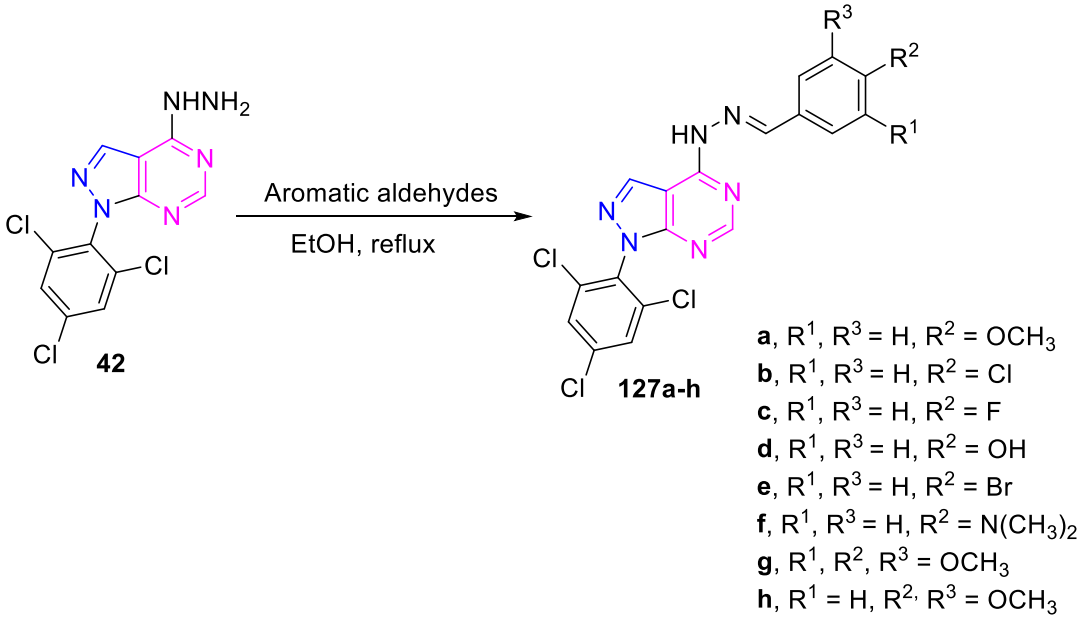
Mechanistically, the reaction proceeds via initial in situ formation of arylidene intermediates (I) through condensation of the aldehyde with the active methylene component in absolute ethanol in the presence of catalytic piperidine, under reflux or solvent-free conditions. Subsequent Michael addition of compound **116** to the activated olefin generates intermediate adducts, which undergo intramolecular cyclization **II** to furnish the desired target compounds **123-126** (Scheme 29).



Scheme 29. Suggested mechanism for the formation of compounds **122–126**.

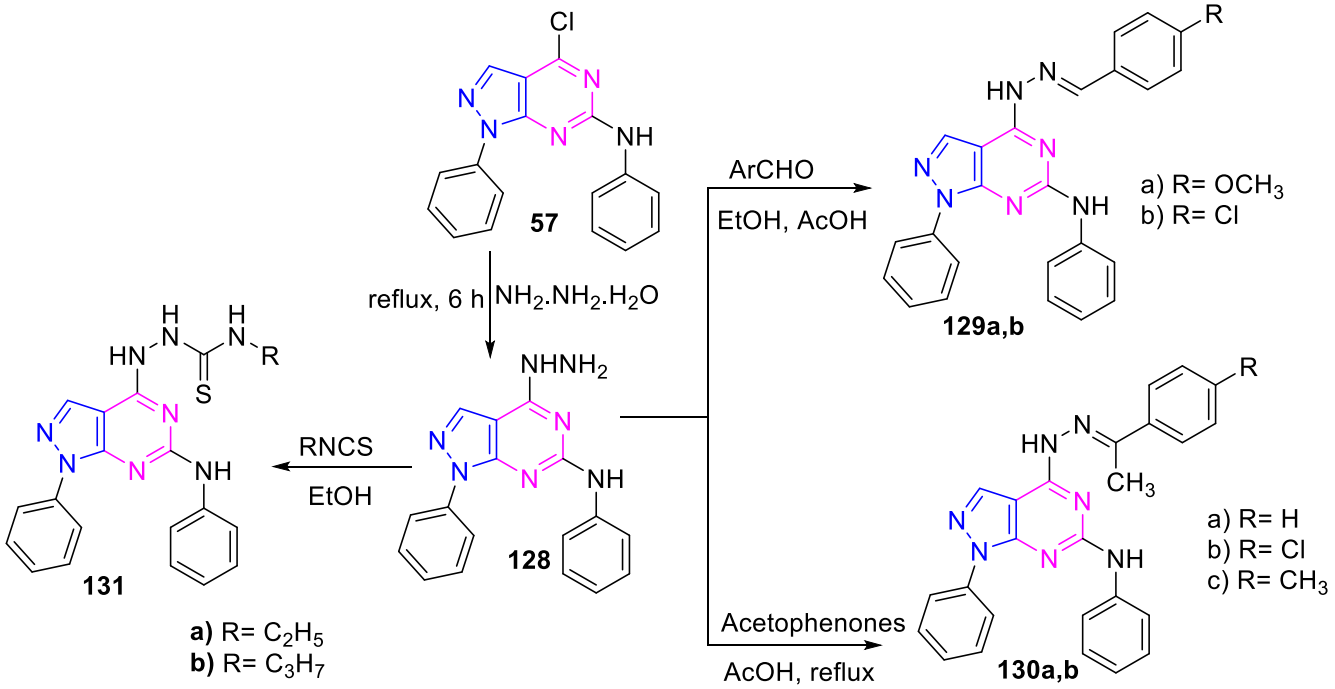
3.5. Reactions of 4-hydrazinopyrazolo[3,4-*d*]pyrimidine

Nemr *et al*. reported that[67] treatment of hydrazino derivative **42** with different aromatic aldehydes in ethanol under reflux afforded the corresponding hydrazone derivatives **127a–h** (Scheme 30).



Scheme 30. Synthesis of 4-imino-1-(2,4,6-trichlorophenyl)-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-amine **127a-h**.

In 2022, compound **57**[52] was converted into the corresponding hydrazine derivative **128** by heating with hydrazine hydrate under reflux. The resulting 4-hydrazinyl-*N*,[1]-diphenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**128**) was subsequently condensed with a series of commercially available aromatic aldehydes and acetophenones in refluxing glacial acetic acid to furnish the desired hydrazone derivatives **129a,b** and **130a–c**, respectively. Similarly, treatment of compound **57** with aliphatic isothiocyanates under reflux afforded the corresponding carbothioamide derivatives **131a, b** (Scheme 31).

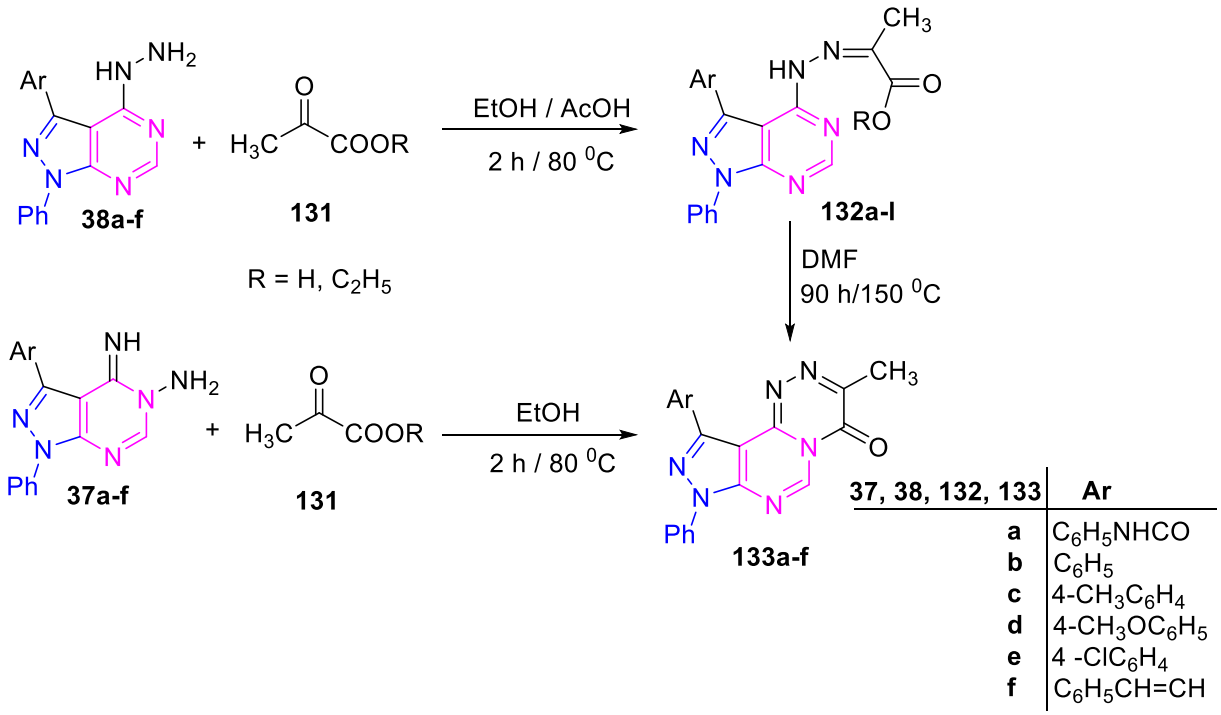


Scheme 31. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **128-131.**

4. Reactions of 5-Amino-4-iminopyrazolo[3,4-*d*]pyrimidine

4.1. Synthesis of pyrazolo[3',4':4,5]pyrimido[6,1-c][1,2,4]triazin-4-one

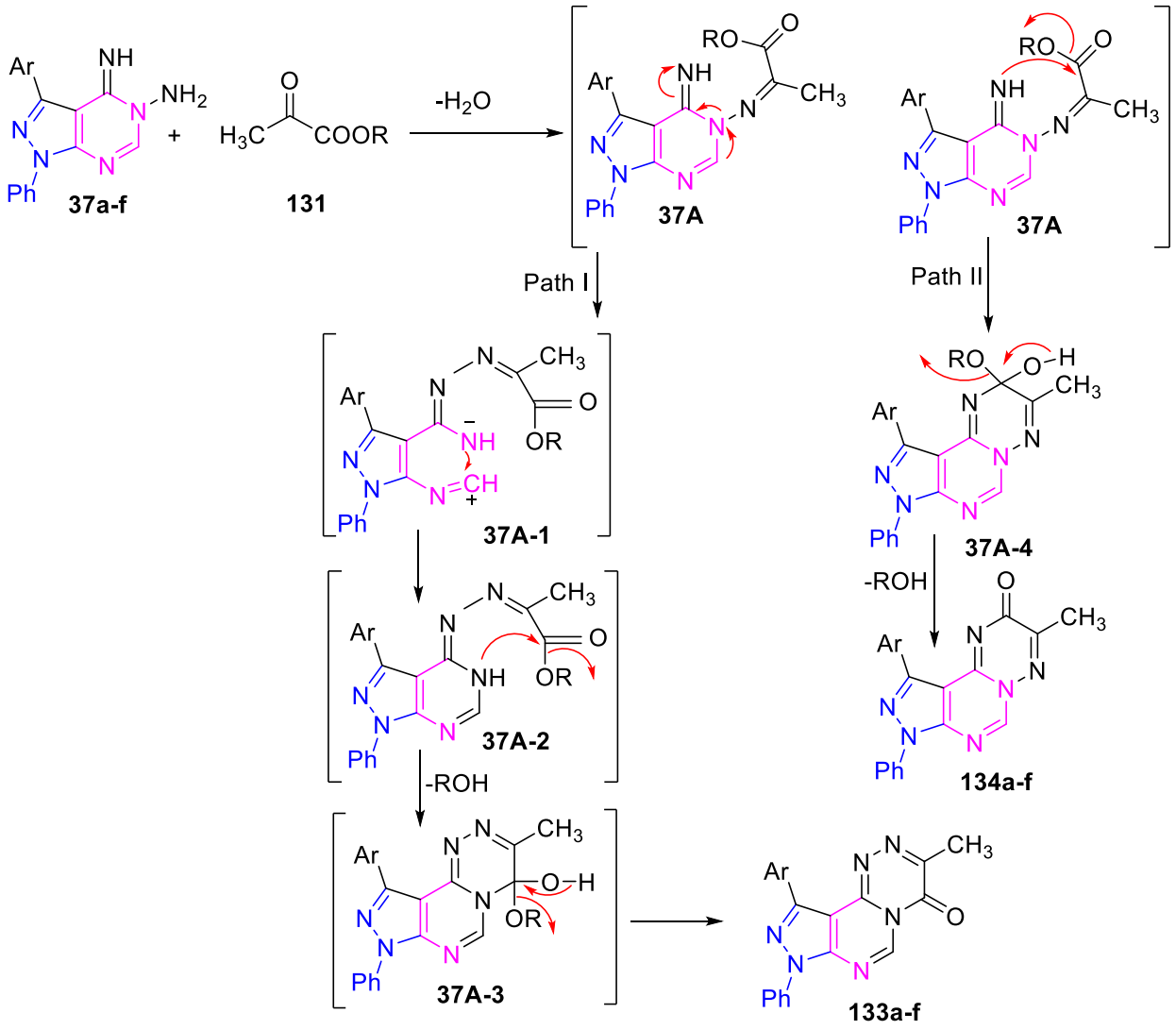
In 2025 it was reported that treatment[63] of hydrazine derivatives **38a–f** with either pyruvic acid or ethyl pyruvate **131** under reflux in ethanol, in the presence of a few drops of acetic acid, afforded the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives **132a–l**. Refluxing of hydrazone derivatives **132a–f** in DMF for 90 h furnished the corresponding 10-aryl-3-methyl-8-phenylpyrazolo[3',4':4,5]pyrimido[6,1-*c*][1,2,4]triazin-4(8*H*)-one derivatives **133a–f**. Alternatively, the latter compounds **133a–f** were synthesized directly through the reaction of aminoimino derivatives **37a–f** with same reagent **131** the in refluxing ethanol (Scheme 32).



37a-f, 38a-f, 132a-f, 133a-f	Ar	**R**	**132g-l**	**Ar**	**R**
a	C_6H_5NHCO	H	g	$4\text{-}CH_3OC_6H_5$	H
b	C_6H_5NHCO	C_2H_5	h	$4\text{-}CH_3OC_6H_5$	C_2H_5
c	C_6H_5	H	i	$4\text{-}ClC_6H_4$	H
d	C_6H_5	C_2H_5	j	$4\text{-}ClC_6H_4$	C_2H_5
e	$4\text{-}CH_3C_6H_4$	H	k	$C_6H_5CH=CH$	H
f	$4\text{-}CH_3C_6H_4$	C_2H_5	l	$C_6H_5CH=CH$	C_2H_5

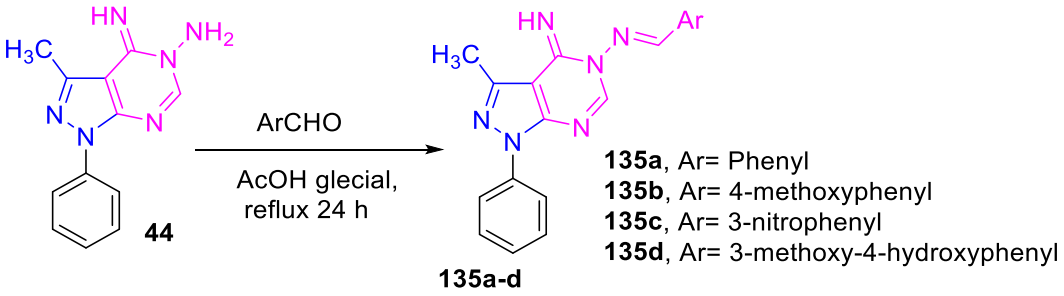
Scheme 32. Synthesis of 10-aryl-3-methyl-8-phenylpyrazolo[3',4':4,5]pyrimido[6,1-*c*][1,2,4]triazin-4(8*H*)-one derivatives **133a–f.**

The proposed alternative mechanism for the formation of compounds **133a–f** is outlined in Scheme 33.[63] Initially, condensation of pyruvic acid or ethyl pyruvate **131** with imino-amino derivative **37a–f** affords intermediate **37A**. In pathway 1, intermediate **37A** undergoes an in situ Dimroth rearrangement followed by intramolecular cyclization and nucleophilic addition to give cycloadduct **37A-3**, which upon elimination of water or ethanol furnishes compounds **133a–f.** Alternatively, direct cyclization of **37A** through pathway 2 would lead to the regioisomers **134a–f** (Scheme 33).



Scheme 33. Proposed mechanism of formation of 3-methyl-8-phenylpyrazolo[3',4':4,5]pyrimido[6,1-*c*][1,2,4] triazine derivatives **133a-f**.

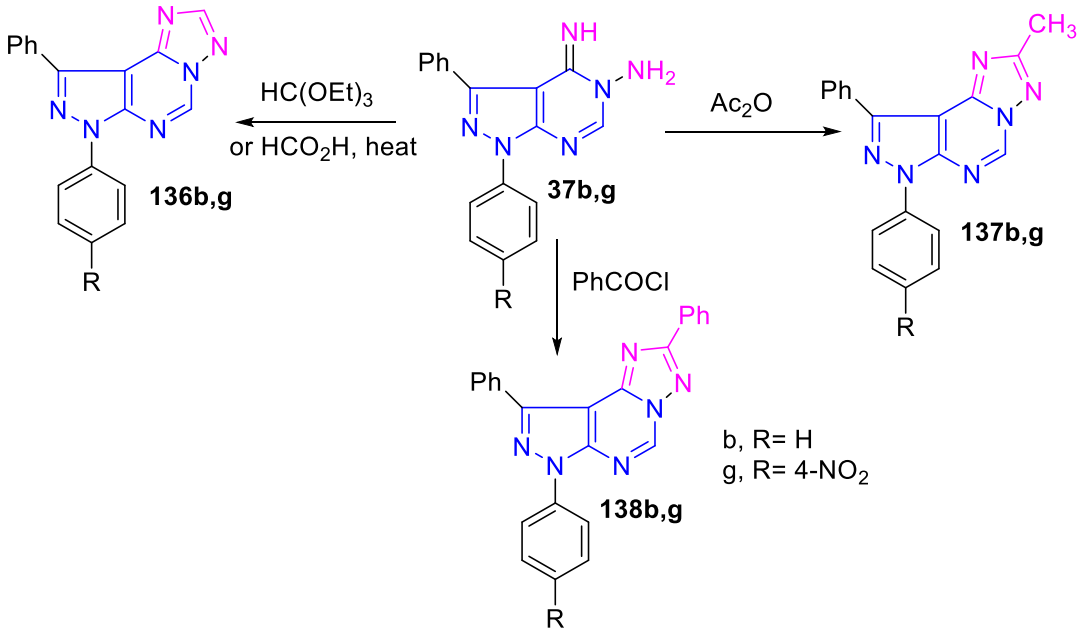
Recently, it was reported that treatment of compound **44** with different aromatic aldehydes gave the desired compounds **135a–d** (Scheme 32) [11]



Scheme 34. Synthetic pathway for the pyrazolo[3,4-*d*]pyrimidine derivatives **135a-d.**

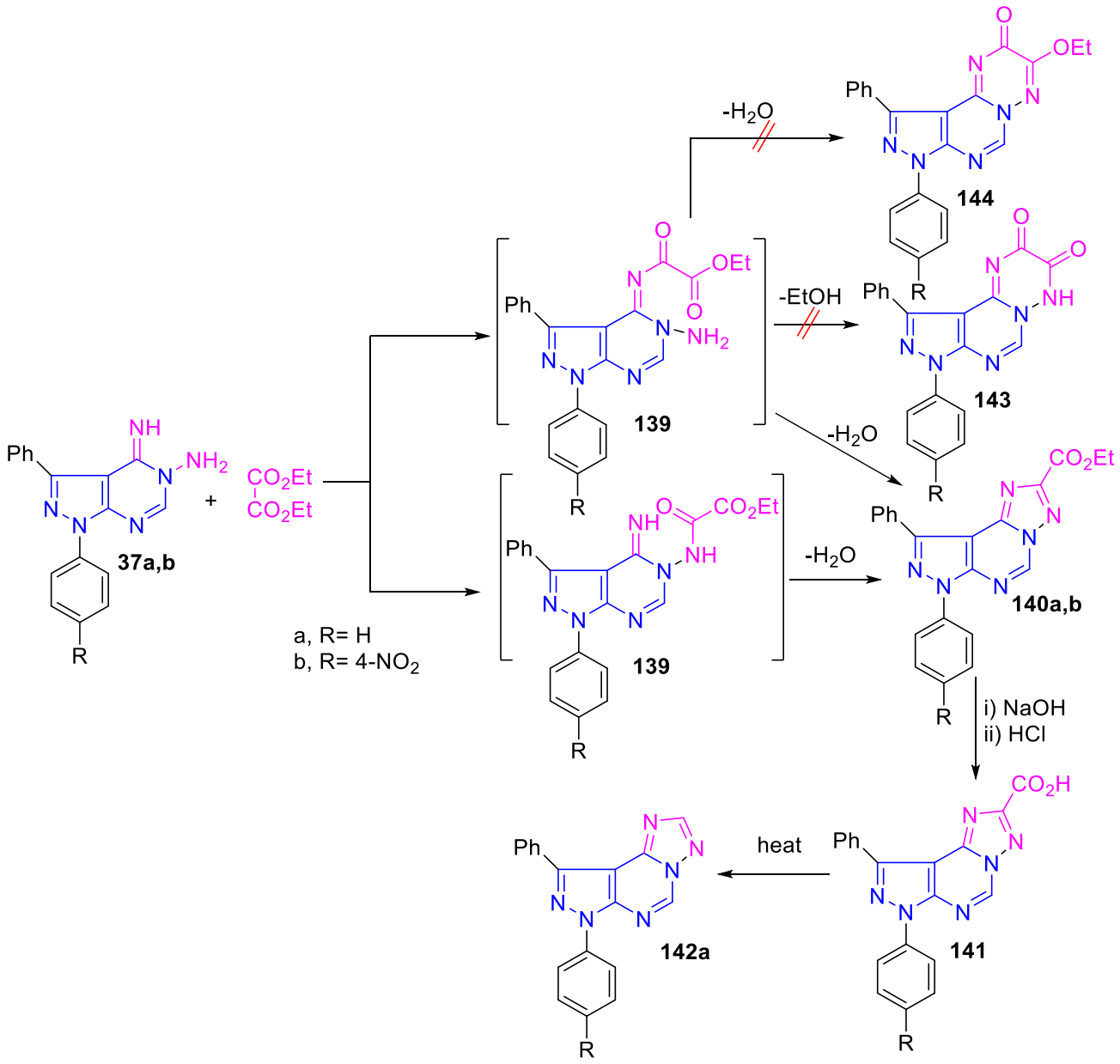
4.2. Synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives

It was reported that refluxing compounds **37b,g** with triethyl orthoformate or formic acid afforded the corresponding 7,9-diaryl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines **136b,g**.[61] In contrast, treatment with acetic anhydride or benzoyl chloride furnished the corresponding 2-methyl derivatives **137b,g** and 2-phenyl derivatives **138b,g,** respectively (Scheme 35).[61]



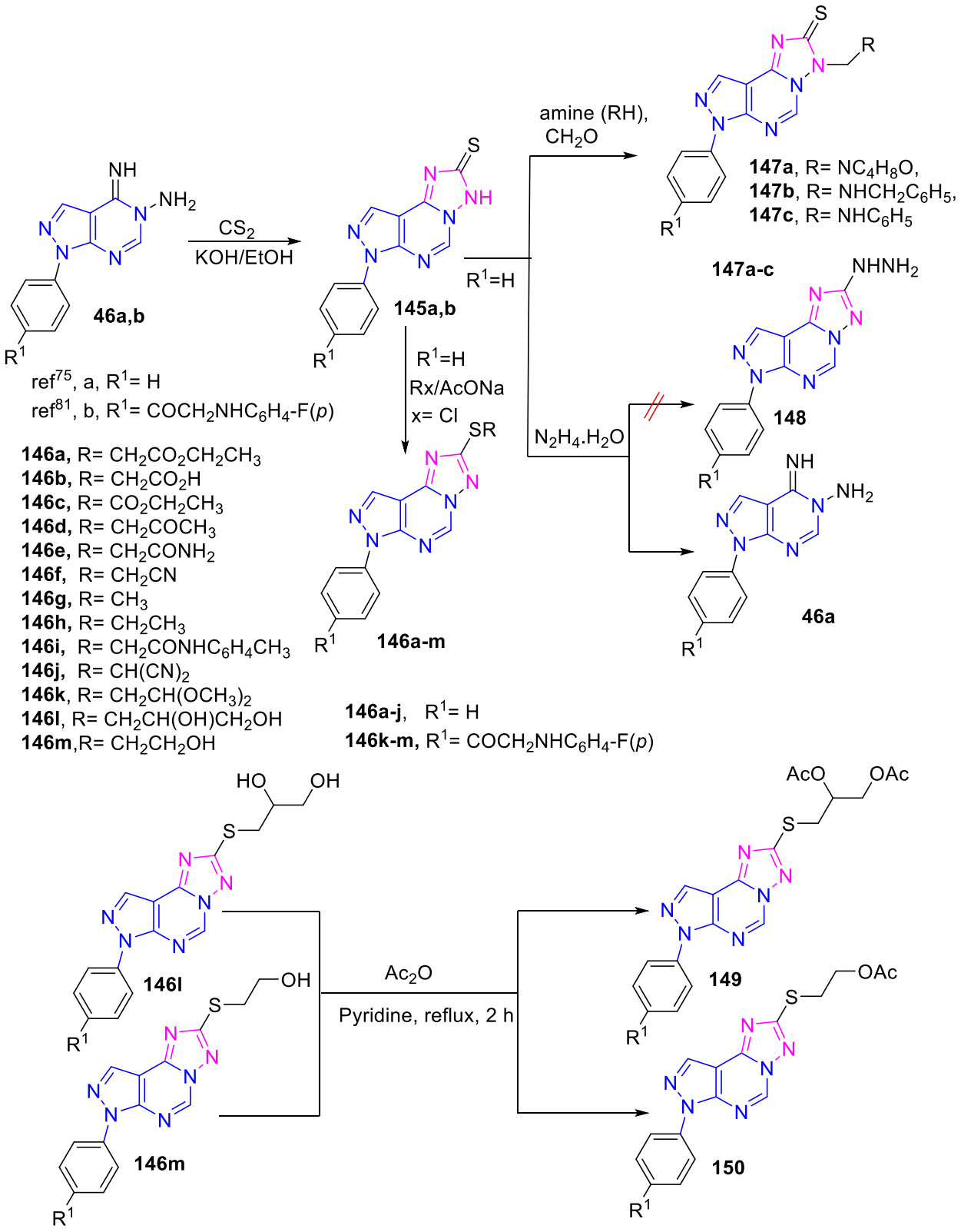
Scheme 35. Synthesis of pyrazolo[4,3-*e*][l,2,4]triazolo[1,5-*c*]pyrimidine derivatives **136-138**.

It was also demonstrated[61] that the reaction of compounds **37a,b** with diethyl oxalate afforded fused pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine derivatives. The reaction initially proceeds through formation of oxalylated intermediates **139** followed by intramolecular cyclization. Elimination of ethanol leads preferentially to compounds **140a,b** while the alternative regioisomeric products **143** and **144** were not isolated, indicating that formation of **140a,b** is the favoured pathway. Hydrolysis of **140a,b** afforded the corresponding carboxylic acid derivative **141**, which upon heating underwent decarboxylation to give compound **142a**. The stability of the fused heteroaromatic system likely drives the cyclization and decarboxylation processes (Scheme 36).[61]



Scheme 36. Synthesis of pyrazolo[4,3-e][1,2,4]triazolo[1,5-*c*]pyrimidines **140-142**.

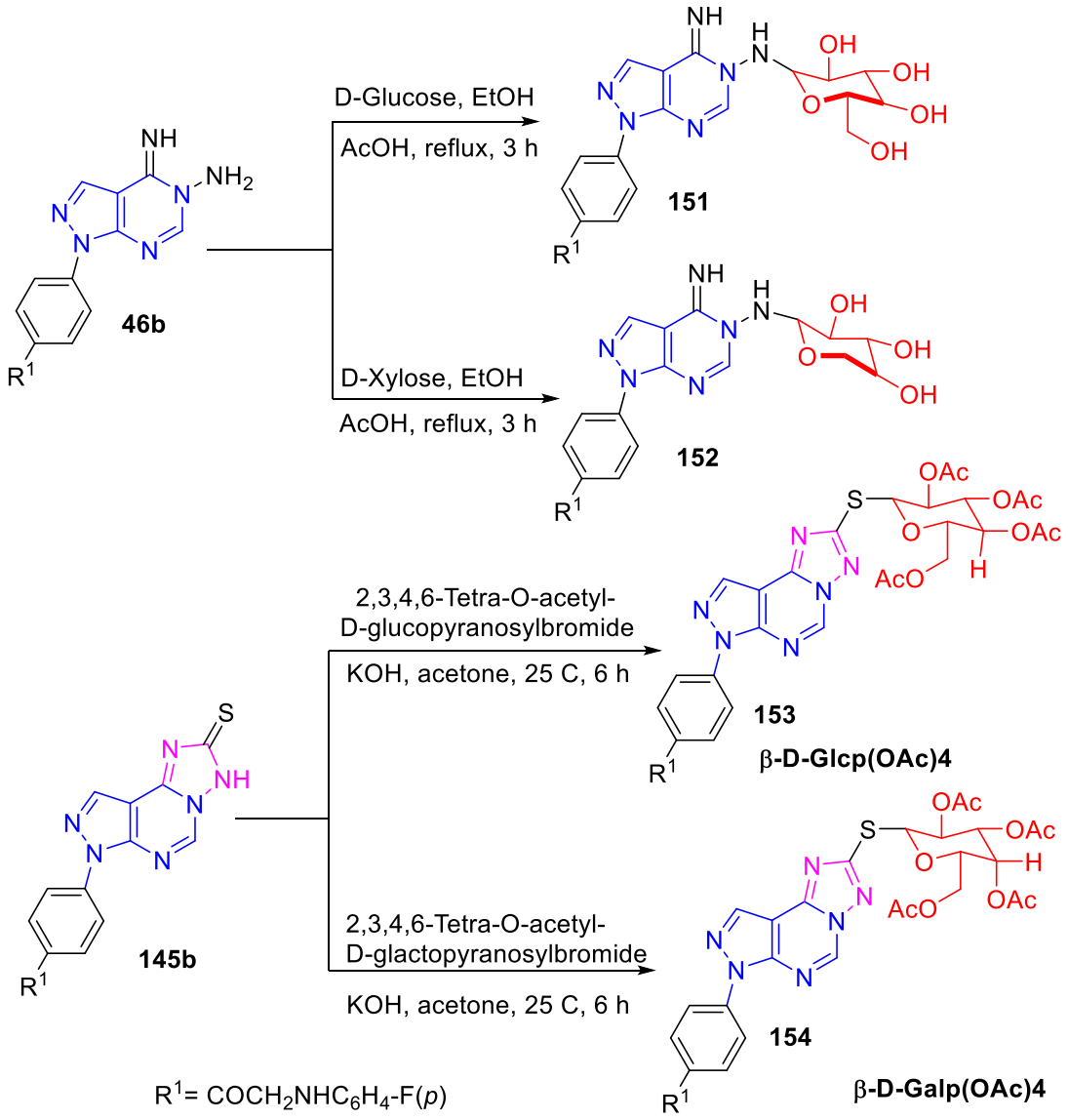
It was reported that treatment of compound **46** with carbon disulfide (CS_2) gave the pyrazolo-triazolo-pyrimidine-2-thione **145**.[75, 81] In its thiol tautomeric form, compound **145** was subsequently alkylated with various alkylating agents under reflux in ethanol in the presence of anhydrous sodium acetate to afford the corresponding derivatives **146a–m**. Moreover, the Mannich bases **147a–c** were synthesized via the reaction of thione **145** with formaldehyde and the appropriate amines. Attempted conversion of **145** into the corresponding hydrazino derivative **148** by treatment with hydrazine hydrate unexpectedly yielded compound **46a** as the sole isolated product. In addition, the hydroxyl compounds **146l** and **146m** were acetylated with acetic anhydride to yield the corresponding O-acetylated acyclic analogs **149** and **150**, respectively (Scheme 37).



Scheme 37. Synthesis of pyrazolotriazolopyrimidine-2-thione derivatives **145-150**.

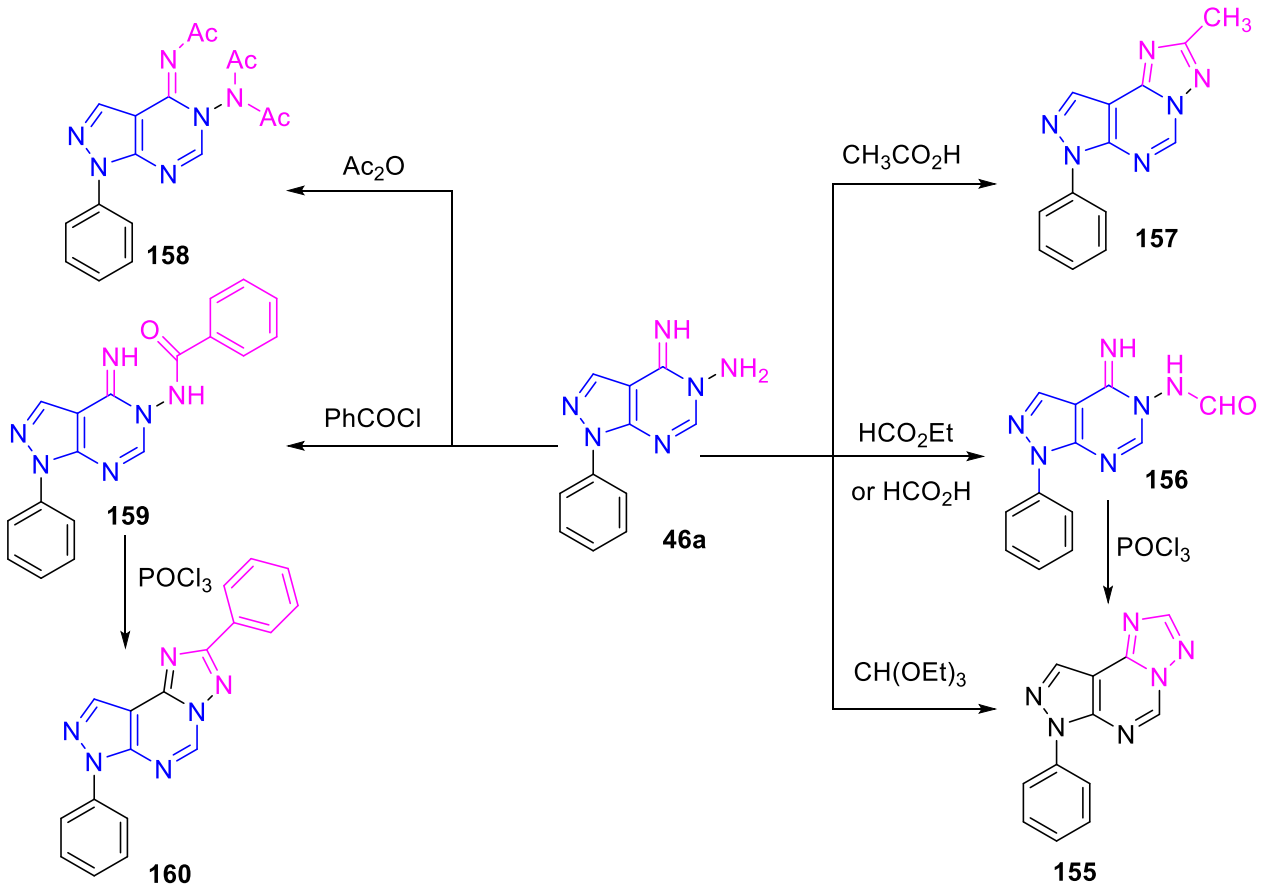
Treatment[81] of pyrazolopyrimidine **46a** with the sugar aldoses D-glucose and D-xylose, in the presence of a catalytic amount of acetic acid, afforded the corresponding amino-sugar derivatives **151** and **152**, respectively. the anomeric configuration of compounds **151** and **152** was not explicitly reported in the original study and is

therefore shown as unspecified. In addition, treatment of the triazolopyrazolopyridine derivative **145b** with the acetylated glycosyl halides tetra-O-acetyl-α-D-glucopyranosyl bromide and tetra-O-acetyl-α-D-galactopyranosyl bromide furnished the corresponding nucleoside analogues, thioglycoside derivatives **153** and **154** respectively. The glycosylation products were assigned where possible according to the reported anomeric configuration. Thus, compounds **153** and **154**, obtained were identified as the corresponding β-glycosides (Scheme 38).



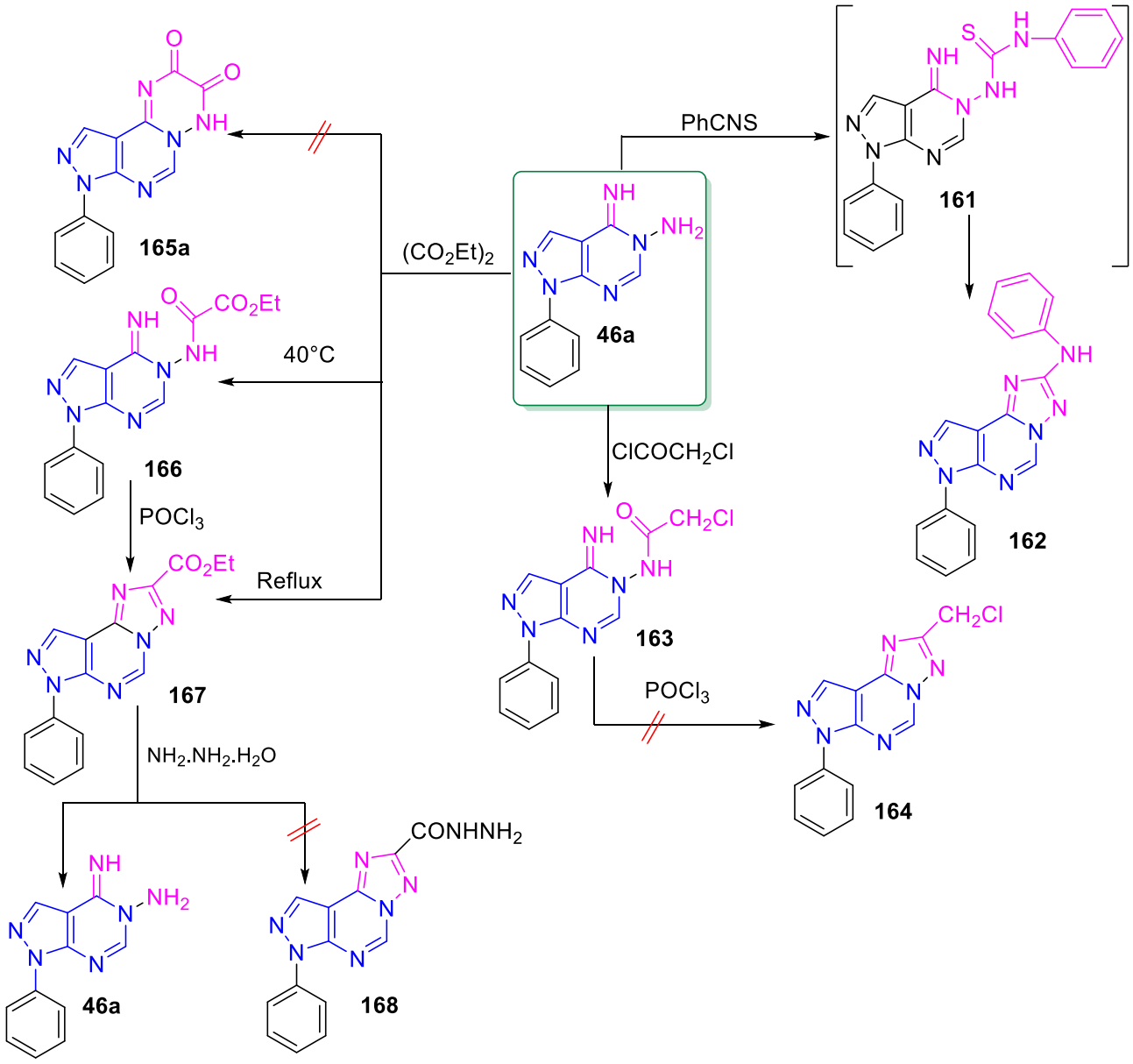
Scheme 38. Reaction of pyrazolopyrimidine **46a** and **145** with sugar aldoses.

Treatment of compound **46a** with triethyl orthoformate afforded 7-phenyl-7*H*-pyrazolo[4,3-*e*][1,2,4] triazolo[1,5-*c*]pyrimidine **155** directly. Reaction of **46a** with ethyl formate gave the corresponding formylamino intermediate **156**, which was subsequently cyclized in refluxing phosphorus oxychloride to furnish compound **155**. Refluxing **46a** in glacial acetic acid afforded the 2-methyl triazole derivative **157**, whereas heating it in acetic anhydride yielded the triacetyl derivative **158**. Similarly, treatment of **46a** with benzoyl chloride produced the benzoylamino derivative **159**, rather than the expected triazolo derivative **160**. However, the triazolo derivative **160** compound was obtained successfully by cyclization of **159** in boiling phosphorus oxychloride (Scheme 39).[75]



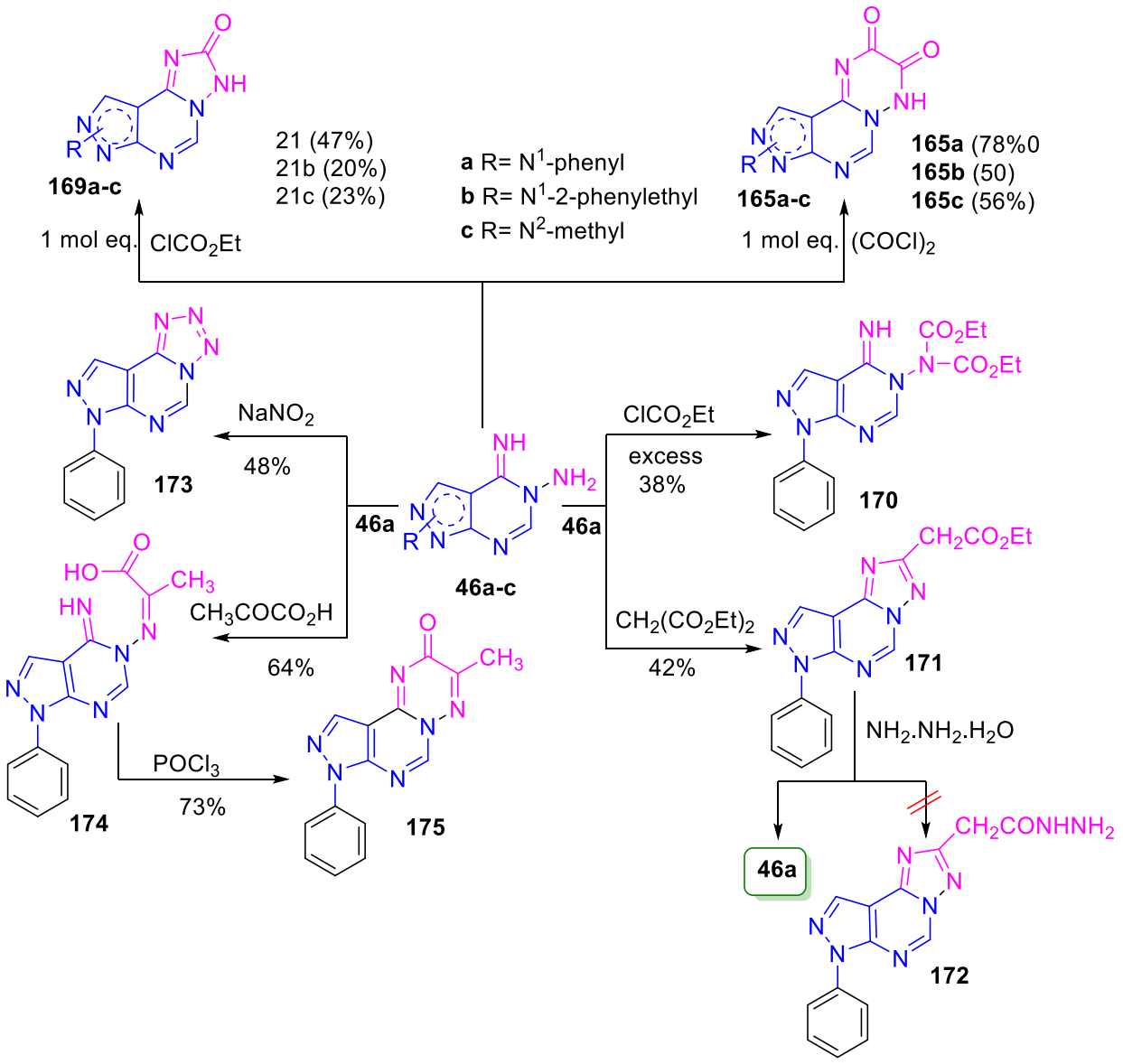
Scheme 39. Synthesis of triazolo-pyrazolo-pyrimidine derivatives **155-159**.

As illustrated in Scheme 40, [75] treatment of compound **46a** with phenyl isothiocyanate in refluxing pyridine afforded the 2-phenylamino derivative **162**, presumably through initial formation of thiourea intermediate **161** followed by dehydrosulfurization. In a similar manner, reaction of **46a** with chloroacetyl chloride yielded the chloroacetylamino derivative **163** rather than the anticipated chloromethyl derivative **164**, and subsequent cyclization attempts in boiling $POCl_3$ were unsuccessful. Furthermore, warming compound **46a** with diethyl oxalate furnished intermediate **166**, which upon treatment with boiling $POCl_3$ underwent cyclization to give ester **167**. The latter compound could also be obtained directly by refluxing **46a** with diethyl oxalate, whereas no evidence for the formation of the possible dioxotriazine derivative **165a** was detected. Notably, hydrazinolysis of ester **167** failed to produce the expected carbohydrazide derivative **168** and instead resulted in cleavage of the triazole ring with regeneration of the amino-imino precursor **46a** (Scheme 40).



Scheme 40. Synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **161-168.**

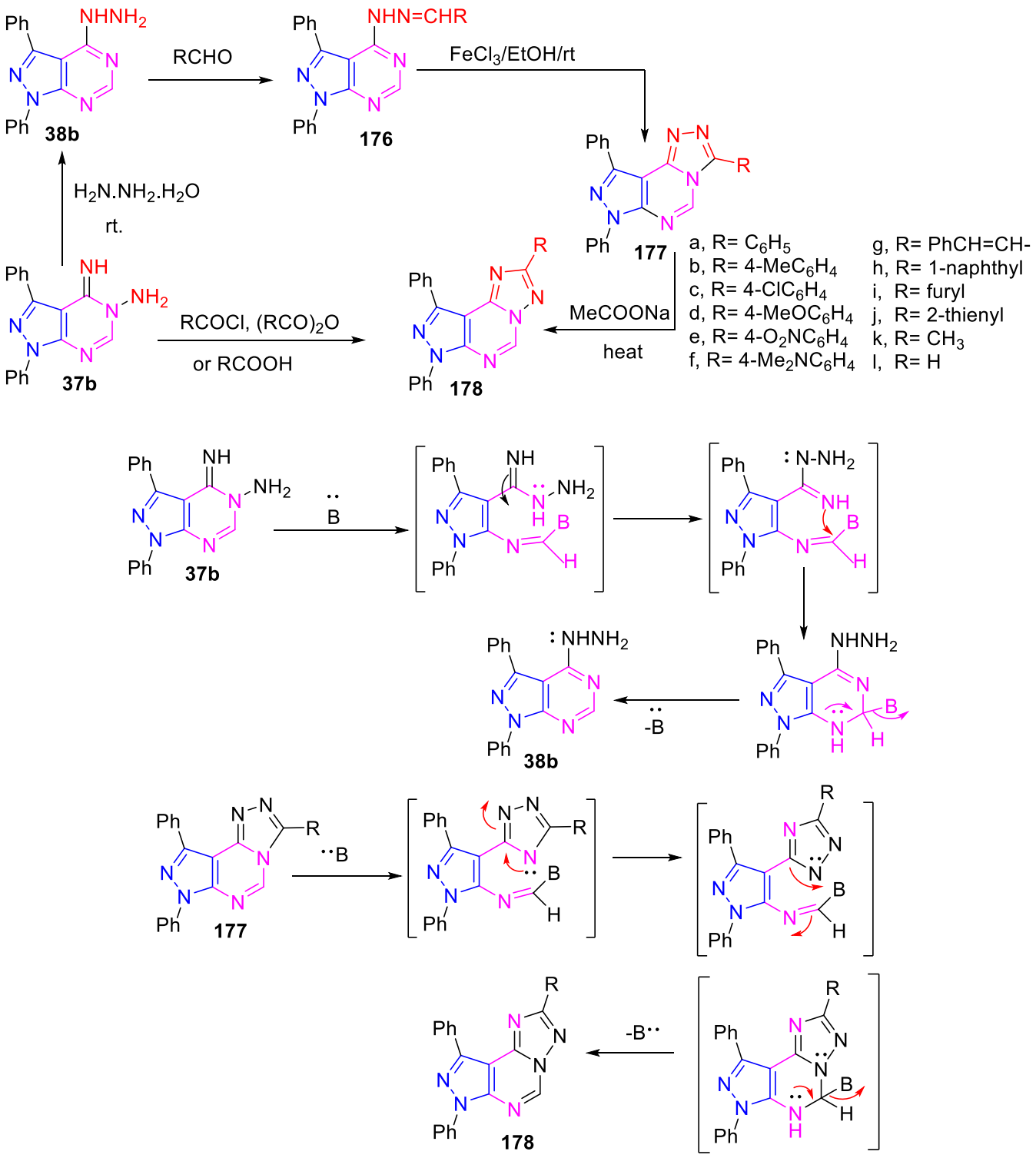
Treatment of compounds **46a–c**[75] with an equimolar amount of ethyl chloroformate afforded the corresponding triazolo derivatives **169a–c**. In contrast, reaction of **46a** with an excess of the same reagent furnished the diethoxycarbonylamino derivative **170**. Similarly, treatment of **46a** with an excess of diethyl malonate directly gave the ester derivative **171**. However, as observed for compound **167**, hydrazinolysis of **171** did not produce the expected hydrazide **172**, the aminoimino compound **46a** was obtained via triazole ring opening. Diazotization of compound **46a** into the tetrazolo derivative **173**. Moreover, refluxing compounds **46a–c** with oxalyl chloride in dry benzene afforded the corresponding dioxotriazine derivatives **165a–c**. Treatment of **46a** with pyruvic acid yielded the intermediate **174**, which underwent cyclization upon heating in phosphorus oxychloride to give the triazinone derivative **175** (Scheme 41).[75]



Scheme 41. Synthesis of pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives **169-175.**

It was reported[17] that the starting material, 5-amino-1,3-diphenyl-4-imino-4,5-dihydro-1*H*-pyrazolo[3,4-d]pyrimidine (**37b**), was prepared according to the reported procedure.[61] Stirring compound **37b** in ethanol with excess hydrazine hydrate at room temperature resulted in a Dimroth-type rearrangement affording 1,3-diphenyl-4-hydrazinopyrazolo[3,4-*d*]pyrimidine (**38b**). The transformation of **37** into **38** is believed to proceed through a base-catalyzed tandem ring opening/ring closure process, as depicted in Scheme 42, in agreement with related rearrangements previously reported.[97] Condensation of hydrazine derivative **38** with the appropriate aldehydes furnished the corresponding *N*-(1,3-diphenylpyrazolo[3,4-*d*]pyrimidin-4-yl)hydrazones **176**. Oxidative cyclization of hydrazones **176** using iron(III) chloride in ethanol afforded the fused pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine derivatives **177**. This transformation is analogous to previously reported $FeCl_3$-mediated oxidative cyclizations of aldehyde *N*-heteroarylhydrazones, which are thought to proceed via in situ generation of nitrilimines followed by 1,5-electrocyclization (Scheme 42).[98]

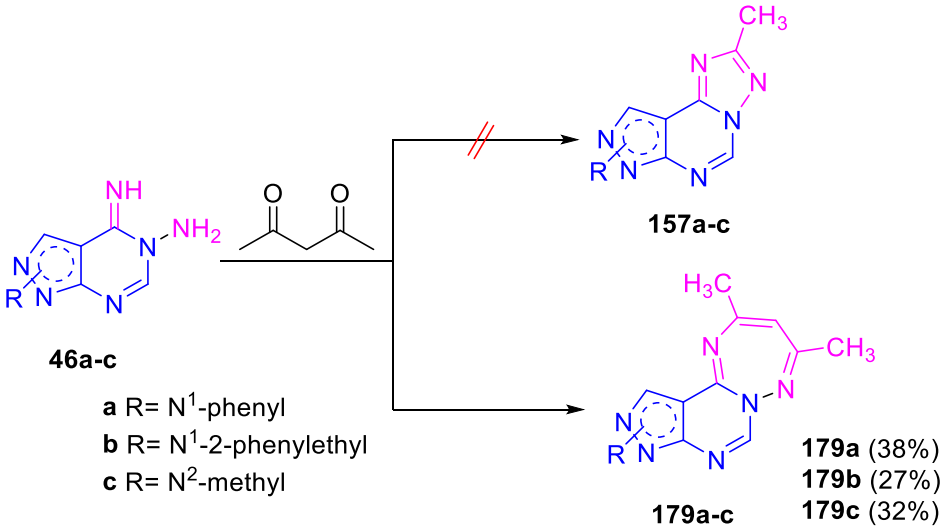
Furthermore, heating compounds **177** in ethanol in the presence of sodium acetate resulted in isomerization to the thermodynamically more stable pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine derivatives **178** through successive ring opening and ring closure reactions (Scheme 42).[97]

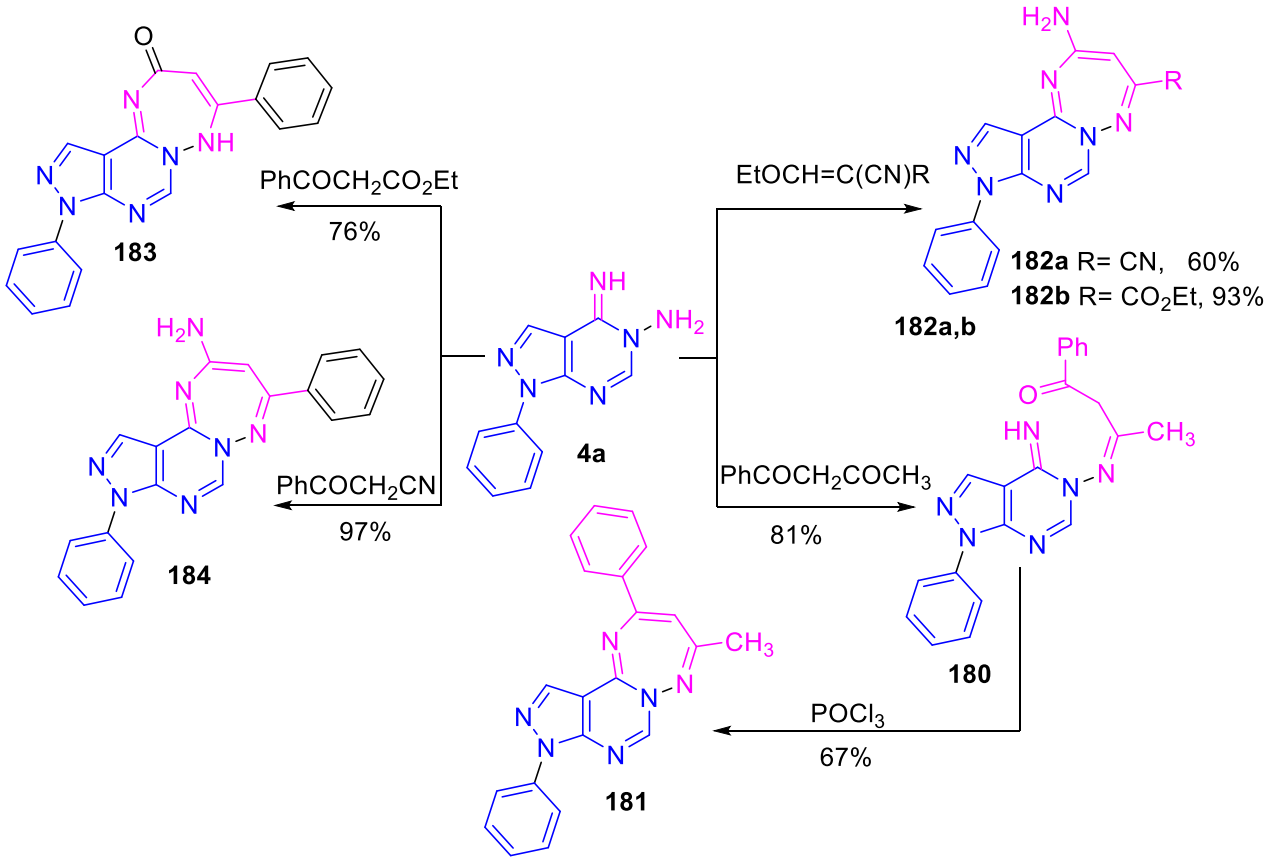


Scheme 42. Synthesis of pyrazolo[4,3-e][1,2,4]triazolo[4,3-c]-pyrimidines **177** and pyrazolo[4,3-e][1,2,4] triazolo[1,5-c]pyrimidines **178**.

4.3. Synthesis of pyrazolo[3',4':4,5]pyrimido[1,6-b][1,2,4]triazepine derivatives

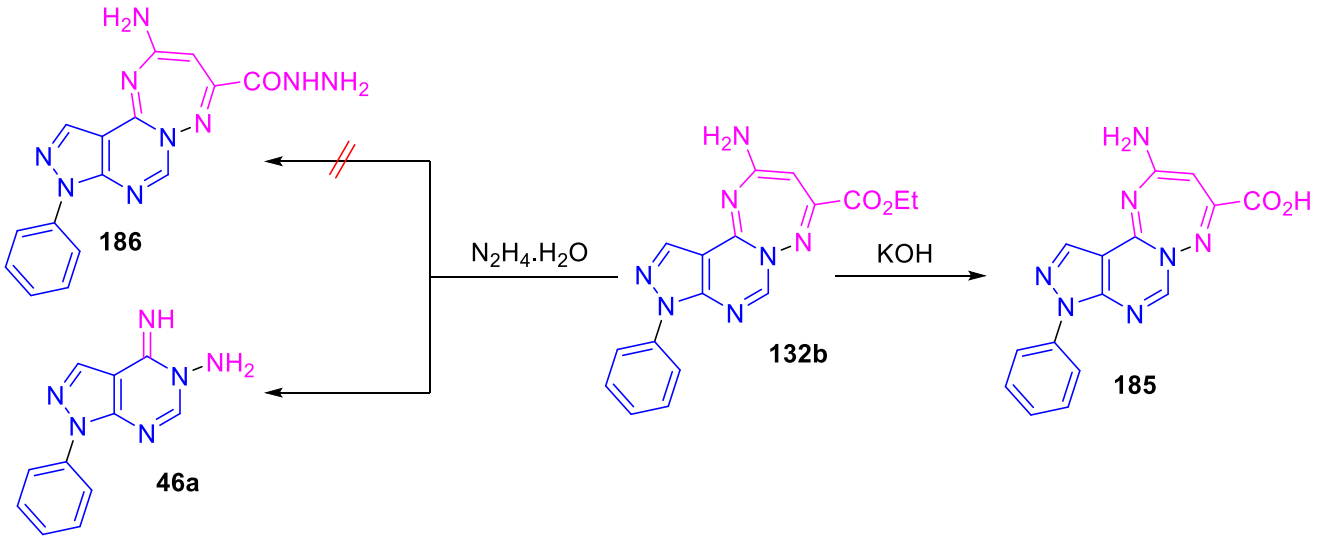
Furthermore,[75] treatment of the aminoimino derivatives **46a–c** with acetylacetone under the reaction conditions previously employed for the above transformation failed to furnish the expected methyl triazoles **157a–c**. Instead, the corresponding 5,7-dimethyl-1(2)-substituted-1(2)*H*-pyrazolo[3',4':4,5]pyrimido[1,6-*b*] triazepines **179a–c** were isolated as the sole reaction products. Interestingly, condensation of compound **46a** with benzoylacetone afforded the azomethine intermediate **180**, which subsequently underwent cyclization upon reflux in phosphoryl chloride to yield the corresponding triazepine derivative **181** (Scheme 43). Likewise, reaction of **46a** with ethyl benzoylacetate provided the triazepine analogue **183**. In contrast to the anticipated triazolo products **155**, treatment of **46a** with ethoxymethylenemalononitrile or ethyl ethoxymethylene-cyanoacetate produced the triazepine aminonitrile and aminoester derivatives **182a** and **182b**, respectively. Finally, compound **184** was obtained through the reaction of **46a** with benzoylacetonitrile (Scheme 43). [75]





Scheme 43. Synthesis of pyrazolo[3',4':4,5]pyrimido[1,6-b][1,2,4]triazepine derivatives **179-184**.

Hydrolysis[75] of the amino ester **132b** smoothly afforded the corresponding amino acid derivative **185**. In contrast, attempted hydrazinolysis of the ester functionality in **182b** did not produce the anticipated amino hydrazide derivative **186**; instead, the reaction unexpectedly regenerated the amino imino compound **46a** (Scheme 44).



Scheme 44. Hydrolysis of the amino ester **132b** to the corresponding amino acid derivative **185**.

Conclusions

In conclusion, pyrazolo[3,4-*d*]pyrimidine derivatives represent an important class of fused heterocycles with broad relevance in medicinal and synthetic chemistry. Significant progress has been achieved in their synthesis through several key reaction strategies, including cyclocondensation of aminopyrazoles with one-carbon and carbonyl reagents, annulation approaches starting from suitably functionalized pyrazole or pyrimidine precursors, nucleophilic substitution on halogenated pyrazolo[3,4-*d*]pyrimidines, alkylation and glycosylation reactions, as well as further ring-fusion and functionalization protocols. These methodologies have greatly expanded the structural diversity of the pyrazolo[3,4-*d*]pyrimidine scaffold and enabled access to a wide range of biologically relevant analogues. Despite these advances, some limitations remain. Many reported methods still require harsh reaction conditions, long reaction times, toxic reagents, or low-yielding steps. In addition, regioselectivity issues, especially during alkylation and glycosylation reactions, may lead to mixtures of isomers that require careful separation and characterization. The scalability of several synthetic routes also remains insufficiently explored, limiting their direct application in pharmaceutical development. Overall, the major synthetic advantages of pyrazolo[3,4-*d*]pyrimidine chemistry include the availability of versatile starting materials, the high reactivity of functionalized intermediates, the possibility of introducing diverse substituents at different positions of the fused ring system, and the ability to construct complex polycyclic and nucleoside-like derivatives. Future work should focus on greener, more selective, and scalable synthetic protocols to further enhance the practical value of this privileged heterocyclic framework in drug discovery and advanced heterocyclic synthesis

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