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Tandem Schiff-Base Formation/Heterocyclization: An Approach to the Synthesis of Fused Pyrazolo–Pyrimidine/Isoxazolo-Pyrimidine Hybrids

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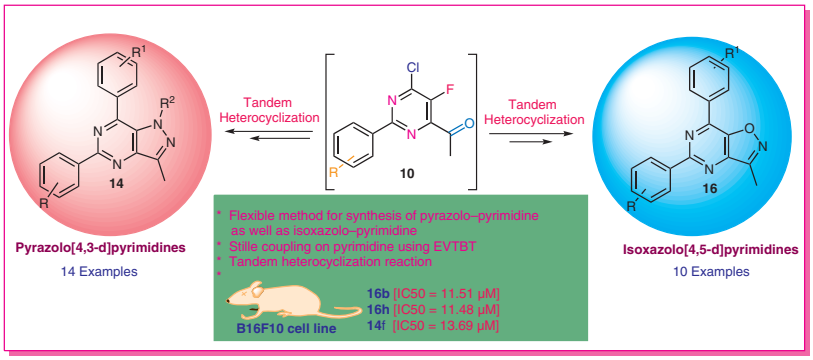

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Abstract A new synthesis of pyrazolo[4,3-d]pyrimidines and isoxazolo[4,5-d]pyrimidines is described. Key steps in the synthesis involve Stille coupling of 4,6-dichloro-2-phenyl-pyrimidine with tributyl(1-ethoxyvinyl)stannane and tandem Schiff-base formation/heterocyclization of 2,6-di-aryl-5-fluoro-4-acetylpyrimidine with hydrazines or hydroxylamine to give pyrazolo[4,3-d]pyrimidines and isoxazolo[4,5-d]pyrimidines, respectively. The position of the fluoro group in the pyrimidine ring is important for the success of heterocylization reaction.

Key words pyrazolo[4,3-d]pyrimidine, isoxazolo[4,5-d]pyrimidine, Stille coupling, Schiff base

Pyrimidine derivatives are privileged heterocyclic structures in medicinal chemistry.[1] Along with the varied biological activities of pyrimidines, other heterocycles fused with pyrimidines play essential roles in several biological processes and have considerable chemical and pharmacological importance.[2] Fused pyrimidines exhibit a broad spectrum of biological properties[3] such as anticancer,[4] antiviral,[5] antibacterial,[6] antioxidant,[7] anxiolytic,[8] and antidepressant activities.[9] Studies indicate that fusion of the pyrimidine moiety with different heterocycle scaffolds gives rise to new classes of hybrid heterocycles with improved biological activity (Figure 1).[10]

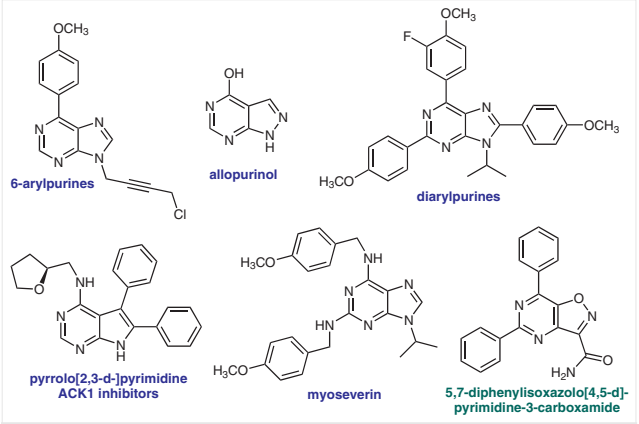
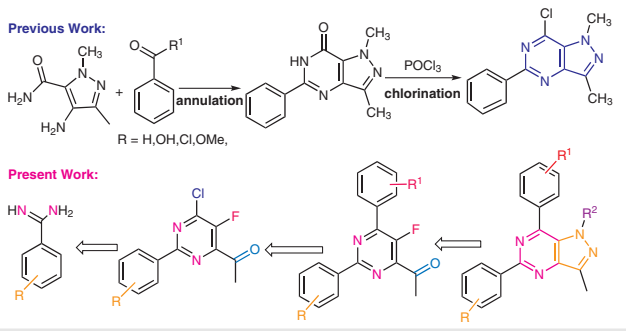


Figure 1 Representative biologically active azolo fused pyrimidines and isoxazolopyrimidine derivatives

Many simple fused pyrimidines such as purines and pteridines are biologically active by themselves or are essential components of important naturally occurring substances, such as nucleic acids.[11] Pyrrolo[2,3-*d*]pyrimidines, also known as deazapurines because of their structural similarity to natural purines, are interesting templates for drug discovery programs in medicinal chemistry[12] and have proved to be valuable scaffolds in organic chemistry.[13] Seminal work on the synthesis of 7-deazapurines derivatives, carried out by Hocek, highlights the applications of this class of biologically active pyrimidine derivatives.[14]

1H-Pyrazolo[4,3-d]pyrimidines are a class of fused heterocycles with potent biological activities.[15] They are selective second-generation phosphodiesterase 5 (PDE-5) inhibitors,[16] and others are known to function as central nervous system depressants,[17] neuroleptic agents,[18] tuberculostatic,[19] and to be active on adenosine receptors.[20] The application of pyrazolopyrimidines as antipyretic and analgesic agents has also been reported.[21,22] A recent report on the synthesis and chemistry of these compounds has appeared (Scheme 1).[23]



Scheme 1 Synthetic approaches for pyrazoleo[4,3-d]pyrimidines

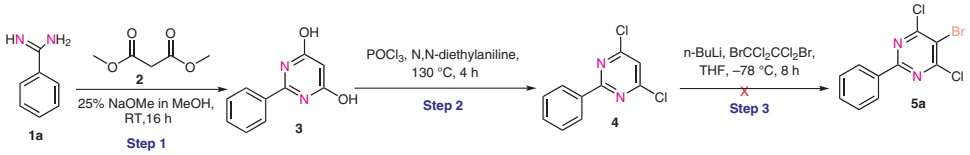
The pyrazolo[4,3-d]pyrimidine ring system has received less attention.[24] As a result the chemical as well as biological properties of this system are less known compared to its isomeric form. Besides, pyrazolopyrimidines, other heterocycle-fused pyrimidine derivatives, such as isoxazolopyrimidine derivatives have shown analgesic, anti-inflammatory, anti-bactericidal, circulatory, and anxiolytic activities.[25] Of the four structural isomers of isoxazolopyrimidines, only the [4,5-d] isomer has not been thoroughly investigated.[26] To date there have been only a few reports concerning the synthesis of derivatives of this isomer.[27–30] Therefore there is a need to develop synthetic methodology for the preparation of isoxazolo[4,5-d]pyrimidine and pyrazolo[4,3-d]pyrimidine derivatives.

Synthesis of hybrid molecules wherein two naturally occurring structures are linked by a C–C bond has received much attention recently.[31–33] Taking into consideration the biological significance of pyrazoles, isoxazole, and pyrimidines, we planned to develop a general methodology for the synthesis of pyrazolopyrimidine and isoxazolopyrimidine hybrids and herein we report our initial results.

The most common method for the preparation of pyrazolo[4,3-d]pyrimidines is the stepwise condensation of a hydrazine with a 2-alkoxy-1,1-dicyanoalkene to obtain a pyrazole derivative, onto which the pyrimidine is fused. This sequence requires multiple discrete steps and the conditions involved are incompatible with a number of functional groups, in particular the nitrile functionality. There is a report for the synthesis of pyrazolo[4,3-d]pyrimidine derivatives by treatment of hydrazine hydrate with 2,4 bis(trichloromethyl)-5-cyano-6-phenyl pyrimidine. A more efficient method for achieving such a transformation would be to condense a substituted hydrazine with 2,6-diaryl-5-fluoro-4-acetyl-pyrimidine allowing formation of the desired pyrazole ring system in a tandem manner. Such a transformation will give 3-methyl-5,7-diaryl-pyrazolo[4,3-d]pyrimidine derivatives selectively, which are otherwise difficult to prepare using existing methodology.

In the course of our research to develop novel hybrid molecules, we have been interested in the synthesis of novel analogues of 1,3- and 5,7-substituted 1H-pyrazolo[4,3-d]pyrimidines **14** as well as 5,7-disubstituted isoxazolo[4,5-d]pyrimidines **16**. Initially our aim was to synthesize the key compound, 5-bromo-4,6-dichloro-2-phenylpyrimidine **5a** (Scheme 2), so that a variety of structural and functional moieties could be introduced by palladium-assisted methodology.

Initially, compound **3** was prepared by condensation of benzamidine with dimethyl malonate in the presence of sodium methoxide in methanol. Subsequent chlorination with $POCl_3$using a catalytic amount of N,N-diethyl aniline at 130 °C gave the 3,6-dichloro-2-phenyl-pyrimidine derivative **4** in good yield. The key bromination reaction was attempted using a variety of brominating reagents as summarized in Table 1 without success. Although bromination at C-5 has been reported with unsubstituted pyrimidines using a variety of methods, the position of two chlorine substituents at adjacent positions may be detrimental for this bromination. After realizing the difficulties in halogenation at the C-5 position of pyrimidine, we decided to construct the pyrimidine ring with suitably substituted halogen derivatives of dimethyl malonate. The synthesis started with the reaction of commercially available 2-fluorodimethyl malonate **6** with benzamidine in the presence of sodium methoxide in methanol. Gratifyingly, we were able to obtain 4,6-dihydroxy-2-phenyl-5-fluoro-pyrimidine (**7a**) in good yield. Compound **7a** was characterized by ^{1}H NMR,



Scheme 2

^{19}F NMR spectroscopy and MS analysis. Compound **7a** was then treated with $POCl_3$using a catalytic amount of *N*,*N*-diethyl aniline at 130 °C to provide the 4,6-dichloro-2-phenyl-5-fluoro-pyrimidine derivative **8a**. Inspired by the statistical Stille coupling[34] on 3,5-dichloropyrimidine by Lehn's group,[35] we went on to explore the Stille coupling on 4,6-dichloro-2-phenyl-5-fluoro-pyrimidine derivative **8a**.

Table 1 Screening the Optimal Conditions for the Bromination Reaction of Compound **4**

Entry	Reaction conditions	Result[a]
1	NBS, DMF, RT, 16 h	SM intact
2	HBr, AcOH, RT, 16 h	no product
3	Br_2, $CHCl_3$, RT, 16 h	SM intact
4	Br_2, AcOH, RT, 16 h	SM intact
5	$C_2Br_2Cl_4$, LDA, THF, –78 °C to RT, 16 h	no product
6	$C_2Br_2Cl_4$, n-BuLi, THF, –78 °C to RT, 16 h	no product
7	$POBr_3$, DCM, RT, 16 h	SM intact
8	PBr_3, DCM, RT, 16 h	no product

[a]*Reaction conditions*: compound **4** (0.44 mmol) and brominating agents (0.53 mmol), results based on LC–MS analysis of crude product mixtures.

Table 2 Screening Optimal Conditions for the Stille Coupling Reaction

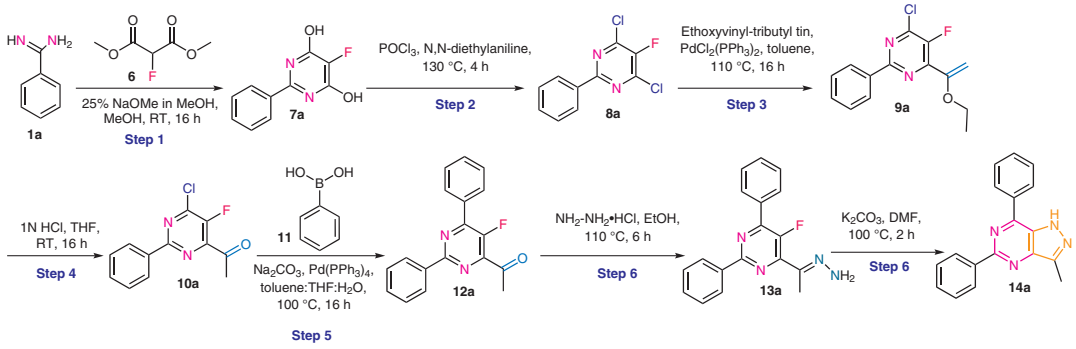
Entry	Reaction conditions	Result[a]
1	$Pd(PPh_3)_4$, THF, 65 °C, 12 h	25% of compound **9a**
2	$Pd(PPh_3)_4$, dioxane, 90 °C, 14 h	33% of compound **9a**
3	$Pd(PPh_3)_4$, toluene, 95 °C, 16 h	6% of compound **9a**
4	$PdCl_2$dppf, THF, 65 °C, 12 h	15% of compound **9a**
5	$PdCl_2$dppf, dioxane, 90 °C,14 h	no product
6	$PdCl_2$dppf, toluene, 95 °C, 16 h	no product
7	$Pd(dba)_2$, THF, 65 °C, 12 h	8% of compound **9a**
8	$Pd(dba)_2$, dioxane, 90 °C,14 h	5% of compound **9a**
9	$Pd(dba)_2$, toluene, 95 °C, 16 h	2% of compound **9a**
10	$Pd(OAc)_2$, TPP, THF, 65 °C, 12 h	no product
11	$Pd(OAc)_2$, TPP, dioxane, 90 °C, 14 h	16% of compound **9a**
12	$Pd(OAc)_2$, TPP, toluene, 95 °C, 16 h	46% of compound **9a**
13	$PdCl_2(PPh_3)_2$, toluene, 100 °C, 16 h	61% of compound **9a** and 10% of compound **10a**

[a]*Reaction conditions*: compound **8a** (0.41 mmol) and 1-ethoxyvinyl tri-n-butyltin (0.44 mmol), results based on LC–MS analysis of crude reaction mixtures.

The Stille reaction of compound **8a** was tried with ethoxyvinyltributyltin in the presence of Pd(0)/THF under reflux, which gave 25% product formation as observed by LC–MS analysis. Various palladium catalysts as well as solvents and temperature were screened for the Stille coupling reaction (Table 2).

The best result was obtained using 1.1 equivalents of 1-ethoxyvinyltri-n-butyltin and $PdCl_2(PPh_3)_2$ as catalyst in toluene (Table 2, entry 13), giving **9a** in 61% yield that was pure enough to proceed to the next step. Compound **10a** was obtained by hydrolysis of compound **9a** using HCl in THF and was purified by flash column chromatography on silica and characterized by ^{1}H NMR spectroscopy and LC–MS analysis. The characteristic peak at δ = 2.8 ppm in the ^{1}H NMR spectrum confirms the presence of an acetyl group at C-5 of the pyrimidine ring (Scheme 3). Compound **10a** was then desymmetrized by Suzuki coupling with various phenylboronic acids to give 2,6-diphenyl-substituted pyrimidines **12**. Finally, we explored the tandem Schiff-base formation/heterocyclization reaction of compound **12a** with hydrazine to obtained fused pyrazolopyrimidine derivatives.[36] Compound **12a** was treated with hydrazine hydrate under refluxing EtOH conditions to give the intermediate hydrazone **13a**, which was subjected to heterocyclization using K_2CO_3/DMF with heating[37] to give the final compound **14a**. Compound **14a** was characterized by ^{1}H NMR, ^{13}C NMR spectroscopy and MS analysis.

To prove the generality of this method various highly substituted aryl, hetero aryl, alkyl benzamidines, as well as polysubstituted hydrazines were examined, and the results are summarized in Figure 2. Similar yields were observed for substrates having electron-withdrawing substituents



Scheme 3

(compounds **14f**, **g**,**i**,**j**) as well as electron-donating substituents (compounds **14j–n**). Alkyl hydrazines (methyl, isopropyl, and benzyl) were also converted into the corresponding novel fused biphenyl-substituted pyrazolo[4,3-d]pyrimidines in good yields.

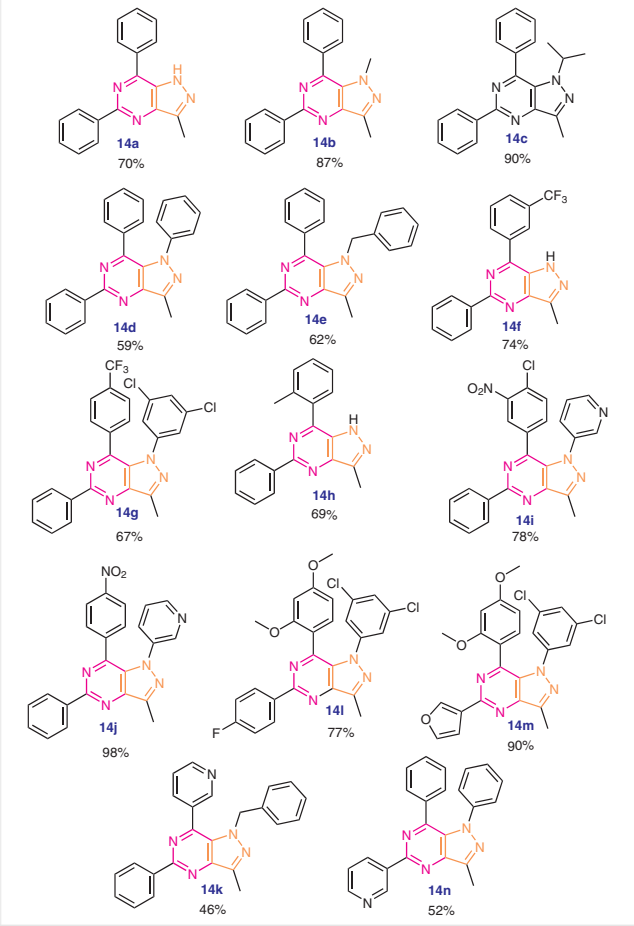
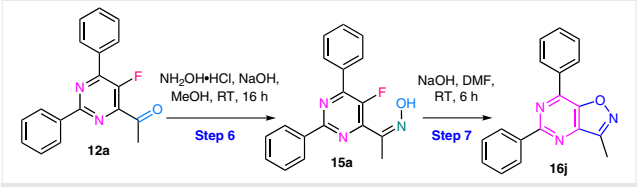


Figure 2 List of fused pyrazolo–pyrimidine hybrids (% isolated yield)

In order to expand further our synthetic approaches towards fused heterocyclic pyrimidine hybrids, we turned our attention to the prepare 3-methyl-5,7-disubstituted isoxazolo[4,5-d]pyrimidines using the same key intermediate **12a**. Thus, **12a** was treated with hydroxylamine hydrochloride in the presence of NaOH/MeOH at room temperature to give the hydrazone **15a** that was subsequently subjected to heterocyclization using K_2CO_3/DMF with heating[38] to give the isoxazolo[4,5-d]pyrimidine **16a** in good yield. This is the first report of diphenylisoxazolo[4,5-d]pyrimidine preparation by a tandem Schiff-base/heterocyclization sequence. Using a similar sequence (Scheme 4) the target compounds **16a–j** (Figure 3) were prepared and fully characterized.[39]



Scheme 4

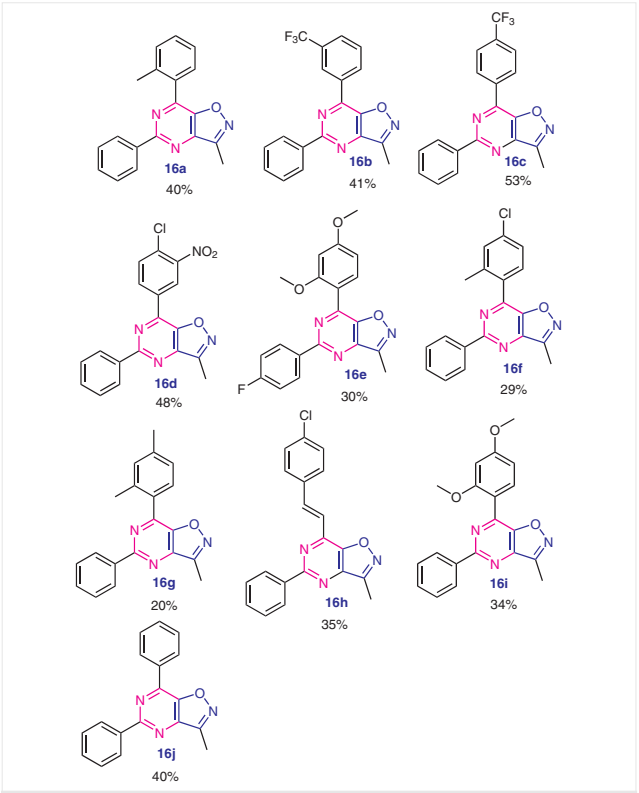


Figure 3 List of fused isoxazolo–pyrimidine hybrids (% isolated yield)

In summary, we have synthesized novel pyrazolopyrimidine as well as isoxazolopyrimidine hybrids using a tandem Schiff-base formation/heterocyclization reaction of 2,6-diaryl-5-fluoro-4-acetyl-pyrimidines. This method has several advantages over previously reported methods as it starts with simple benzamidine; whereas previous methods use 4-amino-5-carboxamide pyrazole derivatives, which are difficult to prepare.

Acknowledgment

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1612081.

References and Notes

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(37) **Typical Experimental Procedures**
3-Methyl-5,7-diphenyl-1H-pyrazolo [4,3-d]pyrimidine (14a)
To a stirred solution of compound **12a** (0.25 g, 0.85 mmol) in ethanol (10 mL) was added hydrazine hydrochloride (0.055 g, 1.71 mmol), and the resulting reaction mixture was heated to 110 °C for 6 h. The progress of the reaction was monitored by TLC (20% ethyl acetate in petroleum ether). The ethanol was evaporated, and the crude the **13a** was dissolved in DMF (10 mL), K_2CO_3 (0.177 g, 1.28 mmol) was added and the reaction mixture stirred at 100 °C for 2 h. After completion of reaction; water was added, and the reaction mixture was extracted with ethyl acetate (X × Y mL). The combined organic extracts were washed with water (X × Y mL), brine (X × Y mL), dried over Na_2SO_4, filtered and evaporated to give the crude product. The crude product was purified by silica gel column chromatography, eluting with 10% ethyl acetate in petroleum ether to afford pure **14a** (180 mg, 73%) as a pale yellow solid; mp 268–272 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.24 (br s, 1 H), 8.67 (dd, J = 8 Hz, 2 H), 8.23 (d, J = 8 Hz, 2 H), 7.63 (m, 6 H), 2.78 (s, 3 H). IR (KBr): 3247, 2920, 1954, 1554, 1465, 1375, 1292, 1203, 990, 930, 755, 685, 542 cm^{-1}. ^{13}C NMR (100 MHz, DMSO-d_6) = 156.2, 148.9, 145.3, 142.3, 137.9, 135.3, 131.2, 129.7, 129.7, 129.6, 129.0, 128.7, 128.5, 128.5, 128.5, 127.5, 127.5, 10.7. MS (EI): *m/z* = 286 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{18}H_{14}N_4$ [M + H]: 287.1218; found: 287.1298.

1-Isopropyl-3-methyl-5,7-diphenyl-1H-pyrazolo[4,3-d]pyrimidine (14c)

Pale yellow solid (0.2 g, 90%); mp 109–113 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (d, J = 8 Hz, 2 H), 7.78 (d, J = 4 Hz, 2 H), 7.75 (m, 3 H), 7.43 (m, 3 H), 4.53 (m 1 H), 2.75 (s, 3 H), 1.37 (d, J = 4 Hz, 6 H). IR (KBr): 3423, 2974, 1996, 1550, 1414, 1369, 1230, 1123, 1070, 926, 767, 603, 544 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$) = 157.1, 151.5, 145.9, 143.1, 138.3, 137.3, 130.0, 130.0, 129.5, 129.5, 129.5, 129.5, 128.8, 128.8, 128.5, 128.5, 128.4, 128.0, 51.4, 22.2, 10.9. MS (EI): *m/z* = 328 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{21}H_{20}N_4$ [M + H]: 329.1688; found: 329.1770.

7-[3-(Trifluoromethyl)phenyl]-3-methyl-5-phenyl-1H-pyrazolo [4,3-d]pyrimidine (14f)

Yellow solid (0.18 g 74%); mp 217–221 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.40 (br s, 1 H), 8.66 (m, 2 H), 8.53 (m, 1 H), 8.45 (d, J = 8 Hz, 1 H), 7.88 (m, 1 H), 7.81 (t, J = 8, 8 Hz, 1 H), 7.54 (m, 3 H), 2.80 (s, 3 H). IR (KBr): 3243, 3069, 1556, 1469, 1425, 1328, 1250, 1166, 1119, 1070, 993, 772, 544 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ =158.4, 148.3, 138.0, 137.1, 132.1, 131.8, 131.5, 130.0, 129.9, 129.9, 129.8, 128.5, 128.5, 128.1, 127.6, 127.6, 125.2, 124.8, 10.8. MS (EI): *m/z* = 354 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{19}H_{13}N_4$ [M + H]: 355.1092; found: 355.1173.

1-(3,5-Dichlorophenyl)-7-[4-(trifluoromethyl)phenyl]-3-methyl-5-phenyl-1H-pyrazolo[4,3-d]pyrimidine (14g)

Off-white solid (0.28 g, 67%); mp 216–220 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65 (m, 2 H), 7.61 (m, 4 H), 7.51 (m, 3 H), 7.21 (m, 1 H), 7.00 (d, J = 1.6 Hz, 2 H), 2.80 (s, 3 H). IR (KBr): 3086, 2323, 1585, 1455, 1326, 1167, 1124, 1069, 1018, 848, 704, 628, 600 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.7, 149.8, 148.7, 146.8, 140.3, 139.1, 137.4, 134.9, 132.4, 132.1, 130.4, 129.7, 129.7, 128.6, 128.6, 128.2, 127.1, 127.0, 127.0, 125.0, 125.0, 124.7, 123.1, 122.5, 10.9. MS (EI): *m/z* = 498 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{25}H_{15}Cl_2N_4$ [M + H]: 499.0626; found: 499.0708.

1-(3,5-Dichlorophenyl)-7-(2,4-dimethoxyphenyl)-5-(furan-3-yl)-3-methyl-1H-pyrazolo[4,3-d]pyrimidine (14m)

Off-white solid (0.19 g, 90%); mp 208–217 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.33 (m, 1 H), 7.73 (d, J = 8 Hz, 1 H), 7.52 (m, 1 H), 7.22 (m, 1 H), 7.16 (m, 1 H), 7.04 (m, 2 H), 6.72 (d, J = 8 Hz, 1 H), 6.08 (s, 1 H), 4.85 (s, 3 H), 3.19 (s, 3 H), 2.81 (s, 3 H). IR (KBr): 3438, 2926, 1736, 1610, 1584, 1504, 1461, 1277, 1111, 1027, 934, 795, 596 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ =163.4, 157.9, 157.9, 155.0, 149.4, 146.7, 146.6, 145.3, 144.2, 143.5, 141.0, 134.2, 131.6, 127.8, 127.2, 126.1, 122.2, 118.6, 109.8, 105.7, 97.3, 55.6, 54.5, 10.8. MS (EI): *m/z* = 480 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{24}H_{18}Cl_2N_4O_3$ [M + H]: 480.0756; found: 481.0845.

3-Methyl-1,7-diphenyl-5-(pyridin-3-yl)-1H-pyrazolo [4,3-d]pyrimidine (14n)

Light brown solid (0.13 g, 52%); mp 181–185 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.86 (s, 1 H), 8.93 (d, J = 4 Hz, 1 H), 8.72 (d, J = 4 Hz, 1 H), 7.48 (m, 3 H), 7.39 (m, 1 H), 7.23 (m, 5 H), 7.12 (m, 2 H), 2.85 (s, 3 H). IR (KBr): 3481, 3036, 2922, 1558, 1498, 1417, 1376, 1227, 1118, 1015, 759, 696, 686 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ =156.1, 152.1, 152.1, 150.7, 150.1, 147.8, 147.8, 145.5, 139.6, 135.6, 133.8, 130.1, 129.6, 129.6, 128.7, 128.7, 127.9, 127.9, 127.7, 127.5, 125.1, 123.4, 11.0. MS (EI): *m/z* = 363 [M + 1]. HRMS (ESI): *m/z* calcd for $C_{23}H_{17}N_5$ [M + H]: 363.1484; found: 364.1571.

(38) **Preparation of 3-Methyl-5,7-diphenylisoxazolo[4,5-d]pyrimidine (16j)**

To a stirred solution of compound **12a** (0.2 g, 0.68 mmol) in MeOH (8 mL) were added hydroxylamine hydrochloride (0.052 g, 0.75 mmol) and powdered NaOH (0.033 g, 0.82 mmol) at RT, then the reaction mixture was stirred under N_2 atmosphere for 16 h. Progress of the reaction was monitored by TLC (5% ethyl acetate in petroleum ether). After completion of the reaction, the methanol was evaporated to give crude **15a** as a solid. This was dissolved in DMF under N_2 and cooled to 0 °C. To this, NaOH (0.033 g, 0.82 mmol) powder was added and the mixture stirred at RT for 6 h. The progress of the reaction was monitored by TLC (5% ethyl acetate in petroleum ether). After completion of reaction, water was added, and the mixture was extracted with ethyl acetate (X × Y mL). The combined extracts were washed with water (X mL), brine (Y mL), dried over Na_2SO_4, filtered, and evaporated to afford the crude product, which was purified by silica column chromatography, eluting with 3% ethyl acetate in petroleum ether to give the pure **16j** (80 mg, 40%) as a pale yellow solid; mp 153–157 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.73 (m, 2 H), 8.71 (m, 2 H), 7.65 (m, 3 H), 7.56 (m, 3 H), 2.77 (s, 3 H). IR (KBr): 3414, 2921, 1589, 1457, 1376, 1274, 1164, 1067, 920, 853, 797, 750, 688 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.2, 156.5, 150.3, 149.0, 147.7, 137.3, 133.5, 132.1, 130.6, 130.5, 129.7, 129.4, 129.0, 128.6, 128.5, 128.3, 127.5, 9.2. MS (EI): *m/z* = 287 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{18}H_{13}N_3O$ [M + H]: 288.3153; found: 288.1143.

3-Methyl-5-phenyl-7-[3-(trifluoromethyl)phenyl]isoxazolo[4,5-d]pyrimidine (16b)

Pale yellow solid (0.12 g, 41%); mp 161–165 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.96 (m, 1 H), 8.91 (d, J = 8 Hz, 1 H), 8.64 (m, 2 H), 7.88 (m, 1 H), 7.79 (t, J = 8, 4 Hz, 1 H), 7.53 (m, 3 H), 2.79 (s, 3 H). IR (KBr): 3073, 2329, 1655, 1594, 1412, 1326, 1231, 1161, 1069, 920, 769, 690, 594 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.4, 161.4, 156.6, 150.0, 149.6, 145.9, 136.9, 134.9, 134.3, 132.8, 132.6, 131.8, 131.5, 130.7, 130.2, 129.6, 128.7, 128.6, 9.2. MS (EI): *m/z* = 355 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{19}H_{12}F_3N_3O$ [M + H]: 356.0932; found: 356.1014.

7-(4-Chloro-3-nitrophenyl)-3-methyl-5phenylisoxazolo[4,5-d]pyrimidine (16d)

Pale yellow solid (0.07 g, 48%); mp 187–191 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.20 (d, J = 4 Hz, 1 H), 8.86 (d, J = 8 Hz, 1 H), 8.62 (m, 2 H), 7.84 (d, J = 8 Hz, 1 H), 7.58 (m, 3 H), 2.79 (s, 3 H). IR (KBr): 3092, 2921, 2850, 2324, 1742, 1600, 1542, 1355, 1272, 1038, 844, 758, 696 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.4, 162.4, 156.7, 154.8, 150.2, 149.6, 143.9, 138.1, 136.6, 133.5, 133.2, 132.6, 131.0, 130.3, 128.8, 128.3, 125.7, 9.26. MS (EI): *m/z* = 366 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{18}H_{11}ClN_4O$ [M + H]: 367.0520; found: 366.9997.

7-(2,4-Dimethylphenyl)-3-methyl-5-phenylisoxazolo[4,5-d]pyrimidine (16g)

Pale yellow solid (0.07 g, 20%); mp 99–103 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.59 (m, 2 H), 7.86 (d, J = 4 Hz, 1 H), 7.54 (m, 3 H), 7.24 (m, 2 H), 2.81 (s, 3 H), 2.61 (s, 3 H), 2.40 (s, 3 H). IR (KBr): 3418, 2919, 2626, 2525, 1591, 1533, 1392, 1354, 1276, 1219, 841, 769, 695 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ =161.0, 161.0, 156.6, 150.9, 150.8, 148.0, 141.0, 138.0, 137.4, 132.6, 131.1, 130.4, 129.9, 129.9, 128.6, 128.3, 126.9, 21.4, 21.0, 9.3. MS (EI): *m/z* = 315 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{20}H_{17}N_3O$ [M + H]: 316.1372; found: 316.1458.

(E)-7-(4-Chlorostyryl)-3-methyl-5-phenylisoxazolo[4,5-d]pyrimidine (16h)

Pale yellow solid (0.07g, 35%); mp 89–93 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.57 (m, 2 H), 8.30 (d, J = 20 Hz, 1 H), 7.68 (d, J = 8 Hz, 1 H), 7.53 (m, 4 H), 7.45 (m, 3 H), 2.75 (s, 3 H). IR (KBr): 3443, 3044, 2920, 2299, 1978, 1586, 1502, 1409, 1176, 1083, 964, 809,

705 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 161.5, 161.5, 156.5, 150.1, 148.1, 147.1, 137.3, 136.0, 134.1, 130.4, 129.2, 129.1, 129.1, 129.1, 128.6, 128.3, 128.3, 128.3, 123.1, 9.26. MS (EI): *m/z* = 347 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{20}H_{14}ClN_3O$ [M + H]: 347.0825; found: 348.0908.

7-(2,4-Dimethoxyphenyl)-3-methyl-5-phenylisoxazolo[4,5-d]pyrimidine (16i)

Pale brown solid (0.05 g, 34%); mp 170–174 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.60 (m, 2 H), 7.92 (d, J = 8 Hz, 1 H), 7.51 (m, 3 H), 6.73 (m, 1 H), 6.65 (d, J = 4 Hz, 1 H), 3.95 (s, 3 H), 3.92 (s, 3 H), 2.75 (s, 3 H). IR (KBr): 3445, 2923, 2841, 1600, 1503, 1374, 1283, 1147, 1025, 922, 821, 770, 696 cm^{-1}. ^{13}C NMR (100 MHz, $CDCl_3$): δ =163.8, 161.0, 161.0, 159.8, 156.2, 151.1, 147.8, 147.5, 137.6, 133.0, 130.2, 130.2, 128.5, 128.3, 128.3, 105.9, 98.7, 55.8, 55.6, 9.3. MS (EI): *m/z* 347 (100) [M + 1]. HRMS (ESI): *m/z* calcd for $C_{20}H_{17}N_3O_3$ [M + H]: 347.1270; found: 348.1352.

(39) See Supporting Information for biological activity graphs, intermediates preparation, and spectroscopic data.