

Synthesis and cytotoxic evaluation for some new 2,5-disubstituted pyrimidine derivatives for anticancer activity

Onteddu Surendranatha Reddy · Ch. Venkata Suryanarayana ·
K. J. P. Narayana · V. Anuradha · B. Hari Babu

Received: 11 June 2014 / Accepted: 28 September 2014
© Springer Science+Business Media New York 2014

Abstract An efficient synthetic approach for 2,5-disubstituted pyrimidines has been reported. The desired 2,5-substituted pyrimidines were obtained by Suzuki coupling of 2-substituted benzyloxy-5-bromopyrimidines with various aryl boronic acids in the presence catalytic amount of $\text{PdCl}_2(\text{PPh}_3)_2$ with 0.5 M aqueous Na_2CO_3 in water at 80 °C. 2-Benzyloxy-5-bromopyrimidines were synthesized, in turn by the reaction of 2-chloro-5-bromopyrimidine with substituted benzyl alcohols in the presence of Cs_2CO_3 in $\text{CH}_3\text{CN}:\text{DMF}$ (1:1). Some of the 2,5-disubstituted pyrimidines have shown moderate in vitro cytotoxic activity against HeLa cell line.

Keywords 2-Chloro-5-bromopyrimidine · Cesium carbonate · Green synthesis · HeLa cell line · $\text{PdCl}_2(\text{PPh}_3)_2$

Electronic supplementary material The online version of this article (doi:10.1007/s00044-014-1276-6) contains supplementary material, which is available to authorized users.

O. S. Reddy · B. H. Babu (✉)
Department of Chemistry, Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur, Andhra Pradesh, India
e-mail: dr.b.haribabu@gmail.com

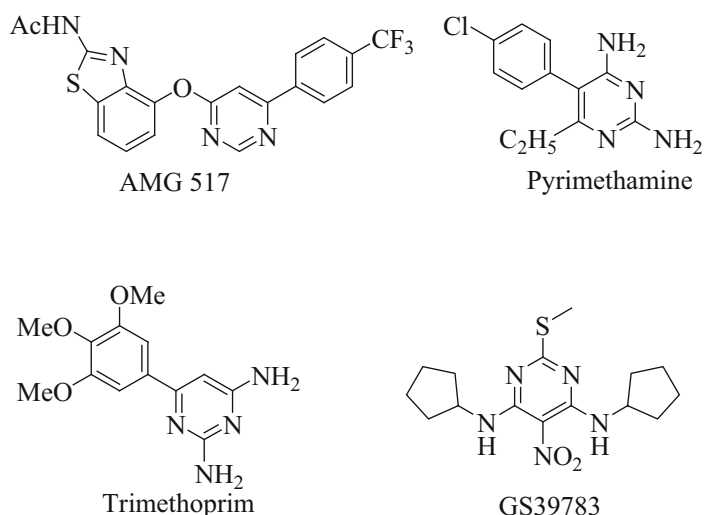
Ch. V. Suryanarayana · V. Anuradha
Department of Chemistry, Vignan School of P.G. Studies,
Guntur, Andhra Pradesh, India

K. J. P. Narayana
Department of Microbiology, Acharya Nagarjuna University,
Nagarjuna Nagar, Guntur, Andhra Pradesh, India

Introduction

Nitrogen-containing heterocycles are widely found in nature and are integral part of several biologically active compounds (García-Valverde and Torroba, 2005) (Fig. 1). Many biologically active compounds including nucleic acids, nucleotides, and corresponding nucleosides have pyrimidine as a core unit (Lagoja, 2005). It was reported that pyrimidines and their derivatives exhibited significant in vitro activity against DNA and RNA (Kappe, 1993). In addition, pyrimidine derivatives were found to possess inhibition properties against polio herpes viruses and as diuretics, antitumor agents, anti HIV agents, and for cardiovascular diseases (Kappe, 1993). Further, pyrimidines substituted with nitro group acted as novel allosteric enhancer of γ -amino butyric acid receptor function (Urwiler *et al.*, 2003). Moreover, heterocyclic compounds containing a CF_3 group exhibit wide range of biological activities (Berber *et al.*, 2002; Jain *et al.*, 2006). Boyd *et al.* (2001) utilized 2-methoxy bromopyrimidine for the syntheses of 5-substituted pyrimidones as inhibitors for lipoprotein-associated phospholipase A. Recently, Xie *et al.* (2011) reported that 2,4,5-trisubstituted pyrimidines as a new class of tubulin polymerization inhibitors and 5-substituted-6-chloro uracils have been reported (Nencka *et al.*, 2007) as efficient inhibitors of human thymidine phosphorylase which plays an important role in angiogenesis. Conventional syntheses of pyrimidines are well documented in the literature, those methods involve double condensation with elimination of water, alcohol, or hydrogen halide between amino and carboxylic acid, acid chloride or condensation of amino to CN groups or to polarized double bonds without elimination (Lagoja, 2005; Herrera *et al.*, 2002). In view of aryl-substituted pyrimidines having great potential for anticancer activity, we have designed

Fig. 1 Representative examples of pyrimidine containing biologically active compounds



2,5-disubstituted pyrimidines that are not reported so far. The palladium-catalyzed Suzuki coupling (Miyaura and Suzuki, 1995) is an important and versatile method for carbon–carbon bond formation. It has been extensively explored for synthesis of unsymmetrical biaryls, as well as aryl pyrimidines (Schomaker and Delia, 2001; Leadbeater and Marco, 2002; Liu *et al.*, 2005; Li *et al.*, 2007; Bardhan *et al.*, 2009). Herein, we report palladium-catalyzed Suzuki coupling for the synthesis of some new 2,5-disubstituted pyrimidines from 2-benzyloxy-5-bromopyrimidines, arylboronic acids, and water, utilizing the approaches described earlier (Saygili *et al.*, 2004; Parry *et al.*, 2002; Isley *et al.*, 2013; Lipshutz and Abela, 2008).

Results and discussion

The present synthesis begins with commercially available 2-chloro-5-bromopyrimidine **1** as a starting material. We selected **1** as starting material on the basis of the following reasons: 1. The chloro group of compound **1** can easily be displaced with appropriately substituted benzyl alcohols in the presence of a base. This is possible because of the electronegative nitrogen atoms induced polarization in the sigma bond frame work of pyrimidine ring (Joule and Mills, 2010; Brown, 1962). The enhanced electron deficiency at the 2, 4, and 6 positions makes these positions more susceptible for the nucleophilic attack. This nucleophilic attack is especially feasible when the substituent is a chloro or bromo (Joule and Mills, 2010; Brown, 1962). 2. The bromopyrimidines (**3**) could serve as suitable candidates for palladium-catalyzed Suzuki coupling.

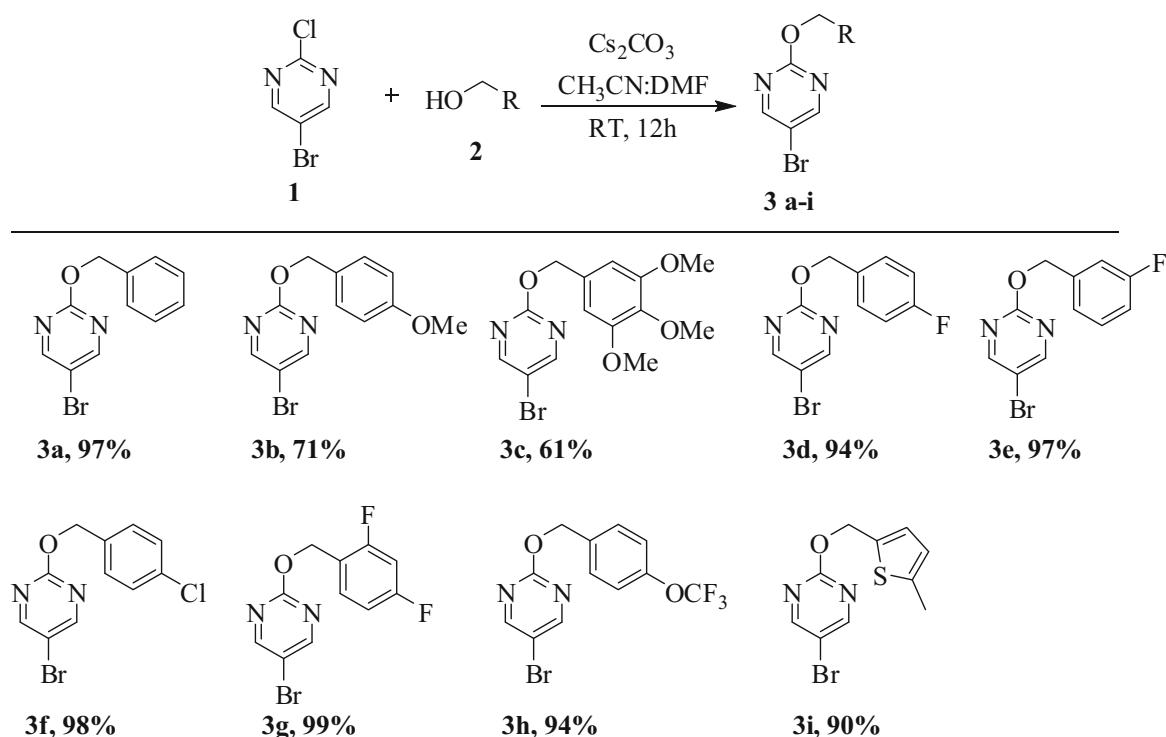
As shown in Scheme 1, treatment of 2-chloro-5-bromopyrimidine (**1**) with 2,4-difluoro benzyl alcohol (**2a**) in the presence of cesium carbonate in CH₃CN and DMF at room temperature for 12 h afforded the desired

2-(benzyloxy)-5-bromopyrimidine (**3a**) in quantitative yield. Similarly, compound **1** was reacted with differently substituted benzyl alcohols (**2b–i**; e.g., OMe, F, Cl, OCF₃) and thiophenylmethanol to give the corresponding 2-benzyloxy-5-bromopyrimidines (i.e., **3b–i**) in good yields. The new compounds (i.e., **3a–i**) thus obtained were characterized well by ¹H NMR, ¹³C NMR, and mass spectral data (See experimental section).

With these intermediates (i.e., **3a–i**) in hand, our next aim was to use these bromopyrimidines for the syntheses of 2,5-disubstituted pyrimidines through Suzuki coupling. As such, we have planned to develop a general synthetic route for synthesis of various 2,5-disubstituted pyrimidines using Suzuki coupling in water as a key step as shown in Scheme 2.

The optimization of suitable coupling conditions for the formation of carbon–carbon bond between 2-(substituted benzyloxy)-5-bromopyrimidine (**3a**) and 3-(methylsulfonyl) phenylboronic acid by evaluation of various Pd catalysts, solvents, and bases was undertaken (See Table 1). Then, coupling of compound **3a** with PdCl₂(PPh₃)₂ with tetrahydrofuran as solvent at 80 °C afforded the coupling product **4a** with only 50 % yield. Performance of this reaction at >80 °C caused decomposition of the methanesulfonyl group in the target molecule. On the other hand, when the solvent was replaced with water under the same conditions produced the desired product **4a** with 67 % yield and >90 % purity. Therefore, replacement of organic solvent with water allowed us to improve the yields of Suzuki coupling. Similar conditions have been adopted for the synthesis of various 2,5-disubstituted pyrimidines (i.e., **4b–i**) as shown in Scheme 2.

In order to establish the generality of reaction conditions developed with other boronic acids of interest, coupling of bromopyrimidines (i.e., **3a–i**) using similar conditions with the appropriately substituted phenylboronic acids containing OMe, CF₃, and NO₂ groups on aromatic system was carried



Scheme 1 Synthesis of 2-benzyloxy-5-bromopyrimidines (**3**)

out and the reactions yielded the desired titled compounds in good yields (Scheme 3). On the other hand, the substituted 2-(benzyloxy) group in 2,5-disubstituted pyrimidines (i.e., **4a–i**, **5a–g**, **i**, **6b**, **d–g**, and **7b**, **f–h**) was found to be cleaved under standard hydrogenolysis (Morgentin *et al.*, 2009) conditions to yield 2-hydroxy-5-substituted pyrimidines. These hydroxy intermediates are useful scaffolds for the synthesis of diverse functionalized pyrimidines.

Conclusions

In conclusion, we have found that 2-(benzyloxy)-5-bromopyrimidines as suitable coupling partners for the green aqueous Suzuki coupling. The desired 2-(benzyloxy)-5-bromopyrimidines were prepared by the treatment of 2-chloro-5-bromopyrimidine with substituted benzyl alcohols in the presence of CS_2CO_3 in $\text{CH}_3\text{CN}:\text{DMF}$. As such, we have developed an efficient methodology for the synthesis of various 2,5-disubstituted pyrimidines by coupling of 2-benzyloxy-5-bromopyrimidines with arylboronic acids in the presence 10 mol% of $\text{PdCl}_2(\text{PPh}_3)_2$ in water at 80 °C. In addition, this catalytic system tolerated broad range of functional groups under mild reaction conditions. The synthetic methodology developed is general and total of 26 new 2,5-substituted pyrimidines could be prepared in high yields and under mild reaction conditions.

Cytotoxic evaluation of 2,5-disubstituted pyrimidines compounds (**4a–7h**)

The compounds (**4a–7h**) were tested on Human cervical cancer cell line (HeLa) using MTT cell proliferation assay. The compounds were screened for anticancer activity at 100 $\mu\text{g}/\text{mL}$ and compounds which showed more than 50 % cell growth inhibition were selected for dose response study using different concentrations (0–100 $\mu\text{g}/\text{mL}$). IC_{50} values were calculated and are presented in Table 2. The compound **4i** showed more potent anticancer activity among the compounds **4a–7h** and followed by **5g**, **5i**, **5e**, and **5b**.

Experimental

General

All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen unless as indicated otherwise. Ethyl acetate and hexanes from Malinckrodt Chemical Co. were dried and distilled from CaH_2 . Tetrahydrofuran from Chemlabs Chemicals were dried by distillation from sodium and benzophenone under an atmosphere of nitrogen. Acetonitrile was purchased from Qualigens Chemical Co, and dimethylformamide was purchased from Merck.

Thin layer chromatography (TLC) was performed on percolated plates (silica gel 60 F₂₅₄), which were purchased from Merck Inc. Purification by gravity column chromatography was carried out by use of Silicycle ultra-pure silica gel (particle size 40–63 µm, 100–200 mesh). Purity of products was checked by High-resolution mass spectra (HRMS) obtained by means of Q-TOF micro mass spectrometer and HPLC (Waters 2695). Proton NMR spectra were obtained on a MR (400 MHz) and Vnmrs (300 MHz) spectrometer by use of dimethylsulfoxide-*d*₆ (DMSO) as solvent and TMS as internal standard. Proton NMR chemical shifts were referenced to residual protonated solvents (δ 2.5 ppm for dimethylsulfoxide), and carbon-13 NMR spectra were obtained on a MR (100 MHz) and Vnmrs (75 MHz) spectrometer by use of dimethylsulfoxide as the solvent and TMS as internal standard. Carbon-13 chemical shifts are referenced to the center of the DMSO septet (δ 39.5 ppm). Multiplicities are recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs broad singlet; bd, broad doublet; and *J*, coupling constant (hertz). Melting points were obtained with a Buchi MP-B540 melting point apparatus.

General procedure for the synthesis of substituted benzyloxy halo pyrimidines (3a–3i, Scheme 1)

2-(Benzyloxy)-5-bromopyrimidine (3a)

In portion wise, Cesium carbonate (6.78 g, 20.6 mmol) was added to a stirred solution of benzyl alcohol (2.25 g, 20.6 mmol) in acetonitrile and dimethylformamide (1:1, 40 mL) under nitrogen atmosphere at room temperature. After 10 min, 5-bromo-2-chloropyrimidine (2.0 g, 10.3 mmol) was added and the mixture was stirred at the same temperature for overnight. The reaction was monitored by TLC; after completion of the reaction, reaction mixture was poured into ice-cold water; the resultant solid was filtered; solid was washed with water (3 × 10 mL) followed by *n*-pentane (2 × 10 mL) and air dried and recrystallized from benzene gave **3a** (2.675 g) as white solid in 97 % yield, mp 102.9–105.6 °C, TLC *R*_f 0.34 (10 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz); δ 8.78 (s, 2H, Pyrimidine H), 7.34–7.47 (m, 5H, ArH), 5.38 (s, 2H, ArCH₂); ¹³C NMR (DMSO, 100 MHz) δ 163.16, 159.83, 136.15, 128.39, 128.02, 127.95, 111.91, 68.95; IR (KBr) 1271 (C–O), 1179 (C–N), 525 (C–Br) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₁H₉BrN₂O) requires *m/z* 264.989, found *m/z* 265.091, 267.301.

5-Bromo-2-(4-methoxybenzyloxy) pyrimidine (3b)

71 % yield as brown solid, mp (recrystallized from benzene) 119.5–124.3 °C, TLC *R*_f 0.27 (10 % EtOAc in

hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.75 (s, 2H, pyrimidine H), 7.39 (d, *J* = 8.4 Hz, 2H, ArH), 6.94 (d, *J* = 6.3 Hz, 2H, ArH), 5.29 (s, 2H, ArCH₂), 3.75 (s, 3H, OMe); ¹³C NMR (DMSO, 100 MHz) δ 163.19, 159.75, 159.15, 129.94, 127.97, 113.74, 111.75, 68.83, 55.05; IR (KBr) 1242 (C–O), 1210 (C–N), 530 (C–Br) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₂H₁₁BrN₂O₂) requires *m/z* 295.000, found *m/z* 295.101, 297.100.

5-Bromo-2-(2, 3, 4-trimethoxybenzyloxy) pyrimidine (3c)

61 % yield as brown solid, mp (recrystallized from benzene) 105.4–107 °C, TLC *R*_f 0.18 (10 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.77 (s, 2H, pyrimidine H), 7.13 (d, *J* = 8.45 Hz, 1H, ArH), 6.81 (d, *J* = 8.4 Hz, 1H, ArH), 5.28 (s, 2H, ArCH₂), 3.76–3.80 (d, *J* = 12.9 Hz, 9H, OMe). ¹³C NMR (DMSO, 75 MHz) δ 163.23, 159.89, 153.94, 151.98, 141.71, 124.88, 121.62, 111.83, 107.63, 64.88, 61.16, 60.37, 55.87; IR (KBr) 1276 (C–O), 1263 (C–N), 528 (C–Br) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₄H₁₅BrN₂O₄) requires *m/z* 355.021, found *m/z* 355.015, 357.030.

5-Bromo-2-(4-fluorobenzyloxy) pyrimidine (3d)

94 % yield as white solid, mp (recrystallized from benzene) 103.2–106.1 °C; TLC *R*_f 0.38 (10 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.78 (s, 2H, pyrimidine H), 7.51 (m, 2H, ArH), 7.22 (m, 2H, ArH), 5.36 (s, 2H, ArCH₂). ¹³C NMR (DMSO, 100 MHz) δ 163.08, 160.65, 159.84, 132.42, 130.38, 115.32, 111.95, 68.26; IR (KBr) 1270 (C–O), 1270 (C–N), 1338 (C–F), 533 (C–Br) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₁H₈BrFN₂O) requires *m/z* 282.980, found *m/z* 283.100, 285.130.

5-Bromo-2-(3-fluorobenzyloxy) pyrimidine (3e)

97 % yield as white solid, mp (recrystallized from benzene) 92–94 °C, TLC *R*_f 0.38 (10 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.79 (s, 2 H, pyrimidine H), 7.44 (m, 1 H, ArH), 7.28 (m, 2 H, ArH), 7.17 (td, *J* = 2.4 Hz, 1 H, ArH), 5.40 (s, 2 H, ArCH₂); ¹³C NMR (DMSO, 100 MHz) δ 163.02, 162.02, 159.89, 139.08, 130.46, 123.74, 114.65, 112.09, 68.08; IR (KBr) 1282 (C–O), 1240 (C–N), 1338 (C–F), 525 (C–Br) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₁H₈BrFN₂O) requires *m/z* 282.980, found *m/z* 283.050, 285.023.

5-Bromo-2-(4-Chlorobenzyloxy) pyrimidine (**3f**)

98 % yield as white solid, mp (recrystallized from benzene) 115.7–116.9 °C, TLC R_f 0.37 (10 % EtOAc in hexanes as the eluent); ^1H NMR (DMSO, 300 MHz) δ 8.78 (s, 2H, pyrimidine H), 7.46 (m, 4H, ArH), 5.37 (s, 2H, ArCH₂); ^{13}C NMR (DMSO, 100 MHz) δ 163.02, 159.83, 135.24, 132.61, 129.74, 128.37, 112.02, 68.09; IR (KBr) 1247 (C–O), 1207 (C–N), 790 (C–Cl), 525 (C–Br) cm^{-1} ; HRMS (ES^+) exact mass calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{11}\text{H}_8\text{BrClN}_2\text{O}$) requires m/z 298.950, found m/z 299.120, 301.200.

5-Bromo-2-(2,4-difluorobenzyloxy) pyrimidine (**3g**)

99 % yield as pale white solid, mp (recrystallized from benzene) 85.3–87.6 °C, TLC R_f 0.36 (10 % EtOAc in hexanes as the eluent); ^1H NMR (DMSO, 300 MHz) δ 8.79 (s, 2H, pyrimidine H), 7.62 (q, $J = 9$ Hz, 1H, ArH), 7.31 (td, $J = 8.1$ Hz, 1H, ArH), 7.13 (td, $J = 2.1$ Hz, 1H, ArH), 5.39 (s, 2H, ArCH₂); ^{13}C NMR (DMSO, 100 MHz) δ 163.70, 162.89, 160.40, 159.91, 132.37, 119.59, 112.15, 111.64, 104.04, 62.70; IR (KBr) 1279 (C–O), 1268 (C–N), 1324 (C–F), 520 (C–Br) cm^{-1} ; HRMS (ES^+) exact mass calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{11}\text{H}_8\text{BrF}_2\text{N}_2\text{O}$) requires m/z 300.97, found m/z 301.09, 303.20.

5-Bromo-2-(4(trifluoromethoxy) benzyloxy) pyrimidine (**3h**)

94 %, yield as white solid, mp (recrystallized from benzene) 91.7–93.6 °C, TLC R_f 0.27 (10 % EtOAc in hexanes as the eluent); ^1H NMR (DMSO, 300 MHz) δ 8.79 (s, 2H, pyrimidine H), 7.59 (d, $J = 8.4$ Hz, 2H, ArH), 7.39 (d, $J = 8.4$ Hz, 2H, ArH), 5.41 (s, 2H, ArCH₂); ^{13}C NMR (DMSO, 100 MHz) δ 163.04, 159.89, 147.96, 135.72, 129.83, 120.99, 112.07, 68.00; IR (KBr) 1271 (C–O), 1216 (C–N), 1340 (C–F), 534 (C–Br) cm^{-1} ; HRMS (ES^+) exact mass calculated for $[\text{M}+\text{H}]^+$ ($\text{C}_{12}\text{H}_8\text{BrF}_3\text{N}_2\text{O}$) requires m/z 348.972, found m/z 349.102, 351.081.

5-Bromo-2-((5-methylthiophen-2-yl) methoxy) pyrimidine (**3i**)

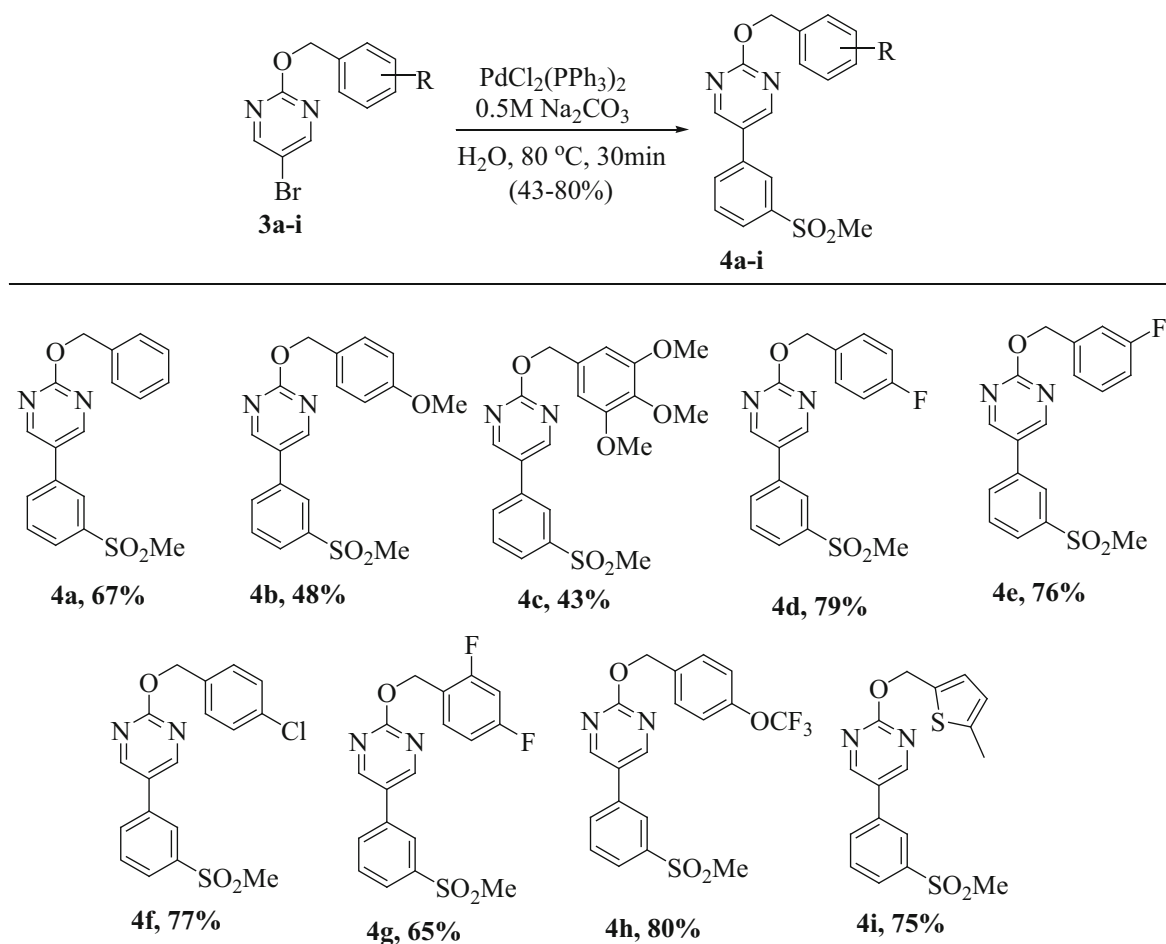
90 % yield as brown solid, mp (recrystallized from benzene) 86–86.8 °C, TLC R_f 0.4 (10 % EtOAc in hexanes as the eluent); ^1H NMR (DMSO, 400 MHz) δ 8.79 (s, 2H, pyrimidine H), 7.03 (d, $J = 3.6$ Hz, 1H, thiophene H), 6.70 (d, $J = 2.8$ Hz, 1H, thiophene H), 6.46 (s, 2H, thiophene CH₂), 2.41 (s, 3H, Me); ^{13}C NMR (DMSO, 100 MHz) δ 162.81, 159.81, 140.99, 135.33, 128.84, 124.91, 111.96, 63.66, 14.94; IR (KBr) 1282 (C–O), 1210 (C–N), 609 (C–S), 516 (C–Br) cm^{-1} ; HRMS (ES^+) exact mass calculated for $[\text{M} + \text{H}]^+$ ($\text{C}_{10}\text{H}_9\text{BrN}_2\text{OS}$) requires m/z 284.961, found m/z 285.130, 287.090.

General procedure for the preparation (benzyloxy)-5-(3-(methylsulfonyl) phenyl) pyrimidine (**4a**)

To a oven dried 25 mL round bottom flask were added 2-(benzyloxy)-5-bromopyrimidine (0.15 g, 0.568 mmol), 3-(methylsulfonyl) phenylboronic acid (0.125 g, 0.625 mmol), and 0.5 N aqueous sodium carbonate (0.240 g, 2.27 mmol in 4.52 mL water) followed by 5 mL water and were degassed by bubbling with nitrogen gas for 15 min. $\text{PdCl}_2(\text{PPh}_3)_2$ (0.039 g, 0.0056 mmol) was added to the above reaction mixture and then heated to 80 °C for 30 min. The reaction mixture was cooled to room temperature, the resultant solid was filtered and solid was washed with water and air dried. The crude product was recrystallized from dichloromethane in petroleum ether to give **4a** (131 mg) in 67 % yield as off-white solid, mp (recrystallized from dichloromethane in petroleum ether) 180.1–184.4 °C; TLC R_f 0.25 (40 % EtOAc in hexanes as the eluent); ^1H NMR (DMSO, 300 MHz) δ 9.06 (s, 2H, pyrimidine H), 8.26 (s, 1H, ArH), 8.10 (d, $J = 7.8$ Hz, 1H, ArH), 7.95 (d, $J = 7.8$ Hz, 1H, ArH), 7.78 (t, $J = 7.5$ Hz, 1H, ArH), 7.49 (d, $J = 6.9$ Hz, 2H, ArH), 7.44–7.34 (m, 3 H, ArH), 5.48 (s, 2H, ArCH₂), 3.30 (s, 3H, SO₂Me); ^{13}C

Table 1 Exploration of various Pd catalysts, solvents, and bases for the Suzuki coupling

Pd catalyst	Ligand	Bases	Solvent	Cosolvent	Temp (°C)	Time(h)	Yield (%)
$\text{Pd}(\text{PPh}_3)_4$	–	K_2CO_3	Benzene	–	70	12	8
$\text{Pd}(\text{dppf})\text{Cl}_2$	–	Na_2CO_3	DME	H_2O	80	6	22
$\text{Pd}(\text{OAc})_2$	TBAB	K_2CO_3	PEG-400	–	110	8	0
$\text{Pd}(\text{PPh}_3)_4$	–	Na_2CO_3	DME	H_2O	80	–	30
$\text{Pd}_2(\text{dba})_3$	PPh_3	Cs_2CO_3	Dioxane	–	80	5	15
$\text{Pd}_2(\text{dba})_3$	$\text{P}(t\text{-Bu})_3$	Cs_2CO_3	Dioxane	–	80	5	20
$\text{PdCl}_2(\text{dppf})$	–	K_3PO_4	DMF	–	60	–	5
$\text{PdCl}_2(\text{PPh}_3)_2$	–	Na_2CO_3	H_2O	–	80	0.5	67
$\text{PdCl}_2(\text{PPh}_3)_2$	–	Na_2CO_3	THF	H_2O	80	2	50



Scheme 2 Green aqueous Suzuki coupling of 2-(substituted benzyloxy)-5-bromopyrimidines with 3-(methylsulfonyl) phenylboronic acid

NMR (DMSO, 100 MHz) δ 164.32, 157.85, 141.84, 136.49, 135.20, 131.40, 130.19, 128.41, 127.95, 126.20, 126.13, 124.72, 68.62, 43.31; IR (KBr) 1149 (C–O), 1298 (SO₂), 1186 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₆N₂O₃S) requires m/z 341.088, found m/z 341.080.

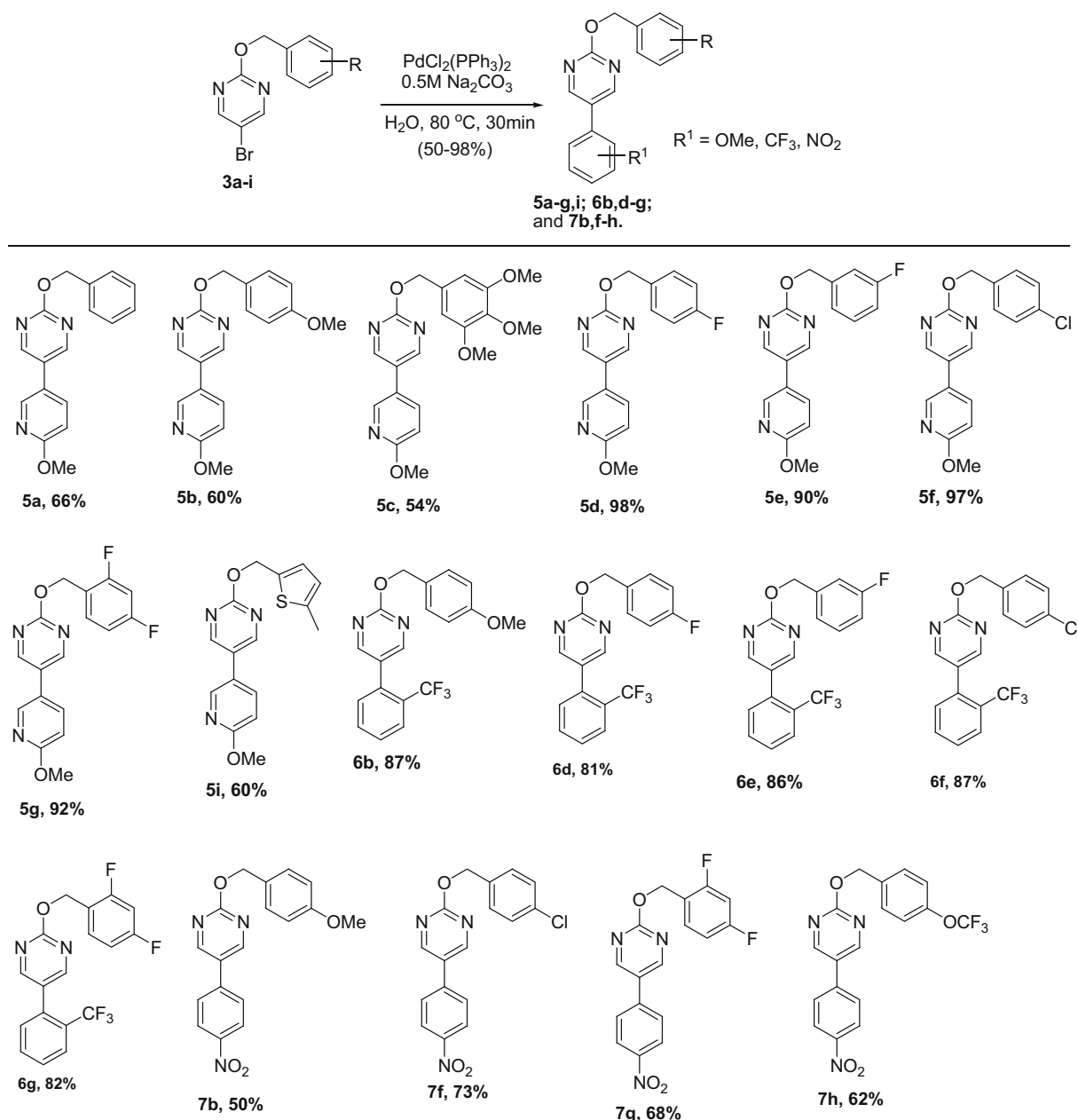
2-(4-Methoxybenzyloxy)-5-(3-(methylsulfonyl) phenyl) pyrimidine (**4b**)

48 % yield as pale yellow solids, mp (recrystallized from dichloromethane in petroleum ether) 155.5–157.0 °C; TLC R_f 0.25 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 9.05 (s, 2 H, pyrimidine H), 8.25 (s, 1H, ArH), 8.10 (d, J = 7.8 Hz, 1H, ArH), 7.95 (d, J = 8.1 Hz, 1H, ArH), 7.79 (t, J = 7.8 Hz, 1H, ArH), 7.43 (d, J = 8.7 Hz, 2H, ArH), 6.96 (d, J = 8.7 Hz, 2H, ArH), 5.39 (s, 2H, ArCH₂), 3.76 (s, 3H, OMe), 3.30 (s, 3H, SO₂Me); ¹³C NMR (DMSO, 100 MHz) δ 164.36, 159.13, 157.78, 141.04, 135.23, 131.37, 130.18, 129.94, 128.31,

126.069, 124.69, 113.78, 68.47, 55.08, 43.31; IR (KBr) 1250 (C–O), 1294 (SO₂), 1176 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₉H₁₈N₂O₄S) requires m/z 371.098, found m/z 371.067.

5-(3-(Methylsulfonyl) phenyl)-2-(3,4,5-trimethoxybenzyloxy) pyrimidine (**4c**)

43 % yield as pale yellow solids, mp (recrystallized from dichloromethane in petroleum ether) 97.9–102.1 °C; TLC R_f 0.14 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 9.02 (s, 2H, pyrimidine H), 8.24 (s, 1H, ArH), 8.06 (bd, J = 6.4 Hz, 1H, ArH), 7.93 (bd, J = 6.8 Hz, 1H, ArH), 7.75 (bd, J = 6.4 Hz, 1H, ArH), 7.14 (d, J = 8 Hz, 1H, ArH), 6.79 (d, J = 8 Hz, 1H, ArH), 5.34 (s, 2 H, ArCH₂), 3.81–3.74 (t, J = 14.8 Hz, 9H, OMe), 3.28 (s, 3H, SO₂Me); ¹³C NMR (DMSO, 100 MHz) δ 164.36, 157.80, 153.83, 151.94, 141.84, 135.27, 131.37, 130.18, 126.11, 124.74, 121.91, 107.64, 64.45, 61.11, 60.32, 55.84, 43.32; IR (KBr) 1201 (C–O), 1289 (SO₂),



Scheme 3 Syntheses of diversely substituted 2,5-disubstituted pyrimidines use of green aqueous Suzuki coupling

Table 2 Selected compounds studied for IC₅₀ by using different concentrations (0–100 µg/mL)

Compounds	4d	4i	5b	5e
IC ₅₀ (µg/mL)	96.1	82.7	89.2	86.2
Compounds	5g	5i	6b	–
IC ₅₀ (µg/mL)	83.1	84.3	95.9	–

1184 (C–N) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₂₁H₂₂N₂O₆S) requires *m/z* 431.119, found *m/z* 431.089.

2-(4-Fluorobenzyloxy)-5-(3-(methanesulfonyl) phenyl) pyrimidine (**4d**)

79 % yield as off white solids, mp (recrystallized from dichloromethane in petroleum ether) 174.3–179.2 °C; TLC *R_f* 0.27 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 9.06 (s, 2H, pyrimidine H), 8.25 (s, 1H, ArH), 8.10 (d, *J* = 7.6 Hz, 1H, ArH), 7.95 (d, *J* = 8.0 Hz, 1H, ArH), 7.78 (t, *J* = 8.0 Hz, 1H, ArH), 7.56 (q, *J* = 5.2 Hz, 2H, ArH), 7.23 (t, *J* = 8.8 Hz, 2H, ArH), 5.45 (s, 2H, ArCH₂), 3.30 (s, 3H, SO₂Me); ¹³C NMR

(DMSO, 75 MHz) δ 164.22, 163.46, 157.85, 141.84, 135.18, 132.71, 131.41, 130.27, 126.24, 126.15, 124.72, 115.37, 67.93, 43.31; IR (KBr) 1218 (C–O), 1295 (C–F), 1309 (SO₂), 1186 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₅FN₂O₃S) requires m/z 359.078, found m/z 359.090.

2-(3-Fluorobenzyloxy)-5-(3-(methylsulfonyl) phenyl) pyrimidine (**4e**)

76 % yield as brown solids, mp (recrystallized from dichloromethane in petroleum ether) 132.4–135.2 °C; TLC R_f 0.4 (50 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 9.09 (s, 2H, pyrimidine H), 8.33 (d, J = 8.7 Hz, 2H, ArH), 8.06 (d, J = 8.7 Hz, 2H, ArH), 7.43 (d, J = 8.7 Hz, 2H, ArH), 6.96 (d, J = 8.4 Hz, 2H, ArH), 5.40 (s, 2H, ArCH₂), 3.76 (s, 3H, SO₂Me); ¹³C NMR (DMSO, 100 MHz) δ 164.16, 163.32, 157.89, 141.84, 139.46, 135.15, 131.42, 130.507, 130.19, 126.35, 124.76, 123.76, 123.73, 114.59, 67.76, 43.31; IR (KBr) 1254 (C–O), 1339 (C–F), 1291 (SO₂), 1183 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₅FN₂O₃S) requires m/z 359.078, found m/z 358.898.

2-(4-Chlorobenzyloxy)-5-(3-(methylsulfonyl) phenyl) pyrimidine (**4f**)

77 % yield as off white solids, mp (recrystallized from dichloromethane in petroleum ether) 159.9–162.2 °C; TLC R_f 0.26 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 9.06 (s, 2 H, pyrimidine H), 8.25 (t, J = 1.6 Hz, 1H, ArH), 8.10 (d, J = 8.4 Hz, 1H, ArH), 7.95 (d, J = 8.4 Hz, 1H, ArH), 7.78 (t, J = 7.6 Hz, 1H, ArH), 7.52 (d, J = 8.4 Hz, 2H, ArH), 7.47 (dd, J = 2.0 Hz, 2H, ArH), 5.47 (s, 2H, ArCH₂), 3.30 (s, 3H, SO₂Me); ¹³C NMR (DMSO, 75 MHz) δ 164.18, 157.86, 141.84, 135.58, 135.15, 132.54, 131.41, 130.19, 129.78, 128.406, 126.30, 126.16, 124.74, 67.78, 43.31; IR (KBr) 1146 (C–O), 1299 (SO₂), 1181 (C–N), 800 (C–Cl) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₅ClN₂O₃S) requires m/z 375.049, found m/z 374.975.

2-(2,4-Difluorobenzyloxy)-5-(3-(methylsulfonyl) phenyl) pyrimidine (**4g**)

65 % yield as pale yellow solid, mp (recrystallized from dichloromethane in petroleum ether) 97.9–102.1 °C; TLC R_f 0.27 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 9.07 (s, 2 H, pyrimidine H), 8.26 (s, 1H, ArH), 8.10 (d, J = 7.8 Hz, 1H, ArH), 7.95 (d, J = 8.1 Hz, 1H, ArH), 7.78 (t, J = 7.8 Hz, 1H, ArH), 7.65 (q, J = 8.4 Hz, 1H, ArH), 7.33 (td, J = 2.4 Hz, 1H, ArH),

7.15 (td, J = 1.5 Hz, 1H, ArH), 5.48 (s, 2H, ArCH₂), 3.30 (s, 3H, SO₂Me); ¹³C NMR (DMSO, 100 MHz) δ 164.03, 163.66, 161.08, 157.90, 141.84, 135.12, 132.32, 131.43, 130.19, 126.40, 126.18, 124.76, 119.89, 111.68, 104.04, 62.37, 43.31; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₄F₂N₂O₃S) requires m/z 377.069, found m/z 396.989.

5-(3-(Methylsulfonyl) phenyl)-2-(4-(trifluoromethoxy) benzyloxy) pyrimidine (**4h**)

80 % yield as brown solids, (recrystallized from dichloromethane in petroleum ether) 149.0–151.1 °C; TLC R_f 0.31 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 9.07 (s, 2H, pyrimidine H), 8.26 (s, 1H, ArH), 8.10 (d, J = 8.1 Hz, 1H, ArH), 7.95 (d, J = 8.1 Hz, 1H, ArH), 7.78 (t, J = 7.8 Hz, 1H, ArH), 7.63 (d, J = 8.7 Hz, 2H, ArH), 7.40 (d, J = 8.4 Hz, 2H, ArH), 5.51 (s, 2H, ArCH₂), 3.31 (s, 3H, SO₂Me); ¹³C NMR (DMSO, 100 MHz) δ 164.17, 157.88, 147.92, 141.84, 136.05, 135.14, 131.42, 130.19, 129.81, 126.34, 126.17, 124.74, 121.02, 118.76, 67.68, 43.31; IR (KBr) 1217 (C–O), 1342 (C–F), 1291 (SO₂), 1217 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₉H₁₅F₃N₂O₄S) requires m/z 425.070, found m/z 425.063.

2-((5-methylthiophen-2-yl) methoxy)-5-(3-(methylsulfonyl) phenyl) pyrimidine (**4i**)

75 % yield as pale yellow solids, mp (recrystallized from dichloromethane in petroleum ether) 72.0–76.0 °C; TLC R_f 0.4 (40 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 9.07 (s, 2H, pyrimidine H), 8.26 (s, 1H, ArH), 8.11 (d, J = 2.81 Hz, 1H, ArH), 7.95 (d, J = 8.1 Hz, 1H, ArH), 7.78 (t, J = 7.8 Hz, 1H, ArH), 7.06 (bd, J = 3.6 Hz, 1H, thiophene H), 6.72 (bd, J = 2.4 Hz, 1H, thiophene H), 5.55 s, 2H, ArCH₂), 3.31 (s, 3H, OMe), 2.42 (s, 3H, Me); ¹³C NMR (DMSO, 100 MHz) δ 163.97, 157.80, 141.84, 140.89, 135.71, 135.16, 131.40, 130.19, 128.73, 126.15, 124.90, 63.35, 43.31, 14.96; IR (KBr) 1294 (C–O), 694 (C–S), 1149 (SO₂), 1211 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₇H₁₆N₂O₃S₂) requires m/z 361.060, found m/z 361.083.

2-(Benzyloxy)-5-(6-methoxypyridin-3-yl) pyrimidine (**5a**)

66 % yield as brown solids, mp (recrystallized from dichloromethane in petroleum ether) 140–142 °C; TLC R_f 0.28 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.94 (s, 2H, pyrimidine H), 8.54 (d, J = 2.4 Hz, 1H, PyH), 8.08 (dd, J = 2.4 Hz, 1H, PyH), 7.48 (d, J = 7.2 Hz, 2H, ArH), 7.42–7.32 (m, 3H, ArH),

6.94 (d, $J = 8.8$ Hz, 1H, PyH), 5.45 (s, 2H, ArCH₂), 3.90 (s, 3H, OMe); ¹³C NMR (DMSO, 100 MHz) δ 163.88, 163.40, 156.99, 144.53, 137.21, 136.60, 128.40, 127.86, 124.84, 123.22, 110.77, 68.44, 53.29; IR (KBr) 1291 (C–O), 1251 (C–N) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₇H₁₅N₃O₂) requires m/z 294.116, found m/z 294.069.

2-(4-Methoxybenzyloxy)-5-(6-methoxypyridin-3-yl)pyrimidine (**5b**)

60 % yield as off white solids, mp (recrystallized from dichloromethane in petroleum ether) 140.1–141.3 °C; TLC R_f 0.4 (30 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.94 (s, 2H, pyrimidine H), 8.54 (d, $J = 2.4$ Hz, 1H, PyH), 8.08 (dd, $J = 2.4$ Hz, 1H, PyH), 7.42 (d, $J = 8.8$ Hz, 2H, ArH), 6.96–6.93 (m, 3H, ArH&PyH), 5.36 (s, 2H, ArCH₂), 3.90 (s, 3H, OMe), 3.76 (s, 3H, OMe); ¹³C NMR (DMSO, 100 MHz) δ 163.92, 163.38, 159.097, 156.95, 144.51, 137.21, 129.85, 128.43, 124.71, 123.26, 113.77, 110.78, 68.30, 55.06, 53.28; IR (KBr) 1293 (C–O), 1245 (C–N) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₇N₃O₃) requires m/z 324.126, found m/z 324.095.

5-(6-Methoxypyridin-3-yl)-2-(3,4,5-trimethoxybenzyloxy) pyrimidine (**5c**)

54.87 % yield as yellow solids, mp (recrystallized from dichloromethane in petroleum ether) 139.3–144.4 °C; TLC R_f 0.35 (30 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.94 (s, 2H, pyrimidine H), 8.54 (d, $J = 2.4$ Hz, 1H, PyH), 8.08 (dd, $J = 2.4$ Hz, 1H, PyH), 7.16 (d, $J = 8.8$ Hz, $J = 1$ Hz, ArH), 6.95 (d, $J = 8.8$ Hz, 1H, ArH), 6.82 (d, $J = 8.0$ Hz, 1H, PyH), 5.34 (s, 2H, ArCH₂), 3.89 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.77 (s, 3H, OMe); ¹³C NMR (DMSO, 75 MHz) δ 163.93, 163.38, 156.97, 153.79, 151.90, 144.53, 141.74, 137.22, 124.65, 123.30, 122.02, 110.77, 109.53, 107.65, 64.28, 61.09, 60.31, 55.84, 53.29; IR (KBr) 1282 (C–O), 1261 (C–N) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₂₀H₂₁N₃O₅) requires m/z 384.148, found m/z 384.115.

2-(4-Fluorobenzyloxy)-5-(6-methoxypyridin-3-yl)pyrimidine (**5d**)

98 % yield as Pale yellow solids, mp (recrystallized from dichloromethane in petroleum ether) 159.6–163.3 °C; TLC R_f 0.44 (30 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.95 (s, 2H, pyrimidine H), 8.54 (s, 1H, PyH), 8.08 (dd, $J = 2.4$ Hz, 1H, PyH), 7.53 (t, $J = 7.6$ Hz, 2H, ArH), 7.23 (t, $J = 8.8$ Hz, 2H, ArH), 6.95

(d, $J = 9.2$ Hz, 1H, PyH), 5.42 (s, 2H, ArCH₂), 3.90 (s, 3H, OMe); ¹³C NMR (DMSO, 100 MHz) δ 163.79, 163.41, 163.03, 157.00, 144.554, 137.23, 132.83, 130.27, 124.89, 123.21, 115.32, 110.78, 67.75, 53.30; IR (KBr) 1222 (C–O), 1316 (C–F), 1247 (C–N) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₇H₁₄FN₃O₂) requires m/z 312.107, found m/z 312.069.

2-(3-Fluorobenzyloxy)-5-(6-methoxypyridin-3-yl)pyrimidine (**5e**)

90 % yield as pale yellow solid, mp (recrystallized from dichloromethane in petroleum ether) 152–154 °C; TLC R_f 0.20 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.95 (s, 2H, pyrimidine H), 8.55 (d, $J = 2.4$ Hz, 1H, PyH), 8.09 (dd, $J = 2.4$ Hz, 1H, PyH), 7.44 (q, $J = 5.6$ Hz, 1H, ArH), 7.31 (t, $J = 8.8$ Hz, 2H, ArH), 7.17 (t, $J = 2.4$ Hz, 1H, ArH), 6.95 (d, $J = 8.4$ Hz, 1H, PyH), 5.47 (s, 2H, ArCH₂), 3.90 (s, 3H, OMe); ¹³C NMR (DMSO, 75 MHz) δ 163.53, 163.41, 156.94, 148.59, 144.54, 140.80, 137.22, 135.87, 128.57, 124.89, 123.17, 110.79, 63.21, 53.29; IR (KBr) 1295 (C–O), 1352 (C–F), 1259 (C–N) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₇H₁₄FN₃O₂) requires m/z 312.107, found m/z 312.086.

2-(4-Chlorobenzyloxy)-5-(6-methoxypyridin-3-yl)pyrimidine (**5f**)

97 % yield as pale yellow solids, mp (recrystallized from dichloromethane in petroleum ether) 155.9–158.5 °C; TLC R_f 0.45 (30 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.95 (s, 2H, pyrimidine H), 8.54 (d, $J = 2.8$ Hz, 1H, PyH), 8.09 (dd, $J = 2.4$ Hz, 1H, PyH), 7.48 (q, $J = 8.4$ Hz, 4H, ArH), 6.95 (d, $J = 9.2$ Hz, 1H, PyH), 5.44 (s, 2H, ArCH₂), 3.89 (s, 3H, OMe); ¹³C NMR (DMSO, 75 MHz) δ 163.73, 163.43, 157.04, 144.57, 137.25, 135.70, 132.28, 129.69, 128.40, 124.97, 123.17, 110.79, 67.62, 53.31; IR (KBr) 1282 (C–O), 1246 (C–N) 807 (C–Cl) cm^{−1}; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₇H₁₄ClN₃O₂) requires m/z 328.077, found m/z 327.965.

2-(2,4-Difluorobenzyloxy)-5-(6-methoxypyridin-3-yl)pyrimidine (**5g**)

92 % yield as off white solids, mp (recrystallized from dichloromethane in petroleum ether) 142.6–144.2 °C; TLC R_f 0.29 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 8.96 (s, 2H, pyrimidine H), 8.53 (d, $J = 1.6$ Hz, 1H, PyH), 8.09 (dd, $J = 2.4$ Hz, 1H, PyH), 7.64 (q, $J = 8.0$ Hz, 1H, ArH), 7.33 (td, $J = 2.4$ Hz, 1H, ArH), 7.15 (td, $J = 4.0$ Hz, 1H, ArH), 6.95 (d, $J = 8.8$ Hz,

¹H, PyH), 5.45 (s, 2H, ArCH₂), 3.90 (s, 3H OMe); ¹³C NMR (DMSO, 100 MHz) δ 163.44, 162.75, 160.301, 157.06, 144.60, 137.25, 132.23, 125.06, 123.16, 119.94, 111.56, 110.79, 110.41, 104.04, 62.22, 53.30; IR (KBr) 1277 (C–O), 1337 (C–F), 1183 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₇H₁₃F₂N₃O₂) requires *m/z* 330.097, found *m/z* 330.079.

5-(6-Methoxypyridin-3-yl)-2-((5-methylthiophen-2-yl) methoxy) pyrimidine (**5i**)

60 % yield as brown solid, mp (recrystallized from dichloromethane in petroleum ether) 109–111.5 °C; TLC *R_f* 0.24 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 400 MHz) δ 9.01 (s, 2H, pyrimidine H), 8.61 (d, *J* = 2.0 Hz, 1H, PyH), 8.15 (dd, *J* = 2.4 Hz, 1H, PyH), 7.10 (d, *J* = 3.2 Hz, thiophene H), 7.00 (bd, *J* = 8.4 Hz, 1H, thiophene H), 6.77 (bd, *J* = 2.4 Hz, 1H, PyH), 5.58 (s, 2H, ArCH₂), 3.95 (s, 3H, OMe), 2.47 (s, 3H, Me); ¹³C NMR (DMSO, 75 MHz) δ 163.53, 163.41, 156.94, 144.54, 140.80, 137.22, 135.87, 128.57, 124.89, 123.17, 110.79, 63.21, 53.29, 14.95; IR (KBr) 1295 (C–O), 656 (C–S), 1261(C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₆H₁₅N₃O₂S) requires *m/z* 314.088, found *m/z* 314.047.

2-(4-Methoxybenzyloxy)-5-(2-(trifluoromethyl) phenyl) pyrimidine (**6b**)

87 % yield as pale yellow solid, mp (recrystallized from dichloromethane in petroleum ether) 67–70 °C; TLC *R_f* 0.25 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.61 (s, 2H, pyrimidine H), 7.90 (d, *J* = 7.8 Hz, 1H, ArH), 7.79 (t, *J* = 7.5 Hz, 1H, ArH), 7.69 (t, *J* = 7.8 Hz, 1 H, ArH), 7.53 (d, *J* = 7.8 Hz, 1H, ArH), 7.45 (d, *J* = 9.0 Hz, 2H, ArH), 6.97 (d, *J* = 8.4 Hz, 2H, ArH), 5.38 (s, 2H, ArCH₂), 3.77 (s, 3H, OMe); ¹³C NMR (DMSO, 75 MHz) δ 164.07, 159.18, 158.54, 133.52, 132.79, 130.03, 129.06, 128.23, 127.77, 126.81, 126.18, 125.80, 113.79, 68.53, 55.07; IR (KBr) 1254 (C–O), 1323 (C–F), 1267(C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₉H₁₅F₃N₂O₂) requires *m/z* 361.108, found *m/z* 361.110.

2-(4-Fluorobenzyloxy)-5-(2-(trifluoromethyl) phenyl) pyrimidine (**6d**)

81 % yield as pale yellow liquid, TLC *R_f* 0.23 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.63 (s, 2H, pyrimidine H), 7.906 (d, *J* = 7.5 Hz, 1H, ArH), 7.80 (t, *J* = 7.2 Hz, 1H, ArH), 7.70 (t, *J* = 7.5 Hz, 1H, ArH), 7.56 (m, 3H, ArH), 7.24 (t, *J* = 6.3 Hz, 2H, ArH), 5.44 (s, 2H, ArCH₂). ¹³C NMR

(DMSO, 75 MHz) δ 163.95, 160.27, 158.588, 133.44, 132.77, 130.49, 129.08, 127.77, 126.98, 126.26, 122.15, 115.37, 67.98; IR (KBr) 1224 (C–O), 1317 (C–F), 1264 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₂F₄N₂O) requires *m/z* 349.088, found *m/z* 349.057.

2-(3-Fluorobenzyloxy)-5-(2-(trifluoromethyl) phenyl) pyrimidine (**6e**)

86 % yield as pale yellow liquid, TLC *R_f* 0.23 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.38 (s, 2H, pyrimidine H), 7.90 (d, *J* = 7.5 Hz, 1H, ArH), 7.79 (t, *J* = 7.5 Hz, 1H, ArH), 7.72 (t, *J* = 7.5 Hz, 1H, ArH), 7.54 (d, *J* = 7.2 Hz, 1 H, ArH), 7.45 (m, 1H, ArH), 7.34 (m, 2H, ArH), 7.19 (m, 1H, ArH), 5.40 (s, 2H, ArCH₂); ¹³C NMR (DMSO, 75 MHz) δ 164.35, 160.98, 159.11, 139.86, 133.89, 133.26, 131.00, 129.59, 128.25, 127.56, 126.73, 124.34, 122.63, 115.21, 68.30; IR (KBr) 1259 (C–O), 1328 (C–F), 1220 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₂F₄N₂O) requires *m/z* 349.088, found *m/z* 349.038.

2-(4-Chlorobenzyloxy)-5-(2-(trifluoromethyl) phenyl) pyrimidine (**6f**)

87 % yield as pale yellow solid, mp (recrystallized from dichloromethane in petroleum ether) 76–79 °C; TLC *R_f* 0.28 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.62 (s, 2H, pyrimidine H), 7.90 (d, *J* = 8.1 Hz, 1H, ArH), 7.79 (t, *J* = 7.5 Hz, 1H, ArH), 7.69 (t, *J* = 7.5 Hz, 1H, ArH), 7.49 (m, 5H, ArH), 5.46 (s, 2H, ArCH₂); ¹³C NMR (DMSO, 75 MHz) δ 163.89, 158.63, 135.48, 133.41, 132.77, 132.65, 129.87, 129.09, 128.42, 127.76, 127.04, 126.18, 122.15, 67.82; IR (KBr) 1261 (C–O), 1317 (C–F), 805 (C–Cl), 1171 (C–N) cm⁻¹; HRMS (ES⁺) exact mass calculated for [M+H]⁺ (C₁₈H₁₂ClF₃N₂O) requires *m/z* 365.059, found *m/z* 364.969.

2-(2,4-Difluorobenzyloxy)-5-(2-(trifluoromethyl) phenyl) pyrimidine (**6g**)

82 % yield as pale yellow liquid, TLC *R_f* 0.27 (20 % EtOAc in hexanes as the eluent); ¹H NMR (DMSO, 300 MHz) δ 8.64 (s, 2H, pyrimidine H), 7.90 (d, *J* = 7.8 Hz, 1H, ArH), 7.80 (t, *J* = 7.2 Hz, 1H, ArH), 7.68 (q, *J* = 8.4 Hz, *J* = 2H, 2H, ArH), 7.54 (d, *J* = 7.2 Hz, 1H, ArH), 7.33 (td, *J* = 2.4 Hz, 1H, ArH), 7.15 (td, *J* = 1.5 Hz, 1H, ArH), 5.47 (s, 2H, ArCH₂); ¹³C NMR (DMSO, 75 MHz) δ 163.75, 163.35, 160.70, 158.64, 133.38, 132.65, 129.11, 127.76, 127.13, 126.18, 122.15, 119.82, 111.69, 104.06, 62.41; IR (KBr) 1286 (C–O), 1286 (C–F), 1175 (C–N) cm⁻¹; HRMS (ES⁺) exact mass

calculated for $[M+H]^+$ ($C_{18}H_{11}F_5N_2O$) requires m/z 367.079, found m/z 367.044.

2-(4-Methoxybenzyloxy)-5-(4-nitrophenyl) pyrimidine (7b)

50 % yield as pale yellow solid, mp (recrystallized from dichloromethane in petroleum ether) 168.5–173.1 °C; TLC R_f 0.17 (20 % EtOAc in hexanes as the eluent); 1H NMR (DMSO, 300 MHz) δ 9.07(s, 2H, pyrimidine H), 8.26 (bt, $J = 8.8$ Hz, 1H, ArH), 8.11 (d, $J = 8.1$ Hz, 1H, ArH), 7.95 (d, $J = 9.6$ Hz, 1H, ArH), 7.78 (t, $J = 7.8$ Hz, 1H, ArH), 7.46 (m, 1H, ArH), 7.32 (m, 2H, ArH), 7.183 (td, $J = 2.1$ Hz, 1H, ArH), 5.50 (s, 2H, ArCH₂), 3.30 (s, 3H, OMe); ^{13}C NMR (DMSO, 100 MHz) δ 164.63, 159.15, 158.04, 146.97, 140.53, 129.94, 128.21, 127.37, 125.39, 124.14, 113.78, 68.60, 55.08; IR (KBr) 1174 (C–O), 1343 (NO₂), 1249 (C–N) cm^{-1} ; HRMS (ES⁺) exact mass calculated for $[M+H]^+$ ($C_{18}H_{15}N_3O_4$) requires m/z 338.106, found m/z 337.953.

2-(4-Chlorobenzyloxy)-5-(4-nitrophenyl) pyrimidine (7f)

73 % yield as brown solid, mp (recrystallized from dichloromethane in petroleum ether) 213.6–219.6 °C; TLC R_f 0.2 (20 % EtOAc in hexanes as the eluent); 1H NMR (DMSO, 400 MHz) δ 9.10 (s, 2H, pyrimidine H), 8.33 (d, $J = 8.8$ Hz, 2H, ArH), 8.06 (d, $J = 8.0$ Hz, 2H, ArH), 7.53–7.46 (m, 4H, ArH), 5.50 (s, 2H, ArCH₂); ^{13}C NMR (DMSO, 100 MHz) δ 164.46, 158.13, 147.03, 140.45, 135.49, 132.59, 129.77, 128.43, 127.46, 125.65, 124.15, 67.88; IR (KBr) 1103 (C–O), 1309 (NO₂), 1274 (C–N), 802 (C–Cl) cm^{-1} ; HRMS (ES⁺) exact mass calculated for $[M+H]^+$ ($C_{17}H_{12}ClN_3O_3$) requires m/z 342.056, found m/z 342.110.

2-(2,4-Difluorobenzyloxy)-5-(4-nitrophenyl) pyrimidine (7g)

68 % yield as brown solid, mp (recrystallized from dichloromethane in petroleum ether) 140.1–144.5 °C; TLC R_f 0.22 (20 % EtOAc in hexanes as the eluent); 1H NMR (DMSO, 400 MHz) δ 9.10 (s, 2H, pyrimidine H), 8.32 (d, $J = 8.0$ Hz, 2H, ArH), 8.06 (d, $J = 8.8$ Hz, 2H, ArH), 7.66 (q, $J = 8.0$ Hz, 1H, ArH), 7.33 (t, $J = 1.6$ Hz, 1H, ArH), 7.14 (td, $J = 1.6$ Hz, 1H, ArH), 5.50 (s, 2H, ArCH₂); ^{13}C NMR (DMSO, 100 MHz) δ 164.30, 163.86, 162.81, 160.34, 158.13, 147.02, 140.41, 132.31, 127.46, 126.13, 125.73, 124.13, 119.74, 115.73, 111.57, 104.04, 62.48; IR (KBr) 1280 (C–O), 1303 (C–F), 1343 (NO₂), 1225 (C–N) cm^{-1} ; HRMS (ES⁺) exact mass calculated for $[M+H]^+$ ($C_{17}H_{11}F_2N_3O_3$) requires m/z 344.076, found m/z 343.948.

5-(4-Nitrophenyl)-2-(4-(trifluoromethoxy) benzyloxy) pyrimidine (7h)

62 % yield as pale yellow solid, mp (recrystallized from DCM in hexane) 164.8–166.2 °C; TLC R_f 0.31 (20 % EtOAc in hexanes as the eluent); 1H NMR (DMSO, 400 MHz) δ 9.10 (s, 2H, pyrimidineH), 8.33 (d, $J = 8.8$ Hz, 2H, ArH), 8.06 (d, $J = 8.8$ Hz, 2H, ArH), 7.63 (d, $J = 8.4$ Hz, 2H, ArH), 7.40 (d, $J = 8.8$ Hz, 2H, ArH), 5.51(s, 2H, ArCH₂); 3.30 (s, 3H, OMe); ^{13}C NMR (DMSO, 100 MHz) δ 164.45, 158.14, 147.96, 147.03, 140.44, 135.96, 129.93, 127.45, 125.68, 124.14, 121.02, 118.76, 67.78; IR (KBr) 1223 (C–O), 1345 (NO₂), 1199 (C–N), 1251 (C–F) cm^{-1} ; HRMS (ES⁺) exact mass calculated for $[M+H]^+$ ($C_{18}H_{12}F_3N_3O_4$) requires m/z 392.077, found m/z 392.120.

Cytotoxic evaluation of 2,5-disubstituted pyrimidines compounds (4a–7h)

The compounds were tested on HeLa cells using MTT cell proliferation assay (Plumb *et al.*, 1989). HeLa cell line was obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's medium (DMEM) (Sigma Life Science, USA) containing 10 % fetal bovine serum (FBS). The cells (2,000 cells per well) were seeded in a 96-well microplate containing 100 μ L of DMEM complete medium per well and incubated at 37 °C with 5 % CO₂.

The cells were treated different concentrations of compounds up to 72 h for every 24 h interval. Controls were maintained with 0.5 % DMSO. After 72 h treatment, 5 μ L of MTT (3-(4,5-dimethyl- thiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent (R&D Systems, USA) along with 45 μ L of phenol red free DMEM (Sigma Life Science, USA) without FBS was added to each well and plates were incubated at 37 °C with 5 % CO₂ for 4 h. Thereafter, 50 μ L of solubilization buffer (R&D Systems, USA) was added to each well to dissolve the colored formazan crystals produced by the reduction of MTT. After 24 h, the optical density was measured at 550 nm using microplate reader (Bio-Rad, USA).

Acknowledgments The authors are highly thankful to Department of Chemistry, Acharya Nagarjuna University, Nagarjunanagar, Guntur, and Andhra Pradesh, India for constant encouragement.

References

- Bardhan S, Wacharasindhu S, Wan Z-K, Mansour TS (2009) Heteroaryl ethers by oxidative palladium catalysis of pyridotriazol-1-yloxy pyrimidines with arylboronic acids. *Org Lett* 11:2511–2514

- Berber H, Soufyane M, Santillana-Hayat M, Mirand C (2002) Unexpected synthesis of (trifluoroethyl)pyrimidines from the heterocyclisation of α -trifluoroacetylpropanenitriles. *Tetrahedron Lett* 43:9233–9235
- Boyd HF, Hammond B, Hickey DMB, Ife RJ, Leach CA, Lewis VA, Macphree CH, Milliner KJ, Pinto IL, Smith SA, Stansfield IG, Theobald CJ, Whittaker CM (2001) The identification of a potent water soluble inhibitor of lipoprotein-associated phosphopase A₂. *Bioorg Med Chem Lett* 11:701–704
- Brown DJ (1962) The chemistry of heterocyclic compounds. In: *The pyrimidines*, vol 16. Wiley-Inter science
- García-Valverde M, Torroba T (2005) Special issue: sulfur-nitrogen heterocycles. *Molecules* 10:318–320
- Herrera A, Martínez-Álvarez R, Chioua M, Chioua R, Sánchez Á (2002) On the regioselectivity in the reaction of aliphatic ketones and Aromatic nitriles. Regiospecific synthesis of alkylarylpyrimidines. *Tetrahedron* 58:10053–10058
- Isley NA, Gallou F, Lipshutz BH (2013) Transforming Suzuki-Miyaura cross-couplings of MIDA boronates into a green technology: no organic solvents. *J Am Chem Soc* 135(47):17707–17710
- Jain KS, Chitre TS, Miniya PB, Kathiravan MK, Bendre VS, Veer VS, Shahane SR, Shishoo CJ (2006) Biological and medicinal significance of pyrimidines. *Curr Sci* 90:793–803
- Joule JA, Mills K (2010) *Heterocyclic chemistry*, 5th edn. Wiley-Blackwell
- Kappe CO (1993) 100 years of the biginelli dihydropyrimidine synthesis. *Tetrahedron* 49:6937–6963
- Lagoja IM (2005) Pyrimidine as constituent of natural biologically active compounds. *Chem Biodivers* 2:1–50
- Leadbeater NE, Marco M (2002) Ligand-free palladium catalysis of the Suzuki reaction in water using microwave heating. *Org Lett* 4:2973–2976
- Li S, Lin Y, Cao J, Zhang S (2007) Guanidine/Pd(OAc)₂ - catalyzed room temperature Suzuki cross-coupling reaction in aqueous media under aerobic conditions. *J Org Chem* 72(11):4067–4072
- Lipshutz BH, Abela AR (2008) Micellar catalysis of Suzuki-Miyaura cross-couplings with heteroaromatics in water. *Org Lett* 10(23):5329–5332
- Liu L, Zhang Y, Wang Y (2005) Phosphine-free palladium acetate catalyzed Suzuki reaction in water. *J Org Chem* 70:6122–6125
- Miyaura N, Suzuki A (1995) Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem Rev* 95:2457–2483
- Morgentin R, Jung F, Lamorlette M, Maudet M, Menard M, Ple P, Pasquet G, Renaud F (2009) An efficient large-scale synthesis of alkyl 5-hydroxy-pyridine- and pyrimidin-2-yl- acetate. *Tetrahedron* 65(4):757–764
- Nencka R, Votruba I, Hřebabeký H, Jansa P, Tloušťová E, Horská K, Masojdková M, Holý A (2007) Discovery of 5-substituted-6-chlorouracils as efficient inhibitors of human thymidine phosphorylase. *J Med Chem* 50:6016–6023
- Parry PR, Wang C, Batsanov AS, Bryce MR, Tarbit B (2002) Functionalized pyridylboronic acids and their Suzuki cross-coupling reactions to yield novel heteroarylpyrimidines. *J Org Chem* 67:7541–7543
- Plumb JA, Milroy R, Kaye SB (1989) Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Res* 49(16):4435–4440
- Saygili N, Batsanov AS, Bryce MR (2004) 5-Pyrimidylboronic acid and 2-methoxy-5-pyrimidyl boronic acid: new heteroarylpyrimidine derivatives via Suzuki cross-coupling reactions. *Org Biomol Chem* 2(6):852–857
- Schomaker JM, Delia TJ (2001) Arylation of halogenated pyrimidines via a Suzuki coupling reaction. *J Org Chem* 66:7125–7128
- Urwiler S, Pozza MF, Lingenhoehl K, Mosbacher J, Lampert C, Froestl W, Koller M, Kaupmann K (2003) *N, N'*-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acidB receptor. *J Pharmacol Exp Ther* 307(1):322–330
- Xie F, Zhao H, Li D, Chen H, Quan H, Shi X, Lou L, Hu Y (2011) Synthesis and Biological Evaluation of 2,4,5-Substituted Pyrimidines as a New Class of Tubulin Polymerization Inhibitors. *J Med Chem* 54:3200–3205