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Modified eggshell catalyzed, one-pot synthesis and antimicrobial evaluation of 1, 4-dihydropyridines and polyhydroquinolines

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ABSTRACT

A green and efficient protocol for synthesis of 1,4-dihydropyridine and polyhydroquinoline was achieved by the condensation of aldehydes, dimedone, ethylacetoacetate and ammonium acetate using environmentally benign Modified Eggshells (MES) as heterogeneous base catalyst. The promising features of reported methodology are use of green solvent system, high product yields, easy work up procedure, recycling of the catalyst and use of natural catalyst obtained from renewable resources. All synthesized compounds have been screened in vitro, against (G^+) bacteria Staphylococcus aureus and (G^-) bacteria Escherichia coli as well as for antifungal activity against fungal strains Aspergillus flavus and Candida albicans using the agar well diffusion method.

Keywords anti-bacterial activity, antifungal activity, dihydropyridines, polyhydroquinoline , MES.

INTRODUCTION

Multicomponent reactions (MCRs) are of prime importance in modern organic synthesis [1-2]. In this type of reactions, three or more components are reacted in one-pot procedure to form single product without isolation of intermediate and modification of reaction condition. MCRs show a facile execution, high atom-economy and high selectivity [3].

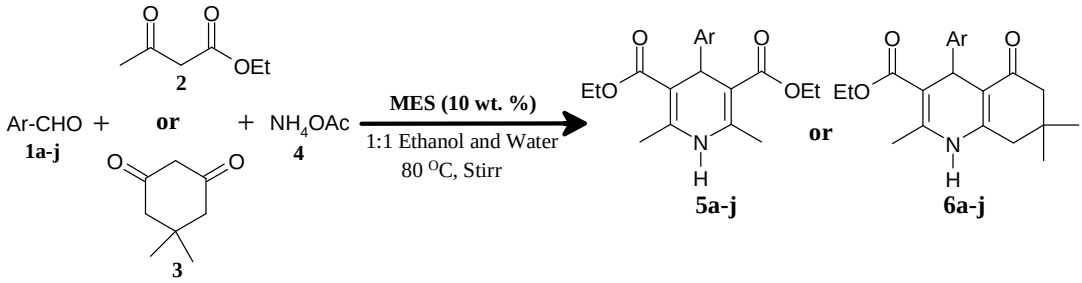
1,4-Dihydropyridine (1,4-DHP) and polyhydroquinoline have a six membered aromatic rings saturated at 1st and 4th position containing nitrogen at first position. Pyridine ring system represents the major class of nitrogen heterocycles and its analogues exhibited diverse biological and physiological activities, as the calcium channel antagonists [4], antianginal [5], antitumor [6], anti-inflammatory [7], anti-tubercular [8], analgesic [9]. It binds to L-type channel and also shows action by binding to N-type channel [10] and other activities like vasodilatation, anticonvulsant, and stress protective effect [11].

1,4-Dihydropyridines with these wide range of applications are initially synthesized by Arthur Rudolf Hantzsch in 1881 [12], widely used for synthesis of symmetrical and unsymmetrical dihydropyridine derivatives which involves cyclocondensation of an aldehyde, β-ketoesters and ammonia either in acetic acid or in refluxing ethanol. The disadvantage of this method is long reaction time which probably leads to low yield. However, this method cannot be applied for the synthesis of different substituted biologically active 1, 4-DHPs. Later on successive substitutions are performed almost at all positions (1 to 6) of 1, 4-dihydropyridines and various methodologies are reported for their synthesis. A few diverse modifications for this classical method have been reported for the synthesis of 1,4-DHP and polyhydroquinoline derivatives catalyzed by CAN [13], silica gel/$NaHSO_4$ [14], $Sc(OTf)_3$ [15], $HClO_4$–SiO_2 [16], HY-zeolites [17], TMSCl-NaI [18], montmorillonite K-10 [19], p-TSA [20], polymer [21-22], heteropolyacid [23], organic catalyst [24], L-proline [25], Bakers' yeast [26], $ZrCl_4$ [27], MCM-41 [28], $Yb(OTf)_3$

[29], Hf $(NPf_2)_4$ [30], $Cu(OTf)_2$ [31], triphenyl phosphine [32]. Several other methods have been reported using ultrasound [33] as well as microwave irradiation [34], grinding technique [35], and use of ionic liquids[36].

In spite of potential utility of these reagents, most of the existing methods suffer from drawbacks such as low yields, long reaction times, high temperature, occurrence of several side products, and the use of expensive, strong oxidant and toxic transition metallic reagents. Therefore, exploring the new catalytic system preferably in an environmentally benign method to overcome these drawbacks is a challenging task to the organic chemists.

Introduction of clean procedures by utilizing eco-friendly green catalysts have attracted great attention of researchers. Easy availability, biodegradability and environmental acceptability of catalysts obtained from natural resources have a value addition in synthetic organic chemistry.



Scheme 1

Herein, we would like to report symmetrical and unsymmetrical Hantzsch condensation for synthesis of 1,4-dihydropyridines and polyhydroquinoline catalyzed by modified eggshell [MES] with mild reaction conditions, using aromatic aldehyde, ethylacetoacetate/dimedone and ammonium acetate **(Scheme 1)**. This method not only preserves the simplicity, but also consistently gives the corresponding products in good to excellent yields.

Recently, waste eggshells have been utilized as humidity adsorbent [37], as a supplement in lime stabilization of clay soil [38], as an effective adsorbent for removal of anionic dye from aqueous solution,[39] as an adsorbent in the removal of chromium from its aqueous solution [40], to remove hazardous Malachite Green dye from aqueous solution [41], as a catalyst for lactose isomerisation to lactulose [42], Eggshell as a heterogenous catalyst for synthesis of chromenones [43], Eggshell powder has also been efficiently used as a natural source of calcium, as calcium supplement in prevention and treatment of osteoporosis [44].

Eggshells contain higher concentration of calcium carbonate, which consequently decomposes due to decarboxylation after thermal treatment and finally get converted into soft powder which comprises higher concentration of calcium oxide. Xu et al. [45], have used calcined eggshells as an active base catalyst for synthesis of dimethyl carbonate by transesterification. This calcined eggshell powder is used as a low-cost solid catalyst for biodiesel production [46], as heterogeneous catalyst for the synthesis of biodiesel from a non-edible feedstock, mahua oil (*Madhuca indica*) [47].

MATERIALS AND METHODS

The ^1H and ^{13}C NMR spectra were recorded on Avance-300 Bruker NMR spectrophotometer in $CDCl_3$. IR spectra were obtained using potassium bromide pallets on Bruker ALPHA FT-IR spectrometer. Melting points were measured on open capillary method on DBK-programmable melting point apparatus. Purity of the substrates and completion of reactions were checked by thin layer chromatography (TLC) using Merck silicagel 60 F_{254} plates. All the chemicals used are commercially available and were used without purification.

Preparation of catalyst

The waste eggshells were collected, washed thoroughly with hot water to remove an adhesive impurities and dried in oven at 80-90^{o}C for 24 hrs. The dried eggshells were ground in an agate mortar until they became a powder form. The raw eggshell powder was then calcined at 900^{o}C temperature at heating rate 2 degree min^{-1} in Muffle furnace. After thermal treatment, most of the organic materials were burnt out and the eggshell got transformed into white soft powder, which was used as Modified Egg Shell.

Typical Procedure for preparation of compounds **(5a-j)**[a]

Mixture of aldehyde (5.0 mmol), ethylacetoacetate (10.0 mmol), ammonium acetate (5.0 mmol) and (10 wt.%) MES catalyst was continuously stirred at 80°C in Ehanol:water system (5 mL). Progress of the reaction was monitored by thin-layer chromatography (TLC) by using ethyl acetate: n-haxane (4:6). After completion of reaction, the product was filtered, washed successively with water and recrystallized from ethanol. Orange colored crude product (Fig. 1) was obtained after completion of reaction, the product was then separated by simple filtration, washed with water and recrystallized from ethanol.

Typical Procedure for preparation of compounds **(6a-j)**[a]
Similiraly mixture of aldehyde (5.0 mmol), ethylacetoacetate (5.0 mmol), dimedone (5.0 mmol), ammonium acetate (5.0 mmol) and (10 wt.%) MES catalyst was continuously stirred at 80°C in Ehanol:water system (5 mL). Progress of the reaction was monitored by thin-layer chromatography (TLC) by using ethyl acetate: n-haxane (4:6). After completion of reaction, the product was filtered, washed successively with water and recrystallized from ethanol

Figure 1. Colour changes during conversion of substrates into products(5c)

RESULTS AND DISCUSSION

Characterization of MES catalyst
DSC-TGA Analysis

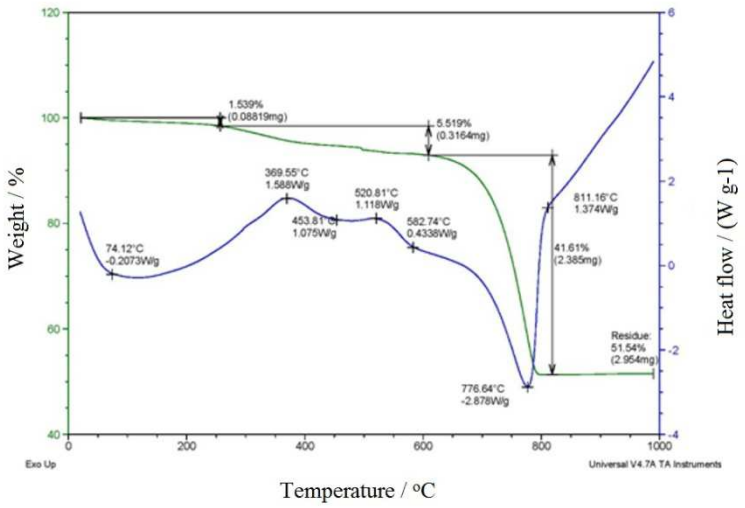


Figure 2. DSC-TGA curves of parent eggshell

The suitable calcination temperature of eggshell was analyzed by thermal gravimetric analysis as shown in Fig.2. The DSC thermal curves representing the carbonate mineral are characterized by exothermic peaks caused by evolution of carbon dioxide. The TGA result showed the temperatures, at which eggshell precursor decomposed when heated in a controlled environment at a given ramp rate. The measured weight loss was 1.539 % below 200^{o}C, 5.519 % below 600^{o}C and reached to 41.61 % between 600^{o}C and 800^{o}C. The weight loss of the eggshell attributed to the decomposition of $CaCO_3$ to CO_2 and CaO.

XRD Analysis

The XRD patterns of MES along with parent eggshells (Fig. 3) were obtained in reflection mode with Cu Ka radiation (λ=1.5418 A^{o}). at 30 kV, 10 mA, a scan speed of 1.0 degree min^{-1}, and a scan range of 10-90^{o}. The data was analyzed in the 2θ / degree range from 2^{o} to 70^{o} with the scanning step of 0.5 per sec. For parent eggshells (a), the main peak was observed at 2θ=29.66^{o} and other peaks were observed at 23.27^{o}, 31.71^{o}, 36.19^{o}, 39.63^{o}, 43.42^{o}, 47.74^{o}, 48.73^{o}, 57.63^{o}, 61.63^{o}, 64.87^{o}, and 65.82^{o} which were characteristics of $CaCO_3$. The peaks for the MES catalyst (b) appeared at 2θ=32.19^{o}, 37.32^{o}, 53.84^{o}, 64.14^{o} and 67.34^{o}, which were characteristics of CaO. Another peak was also observed at 2θ= 18.04^{o} due to $Ca(OH)_2$. It is not possible to prevent the complete removal of the OH group from surface of catalyst by calcination because, after cooling the MES catalyst to room temperature, surface moisture is enough to cover the surface of the catalyst by layer of $Ca(OH)_2$

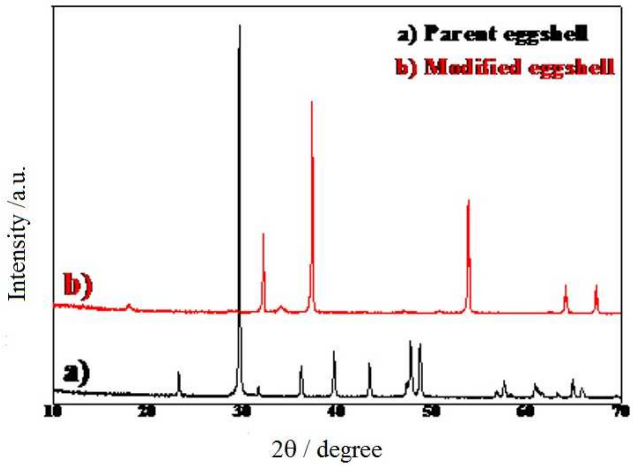


Figure 3. XRD spectra of (a) Parent eggshell and (b) MES catalyst

FT-IR Analysis

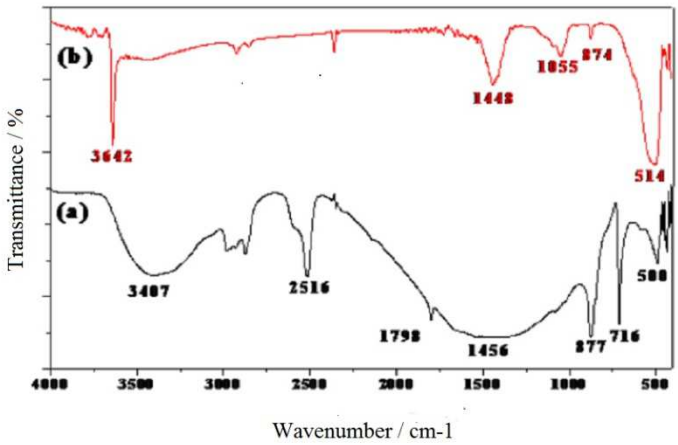


Figure 4. FT-IR spectra of (a) parent eggshell and (b) MES catalyst

The observation of major absorption bands due to CO_3^{2-} molecules, occurred at 1456, 877 and 716 cm^{-1} in parent eggshell are attributed to asymmetric stretch, out-of plane bend and in plane bend vibration mode respectively. After modification by calcinations, absorption bands of CO_3^{2-} molecules shift to higher frequency and are observed at 1448, 1055, and 874 cm^{-1}. A sharp stretching band is observed at 3642 cm^{-1}due to OH group in IR spectrum of MES, which is in parent eggshell displayed at 3407 cm^{-1}. The finding of our study on parent eggshell and MES-catalyst agree with that of observed by Engin et al. [48].

SEM Analysis

The apparent morphology of MES examined by SEM images and are shown in (Fig.5). As observed from SEM micrographs, smaller and homogeneous particle size may be achieved with calcinations of eggshells. In general, the smaller particle size of catalyst provides large contact area for catalyzing the reaction.

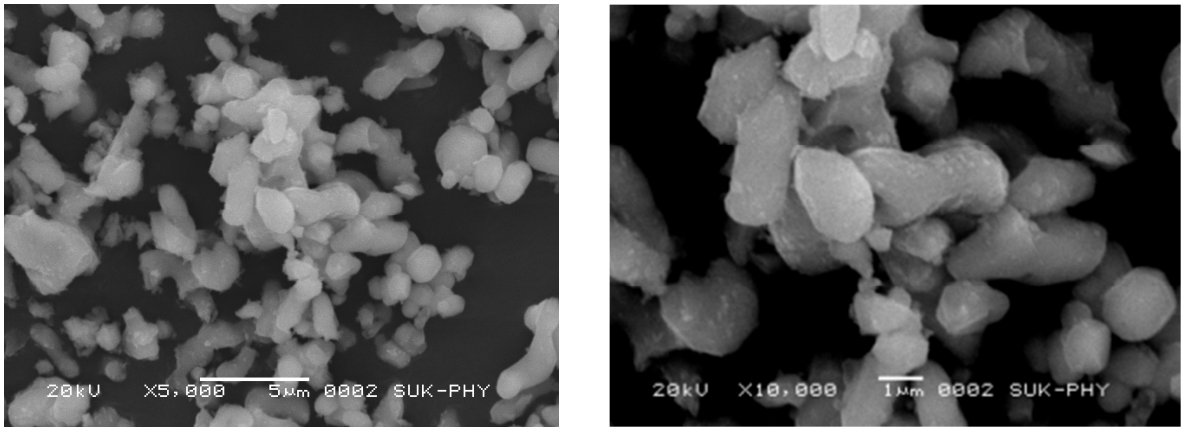


Figure 5. SEM micrographs of MES catalyst

Application of MES catalyst for synthesis of 1, 4-Dihydropyridines

To optimize reaction conditions, reaction of 4-chlorobenzaldehyde (5.0 mmol), ethylacetoacetate (10.0 mmol), ammonium acetate (5.0 mmol) was selected as the model reaction. Initially the model reaction was carried out without any catalyst and under solvent free condition at room temperature but the product formation was not observed on TLC after 18 h (Table 1, entry 1). When the reaction was performed in water as a solvent at room temperature as well as at 80^{o}C for 18 hrs only trace amount of desired product was obtained. Therefore, the catalyst is required for the success of the reaction in terms of rate and yields of Hantzsch 1,4-DHP and polyhydroquinoline derivatives. The catalytic amount of MES was first investigated for model reaction under aqueous conditions at room temperature. The result revealed that 10 wt.% of MES gave good result for model reaction with 84 % yield (Table 1, entry 5). Also, among the different organic solvents tried to optimize the reaction conditions, alcoholic solvents seemed to be better choice in terms of yield of the isolated product. The same trend is also observed when equal amount of water is used along with ethanol. Hence, it is proposed to synthesize these heterocyclic compounds in alcohol-water (1:1) solvent system. Next, we investigated the appropriate loading amount of catalyst. It was found that 10 wt.% of MES was enough to promote the reaction efficiency(Table 1, entry 14).For comparison purpose, the reaction was also investigated with common laboratory reagent CaO as catalyst and very low amount of product was obtained after 5 h (Table 1, entry 17). Therefore, it is evident that MES plays important role in transformation of reactant into product in Hantzsch multicomponent reaction.

Table 1: Optimization of reaction conditions for synthesis of 5c[a]

Entry	Catalyst / (wt.%)	Solvent	Tempreture / (°C)	Time	Yield / (%)[b]
1	None	None	RT	18 h	None
2	None	H_2O	RT	18 h	Trace[c]
3	None	H_2O	80	18 h	Trace[c]
4	MES (10)	None	RT	4 h	Trace[c]
5	MES (10)	H_2O	80	60 min	84
6	MES (10)	Methanol	80	60 min	84
7	MES (10)	Ethanol	80	60 min	86
8	MES (10)	THF	80	2 h	70
9	MES (10)	Acetonitrile	80	2 h	74
10	MES (10)	DCM	80	2 h	60
11	MES (10)	H_2O:Ethanol(1:1)	RT	1 h	Trace[c]
12	MES (10)	H_2O:Ethanol(1:1)	60	1 h	90
13	MES (10)	H_2O:Ethanol(1:1)	70	50 min	92
14	MES (10)	H_2O:Ethanol(1:1)	80	45 min	96
15	MES (20)	H_2O:Ethanol(1:1)	80	45 min	96
16	MES (30)	H_2O:Ethanol(1:1)	80	45 min	96
17	CaO (10)	H_2O:Ethanol(1:1)	80	5 h	64

[a] *All the reactions were carried out using 4-chlorobenzaldehyde* ***1c*** *(5.0 mmol), ethylacetoacetate (10.0 mmol), ammonium acetate (5.0 mmol), and 5 mL solvent.*

[b] *Isolated pure yields.*

[c] *Knoevenagel condensation product was obtained as a major one.*

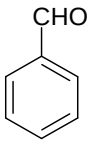
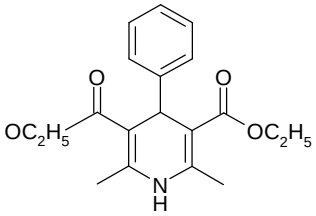
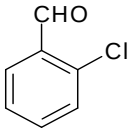
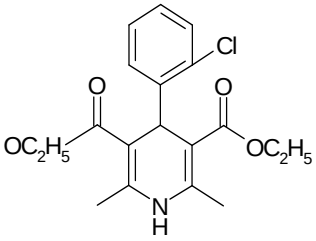
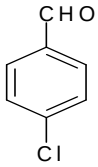
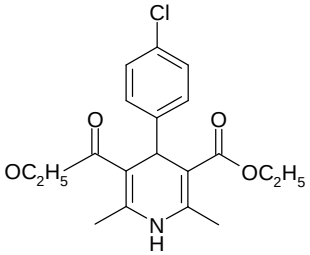
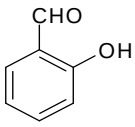
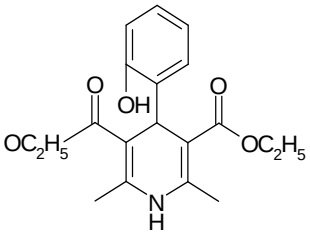
A comparison of the efficiency of catalytic activity of the MES with several previous reported methods is presented in Table 2. The results show that the present method is better compared to some of the earlier reports in terms of yield and other reaction conditions.

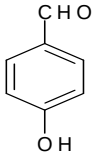
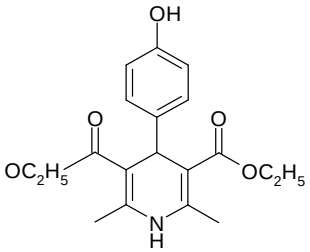
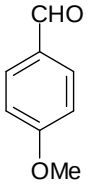
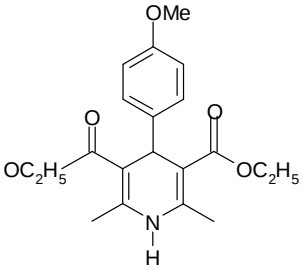
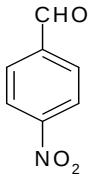
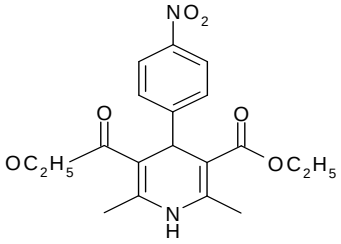
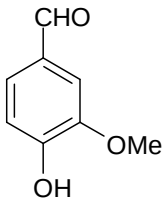
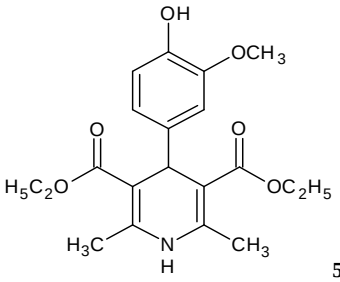
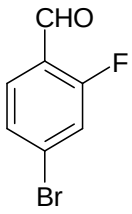
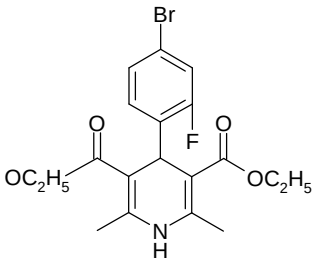
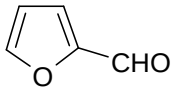
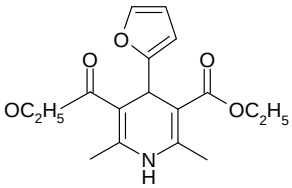
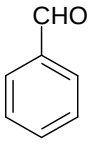
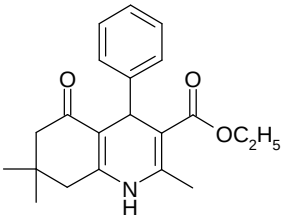
Table 2: Comparison of catalytic activity MES with several reported catalysts

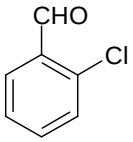
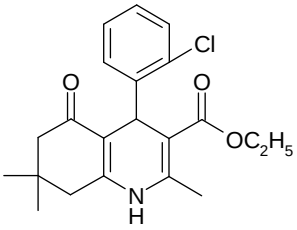
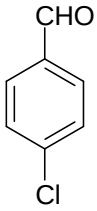
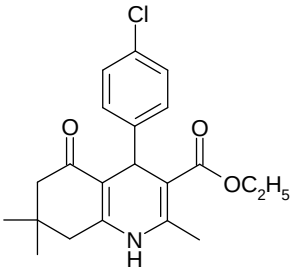
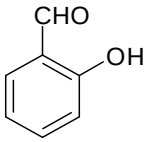
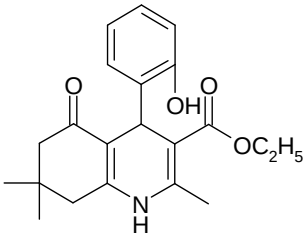
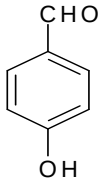
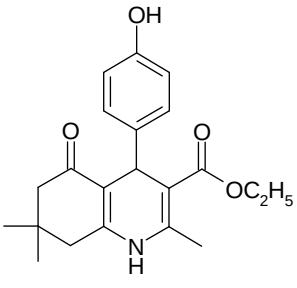
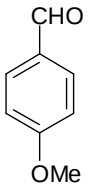
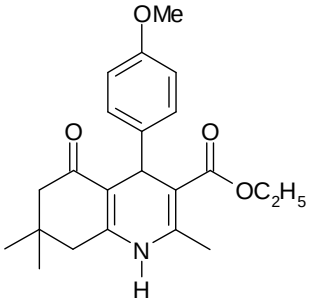
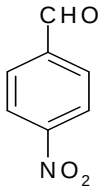
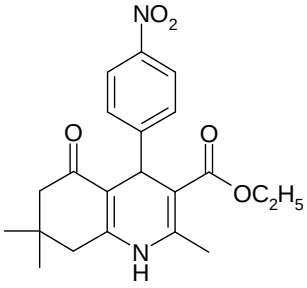
Entry	Reaction conditions	Yield / (%)	Ref
1	CAN, EtOH, 1 h	92	13
2	$Sc(OTf)_3$, EtOH, 4 h	93	15
3	HY-Zeolite, CH_3CN, 2 h	93	17
4	p-TSA, EtOH, 2 h	93	20
5	$Yb(OTf)_3$, EtOH, 5 h	90	29
6	MES, H_2O:EtOH, 45 min.	96	-

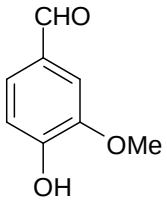
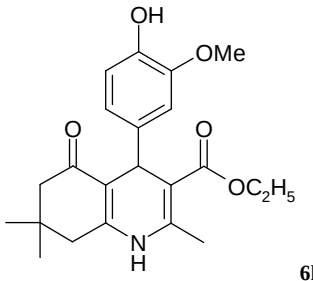
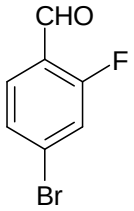
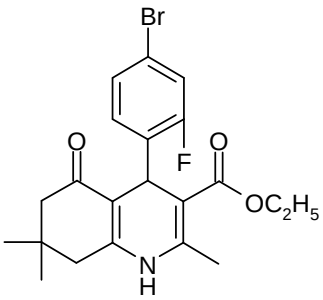
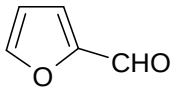
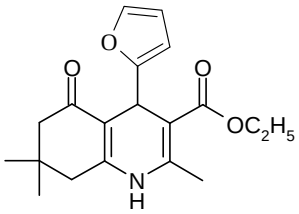
Encouraged by all these remarkable results and after the selection of optimum reaction conditions, we next examined the extent and feasibility of the MES catalyzed condensation for the synthesis of various symmetrical 1,4-dihydropyridines and polyhydroquinolines. We have synthesized symmetrical 1,4-dihydropyridines (Table 3, entry 1-10) by the condensation of various aromatic aldehydes, ethylacetoacetate and ammonium acetate (in 1:2:1 proportion) and polyhydroquinolines (Table 3, entry 11-20) by the condensation of aromatic aldehydes, ethylacetoacetate, dimedone and ammonium acetate (in 1:1:1:1 proportion). It was observed that the aldehydes containing electron-withdrawing substituents reacted faster and gave a high yield of the product as compared to those containing electron-donating substituents.

Table 3: MES catalyzed synthesis of 1,4-dihydihydro pyridines (5a-j)[a] and polyhydroquinolines (6a-j)[b]

Entry	Aldehyde	Product	Time / (min)	Yield/ (%)[c]	(m.p.[o])
1		 **5a**	60	80	158-160
2		 **5b**	60	85	130-132
3		 **5c**	45	96	148-150
4		 **5d**	50	90	146-148

5		 **5e**	50	90	223-225
6		 **5f**	45	85	155-156
7		 **5g**	45	94	128-130
8		 **5h**	60	94	160-162
9		 **5i**	45	93	168-170
10		 **5j**	60	87	162-164
11		 **6a**	60	85	203-205

12		 **6b**	45	85	208-210
13		 **6c**	70	90	245-246
14		 **6d**	60	90	238-240
15		 **6e**	60	83	233-235
16		 **6f**	60	87	252-254
17		 **6g**	45	90	238-240

18		 **6h**	60	90	211-213
19		 **6i**	45	91	208-210
20		 **6j**	60	84	246-248

[a]*Aldehydes (5.0 mmol), ethylacetoacetate (10.0 mmol), ammonium acetate (5.0 mmol), MES catalyst (10 wt.%) in 1:1 water and ethanol (5 mL) at 80°C.*

[b]*Aldehydes (5.0 mmol), ethylacetoacetate (5.0 mmol) , dimedone (5.0 mmol), ammonium acetate (5.0 mmol), MES catalyst (10 wt.%) in 1:1 water and ethanol (5 mL) at 80°C*

[c]*Isolated pure yields based on aldehyde.*

Pharmacology

Antibacterial activity

In-vitro evaluation of antibacterial activity of newly synthesized compounds 5^{a-j} and 6^{a-j} was carried out and results are summarized in Table 5. Different concentrations of test compounds were prepared using DMSO and tested against *Staphylococcus aureus* and *Escherichia coli* bacterial strains by agar well diffusion method [49] using ciprofloxacin as standard. The zone of inhibition if any was then measured in mm for the particular compound and specific organism.

The bacterial inoculums was uniformly spread using sterile cotton swab on a sterile Petri dish MH agar. Five serial dilutions were prepared in different concentrations of 500, 300, 20o, 100 and 50 µg mL^{-1} for synthesized derivatives. Similarly serial dilutions for pure substance were prepared with same concentration. 50 µL of compounds were added to each of the 5 wells (5 mm diameter holes cut in the agar gel, 25 mm apart from one another). The systems were incubated for 24 h at 36ºC ± 1ºC, under aerobic conditions. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm. The bacterial strains used were of non-invasive species of their genera and thus applicable for analytical work.

Staphylococcus aureus and *Escherichia coli* are among the most prevalent species of gram-positive and gram-negative bacteria, respectively, that induces clinical mastitis and are the main causes of blood stream infections (BSIs) in humans. They are also common bacteria which causing food poisoning. Therefore, for this study we have selected these two bacteria and results of study i.e. zone of inhibition have been incorporated in Table 5.

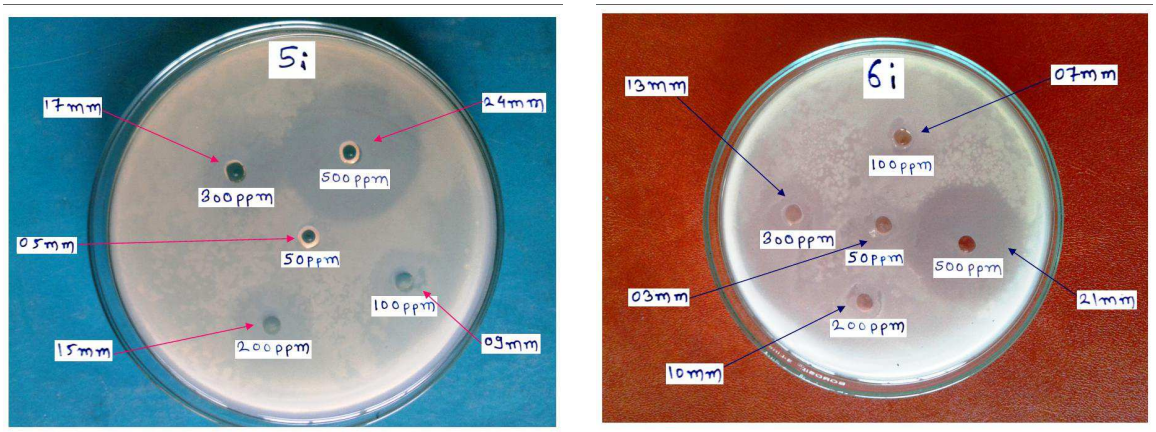


Figure 6. Zone of inhibition against *S. aureus* (5i) and *E. coli* (6i)

Among synthesized compounds **5b**, **5c**, **5f**, **5g**, **5i, 6b, 6c, 6f , 6g, 6i** were found to have equipotent antibacterial activity amongst these **5c**, **6c** and **5i , 6i** (Fig.6) bearing chloro and bromo substituent at *para* position of phenyl ring, respectively showed excellent antibacterial activity against gram positive *S. aureus* and gram negative *E.coli*. Further, their *ortho* substitution with different size and electronic properties were also evaluated to study if steric and electronic factors have any effect on activity., compounds **5b, 5f, 6b, 6f** having *ortho*-chloro and *ortho*-nitro substituent retained the antimicrobial activity whereas compounds **5a, , 5j, 6a, 6j** emerged as less active against both *S. aureus* as well as *E. coli* . Compounds **5d, 5e, 5h, 6d, 6e, 6h** shows moderate activity against both bacterial strains. From the results it was observed that compounds containing chloro, bromo, and nitro group at phenyl ring of 1,4-Dihydropyridine and polyhydroquinoline moiety showed promising antibacterial activity. These are initial screening results to check the antimicrobial potential and further work would be done to understand their mechanism of action.

Table 4. Antibacterial activity of synthesized compounds

Comp No.	Zone of Inhibition (diameter in mm)									
	S. aureus					*E.coli*				
	µg mL^{1}									
	50	100	200	300	500	50	100	200	300	500
5a	--	--	03 ± 0.2	04±0.2	06±0.2	--	--	--	--	-
5b	O4±0.3	06±0.3	07±0.2	12±0.2	16±1.1	--	04±0.2	07±0.4	10±0.5	13±0.7
5c	05±0.3	08±0.3	12±0.4	15±0.6	20±1.5	--	06±0.2	10±0.5	12±0.7	18±1.2
5d	--	03±0.2	05±0.2	06±0.3	09±0.2	--	--	04±0.2	06±0.2	08±0.2
5e	--	--	03±0.3	03±0.3	05±0.2	--	--	--	05±0.2	07±0.2
5f	--	07±0.3	10±0.3	12±0.3	14±0.2	--	--	08±0.2	10±0.2	12±0.2
5g	--	05±0.2	08±0.2	10±0.2	16±0.6	--	05±0.2	08±0.3	12±0.2	14±0.5
5h	--	--	02±0.2	07±0.2	09±0.3	--	--	05±0.2	07±0.2	09±0.2
5i	05±0.2	09±0.4	15±0.9	17±1.1	24±1.2	08±0.3	10±0.2	12±0.4	18±0.2	22±1.5
5j	--	--	--	04±0.3	06±0.2	--	--	03±0.2	06±0.2	10±0.2
6a	--	--	--	--	03±0.3	--	--	--	--	02±0.2
6b	--	05±0.3	09±0.2	13±0.4	18±1.4	--	06±0.2	10±0.2	12±0.5	14±1.3
6c	03±0.2	07±0.2	10±0.4	14±0.9	24±1.8	02±0.3	07±0.2	11±0.4	14±0.8	20±1.7
6d	--	05±0.3	09±0.2	11±0.4	13±0.2	--	03±0.2	04±0.2	09±0.2	12±0.2
6e	--	09±0.2	11±0.2	14±0.3	14±0.2	--	--	08±0.2	10±0.2	12±0.2
6f	--	07±0.2	09±0.3	12±0.2	14±0.3	--	07±0.2	10±0.2	12±0.2	14±0.2
6g	--	08±0.2	15±0.2	16±0.2	20±0.2	--	06±0.2	14±0.2	15±0.2	16±0.2
6h	--	--	03±0.2	04±0.2	08±0.2	--	--	--	03±0.2	06±0.2
6i	08±0.3	12±0.6	14±0.8	15±0.9	22±1.2	03±0.2	07±0.6	10±0.6	13±0.8	21±1.8
6j	--	--	--	02±0.2	04±0.2	--	--	--	03±0.2	06±0.2
Std. (ciprofloxacin)	10±0.6	15±1.2	16±1.2	18±1.8	23±2.1	10±0.8	15±1.5	18±1.5	20±2.4	21±2.1
Control (DMSO)	--	--	--	--	--	--	--	--	--	--

Antifungal activity

The fungal strains used in this study were *Aspergillus flavus* and *Candida albicans*. *A. flavus* is saprotropic and also human and animal pathogenic whereas *C.albicans* is human pathogenic fungal strain. The study was determined by use agar well diffusion method. Among the tested compounds **5i and 6i** having *p*-bromo group in the phenyl ring of

1,4-Dihydropyridine and polyhydroquinoline series were found to be most active against *A. flavus* and *C. albicans.* Compound **5b, 5c, 6b** and **6c,** having chloro in the phenyl ring showed moderate antifungal activity against *A. flavus* and *C. albican*. Rest of the compounds were found to be less active in comparison to standard drug fluconazole. These results indicate that compounds having electron withdrawing group at the phenyl ring moiety of 1,4-Dihydropyridine and polyhydroquinoline were more active against fungal strain. Moreover, it was also found that compounds having electron withdrawing group at *para* position (**6c)** were more effective than the compounds having electron withdrawing groups at *ortho* position (**6b**). The result of antifungal studies have been summarized in Table 6.

Table 5. Antifungal activity of synthesized compounds

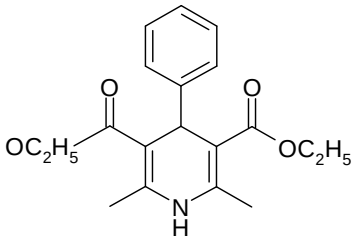
Comp. No.	Zone of Inhibition (diameter in mm)									
	Aspergillus flavus					*Candida albicans*				
	µg mL^{1}									
	50	100	200	300	500	50	100	200	300	500
5a	--	--	--	03±0.3	07±0.2	--	--	--	--	03±0.3
5b	--	05±0.2	09±0.5	10±0.7	15±1.9	--	03±0.3	09±0.5	12±0.5	14±0.8
5c	07±0.2	10±0.5	13±0.8	15±1.6	16±2.1	--	07±0.2	12±0.5	14±1.1	15±2.1
5d	--	--	03±0.2	04±0.2	06±0.5	--	--	--	04±0.2	07±0.2
5e	--	--	--	02±0.2	06±0.2	--	--	--	03±0.2	06±0.2
5f	--	--	03±0.2	05±0.2	07±0.2	--	--	03±0.2	05±0.2	08±0.5
5g	--	03±0.2	05±0.2	09±0.2	12±0.7	--	03±0.2	06±0.3	10±0.7	12±0.5
5h	--	--	02±0.2	05±0.2	07±0.2	--	--	03±0.2	06±0.2	09±0.2
5i	06±0.2	09±0.5	13±1.2	16±1.7	23±2.2	04±0.2	07±0.2	11±0.2	13±0.5	20±0.9
5j	--	--	--	04±0.2	06±0.2	--	--	03±0.2	06±0.2	10±0.5
6a	--	--	--	--	03±0.2	--	--	--	--	02±0.2
6b	--	05±0.3	09±0.5	10±0.5	14±0.9	--	06±0.2	10±0.6	14±1.1	15±1.5
6c	03±0.2	07±0.5	10±0.9	12±1.1	16±1.9	02±0.2	07±0.2	11±0.8	14±0.9	17±1.8
6d	--	--	02±0.2	03±0.2	03±0.2	--	--	02±0.2	04±0.3	06±0.2
6e	--	--	02±0.2	04±0.2	06±0.2	--	--	04±0.2	05±0.3	08±0.2
6f	--	03±0.2	05±0.2	07±0.5	08±0.5	--	03±0.3	05±0.2	07±0.2	08±0.8
6g	--	02±0.2	05±0.2	08±0.4	15±0.8	--	03±0.2	06±0.2	09±0.2	12±0.6
6h	--	--	03±0.2	05±0.2	08±0.3	--	--	--	03±0.2	07±0.2
6i	02±0.2	06±0.3	11±0.8	14±0.9	17±1.7	07±0.2	11±0.2	14±0.3	16±0.4	21±1.8
6j	--	--	--	02±0.2	04±0.2	--	--	--	03±0.2	06±0.2
Std. (Flucanazole)	07±0.3	11±0.9	15±1.2	19±1.7	26±2.3	05±0.2	09±0.2	12±0.3	17±1.6	22±2.1
Control (DMSO)	--	--	--	--	--	--	--	--	--	--

CONCLUSION

In conclusion, a very simple, green, energy-efficient, and atom economical protocol has been developed for the synthesis of 1,4-dihydropyridines and polyhydroquinoline derivatives with a cheap, environmentally benign heterogeneous base catalyst derived from renewable resources. The advantage of the present protocol is the elimination of corrosive catalysts, conventional organic solvents, and toxic reagents. The preliminary in-vitro antimicrobial screening of synthesized compounds from both the series have emerged as potent antibacterial and antifungal agents endowed with moderate to good activity. Among these, interestingly, the **5b, 5c, 5f, 5g, 5i, 6b, 6c, 6f, 6g and 6i** shows remarkable antibacterial activity. Compound **5i**, **6i** shows promising broad spectrum antifungal activity against *Aspergillus flavus* and *Candida albicans.* Compounds **5b, 5c, 6b** and **6c,** having halogen substituent in the phenyl ring also showed moderate to good antifungal activity.

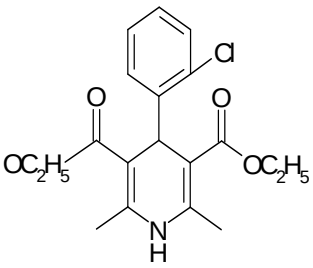
Spectral data for synthesized compounds:

2,6-Dimethyl-4-phenyl-1,4-dihydropyrid2,6-Dimethyl-4-phenyl-1,4-dihydropyridine 3,5-dicarboxylate(5a)



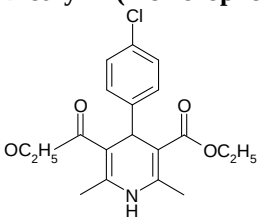
IR(KBr, ν cm^{-1}) 3340, 3072,1704; ^{1}H NMR (300MHz,$CDCl_3$, δ ppm) 1.29 (6H, t, 2 –OCH_2CH_3), 2.30 (6H, s, 2 -CH_3), 4.19 (4H, q, 2 –OCH_2CH_3), 5.21 (1H, s, -CH), 7.27 (1H, bs, -NH), 7.49 to 7.52 (5H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.7, 16.5, 42.9, 62.1, 102.1, 126.1, 128.6, 129.2, 142.9, 150.3, 167.7

Diethyl-4-(2-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(5b)



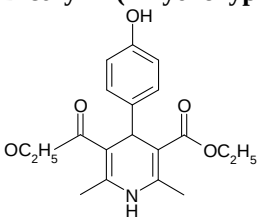
IR(KBr, ν cm^{-1}) 3277, 3079, 2972, 1711; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 1.32 (6H, t, 2 –OCH_2CH_3), 2.28 (6H, s, 2 -CH_3), 4.17 (4H, q, 2 –OCH_2CH_3), 5.19 (1H, s, -CH), 7.24 (1H, bs, -NH), 7.33 to 7.37 (4H, m, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.2, 16.7, 39.9, 62.0, 102.4, 126.5, 127.9, 128.9, 130.4, 134.2, 143.4, 151.0, 167.9

Diethyl-4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(5c)



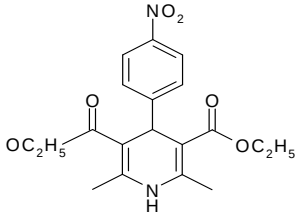
IR(KBr, ν cm^{-1}) 3272, 3084, 2948, 1710; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 1.36 (6H, t, 2 –$OCH_2C\mathbf{H}_3$), 2.35 (6H, s, 2 -$C\mathbf{H}_3$), 4.22 (4H, q, 2 –$OC\mathbf{H}_2CH_3$), 5.24 (1H, s, -C**H**), 7.09 (1H, bs, -N**H**), 7.12 to 7.16 (2H, m, Ar-**H**), 7.21 to 7.27 (2H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 13.9, 16.5, 42.9, 62.3, 102.9, 128.2, 130.2, 131.3, 140.6, 150.1, 167.5

Diethyl-4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(5e)



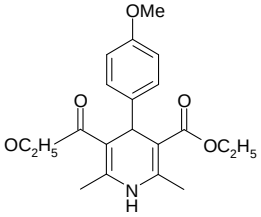
IR(KBr, ν cm^{-1}) 3312, 3054, 2952, 1705, 1455; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 1.27 (6H, t, 2 –$OCH_2C\mathbf{H}_3$), 2.21 (6H, s, 2 -$C\mathbf{H}_3$), 4.26 (4H, q, 2 –$OC\mathbf{H}_2CH_3$), 5.04 (1H, s, -OH), 5.32 (1H, s, -C**H**), 6.59 (1H, bs, -N**H**), 7.07 to 7.11 (2H, m, Ar-**H**), 7.14 to 7.19 (2H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.1, 16.1, 43.5, 61.9, 102.6, 115.1, 130.8, 136.5, 150.2, 155.1, 167.5

Diethyl-2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate(5g)



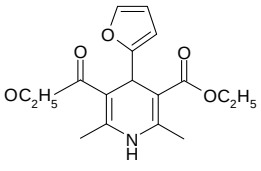
IR(KBr, ν cm^{-1}) 3314, 3076, 2921, 1725, 1534, 1386; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 1.12 (6H, t, 2 –$OCH_2C\mathbf{H}_3$), 2.09 (6H, s, 2 -$C\mathbf{H}_3$), 4.01 (4H, q, 2 –$OC\mathbf{H}_2CH_3$), 5.12 (1H, s, -C**H**), 7.12 (1H, bs, -N**H**), 7.64 to 7.69 (2H, m, Ar-**H**), 7.71 to 7.74 (2H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.6, 16.1, 44.1, 62.4, 103.1, 121.2, 130.4, 145.1, 148.4, 167.5

Diethyl-2,6-dimethyl-4-(4-methoxyphenyl)-1,4-dihydropyridine-3,5 dicarboxylate(5f)



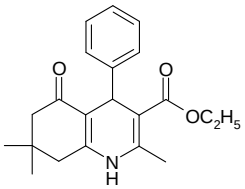
IR(KBr, ν cm^{-1}) 3342, 3032, 2964, 1702; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 1.06 (6H, t, 2 –$OCH_2C\mathbf{H}_3$), 2.01 (6H, s, 2 -$C\mathbf{H}_3$), 3.82 (3H, s, -$OC\mathbf{H}_3$), 3.97 (4H, q, 2 –$OC\mathbf{H}_2CH_3$), 5.11 (1H, s, -C**H**), 7.11 (1H, bs, -N**H**), 7.04 to 7.09 (2H, m, Ar-**H**), 7.12 to 7.14 (2H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.1, 16.2, 45.0, 55.9, 62.4, 102.5, 115.1, 130.4, 134.6, 151.0, 157.1, 167.5*:*

Diethyl 4-(furan-2-yl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate(5j)



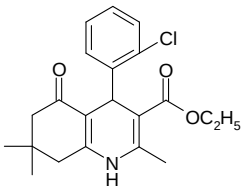
IR(KBr, ν cm^{-1}) 3346, 3082, 1700, 1650, 1488; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 1.26 (6H, t, 2 –$OCH_2C\mathbf{H}_3$), 2.32 (6H, s, 2 -$C\mathbf{H}_3$), 4.16 (4H, q, 2 –$OC\mathbf{H}_2CH_3$), 5.19 (1H, s, C**H**), 5.94 (2H, d, furyl ring **H**s), 6.20 (1H, s, furyl ring **H**), 7.20 (1H, bs, -N**H**) (figure 3); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.9, 15.4, 31.5, 61.1, 102.0, 107.7, 111.2, 142.5, 150.9, 152.2, 167.1

Dithyl-2,7,7-trimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate(6a)



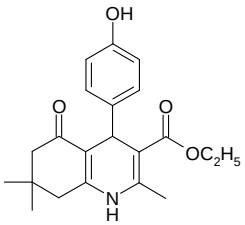
IR(KBr, ν cm^{-1}) 3282, 2974, 1690, 1599, 1481; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 0.89 (3H, s, -C**H**$_3$), 1.15 (3H, s, -C**H**$_3$), 1.21 (3H, t, -OCH$_2$C**H**$_3$), 2.15-2.32 (4H, m, 2 -C**H**$_2$), 2.39 (3H, s, -C**H**$_3$), 4.12 (2H, q, -OC**H**$_2$CH$_3$), 5.21 (1H, s, -C**H**), 5.72 (1H, bs, -N**H**), 7.21 (5H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.1, 16.4, 27.1, 32.4, 41.2, 43.3, 51.1, 61.9, 102.9, 112.1, 126.2, 129.1, 130.2, 142.4, 149.7, 151.1, 167.4, 198.7

Ethyl 4-(2-chlorophenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate(6b)



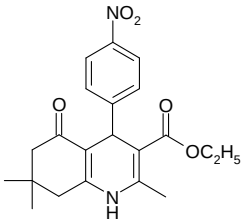
IR(KBr, ν cm^{-1}) 3277, 2992, 1701, 1609, 1423; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 0.91 (3H,s,-C**H**$_3$), 1.17 (3H,s,-C**H**$_3$), 1.26 (3H, t, -OCH$_2$C**H**$_3$), 2.17-2.26 (4H, m, 2 -C**H**$_2$), 2.41 (3H, s, -C**H**$_3$), 4.22 (2H, q, -OC**H**$_2$CH$_3$), 5.17 (1H, s, -C**H**), 6.09 (1H, bs, -N**H**), 6.96-7.12 (4H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.9, 16.3, 27.1, 31.5, 38.4, 43.2, 50.9, 62.1, 102.9, 111.7, 126.4, 127.9, 129.1, 130.5, 135.2, 144.1, 149.2, 150.2, 166.9, 198.1

Ethyl 4-(4-hydroxyphenyl)-2,7,7-trimethyl-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate(6e)



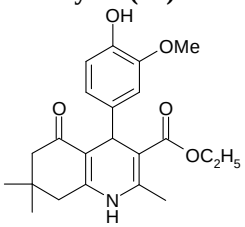
IR(KBr, ν cm^{-1}) 3320, 2984, 1710, 1612, 1494; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 0.95 (3H, s, -C**H**$_3$), 1.19 (3H, s, -C**H**$_3$), 1.31 (3H, t, -OCH$_2$C**H**$_3$), 2.24-2.32 (4H, m, 2 -C**H**$_2$), 2.45 (3H, s, -C**H**$_3$), 4.25 (2H, q, -OC**H**$_2$CH$_3$), 5.24 (1H, s, -O**H**), 5.52 (1H, s, -C**H**), 5.72 (1H, bs, -N**H**), 7.01-7.04 (2H, d, Ar-**H**), 7.12-7.15 (2H, d, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 13.8, 17.1, 27.6, 31.7, 41.7, 43.2, 51.3, 61.9, 102.1, 112.0, 115.0, 130.9, 135.1, 149.7, 151.2, 156.5, 167.6, 197.2

Ethyl 2,7,7-trimethyl-4-(4-nitrophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate(6g)



IR(KBr, ν cm^{-1}) 3314, 2978, 1704, 1624, 1497; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 0.92 (3H, s, -C**H**$_3$), 1.22 (3H, s, -C**H**$_3$), 1.29 (3H, t, -OCH$_2$C**H**$_3$), 2.35-2.39 (4H, m, 2 -C**H**$_2$), 2.45 (3H, s, -C**H**$_3$), 4.24 (2H, q, -OC**H**$_2$CH$_3$), 5.26 (1H, s, -C**H**), 6.12 (1H, bs, -N**H**), 7.76-7.82 (2H, d, Ar-**H**), 7.90-7.97 (2H, d, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.1, 16.5, 27.4, 32.5, 41.9, 43.7, 51.4, 62.1, 104.2, 115.2, 121.9, 130.1,145.1 147.3, 149.1, 150.2, 167.9, 197.7

Ethyl 2,7,7-trimethyl-4-(4-hydroxy-3-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate(6h)



IR(KBr, ν cm^{-1}) 3279, 3014, 1715, 1487, 1392, 1214; ^{1}H NMR (300MHz, $CDCl_3$, δ ppm) 0.92 (3H, s, -C**H**$_3$), 1.22 (3H, s, -C**H**$_3$), 1.31 (3H, t, -OCH$_2$C**H**$_3$), 2.35-2.42 (4H, m, 2 -C**H**$_2$), 2.52 (3H, s, -C**H**$_3$), 3.79 (3H, s, -OC**H**$_3$), 4.52 (2H, q, -OC**H**$_2$CH$_3$), 5.12 (1H, s, -O**H**), 5.82 (1H, s, -C**H**), 6.02 (1H, bs, -N**H**), 7.04-7.09 (3H, m, Ar-**H**); ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm) 14.9, 17.3, 27.6, 31.5, 41.9, 43.1, 52.9, 57.4, 62.1, 102.9, 112.1, 114.2, 116.5, 123.1, 137.2, 142.9, 149.1, 150.4, 152.3, 167.4, 197.9

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