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Simple, fast and efficient synthesis of β-keto esters from the esters of heteroaryl compounds, its antimicrobial study and cytotoxicity towards various cancer cell lines

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ABSTRACT

A series of β-keto esters were synthesized from heteroaryl esters and ethyl acetate using LiHMDS as base at −50 to −30 °C. The increase in yields of cross condensed product were observed and the percentage of self condensed product was reduced drastically by applying the suitable base (LiHMDS), solvent and the minimum amount of ethyl acetate. All these β-keto esters were characterized using ^{1}H NMR, ^{13}C NMR and mass spectral data. A plausible mechanism is also depicted to prove the formation of trans-esterified products. All the synthesized compounds were subjected to test for their cytotoxicity towards various cancer cell lines and also tested for their antimicrobial activity towards various bacterial and fungal strains and some of them were found to have promising activity.

Many hetero cyclic compounds such as pyrazolones, pyrimidines, thiazoles, oxazoles and isooxazoles were prepared traditionally from their corresponding β-keto esters. There are methods to synthesize β-keto esters from esters[1,2], aldehydes[3], but these methods have main limitation in varying the substituents. A number of methods such as acylation of enolates of malonates[4,5], acylation of meldrum's acid[6–9], mixed malonate esters[10,11] and bistrimethylsilylmalonate[12,13], often, a chelating effect has been employed to lock the enolate anion of malonate using lithium and magnesium salts[14,15], however these methods suffer by inconsistent yield in case of aliphatic acylation. The synthesis of β-keto esters from ketones like caboxylation of ketone enolates[16,17] using carbon dioxide and carbon monoxide sources in the presence of palladium or transition metal catalysts. The most general and simple method to synthesize β-keto esters in these days is the reaction of dimethyl or ethyl carbonate with ketone in the presence of strong base also suffers by long reaction time, inconsistent yield and use of excess reagent.

Ohta et al.[18] synthesized β-keto esters by treating the esters with the enolate of ethyl acetate (or) *tert*-butyl acetate in the refluxing conditions but these transformations also require the large quantity of ethyl acetate, excess of strong base (sodium ethoxide, *tert*-butoxide, NaH) and refluxing conditions with longer time under the usual reaction conditions. Apart from that these transformations suffer by large quantity of by products (formed by the self condensation of ethyl acetate in particular) and require tedious work up procedures to remove the same. β-Keto esters of N-protected amino acids were efficiently prepared by treating the corresponding esters with the enolate of *tert*-butyl acetate (prepared using LDA in THF) at −50 to −78 °C.[19] Reactions of these β-keto-*tert*-butyl esters in the preparation of pyrazolones, pyrimidones, isooxazolones with hydrazines, amidines and hydroxylamine respectively, always suffers by the steric effect of *tert*-butyl group. β-Keto methyl (or) ethyl esters are the most preferred esters for these transformations.

Unfortunately the existing methods do not produce the β-keto ester of heteroaryls from the heteroaryl esters in good yield. In most of the cases we did not get the product when we tried to synthesize β-keto esters of some heteroaryl esters by applying the existing methods. Reactivity of the esters mainly depends upon the electrophilicity. Electrophilicity of the heterocyclic esters will change drastically by changing the substituents. So it is not easy to avoid the self-condensed product. The importance of these esters, led us to develop a simple method to synthesize β-keto ethyl esters of heteroaryls in good yield by changing the base, reagent, equivalence of base reagent and reaction conditions.

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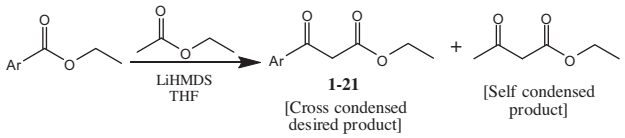


Scheme 1. Efficient synthesis of β-keto esters of heteroaryl compounds **1**–**21**.

Table 1
Effect of solvent and base on the yield of **8**

Base	Ethylacetate (equiv)	Solvent	Temp	Yield (%)
LiHMDS (1.0 M THF) (1 equiv)	3	THF	−78 °C	18
LDA (1.0 M THF) (3 equiv)	3	THF	−78 °C	56
LiHMDS (3 equiv)	7	Toluene	−78 °C	78
NaH (2 equiv)	50	THF	−78 °C	9
NaOMe (2 equiv)	75	THF	Reflux	0
KOtBu (3 equiv)	10	THF	25 °C	0
LiHMDS (1.0 M THF) (3.5 equiv)	7	THF	−50 to −30 °C	94

To overcome the difficulties present in the synthesis of β-keto esters of heteroaryl esters as the continuation of our interest towards the synthesis of β-keto esters and pyrazolones[20–30], we attempted the synthesis of **1–21** from their corresponding heteroaryl esters and ethyl acetate using Scheme 1. Then the yield of cross condensed product was optimized with reference to the compound **8** by changing the equivalence of base, solvents, ethyl acetate and the results are summarized in Table 1.

Our initial attempt with 1 equiv of LiHMDS as base was disappointing and produced only 18% of product. Reaction with 3 equiv of LDA and 3 equiv of ethyl acetate gave the moderate yield. When the reaction was carried out with hydrocarbon solvent like toluene and LiHMDS as base, yield got improved to 78%. Reaction using NaH gave only 9% yield. Other bases NaOMe and *tert*-BuOK did not give the product at all. Finally a reaction with 3.5 equiv of LiHMDS and 7 equiv of ethyl acetate at −50 to −30 °C produced the better yield. By applying the suitable base, solvent and the minimum amount of ethyl acetate we have increased the yields of the cross condensed product and the percentage of self condensed product was reduced drastically. After finding the optimized

Table 2
Synthesis of β-keto esters by cross-Claisen condensation

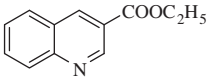
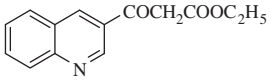
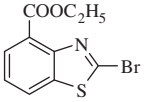
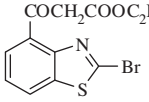
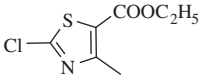
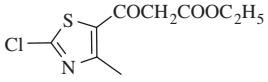
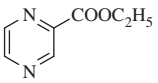
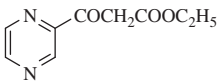
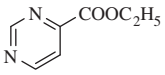
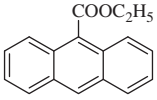
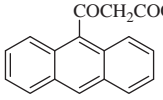
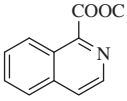
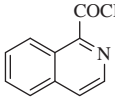
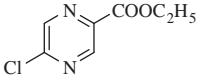
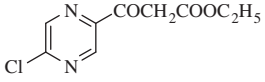
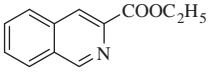
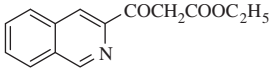
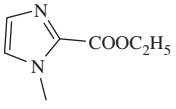
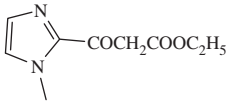
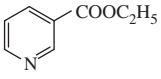
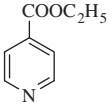
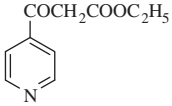
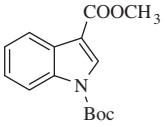
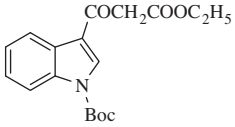
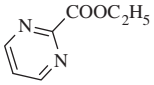
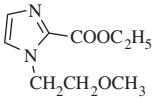
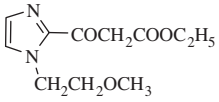
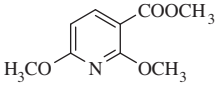
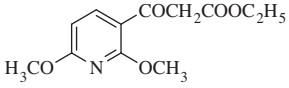
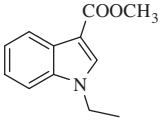
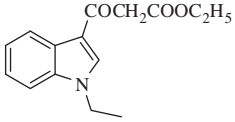
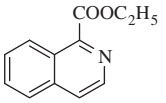
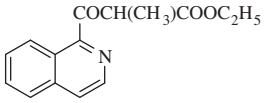
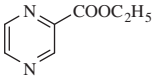
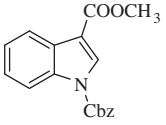
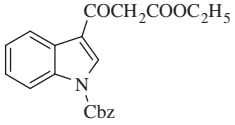
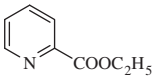
Compd	Ester	Product	Yield (%)
1			94
2			97
3			91
4			88
5			0 [a]
6			0 [a]
7			85
8			83
9			72
10			66
11			92
12			87

Table 2 (*continued*)

Compd	Ester	Product	Yield (%)
13			56
14			85
15			76
16			0[a]
17			0[a]
18			89
19			84
20			0[a]
21			78

[a] Percentage of products in crude LC–MS.

condition, the same methodology has been applied to synthesis various β-keto esters (**1–21**) using different heterocyclic esters to prove the generality of the reaction (Table 2). All these β-keto esters (**1–21**) were characterized through ^{1}H NMR, ^{13}C NMR and LC–MS spectral data. The experimental results clearly reveal that heterocyclic esters with even lower to moderate electrophilicity undergoes the condensation smoothly without unwanted by-products to the greater extent. Esters of different heterocyclic compounds (quinoline, isoquinoline, benzo-thiazole, thiazole, pyridine, pyrazine, pyrimidine, imidazole, indole) were converted in to the corresponding β-keto esters in good yield. In the case of **16** and **17** the unexpected and interesting trans-esterification was observed instead of desired β-keto esters.

This may be due to the weaker electrophilicity of the ester. Presence of two methoxy groups in **16** decreases the electrophilicity of the esters by donating the electrons. In case of **17** the electron donating nature of *N*-ethyl group increases the electron density in the molecule, causes the complete trans-esterification in this particular case. In case of **13** and **20**, we observed unexpected deprotection of the Boc and Cbz groups. In compound **13** the Boc group was partially deprotected and produced the Boc-cleaved starting material and the desired product in 56% yield, whereas in the case of **20** the Cbz group was completely cleaved and gave the Cbz-cleaved starting material. Cleavage of the Boc and Cbz groups were observed within 10 min. This may be due to the labile nature of the protecting groups particularly in indole systems. In

Table 3
Antibacterial activity of the newly synthesized compounds (zone of inhibition in mm, MIC in mg/mL given in parenthesis, standard = ciprofloxacin)

Compd	*S. aureus*	*E. coli*	*P. aeruginosa*	*K. pneumonia*
2	21 (6.25)	22 (6.25)	22 (6.25)	23 (6.25)
7	22 (6.25)	19 (6.25)	20 (6.25)	22 (6.25)
8	23 (6.25)	24 (6.25)	21 (6.25)	20 (6.25)
9	16 (12.5)	19 (12.5)	22 (12.5)	23 (12.5)
14	22 (6.25)	24 (6.25)	22 (6.25)	23 (6.25)
Standard	23 (6.25)	32 (6.25)	28 (6.25)	23 (6.25)

Table 4
Antifungal activities of the newly synthesized compounds

Compd	*Trichophyton*	*Penicillium*	*A. flavus*	*A. fumigates*
2	17 (12.5)	17 (12.5)	19 (12.5)	18 (12.5)
7	17 (12.5)	18 (12.5)	15 (12.5)	18 (12.5)
8	25 (6.25)	24 (6.25)	22 (6.25)	26 (6.25)
9	23 (6.25)	25 (6.25)	27 (6.25)	23 (6.25)
14	18 (12.5)	17 (12.5)	22 (12.5)	25 (12.5)
Standard	27 (3.125)	23 (6.25)	27 (3.125)	26 (6.25)

Zone of inhibition in mm, MIC in mg/mL given in parenthesis, standard = ciclopiroxolamine.

Table 5
Cytotoxic activity of the cross condensed β-keto esters (**1**–**21**)

Compd	Screening concentration in μM	H460 (non-small cell lung cancer)	HCT116 (colon cancer)	ACHN (renal cancer)	Calu1 (lung cancer)	Panc-1 (pancreatic cancer)
1	10	20	28	20	23	26
2	10	14	39	31	19	27
3	10	24	48	37	32	29
4	10	20	30	34	16	27
5	10	13	20	15	12	35
7	10	34	26	30	30	35
8	10	34	40	30	31	14
9	10	17	29	30	21	23
10	10	14	11	30	30	35
11	10	19	30	30	30	35
12	10	10	39	31	12	10
13	10	39	33	30	20	23
14	10	20	23	10	17	37
15	10	14	28	21	24	26
18	10	19	24	25	20	30
19	10	87	90	93	94	97
21	10	36	21	25	22	27
Std 1	Flavopiridol (1000 nM)	71	78	71	88	74
Std 2	Gemcitabine (1000 nM)	73	74	73	71	79

Table 6
Cytotoxic activity of the cross condensed β-keto ester **19** at varying concentrations and its IC_{50} (μM)

Concn (μM)	H460	HCT116	ACHN	Calu1	Panc-1	MCF10A
0.01	3.6	9.4	−23.4	1.5	8.3	−15.2
0.03	6.7	18.8	−28.9	2.9	10.0	−14.1
0.1	14.2	26.9	19.0	6.7	13.6	−9.5
0.3	38.6	32.3	27.9	9.4	15.9	−6.8
1	44.6	44.8	46.2	22.8	29.2	6.3
3	55.0	92.2	51.6	60.8	67.8	24.0
10	100.0	100.0	67.4	85.3	92.1	39.1
30	100.0	100.0	82.3	99.2	100.0	82.8
IC_{50} (μM)	3	1.3	3.0	2.8	2.9	21.2

these cases (**13** and **20**), trans-esterified products were not observed even in trace amount.

We have investigated newly synthesized β-keto esters for their antibacterial activity against *Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonas aeruginosa* (ATTC-27853), and *Klebsiella pneumonia* (recultured) bacterial strains by the disc diffusion method.[31–33] Among the tested compounds, compounds **2**, **7**, **8** and **14** are highly active against all the bacterial stains tested (though all the compounds are subjected to the study, only the compounds with good inhibition results given in Table 3). Compound **9** was moderately active against all the stains and the β-keto esters of heterocyclic compounds shown a good bacterial activity in particular β-keto esters of benzothiazole, isoquinoline, pyrazine are equipotent or even higher than the standard used in some cases. Pyrimidine 2-β-keto ester is moderately active. From the impressive results of the biological activity further conversion of these β-keto esters into the heterocyclic compounds are required.

Similarly all these newly synthesized β-keto esters were screened for their antifungal activity against *Aspergillus flavus* (NCIM No. 524), *Aspergillus fumigates* (NCIM No. 902), *Penicillium marneffei* (recultured) and *Trichophyton mentagrophytes* (recultured) in DMSO by the serial plate dilution method.[34,35] Antifungal activity was determined by measuring the inhibition zone and compared with the standard ciclopiroxolamine (though all the compounds are subjected to the study only the compounds with good inhibition results given in Table 4). Among the tested compounds, compounds **8** and **9** are highly active against all the stains tested. Compounds **2**, **7** and **14** were moderately active against all the stains.

The fungicidal activity of β-keto esters of heterocyclic compounds are very good, in particular β-keto esters of isoquinoline

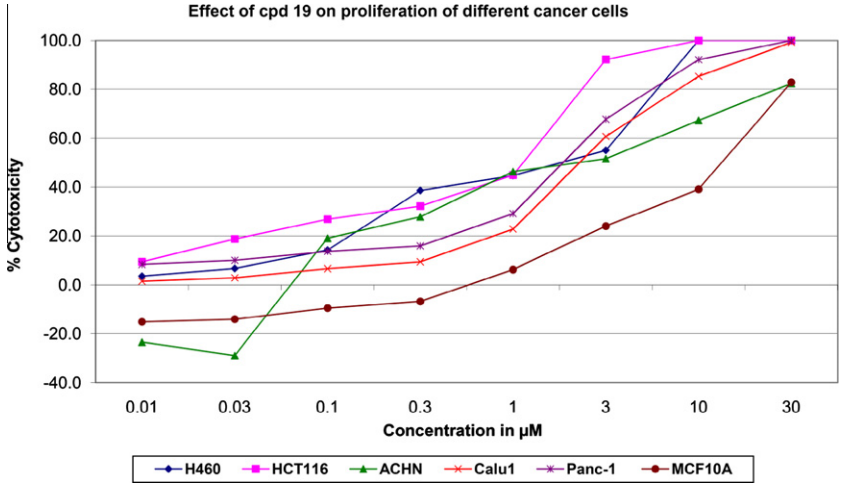


Figure 1. Effect of compound **19** on proliferation of different cell lines.

and pyrazine are equipotent or even higher than the standard used. Benzothiazole and 2-pyrimidine β-keto esters were moderately active. Because of the good fungicidal activity further conversion of these β-keto esters into the heterocyclic compounds are required.

All the synthesized compounds **1–21** have been subjected to WST-1 cytotoxicity assay. A panel of five cancer cells representing multiple cancers of clinical relevance were obtained from ATCC (American Type Culture Collection), namely; ACHN (human renal cell carcinoma), Panc-1 (human pancreatic adenocarcinoma), H460 (human non-small cell lung carcinoma), Calu-1 (human lung carcinoma) and HCT-116 (human colon cancer). Cells were maintained in DMEM (Dulbecco's modified Eagle's medium) medium containing 10% heat inactivated Fetal Bovine Serum and kept in humidified 5% CO_2 incubator at 37 °C. Logarithmically growing cells were plated at a density of 5×10^3 cells/well in a 96-well tissue culture grade micro-plate and allowed to recover overnight. The cells were challenged with varying concentration of compounds for 48 h. Control cells received standard media containing dimethylsulfoxide vehicle at a concentration of 0.2%. After 48 h of incubation, cell toxicity was determined by CCK-8 (Cell Counting Kit-8) reagent (Dojindo Molecular Technologies, Inc. Maryland, Japan); (WST-1 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfo phenyl)]-2*H*-tetrazolium, monosodium salt assay). In accordance with the manufacturer's instructions[36], 5 μL/well CCK-8 reagent was added and plates were incubated for 2 h. Cytotoxicity of all the compounds have been determined by measuring the absorbance on Tecan Sapphire multi-fluorescence micro-plate reader (Tecan, Germany, GmbH (Gesellschaft mit beschränkter Haftung; English: company with limited liability) at a wavelength of 450 nm corrected to 650 nm and normalized to controls. Each independent experiment was performed thrice and tabulated in Table 5. The compound **19** was found to be inhibitive against all these cell lines and hence its % cytotoxicity for various concentrations has been observed (Table 6). By plotting these values (Fig. 1) the IC_{50} value for cancer cell lines has been found as 3, 1.3, 3, 2.8, 2.9 and 21.2 μM for H460, HCT116, ACHN, Calu-1, Panc-1 and MCF10A cancer cell lines respectively. The study reveals the promising activity of **19** towards the above mentioned cancer cell lines and further investigations are in need.

In summary simple, fast and an efficient method has been developed to synthesis β-keto esters from their corresponding heteroaryl esters in mild conditions using LiHMDS as a base. All these compounds have been subjected to WTS-1 cytotoxicity assay and antimicrobial screening; compounds **19** and **8** have been found to be the promising candidates for anti cancer activity and microbial agent respectively and hence further attention of these compounds are in need.

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Supplementary data

Supplementary data (the experimental data along the spectral evidence) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.04.008.

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