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Synthesis and cytotoxicity evaluation of alpha beta unsaturated carboxylic acids of thiophene analogs by using polymer supported microwave-assisted method

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Abstract: α- β unsaturated carboxylic acids containing a heterocyclic moiety is one of the most potent class of bioactive compounds whose speedy generation through novel synthetic techniques has become an enigma for the synthetic chemists. This research project demonstrates a novel method for the synthesis of these compounds using polymer-supported microwave-assisted methodology carried out through one-pot multicomponent reaction. Both soluble and insoluble polymers have been used and their results are comprehensively analyzed. Moreover, the compounds are characterized through spectral analysis like FTIR, GC-MASS, ^{1}HNMR **S**pectroscopy. The cytotoxicity of synthesized compounds is evaluated through MTT assay using HEPG 2 cells.

Keywords: αβ unsaturated carboxylic acids, polymer-supported, microwave-assisted, solvent free, one-pot, multicomponent, anti-cancer activity.

INTRODUCTION

Heterocyclic compounds present such a class of pharmacophores that are chemotherapeutic in nature. A further therapeutically significant division of these compounds are the α-β unsaturated acids that possess importance in both medicine as well as biochemistry. Indeed, these acids are the essential units present in a lot of natural sources such as foods, fruits, pheromones, herbs etc (Mishra *et al*., 2012).

Heterocyclic moieties can enhance the potency of unsaturated carboxylic acid. As a result of it, the industrial, medical and biochemical applications of these compounds increases triple fold which has led it to form the basis of organic synthetic chemistry (Ehsan *et al*., 2016). However, there is a need to focus on the development of better routes for the efficient and eco-friendly synthesis of these compounds as the old techniques that basically relied on the slow conventional heating system and the use of carcinogenic solvents are not acceptable anymore. Moreover, another hazard of these synthetic methodologies is depicted by the numerous isolation steps that is required for the isolation of the final products and their purification (Ehsan *et al*., 2018).

Thus, to overcome these problematic issues that goes against the norms of green chemistry and harms environment to no extent, it is tried to devise such a methodology that focuses on the conservation of time, energy and healthy environment (Ehsan *et al*., 2018). To achieve this purpose, firstly we have switched our mode of synthetic route from the traditional heating system to the one that employs the use of microwaves (Gedye *et al*., 1986). Indeed, the protocol will be carried out using both conventional and microwave-assisted synthesis so a comparison between the two methodologies can be done in terms of time, purity and percentage yield (Polshettiwar *et al*., 2008). In case of microwaves, the time usually reduces from many hours to only a few minutes and seconds (kappe *et al*., 2009).

Moreover, the product formed is higher in purity as nearly no side products are formed due to which the amount of desired product formed is greater in quantity too (Sagheer *et al*., 2018).

Moreover, in the present study microwave-assisted and polymer supported organic synthesis is carried out succesfully. The use of polymers has improved the isolation of the products. (Leznoff *et al*., 974). Polymer supports can be easily removed by simple filtration leaving behind the pure product. Other processes such as solvent extraction, distillation or column chromatography are not required etc (Gravert *et al*., 1997). Filteration process can simply be done using distilled water without any other solvent (Larhed *et al*., 2011).

The polymer supports have definite properties for organic synthesis. (kobayashi *et al*., 1998). The polymer supported synthesis has no side reactions. (Das *et al*., 2002).

The most commonly used polymer supports are polymerase, polyvinylalcohol, polystyrene, polyvinylchlorides polyethylene glycol poly amino acids etc. (Grag *et al*., 2018). This protocol is useful for healthy environment (Haag *et al*., 2001) as well as to give good yield, improved reaction rate and maximum catalytic activity with minimum use of energy sources. This mode of synthesis also offers a chance to improve

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transformation of specific functional groups .Also synthesis is conducted in open vessels that releases the high pressure developed in reaction vessels which is another major advantage of polymer supported MW assisted methods. The cytotoxicity of Thiophene and its derivatives is evaluated through MTT assay (Gangarapu *et al* 2012, Aguiar *et al* (2016).

To sum up, this research based on the use of and comparison between four methodologies that are: conventional methodology, MW assisted protocol, MW assisted with soluble polymer support and MW assisted with insoluble polymer support (Buchmeiser *et al*., 2006).

MATERIALS AND METHODS

Chemicals and instrumentation

Chemicals were purchased from Merck. Microwave oven was used for reactions (DW-180, 2450 MHz). Gallenkamp melting point apparatus was used for melting points. UV spectras were recorded on“HitachiU-2800” double beam spectrophotometer. Midac FT-IR spectrophotometer (M2000) the range of 400-4000cm-^{1} was used Mass spectral analysis of the synthesized compounds was taken by GCMS SchimadzoQP-2010 Spectrometer.

General procedure for the conventional synthesis of 3-(2-thienyl) propen-2-oic acid and its derivatives

In a two-neck, round bottom flask, equal amounts of thiophene carboxaldehyde derivatives (0.01M) and malonic acid (0.01M) were dissolved in ethyl alcohol (10 ml). The reaction mixture was then refluxed for 8 hours. Silica gel plates were used to perform thin layer chromatography at regular intervals to check the progress of reaction. On the completion of the reaction as indicated by TLC, the precipitates formed were filtered, washed and recrystallized using ethanol.

General procedure for the solvent mediated microwave-assisted synthesis of 3-(2-thienyl) propen-2-oic acid and its derivatives

Equal amounts of thiophene carboxaldehyde derivatives (0.01M) and malonic acid (0.01M) were dissolved in ethyl alcohol (10ml). It was then subjected to microwave radiations for 2-4 minutes. TLC was used to monitor the progress of the reaction at regular intervals. The crude product obtained after the completion of reaction was eluted with ethanol (5.15ml) and the product in solid state was obtained by distillation under reduced pressure. The solvent used was also recovered. The solid product obtained was recrystallized from ethanol.

General procedure for the solvent free microwave-assisted synthesis of 3-(2-thienyl) propen-2-oic acid and its derivatives using soluble polymer support

Thiophene carboxaldehyde derivatives (0.01M) and malonic acid (0.01M) were mixed in equal amounts in soluble polymer support i.e. polyethyleneglycol (10ml) and reaction mixture was heated in microwave oven for 2-4 minutes. TLC was used to monitor the progress of reaction. The crude product obtained was eluted with ethanol (5.15ml). The product in solid state was obtained by distillation under reduced pressure which was recrystallized to get the pure product.

General procedure for the solvent free microwave-assisted synthesis of 3-(2-thienyl) propen-2-oic acid and its derivatives using insoluble polymer support

Thiophene carboxaldehyde (0.01M) and malonic acid (0.01M) were mixed in equal amounts in insoluble polymer support i.e. polystyrene (10g) and reaction mixture was heated in microwave oven for 2-4 minutes. TLC was used to monitor the progress of reaction. The crude product obtained was eluted with ethanol (5.15ml). The product in solid state was obtained by distillation under reduced pressure which was recrystallized to get the pure product.

Spectroscopic analysis of the compounds fabricated through solid-supported microwave-assisted protocol

Compound 3a: 3-(2-thienyl) propen-2-oic acid

Light yellow solid; m.p: 143°C; Molecular formula: $C_7H_6O_2S$; Molecular weight: 154 gmol-1; IR (KBr, cm-1): v 3352 (-OH stretching), 2974 (C-H str. of aromatic ring), 1732 (C=O), 1635(C=C), 1504, 1442 (C=C str. of aromatic). 1H NMR [$CDCl_{3,}$ 600 MHz, δ in ppm):]: 6.88 (d, 1H, CH),7.4 (d, 1H,CH), 7.5 (m, 1H, CH), 7.7 (d, 1H, CH), 7.9 (doublet, 2H, CH), 11.6 (s, 1H, OH); MS (m/z): 154 (M+), 155 (M+1), $[C_3H_3O_2]^+$, 60$[C_2H_4O_2]$, 43$[C_3H_7]^+$

Compound 3b: 3-(5-methyl-2-thienyl) propen-2-oic acid

Pale yellow solid; m.p: 165°C; Molecular formula: $C_8H_8O_2S$; Molecular weight: 168 gmol-1; IR (KBr, cm-1): v 3356 (-OH stretching), 2974 (C-H str. of aromatic ring), 1732 (C=O), 1651(C=C), 1442 (C=C str. of aromatic). 1H NMR [$CDCl_{3,}$ 600 MHz, δ in ppm):]: 6.89 (d, 1H, CH), 7.2 (d, 1H,CH), 7.4 (m, 1H, CH), 7.8 (d, 2H, CH), 11.6 (s, 1H, OH); MS (m/z): 168 (M+), 169 (M+1), 122 $[C_7H_6S]$, 57$[C_4H_9]^{+\cdot}$

Compound 3c: 3-(5-chloro-2-thienyl) propen-2-oic acid

Yellow solid; m.p: 180°C; Molecular formula: $C_7H_5SO_2Cl$; Molecular weight: 188 gmol-1; IR (KBr, cm-1): v 3350 (-OH stretching), 2974 (C-H str. of aromatic ring), 1732 (C=O), 1655(C=C), 1502 (C=C str. of aromatic). 1H NMR [$CDCl_{3,}$ 600 MHz, δ in ppm):]: 6.88 (d, 1H, CH), 7.5 (m, 1H, CH), 7.9 (d, 2H, CH), 11.6 (s, 1H, OH); MS (m/z): 188 (M+), 189 (M+1). 117$[C_4H_2SCl]$

Compound 3d: 3-(5-nitro-2-thienyl) propen-2-oic acid

Yellow solid; m.p: 185°C; Molecular formula: $C_7H_5O_2NO_2$; Molecular weight: 199 gmol-1; IR (KBr, cm-1): v 3350 (-OH stretching), 2974 (C-H str. of aromatic ring), 1732 (C=O), 1655(C=C), 1502 (C=C str. of aromatic). 1H NMR [$CDCl_{3,}$ 600 MHz, δ in ppm):]:

6.88 (d, 1H, CH), 7.5 (m, 1H, CH), 8.0 (d, 1H, CH), 8.4 (d, 1H, CH), 11.6 (s, 1H, OH); MS (m/z): 199 (M+), 183 [$C_6H_5NO_2$], 45[COOH].

Anticancer activity

MTT assay was used to determine anticancer activity of synthesized compounds. The tetrazolium-based colorimetric assay (MTT test) measures only *in vitro* living cells and the results are directly related to the number of viable cultured cells. (Gulipalli *et al.*, 2017).

Liver cells were seeded in 96 microplates. Different concentrations of synthesized derivatives were added along with control. In each well MTT prepared solution was added for three to four hours. In live healthy cells MTT was converted in to purple formazine. The resultant compounds were dissolved in DMSO solvent. Their absorbance was measured at 570nm and 620nm. Proliferation was measured by using this formula

$$\%\ \text{cell viability} = \frac{\text{Treated570nm-620nm}}{\text{Control 570nm-620nm}} \times 100$$

RESULTS

This research project describes the organic synthesis of alpha and beta unsaturated carboxylic acid of thiophene and its derivatives 3a-d by the use of three different methods of synthesis i.e. (schem1).

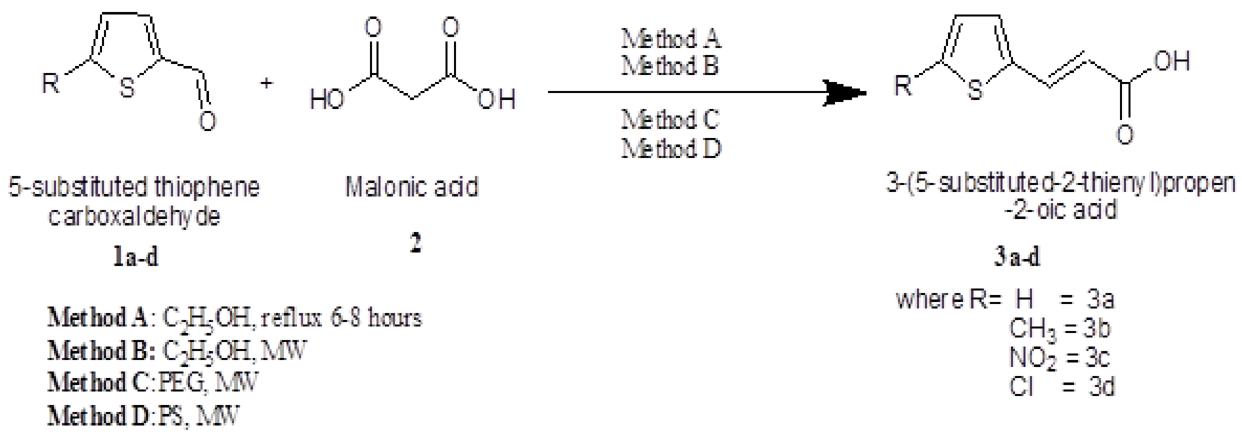
The compounds generated had high medicinal value and have been reported as highly potent in the literature. The formation of these compounds were broadly classified in three categories as described above in order to do a comparative analysis between these modes of synthesis and distinguish the most suitable route in terms of better yield and lower environmental pollution along with the conservation of time and energy (table 1). Amongst these methods of synthesis, the polymer supported methodology was found to be the best one as it diminished the use of solvent and also gave higher yields in minimum time. Furthermore, there were two polymer supports used namely polyethyleneglycol which was a soluble support and polystyrene which was an insoluble support. The amount of energy required to carry out the reactions and the span of time were same in both supports but a higher yield was obtained in case of soluble in contrast to insoluble support.

DISCUSSION

Alpha beta unsaturated acids containing heterocyclic ring are synthesized by polymer supported microwave assisted synthesis with good yields. Structures of the compounds were confirmed by 1HNMR, Mass and FTIR spectroscopy. FTIR spectroscopic data confirmed the formation of product peak due to peak between 1630-1660cm^{-1} shows the presence of conjugated alkene. The characteristic stretching of OH of carboxylic acid was also observed around 3350cm^{-1} whereas the C=O stretching was found at values near to 1730cm^{-1}. In addition, peak in between 2970 resulted due to the presence of =C-H group while the ones at 600-650 were as a result of the C-S bond present in the thiophene ring. In HNMR peak around 11.6 is due to carboxylic acid proton while peak near 6.8 and 7.2-7.8 is due to presence of proton attached to double bonded carbon. On the other hand, the mass spectra of the synthesized compounds showed not only molecular ion peak but also characteristic fragment ion peaks including the loss of side chain from thiophene ring in nearly all the compounds.

The anticancer acitivity carried out depicted that cell underwent cell lysis when they were exposed to various dilutions of synthesized compounds. As a result, their number reduced significantly in contrast to control cells. It was also observed that the higher the dilution was, the

1. Conventional 2. Microwave-assisted 3. Polymer-support (soluble and insoluble)



Scheme 1: Conventional synthesis of 3-(2-thienyl) propen-2-oic acid and its derivatives

lower stress the cells had to face. The cells got impaired and were destroyed at six different concentrations of all derivatives which proved their cytotoxic nature towards cancer cells (table 2, fig 1).

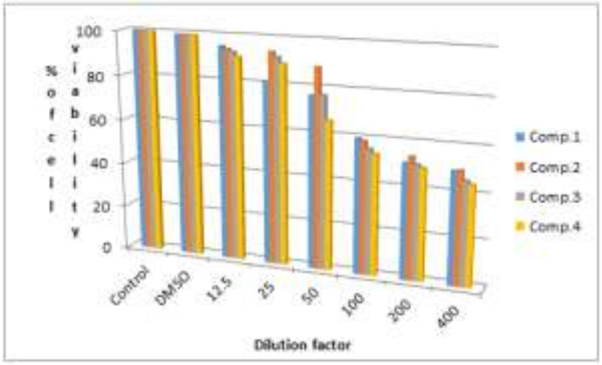


Fig. 1: Cytotoxicity on HepG2 cells after treatment

CONCLUSION

This research work focused on determining the advantages of using polymer support in the synthesis of versatile derivatives of alpha beta unsaturated carboxylic acid of thiophene. The results obtained at the end of present study demonstrated that the use of polymer support in synthesis presents a beneficial methodology in organic synthesis as it omits the use of solvent and the work was speeded up by the use of microwaves to carry out the reaction. Moreover, the yields obtained were quite better and free of impurities as well. Furthermore, two different polymer supports were used in our study and the use of soluble support resulted in slight increase of the yield as compared to the insoluble support while all the other factors were kept constant such as reaction time, energy and the absence of solvent. The compounds formed were also characterized through spectral analysis to confirm their formation and their anticancer activity is investigated.

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Table 1: Melting point, percentage yield and reaction time of the synthesized compounds

Compounds	Conventional Method			MW-assisted Method			MW-assisted Polymer supported Method					
							Soluble			Insoluble		
	Time (hrs)	Yield (%)	M.P (ºC)	Time (mins)	Yield (%)	M.P (ºC)	Time (mins)	Yield (%)	M.P (ºC)	Time (mi)	Yield (%)	M.P (ºC)
3a	8	75	144	2	89	143	2	91	143	2	90	144
3b	8	76	165	2	91	164	2	92	165	2	90	165
3c	8	79	180	2	92	181	2	95	180	2	92	180
3d	8	78	185	2	95	185	2	96	184	2	95	185

Table 2: Anticancer activity of the synthesized compounds

Dilution factor	%age 0f cell viability Compound 1	%age 0f cell viability Compound 2	%age 0f cell viability Compound 3	%age 0f cell viability Compound 4
Control	100	100	100	100
DMSO	99.24	99.24	99.24	99.24
12.5	95.18	94.05	93.02	91.08
25	81.31	93.99	91.89	89.31
50	76.48	88.67	77.09	66.49
100	59.82	58.90	55.89	53.82
200	51.12	54.01	50.90	49.62
400	49.60	50.09	46.08	44.66

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