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Microwave-assisted one step synthesis of 8-arylmethyl-9*H*-purin-6-amines

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Abstract

Molecular chaperone heat shock protein 90 (Hsp90) is an important target in cancer and neurodegenerative diseases, and has rapidly become the focus of several drug discovery efforts. Among small molecule Hsp90 inhibitors with clinical applicability are derivatives of 8-arylmethyl-9*H*-purin-6-amine class. Here we report the use of microwave-assisted chemistry for the successful one-pot delivery of 8-arylmethyl-9*H*-purin-6-amines. We discuss the applicability as well as the limitations of this method towards the creation of a large chemical diversity in the 8-arylmethyl-9*H*-purin-6-amine series.

Molecular chaperone heat shock protein 90 (Hsp90) is an important target in cancer, and has rapidly becoe the focus of several drug discovery efforts.[1] Indeed, Hsp90 has emerged as a cancer target because of its key participation in regulating clinically validated oncogenic proteins such as Her-2, ER, and Bcr-Ab1, as well as other signaling proteins that play a role in malignancy, such as Raf-1, p-Akt, Cdk4, mutant p53 and Flt-3.[2]

Purine-scaffold Hsp90 inhibitors are the first synthetic small molecule Hsp90 inhibitors to be discovered and to be translated to clinic in patients with advanced cancers.[3] Among purines with Hsp90 inhibitory activities are the 8-arylmethyl-9*H*-purin-6-amine derivatives, such as PU3, PU24FCl and PU-DZ8 (Figure 1).[3a] PU3 is the first discovered synthetic Hsp90 inhibitor, and was identified through empirical structure-based design.[3b] PU24FCl, a derivative of PU3, is the first synthetic Hsp90 inhibitor reported to exhibit anti-tumor activity and biological activity in a widerange of tumor types.[3c]

PU-DZ8 has recently been reported to reduce aberrant neuronal proteins and diminish toxic tau aggregates in transgenic mouse models of dementias caused by tau protein toxicity, such as Alzheimer's disease and frontotemporal dementias[3d]. Considering their biological effects in both cancer and neurodegenerative diseases, there is an increased interest in their development and optimization towards a clinical candidate.[3]

Attempts to result in higher chemical diversity in the 8-arylmethyl-9*H*-purin-6-amine series remain limited by the somewhat cumbersome synthesis on which building of the 8-benzyl purine core relies.[4] Its synthesis continues to employ a three-step protocol consisting of generating the acid fluoride of the corresponding phenyl acetic acid, coupling it with pyrimidine-4,5,6-triamine or pyrimidine-2,4,5,6-tetraamine in the presence of K_2CO_3, followed by condensation of the intermediate amide product (Scheme 1).[4]

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These condensation reactions require harsh dehydration conditions and/or long reaction times, such as refluxing overnight in ethanol in the presence of 15 equivalents of NaOMe,[4a] or refluxing for 5 hr in a 25% solution of NaOMe in methanol/isobutanol,[4b] which limit the spectrum of suitable starting materials. Reliance on the multi-step protocol results in lower overall yields, with a 20% isolated yield reported for the precursor of PUDZ8.[4b] Dymock *et al* utilized a two-step protocol in which the carboxylic acid is directly coupled with the tri- or tetra-aminopyrimidine in the presence of *i*-Pr_2NEt and HBTU in DMF, followed by condensation of the intermediate amide product by refluxing overnight with NaOMe (15 equivalents) in EtOH or by microwave irradiation for 30 min at 130°C.[4a] Unfortunately, yields were not reported so that a satisfactory comparison between each of these methods cannot be made.

To limit the number and length of the synthetic steps, and to increase the deliverable chemical variability in the 8-arylmethyl-9*H*-purin-6-amine series, we investigated the use of microwave irradiation. Microwave-assisted organic synthesis continues to grow in importance in synthetic chemistry by enabling rapid chemistry development and numerous reactions, including condensations and heterocycle formations have been explored under these conditions.[5] Herein, we report the use of microwave-assisted chemistry for the successful one-pot delivery of 8-arylmethyl-9*H*-purin-6-amines. In addition, we briefly discuss cases in which the reaction fails to deliver the desired product.

Previously, Lin *et al* have reported a microwave-assisted synthesis of benzimidazoles and other heterocycles from condensation of a diamine with a carboxylic acid.[6] We probed the feasibility of adapting this method[7] to more functionally complex amino-pyrimidines in an attempt to produce the desired condensation product in a single step. In an initial effort to prepare several PU-DZ8 derivatives, 2-(benzo[d][1,3]dioxol-5-yl)acetic acid (1.0 equiv) and pyrimidine-4,5,6-triamine (1.2 equiv) were irradiated at 220°C for 15 min in the presence of $P(OPh)_3$ (1.2 equiv) in pyridine, to result in 8-(benzo[d][1,3]dioxol-5-ylmethyl)-9*H*-purin-6-amine (**3a**) in 87% isolated yield. High recovered yields were also noted when the phenyl ring was replaced with a pyridine moiety (Entry 3, Table 1).

When the triamine-pyrimidine substrate (**1a**) was replaced with its tetraamine-equivalent (**1b**), which is required for the building of PU-DZ8 derivatives, the recorded yields decreased under the Lin conditions (87% yield, Entry 1, Table 1 *versus* 62% yield, Entry 1, Table 2). The relatively lower solubility of **1b** in pyridine, when compared to **1a**, could be partly accountable for poorer yields. Increasing the reaction time to 20 min did not significantly improve the yield (65% yield, Entry 2, Table 2). Addition of DMSO or DMF, solvents which in our hands usually increase the solubility of purines and pyrimidines, had an adverse effect on the yield (Entries 3 and 4, Table 2). As expected, addition of a strong base, NaO*t*Bu, augmented the yield to 78% of recovered material, possibly by a two pronged beneficial effect, on both solubility and coupling and condensation efficacy (Entry 5, Table 2).

The free base form of pyrimidine-2,4,5,6-tetraamine is commercially unavailable due to its propensity to oxidize and decompose, and therefore short shelf life. The compound is instead sold as a sulfate salt of very poor solubility in organic solvents. The base is usually generated by recrystallization from 10% NaOH aqueous solution before its use in the coupling with carboxylic acids **2**. To eliminate the extra step, we probed the *in situ* conversion of the sulfate into free base. Surprisingly, pyrimidine-2,4,5,6-tetraamine sulfate remained poorly soluble in pyridine even when heated at 220 °C. Addition of NaOH (4 equiv), rendered pyrimidine-2,4,5,6-tetraamine sulfate completely soluble in pyridine, however, increased the yield to only 25% (Entry 7, Table 2). We assumed that one reason could be the *in situ* generated water. This however, can only partly be true, because replacement of NaOH with NaO*t*Bu had only a marginal benefit on the recovered yield (Entry 8, Table 2).

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To evaluate the limitations of this procedure towards the formation of 8-arylmethyl-9*H*-purin-6-amines, we chose to investigate the addition of functionalities at positions more likely to result in interference with the condensation reaction (Table 3). These were estimated to be functionalities either in the ortho position of the aryl ring (R^2 Table 3) or adjacent to the carboxyl functionality (R^1 Table 3). We found that the reaction was sensitive to the nature of the ortho functionality, as demonstrated by lower yields when R^2=H was exchanged with a trifluoromethyl or cyano functionality (23% recovered yield for **3e** and traces detectable by mass spectrum for **3f**). This outcome could be due to steric effects, because attempts to increase the solubility of the pyrimidine **1b** and to facilitate coupling and condensation by addition of a base, NaO*t*-Bu (2 equiv), failed to result in improved yields (Entry 4, Table 3). We found that insertion of a substituent at the α-position of the acetyl carboxylic acid (R^1) also played an important role in this reaction. The transformation tolerated the addition of a methyl (Entry 7, Table 3), however, no product was detected when hydroxyl or methoxy derivatives were employed (Entries 5 and 6, Table 3). It is unclear whether a lack of product in the case of hydroxyl and methoxy derivatives **2** is due to decomposition of the carboxylic acid **2**, or to electronic effects which reduce the electrophilicity of the carboxyl group.

In summary, we present a simplified one-pot synthesis of 8-arylmethyl-9*H*-purin-6-amines which can generate the desired product with yields comparable or significantly higher, depending on the reaction set-up, to the previously published route. The procedure is amenable for structure-activity investigation efforts in the pursuit of a higher chemical diversity in the 8-arylmethyl-9*H*-purin-6-amine Hsp90 inhibitor series.

Acknowledgments

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7. General reaction procedure for the synthesis of 8-arylmethyl-9H-purin-6-amines 3: In a conical-bottomed Smith Process vial, the mixture of aryl acetic acid (0.2 mmol), amino pyrimidine (0.24 mmol) and triphenyl phosphite (63 μL, 0.24 mmol) in 1 mL anhydrous pyridine were charged. The sealed vial was irradiated in the microwave for 15 min at 220°C. The pressure reading at this temperature

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was around 8 bar. After cooling, the reaction mixture was concentrated under vacuum and the residue purified by preparative TLC ($CHCl_3$/ammonia MeOH = 25:1) to give the desired product **3**.

8. 8-(Benzo[d][1,3]dioxol-5-ylmethyl)-9 *H*-purin-6-amine **(3a)**: ^{1}H-NMR: 12.61 (br s, 1H), 7.94 (s, 1H), 6.87 (br s, 2H), 6.83 (s, 1H), 6.74 (d, *J* = 7.9 Hz, 1H), 65 (d, *J* = 7.9 Hz, 1H), 5.86 (s, 2H), 3.91 (s, 2H); ^{13}C-NMR: 155.0, 151.9, 151.1, 150.5, 147.3, 145.9, 130.9, 121.7, 109.1, 108.2, 100.8, 34.6; MS m/z 269.9 $(M+H)^+$.8-(3,4,5-Trimethoxybenzyl)-9H-purin-6-amine **(3b)**: ^{1}H-NMR: 12.50 (br s, 1H), 7.82 (s, 1H), 6.76 (s, 2H), 6.42 (s, 2H), 3.80 (s, 2H), 3.79 (s, 6H), 3.67 (s, 3H); ^{13}C-NMR: 157.3, 152.8, 151.9, 136.2, 132.7, 129.3, 118.8, 115.2, 106.1, 59.9, 55.8, 35.3; MS m/z 316.08 $(M+H)^+$.8-(pyridin-3-ylmethyl)-9*H*-purin-6-amine **(3c)**: ^{1}H-NMR: 12.77 (br s, 1H), 8.57 (d, *J* = 1.5 Hz, 1H), 8.46 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.07 (s, 1H), 7.72-7.70 (m, 1H), 7.35 (dd, *J* = 7.5, 4.8 Hz, 1H), 7.03 (s, 2H)4.18 (s, 2H); ^{13}C-NMR: 154,5, 152.0, 151.4, 150.2, 149.7, 136.4, 132.9, 123.7, 32.0; MS m/z 226.99 $(M+H)^+$.
9. 8-(Benzo[d][1,3]dioxol-5-ylmethyl)-9*H*-purine-2,6-diamine **(3d)**: ^{1}H-NMR: 11.97 (s, 1H), 6.91 (s, 1H), 6.88 (d, *J* = 7.9 Hz, 1H), 6.78 (d, *J* = 7.9 Hz, 1H), 6.55 (s, 2H), 5.62 (s, 2H), 3.94 (s, 2H); ^{13}C-NMR: 159.8, 155.2, 153.3, 147.2, 146.7, 145.7, 131.6, 121.5, 112.8, 109.0 108.1, 100.8, 34.5; MS m/z 284.99 $(M+H)^+$.8-((6-(Trifluoromethyl)benzo[d][1,3]dioxol-5-yl)methyl)-9*H*-purine-2,6-diamine **(3e)**: ^{1}H-NMR: 11.96 (br s, 1H), 7.26 (s, 1H), 6.95 (s, 1H), 6.43 (s, 2H), 6.14 (s, 2H), 5.96 (s, 2H), 5.56 (s, 2H), 4.11 (s, 2H); ^{13}C-NMR: 159.8, 155.2, 153.3, 150.1, 146.2, 145.3, 131.0, 129.3, 115.2, 113.0, 111.7, 106.0, 102.3, 31.2; MS m/z 352.99 $(M+H)^+$.8-(1-Phenylethyl)-9*H*-purine-2,6-diamine **(3i)**: ^{1}H-NMR: 11.95 (br s, 1H), 7.38-7.33 (m, 1H), 7.34 (s, 2H), 7.27-7.20 (m, 1H), 6.82-6.80 (m, 1H), 6.52 (s, 2H), 5.60 (s, 2H), 4.25 (q, *J* = 7.3 Hz, 1H), 1.67 (d, J = 7.3 Hz, 3H); ^{13}C-NMR: 159.8, 155.2, 150.5, 143.9, 129.3, 128.4, 127.1, 126.4, 118.8, 115.2, 20.2; MS m/z 254.99 $(M+H)^+$.8-(3,4,5-Trimethoxybenzyl)-9*H*-purine-2,6-diamine **(3j)**: ^{1}H-NMR:11.73 (br s, 1H), 6.38 (s, 2H), 6.32 (s, 2H), 5.35 (s, 2H), 3.53 (s, 6H), 3.40 (s, 3H), 2.94 (s, 2H); ^{13}C-NMR: 159.8, 157.3, 152.8, 146.6, 136.1, 129.3, 118.8, 115.2, 106.0, 59.9, 55.8, 35.3; MS m/z 331.12 $(M+H)^+$.

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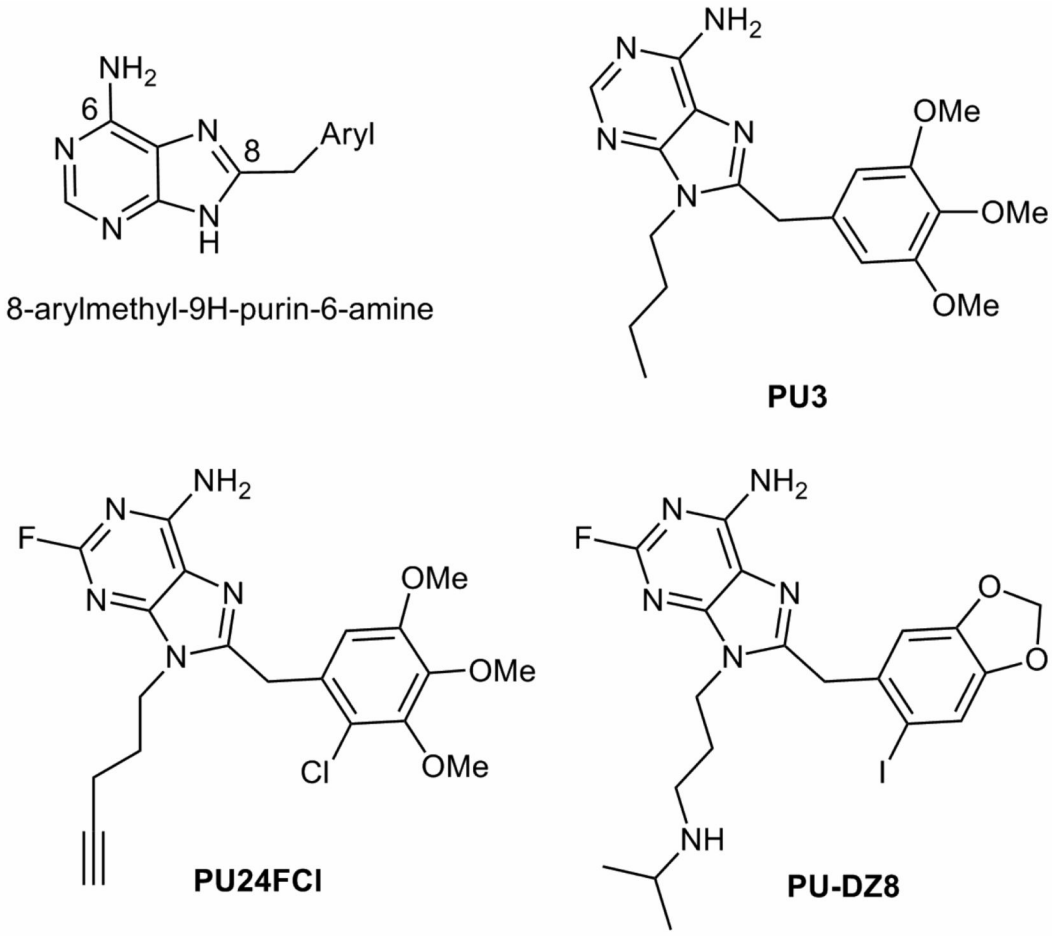

Figure 1.
Hsp90 inhibitors containing the 8-arylmethyl-9*H*-purin-6-amine core.

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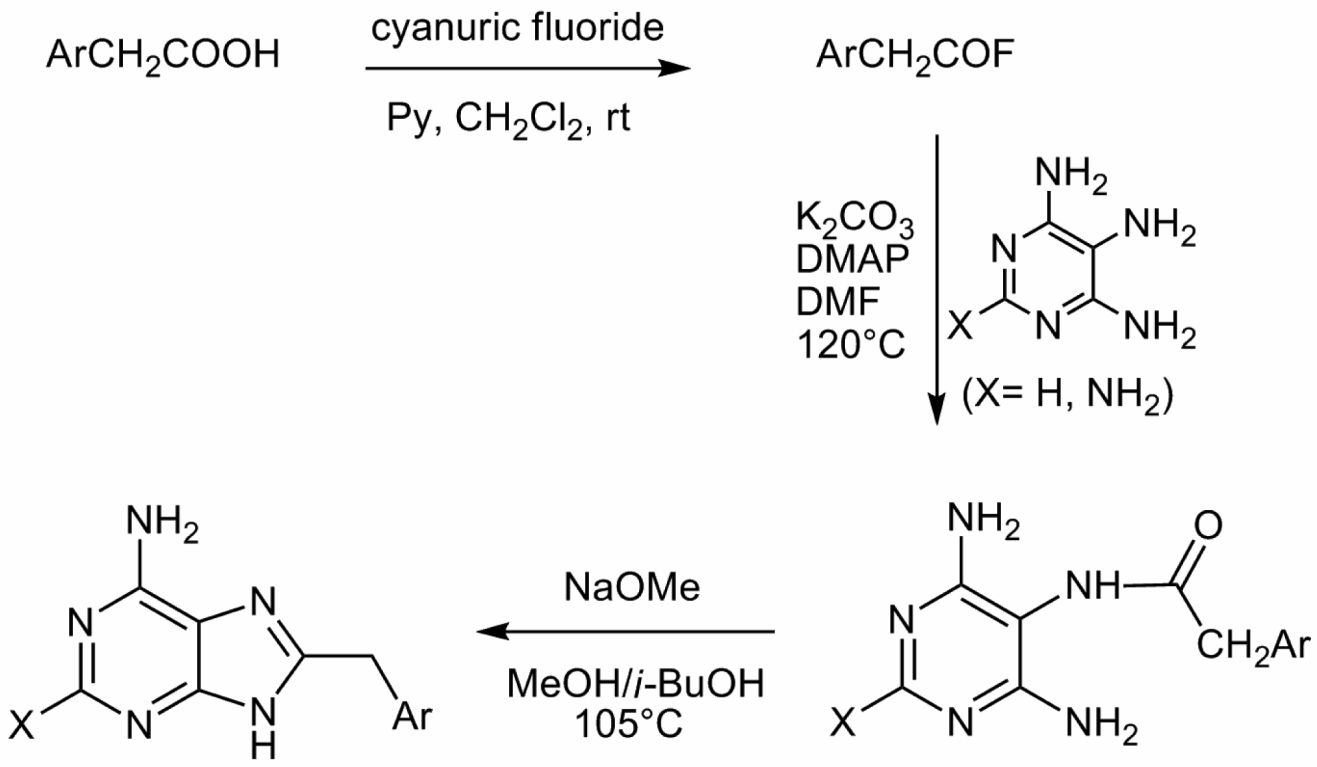

Scheme 1.
Three-step synthesis of 8-arylmethyl-9*H*-purin-6-amines.

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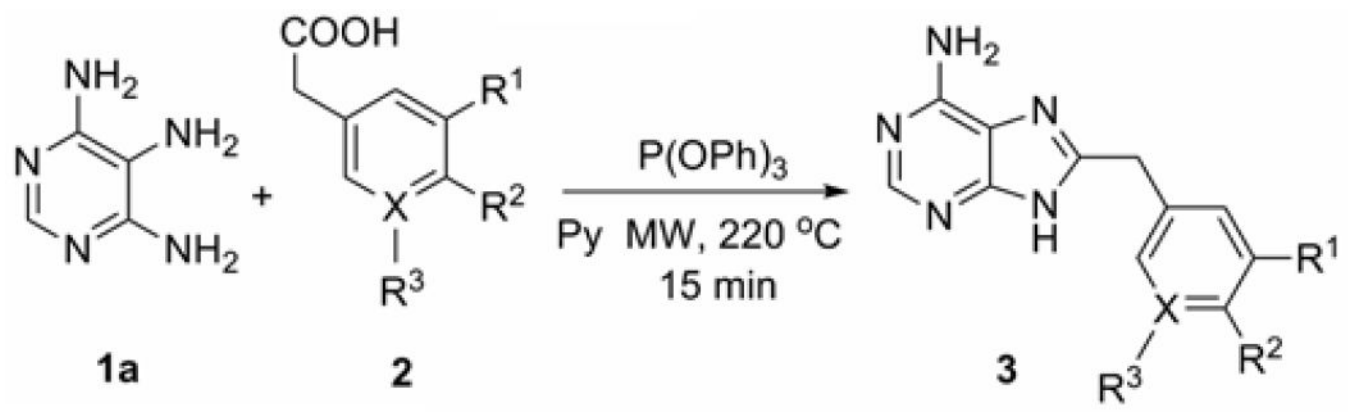
Table 1

Evaluation of carboxylic acids and pyrimidine-4,5,6-triamine.[8]



Entry	3	R^1	R^2	R^3	X	Yield[a]
1	**3a**	OCH_2O		H	C	87
2	**3b**	OMe	OMe	OMe	C	98
3[b]	**3c**	H	H	-	N	93

[a]Isolated yields by preparative TLC

[b]3-Pyridylacetic acid hydrochloride was used in this reaction.

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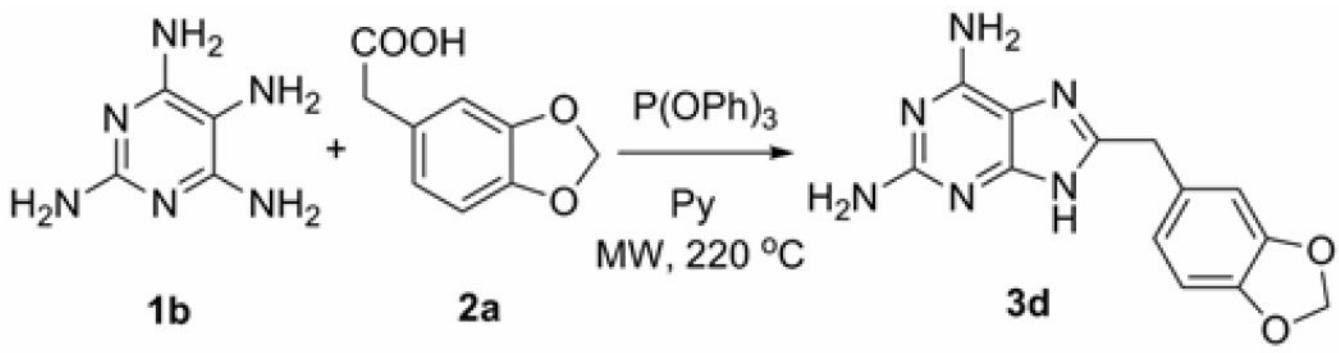
Table 2

Evaluation of methodology for the synthesis of PU-DZ8.



Entry	1b	solvent	co-solvent (50%)	base (2 eq.)	t (min)	yield[a]
1	base	Py	N/A[b]	N/A	15	62
2	base	Py	N/A	N/A	20	65
3	base	Py	DMF	N/A	15	18
4	base	Py	DMSO	N/A	15	11
5	base	Py	N/A	NaO*t*Bu	15	78
6	sulfate	Py	N/A	N/A	15	5
7	sulfate	Py	N/A	NaOH	15	25
8	sulfate	Py	N/A	NaO*t*Bu	15	31

[a]Isolated yields by preparative TLC

[b]None used

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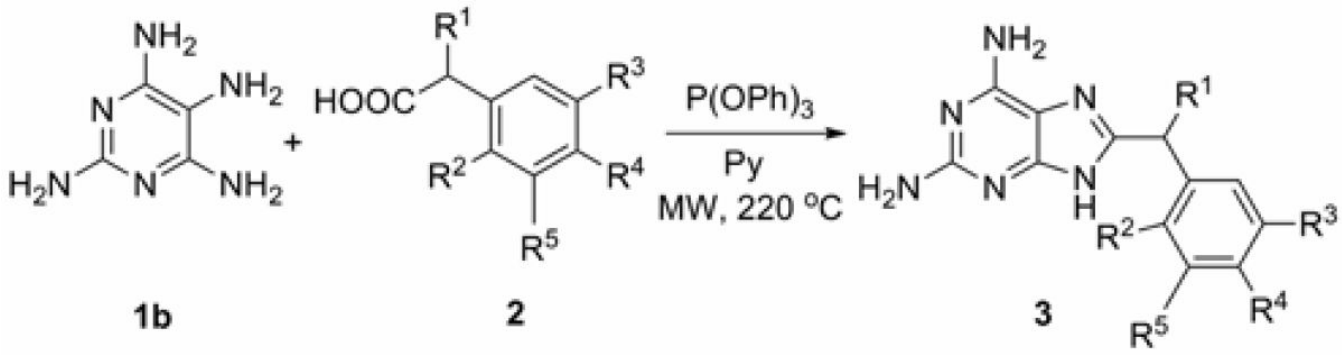
Table 3

Evaluation of carboxylic acids on the reaction yield.[9]



Entry	3	R^1	R^2	R^3, R^4	R^5	base	yield[a]
1	**3d**	H	H	OCH_2O	H	N/A[b]	62
2	**3e**	H	CF_3	OCH_2O	H	N/A	23
3	**3f**	H	CN	OCH_2O	H	N/A	<5
4	**3f**	H	CN	OCH_2O	H	NaO*t*Bu	<5
5	**3g**	OH	H	OCH_2O	H	N/A	NR
6	**3h**	OMe	H	OCH_2O	H	N/A	NR
7	**3i**	Me	H	H, H	H	N/A	48
8	**3j**	H	H	OMe, OMe	OMe	N/A	53

[a]Isolated yields by preparative TLC

[b]None used