2013

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Citation/Publisher Attribution
Rao, V.K., & Tiwari, R., & Chhikara, B.S., & Shirazi, A.N., & Parang, K., & Kumar, A. (2013). Copper(II)triflate-mediated synthesis of 1,3,5-triarylpyrazoles in [bmim][PF_6] ionic liquid and evaluation of their anticancer activities. *Royal Society of Chemsitry Advances, 35,* 15396-15403. doi: 10.1039/C3RA41830H
Available at: http://dx.doi.org/10.1039/C3RA41830H

Copper(II) triflate-mediated synthesis of 1,3,5-triarylpyrazoles in [bmim][PF_6] ionic liquid and evaluation of their anticancer activities

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This is where the receipt/accepted dates will go; Received Month XX, 2000; Accepted Month XX, 2000 [BMCL RECEIPT]

Abstract—A simple, efficient, and environmentally friendly protocol for the synthesis of 1,3,5-triarylpyrazole in [bimm][PF_6] ionic liquid mediated by $Cu(OTf)_2$ is described. The reaction protocol gave 1,3,5-triarylpyrazoles in good to high yields (71-82%) *via* a one-pot addition–cyclocondensation between hydrazines and chalcones, and oxidative aromatization without requirement for an additional oxidizing reagent. The catalyst can be reused up to four cycles without much loss in the catalytic activity. The pyrazoles (**4a-o**) and pyrazolines (**3a-n**) were evaluated for antiproliferative activity in SK-OV-3, HT-29, and HeLa human cancer cells lines. Among all compounds, **3b** inhibited cell proliferation of HeLa cells by 80% at a concentration of 50 μM.

Pyrazoles and their derivatives are well recognized as an important class of heterocyclic compounds that have been found with extensive use in the pharmaceutical, material, and agrochemical industries.[1] Compounds containing pyrazole moiety have exhibited diverse biological activities. For example, 4-substituted 1,5-diaryl-1*H*-pyrazole-3-carboxylate derivatives can act as cannabinoid-1 (CB1) receptor antagonists,[2-7] Iκβ kinase β (IKKβ or IKK-2) inhibitors,[8] and anti-inflammatory agents.[9] Pyrazole derivatives have been shown to have good binding affinity towards estrogen receptor.[10-12] Some of the pyrazole derivatives have been reported to possess antidepressant, anticonvulsant,[13] anti-inflammatory, and arthritics[14] activities. Pyrazole scaffold constitutes the basic framework of several drug molecules such as celecoxib (a non-steroidal anti-inflammatory drug)[14] and rimonabant (an anorectic antiobesity drug) (Figure 1).

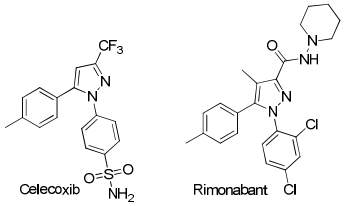


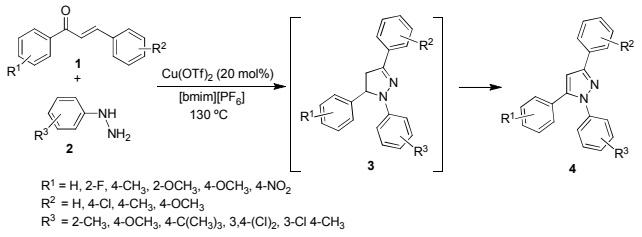
Figure 1. Chemical structures of drug molecules, celecoxib and rimonabant containing pyrazole scaffold.

Because of their diverse bioactivities, pyrazoles have received considerable attention of chemists. Thus, a number of synthetic strategies have been developed for their synthesis.[15, 16] The most common approach for synthesis of substituted pyrazoles is the condensation of α,β-unsaturated carbonyl compounds with hydrazines. However, this strategy results in the formation of 4,5-dihydro-1*H*-pyrazoles (pyrazolines) that need to be further oxidized to corresponding pyrazoles. For this oxidative aromatization of pyrazolines to pyrazoles, various reagents have been employed such as I_2[17], $Bi(NO_3)_3.\ 5H_2O$[18], MnO_2[19], DDQ[20], Pd/C[21], NaOEt[22], $PhI(OAc)_2$[23], and TBBDA[24]. However, many of these oxidative methods suffer from relatively high oxidant loading, use of strong oxidants and chlorinated organic solvents, harsh conditions, poor yields, and longer reaction time. Thus, the development of environmentally benign processes with the use of alternative solvents such as ionic liquids in place of organic solvent and a catalytic amount of ecofriendly catalysts that avoid harsh oxidizing reagents are desirable.

As part of our ongoing work on the development of novel reaction methodologies using metal triflates,[25-27] and evaluation of small molecules as anticancer agents,[27-30] herein we report copper (II) triflate-mediated protocol for the synthesis of pyrazoles by one-pot reaction of hydrazines with α,β-unsaturated ketones in 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim][PF_6]) ionic liquid (Scheme 1) and evaluation of antiproliferative activity against different cancer cell lines.

In the standardization experiment, when 4-*tert*-butylphenylhydrazine hydrochloride (**2**) and 1,2-diphenylprop-2-en-1-one (**1**) were reacted in ethanol at 130 °C in the presence of $Cu(OTf)_2$ (20 mole %), 1-(4-*tert*-butylphenyl)-3,5-diphenyl-4,5-dihydro-1H-pyrazole (**3a**) was obtained in 62% yield (Table 1, entry 8).

Further optimization of reaction condition was carried out by changing solvents, catalysts, and catalyst loading. As shown in Table 1, the use of 20 mol% $Cu(OTf)_2$ in [bmim][PF_6] gave the desired product **4a** in excellent yield (82%) (Table 1, entry 2). When $Cu(OTf)_2$ was changed with other catalysts such as *p*TSA, $Sc(OTf)_3$, $Ce(OTf)_3$, $Zn(OTf)_2$, AgOTf, or $Yb(OTf)_3$, a mixture of **3a** and **4a** was observed. Use of $Ce(OTf)_3$ in [bmim][PF_6] resulted in 75% of **3a** along with 10% of **4a** whereas *p*TSA in [bmim][PF_6] gave 69% of **3a** (Table 1, entry 11-12). There was not much increase in yield of **4a** on changing the amount of $Cu(OTf)_2$ from 20 mol% to 30 mol%. However, reducing the amount of $Cu(OTf)_2$ to 10 mol% decreased the yield of **4a** to 64% along with the formation of **3a** in 15% (Table 1, entries 1-3). These data indicate that $Cu(OTf)_2$ was involved in aerobic oxidation of **3a** to **4a**. It is necessary to mention that **4a** was not formed in the absence of $Cu(OTf)_2$ in [bmim[PF_6] ionic liquids, and only **3a** was isolated along with starting material.



Scheme 1. Synthesis of substituted 1,3,5-triarylpyrazoles.

Table 1. Optimization of reaction conditions for the synthesis of **4a**.[a]

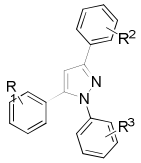
S. No	Catalyst	Mol (%)	Solvent	Yield (**3a**) (%)[b]	Yield (**4a**) (%)[b]
1	$Cu(OTf)_2$	10	[bmim][PF_6]	15	64[c]
2	$Cu(OTf)_2$	20	[bmim][PF_6]	-	82
3	$Cu(OTf)_2$	30	[bmim][PF_6]	-	84
4	$Cu(OTf)_2$	20	[bmim][BF_4]	35	50
5	$Cu(OTf)_2$	20	[bmim][Br]	50	21
6	$Cu(OTf)_2$	20	DMSO	-	15
7	$Cu(OTf)_2$	20	DMF	-	33
8	$Cu(OTf)_2$	20	Ethanol	62[d]	-
9	$Cu(OTf)_2$	20	PEG	60	-
10	$Sc(OTf)_3$	20	[bmim][PF_6]	61	19
11	$Ce(OTf)_3$	20	[bmim][PF_6]	75	10
12	*p*TSA	20	[bmim][PF_6]	69	-
13	$Zn(OTf)_2$	20	[bmim][PF_6]	55	30
14	AgOTf	20	[bmim][PF_6]	15	65
15	$Yb(OTf)_3$	20	[bmim][PF_6]	58	22

[a]Reaction condition: Chalcone (1.0 mmol), arylhydrazine (1.2 mmol), 130 °C, 2 h.
[b]Isolated yield.
[c]Only 20% of **3a** was formed after 30 min in the absence of $Cu(OTf)_2$ under similar conditions.
[d]Reflux condition.

The structure of compound **4a** was confirmed by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectroscopy. In the ^{1}H NMR spectra, a singlet was observed at δ 6.81 for the proton at C-4 position of pyrazole ring along with other protons on aryl substituents. In ^{13}C NMR, a peak appeared at δ 104.97 for the C-4 carbon of pyrazole ring. HRMS of **4a** showed a peak at m/z for molecular ion. Thus, NMR and mass analysis confirmed that the product was pyrazole and not pyrazoline.

To explore the synthetic scope and versatility of the protocol, a series of aryl hydrazines **2** were reacted with different α,β-carbonyl compounds (**1**) under the optimal reaction conditions. The results are summarized in Table 2. Various groups, such as F, Cl, NO_2, OCH_3, CH_3 and -$C(CH_3)_3$ on aryl hydrazines and chalcones were well tolerated in these conditions affording the corresponding 1,3,4-substituted pyrazoles (**4a-o**) in good to high yields (71–82%).

Table 2. Synthesized 1,3,5-triarylpyrazoles (**4a-o**).



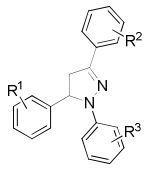
Compd	R^1	R^2	R^3	Time (Min.)	Yield (%)[a]
4a	H	H	4-$C(CH_3)_3$	120	82[b]
4b	H	H	2-CH_3	120	81
4c	H	H	3,4-Cl	180	80
4d	H	H	3-Cl, 4-CH_3	150	72
4e	4-OMe	H	3,4-Cl	120	71
4f	4-OMe	H	3-Cl, 4-CH_3	120	77
4g	3-OMe	4-CH_3	3,4-Cl	120	79
4h	4-CH_3	4-CH_3	3,4-Cl	120	74
4i	4-CH_3	4-CH_3	3-Cl, 4-CH_3	120	77
4j	4-CH_3	4-CH_3	4-$C(CH_3)_3$	105	77
4k	2-F	4-Cl	4-$C(CH_3)_3$	120	84
4l	2-F	4-Cl	2-CH_3	120	82
4m	4-NO_2	4-OMe	4-$C(CH_3)_3$	60	75
4n	4-NO_2	4-OMe	3-Cl, 4-CH_3	60	81
4o	H	H	4-OMe	90	78

[a]Isolated yield
[b]In four consecutive recycle experiment **4a** was observed in 82, 80, 78, and 79% yield, respectively.

By monitoring the model reaction between 1,2-diphenylprop-2-en-1-one (**1**) and *tert*-butylphenyl-hydrazine hydrochloride (**2**) in the presence of 20 mol% $Cu(OTf)_2$ in [bmim][PF_6] at different time interval it was found that in first 30 minutes pyrazoline (**3a**) was the major product, which got oxidized to pyrazole in the reaction as time progresses. We thus decided to synthesize the pyrazolines using this protocol in order to evaluate them in our biological assay. The reaction of **1**

and hydrazines **2** afforded 1,3-pyrazolines **3a–o** *via* a one-pot addition–cyclocondensation process in good to high yields (60-84%). Several α,β-unsaturated carbonyl compounds with both electron-rich and electron-deficient arenes were successfully applied to this reaction. The results of pyrazoline synthesis are summarized in Table 3. The chemical structures of all synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR, and high-resolution mass spectroscopy (Supporting information).

Table 3. Synthesized 1,3,5-triarylpyrazolines (**3a-n**).

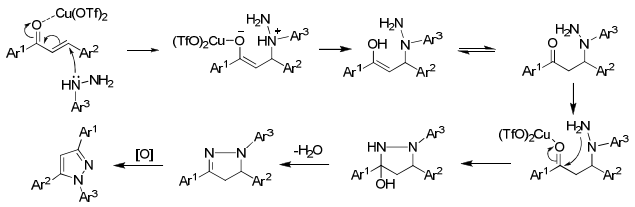


Compd	R^1	R^2	R^3	Time (Min.)	Yield (%)[a]
3a	H	H	4-$C(CH_3)_3$	120	84
3b	H	H	2-CH_3	120	66
3c	H	H	3,4-Cl	90	77
3d	H	H	3-Cl, 4-CH_3	180	68
3e	4-OMe	H	3,4-Cl	150	72
3f	4-OMe	H	3-Cl, 4-CH_3	120	78
3g	3-OMe	4-CH_3	3,4-Cl	120	60
3h	4-CH_3	4-CH_3	3,4-Cl	120	72
3i	4-CH_3	4-CH_3	3-Cl, 4-CH_3	120	74
3j	4-CH_3	4-CH_3	4-$C(CH_3)_3$	120	79
3k	2-F	4-Cl	4-$C(CH_3)_3$	105	63
3l	2-F	4-Cl	2-CH_3	120	65
3m	4-NO_2	4-OMe	4-$C(CH_3)_3$	120	72
3n	4-NO_2	4-OMe	3-Cl, 4-CH_3	60	80

[a]Isolated yield

Based on the intermediate formed (**3a**) and structure of the product (**4a**) as analyzed by NMR, the reaction is proposed to proceed through the sequential steps as shown in Scheme 2. The first step is believed to be 1,4-addition of hydrazine to chalcone mediated by copper (II) triflate. The 3-hydrazino ketone undergoes nucleophilic addition followed by elimination to give 1,3,5-triarylpyrazolines derivative. 1,3,5-Triaryl-pyrazolines derivatives undergo oxidative aromatization in the presence of copper (II) triflate to yield corresponding 1,3,5-triarylpyrazole derivatives. It appeared that ionic liquid helps in stabilization of charged intermediate generated by coordination of $Cu(OTf)_2$ to carbonyl of chalcone and thereby increases electrophilicity of chalcone.

Further, we investigated the possibility of recycling of the catalyst. After performing the first cycle, the product was extracted with ethyl acetate/hexane mixture, and $Cu(OTf)_2$ in ionic liquid was properly dried under pressure. Furthermore, the fresh chalcone and 4-tert-butyl phenylhydrazine hydrochloride were charged under same conditions. The above procedure was repeated four times to give **4a** in high yields (82, 80, 78, and 79%) without much loss of catalytic activity.



Scheme 2. Proposed mechanism for synthesis of 1,3,5-triarylpyrazole.

To evaluate the anticancer activity of synthesized compounds, all derivatives (**4a-o** and **3a-n**) were evaluated for their inhibitory activity on the proliferation of human ovarian adenocarcinoma (SK-OV-3), human colon adenocarcinoma (HT-29), and human cervical adenocarcinoma (HeLa) cells. Doxorubicin (Dox) and DMSO were used as positive and negative controls, respectively. The antiproliferative activity results of compounds **4a-o** and **3a-n** at 50 µM after 72 h incubation are shown in Figures 2 and 3, respectively.

Figure 2 shows that among all 1,3,5-triarylpyrazoles derivatives, compounds **4c**, **4e**, **4f**, **4g**, **4h**, **4i**, and **4k** inhibit the proliferation of HeLa cells by 50%, 55%, 45%, 39%, 54%, 42%, and 50%, respectively. However, they did not exhibit significant inhibitory potency in HT-29 and SK-OV-3 cells.

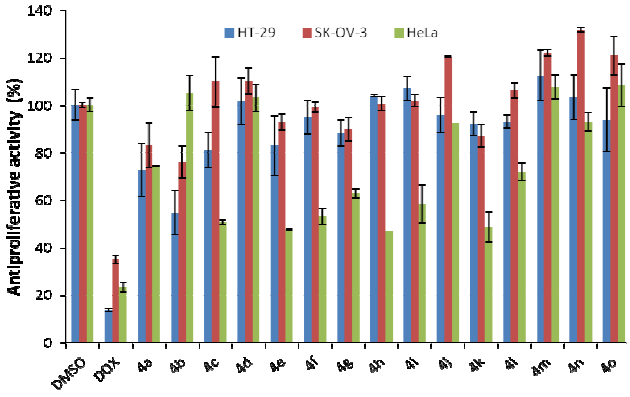


Figure 2. Antiproliferative activity of **4a-o**.

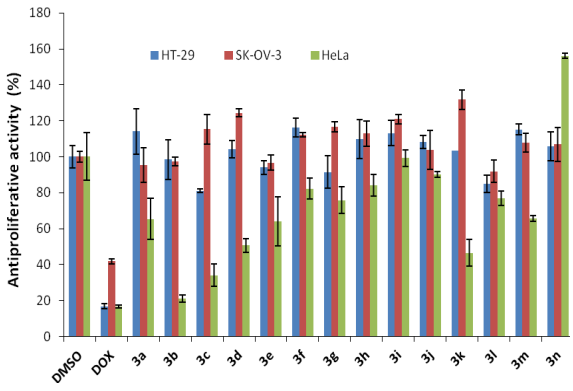


Figure 3. Antiproliferative activity of **3a-n**.

Furthermore, 1,3,5-triarylpyrazolines derivatives (**3a-n**) were examined for their inhibitory activity against the proliferation of HT-29, SK-OV-3, and HeLa cells. Most of the derivatives showed cell specific dependent inhibitory activity. Several derivatives showed high to weak antiproliferative activity against HeLa cells after 72 h incubation. Compounds **3c**, **3d**, **3e**, **3k**, **3l**, and **3m** inhibited the proliferation of HeLa cells by 62%, 50%, 35%, 58%, 23%, and 40%, respectively. 2-Methylsubstituted compound **3b** showed the highest potency by 80% inhibition of HeLa cells. 1,3,5-Triarylpyrazolines derivative showed modest to weak potency in SK-OV-3 and HT-29 cells. Among all derivatives, compound **3b** showed comparable potency to Dox in HeLa cells. Further modification on the chemical structure of this derivative could lead to the synthesis of a promising candidate that selectively target HeLa cells.

In summary, we have developed a simple and efficient and environmentally friendly protocol for the synthesis of 1,3,5-triarylpyrazole in [bimm][PF_6] ionic liquid mediated by $Cu(OTf)_2$. The reaction protocol exhibited tolerance with different functional groups, generating pyrazoles in good to high yields (71-82%) without any requirement for additional reagent for the oxidation of *in situ* generated pyrazolines. The catalyst can be reused up to five cycles without much loss in catalytic activity. The pyrazoles (**4a-o**) and pyrazolines (**3a-n**) were evaluated for antiproliferative activity. Compound **3b** inhibited cell proliferation of HeLa cells by 80% at a concentration of 50 μM. All other synthesized derivatives exhibited a modest inhibition against the proliferation of SK-OV-3, HT-29 and HeLa cells. Further structure-activity relationship studies are required for optimizing antiproliferative activities of these classes of compounds.

Acknowledgments

We thank University Grant Commission, New Delhi Project # 39-733/2010 (SR) and the National Science Foundation, Grant Number CHE 0748555, and American Cancer Society grant number RSG-07-290-01-CDD American Cancer Society Grant #RSG-07-290-01-CDD for the financial support. VKR is thankful to CSIR, New Delhi for SRF. We also thank the National Institute of General Medical Sciences of the National Institutes of Health under grant number 8 P20 GM103430-12 for sponsoring the core facility.

Supplementary data

Supplementary data containing experimental procedures for cell culture, and physical and spectral for compounds (**4a-o** and **3a-n**) can be found in the online version of this article.

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