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$TiCl_4$/nano-sawdust as an Efficient Biocatalyst for the Synthesis of Highly Substituted Dihydro-2-oxopyrroles as Antimicrobial Agents

Ahmad Gholami[1], Soghra Khabnadideh[1], Younes Ghasemi[1], Bi Bi Fatemeh Mirjalili[2], Roozbeh Shahmoradi[1] and Leila Zamani[2*]

[1]Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Chemistry, College of Science, Yazd University, Yazd, P.O.Box 8915813149, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors LZ, SK, BBFM and RS designed, synthesized and analyzed the data of the 2-oxopyrroles. Authors YG, AG and RS prepared the antimicrobial test. Authors LZ, AG, SK and BBFM wrote the paper. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Sawdust nano particles was prepared from the pine wood and applied for the synthesis of $TiCl_4$/nano-sawdust as a readily available, inexpensive, biodegradable and environmentally benign heterogeneous solid acid biocatalyst. The catalyst was used for the one pot cascade synthesis of highly functionalized dihydro-2-oxypyrroles (*S1-S18*). On the other hands, fungal and bacterial infections are always regarded as a dangerous pathogen. Because of increased resistance to microbial strains need to discover new antimicrobial drugs. Here we evaluated the antimicrobial activities of these compounds against different species of micro organisms including gram-positive and gram-negative bacteria as well as fungi.
Methods: Four-component reactions (4CRs) of the dialkyl acetylenedicarboxylates, amines and aldehydes were used for the synthesis of highly functionalized dihydro-2-oxypyrroles under thermal

**Corresponding author: E-mail: dr.l.zamani@gmail.com;*

conditions. A micro-dilution susceptibility test and disk diffusion methods were used to study the antimicrobial activity of the new compounds against different species of gram-positive and gram-negative bacteria and also *Candida albicans* microorganisms.

Results: The results showed that catalytic activity of $TiCl_4$/nano-sawdust for the synthesis of dihydro-2-oxopyrroles *via* 4CRs of the dialkyl acetylenedicarboxylates, amines and aldehydes. Also, dihydro-2-oxopyrrole derivatives had moderate antimicrobial activity against pathogenic bacteria and fungi. The most of compounds are nearly antifungal active in comparison to the control drugs. Also, MIC values of the synthetic compounds showed ethyl 1-(4-ethylphenyl)-4-((4-ethylphenyl)amino)-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate (***S6***), ethyl 5-oxo-1-(p-tolyl)-4-(p-tolylamino)-2,5-dihydro-1*H*-pyrrole-3-carboxylate (***S7***) and ethyl 1-(4-methoxyphenyl)-4-((4-methoxyphenyl)amino)-5-oxo-2,5-dihydro-1*H*-pyrrole-3- carboxylate (***S8***) exhibited strong inhibitory activities against gram positive bacteria.

Conclusion: In summary, $TiCl_4$/nano-sawdust as a green, cheap, natural, biodegradable and readily available biopolymer solid acid catalyst introduced for synthesis of dihydro-2-oxopyrrole derivatives. Comparing the structure and activity of these compounds showed that adding ethyl residue in the 4-position of phenyl ring of dihydro-2-oxopyrroles increased their antibacterial activities.

Keywords: Antimicrobial activity; Dihydro-2-oxopyrrole; $TiCl_4$/nano-sawdust; multicomponent reaction; nano-sawdust; solid acid catalyst.

1. INTRODUCTION

Dihydro-oxo-pyrrole (DPO) derivatives are important azaheterocycle structures exhibit biological activities such as herbicidal [1], antitumor [2], pesticidal [3], anti-HIV [4], antibiotics [5], antimalarial [6], and serving as crucial core structures to construct many natural products like bilirubins [7], oteromycin [8], ypaoamide [9] and pyrrocidine A (Fig. 1) [10].

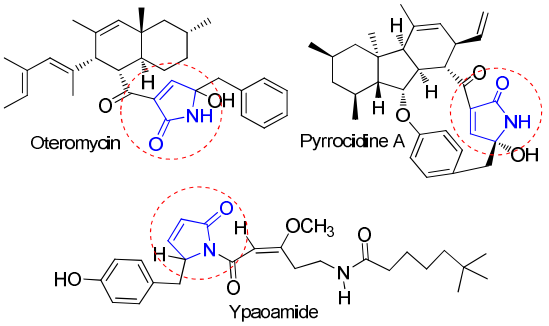


Fig. 1. The structure of some natural compounds with dihydro-2-oxopyrrole motif

Due to the wide range application of dihydro-2-oxopyrroles derivatives in pharmaceuticals, agrochemicals, and natural products, their synthesis remains an area of intense current interest in synthetic chemistry. Therefore, a number of synthetic routes have been developed for the synthesis of highly functionalized dihydro-2-oxypyrroles, including ruthenium-catalyzed reaction of α,ß-unsaturated imines with carbon monoxide and ethylene [11], reaction of isocyanides, dialkyl acetylenedicarboxylates, and benzoyl chlorides [12], carboamination/oxidative cyclization of C-acylimines with alkenes [13], transannulation of 1-sulfonyl-1,2,3-triazole with ketene silylacetal [14], reaction of acetylene with imines and CO_2 [15], Pd-catalyzed cyclization of ethyl glyoxalate and amines [16], and reaction of α-cyanomethyl-ß-ketoesterswith alcohols [17]. Among these versatile synthetic methods, multicomponent reactions (MCRs) have attracted particular attention [18]: A few methods have been reported for the synthesis of dihydro-2-oxopyrroles using MCRs such as four component reaction of dialkyl acetylenedicarboxylates, aldehydes and amines. Previously, this protocol have been catalyzed by TiO_2-nanopowder [19], I_2 [20], *p*-toluenesulfonic acid [21], $Cu(OAc)_2{\cdot}H_2O$ [22], AcOH [23], 1-methyl-2-oxopyrrolidinium hydrogen sulfate ([Hpyro][HSO_4]) [24], $InCl_3$ [25], and [n-Bu_4N][HSO_4] [26].

Some of these catalysts have many limitations such as inefficient separation of the catalyst from homogeneous reaction mixtures [20-23], unrecyclable and environmentally limitations [20-25]. Hence, development of new solid acids with numerous advantages such as cost effectiveness, environmentally benign, easy workup and good stability for one-pot multicomponent synthesis of highly substituted dihydro-2-oxopyrroles scaffolds is still in demand. In this regard, our aim is developing cheap and biomaterial solid acid catalysts for this transformation.

Cellulose is one of the most abundant natural carbon based biopolymers containing free OH groups with nucleophilic character. It has been used for synthesis of some compounds which used in enantioselective chromatography [27], protein immobilization [28], antibodies [29], and retarded drug release [30].

Sawdust is a biodegradable, natural, cheap, renewable and readily available source of cellulose. In this work, we have investigated the synthesis of sawdust based catalyst by bonding Lewis acids to OH groups of D-glucose units. Since, sawdust is containing cellulose with other substances such as pectin, tannin, proteins, minerals and lignin which caused leaching in organic mediums. Therefore, pectin, lignin, proteins and minerals must be removed. For this purpose the pine sawdust was treated respectively with NaOH, NaOCl, and H_2O_2. For preparation of nano-sawdust, the sawdust has been treated with concentrated H_2SO_4 for partial hydrolysis of its cellulose. Then, the nano-sawdust was used to synthesis of $TiCl_4$/nano-sawdust as a new, biodegradable and green catalyst.

In relation to this concept, we have aimed at preparation of $TiCl_4$/nano-sawdust as a new carbon based solid Lewis acid. We have evaluated its catalytic behaviour for the synthesis of highly functionalized dihydro-2-oxypyrroles *via* 4CRs of the dialkyl acetylenedicarboxylates, amines and aldehydes under thermal conditions.

As antibacterial and antifungal potencies are the most important activities which reported for dihydro-2-oxypyrroles derivatives, we have also intended to evaluate the synthesized compounds in these regards. Disc plate method and serial dilution assay were used to establish the minimum inhibitory concentration of the above compounds against four pathogenic bacteria and fungi compared to standard antimicrobial agents.

2. MATERIALS AND CHARACTERIZATION

All chemicals and solvents were purchased from the Merck and Fluka chemical companies in high purity. Materials were used from the commercial reagent grade. FT-IR spectra were recorded on an attenuated total reflectance fourier transform infrared (ATR-FTIR) spectrophotometer (Bruker, Eqinox 55). ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a BrukerDXR-400 spectrometer using $CDCl_3$ as solvent and tetramethylsilane as internal standard. Mass spectra (MS) were recorded on a FINNIGAN-MAT 8430 mass spectrometer, operating at an ionization potential of 70 eV. Melting points were obtained with a Buchi melting point B-540 B.V.CHI apparatus. Quantitative elemental information (EDS) of $TiCl_4$/nano-sawdust was measured by SEM/EDS instrument, Phenom pro X.

2.1 Preparation of Nano-sawdust

Pine sawdust (4 g), was first treated with a solution of 17.5% w/v sodium hydroxide in water bath maintained at 100°C for 12 hours to remove tannin, pectin, proteins and minerals. The residue was alpha cellulose that is not soluble in 17.5% w/v sodium hydroxide solution. The alkali treated fibers were washed repeatedly. The stock was then bleached with 100 ml of 1:1 diluted the 5% w/v sodium hypochlorite solution at 80°C for 8 hours. The resulting was then treated with 10 ml of 20% v/v hydrogen peroxide at 50°C for 2 hours due to the remove of the insoluble lignin. The resultant was hydrolyzed by refluxing with sulphuric acid (65% H_2SO_4 with fibber to liquor ratio of 1:20) for 2 hours at 60°C with strong agitation. Resulting mixture was cooled to room temperature and diluted by adding an excess of distilled water. Then, the suspension was repeatedly centrifuged at 12000 rpm for 8 minutes using a refrigerated centrifuge (Appendorf Centrifuge 5417R). After each run, the nano-sawdust (as white powder) was washed with distilled water and centrifuged until the supernatant was become neutral.

2.2 Preparation of $TiCl_4$/Nano-sawdust

In a ventilated cabin, a 25 mL suction flask equipped with a constant-pressure dropping funnel containing $TiCl_4$ (1 mL) and gas inlet tube for conducting HCl, charged with 1 g nano-sawdust and chloroform, $TiCl_4$ was added drop wise over a period of 3 min at room temperature. The mixture was stirred for one hour at room temperature. The resulted mixture was filtered. The obtained white solid was washed with chloroform and dried at room temperature.

2.3 Typical Procedure for the Synthesis of Dihydro-2-oxopyrroles

In a round-bottom flask (50 mL) equipped with a reflux condenser, firstly, a mixture of primary amine (1 mmol) and dialkyl acetylenedicarboxylate (1 mmol) in absolute

ethanol (4 mL) was stirred for 15 min. Then, substituted aniline (1 mmol), formaldehyde 37% (3 mmol) or aromatic aldehydes (2 mmol) and $TiCl_4$/nano-sawdust (0.07 g) in ethanol (3 mL) were added to the above mixture and stirred in 70°C. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was allowed to be cooled, filtered off and washed with EtOH (3×10 mL) to remove all unreacted substrates. For separation of catalyst from solid product, it was washed with chloroform (15 mL). The chloroform was evaporated and the crude solid product wasrecrystallized from ethanol to give the corresponding pure dihydro-2-oxopyrroles.

2.4 Evaluation of Antibacterial Activity

A combination of gram-negative and gram-positive bacteria and fungi were used in this study. The gram-positive microorganisms were *Staphylococcus aureus* (ATCC29737), *Enterococcus faecalis* (ATCC29212), the gram-negative ones were *Escherichia coli* (ATCC15224) and *Pseudomonas aeroginosa* (ATCC27853) and the fungi strain was *Candida albicans* (ATCC10231). The microorganisms were cultured in brain heart Infusion (BHI) broth overnight and stored in glycerol/Media (20%) at -70ºC. The pure cultures of microorganisms were sub-cultured in BHI broth at 37°C in a shaker incubator 24 h before experiment. The antibacterial evaluation was done using following methods.

2.4.1 Disc diffusion method

All procedures were carried out according to the guidelines of Clinical and Laboratory Standards Institute for the primary evaluation of compounds. Briefly, the bacterial culture that was spectrophotometerically adjusted to match the turbidity of 1.5×10^8 CFU (in OD600) were added to Muller-Hinton agar plates. The paper discs containing certain concentration of compounds, vancomycin and cefazolin antibiotics (standard control) and solvents (DMSO as negative control) were inoculated to the Mueller-Hinton agar. The plates were evaluated for the induced inhibition zones after a 24-h incubation period at 37°C. The zones of inhibitions were measured in diameter. All experiments were performed in six replicates.

The primary antifungal effect of compounds was done in similar way except the media was sabouraud-dextrose agar and the CFU of *Candida albicans (C. albicans)* was adjusted to 2×10^5.

2.4.2 Micro-dilution method

The minimum inhibitory concentration (MIC) of compounds was evaluated by a common broth microdilution method according to the guidelines presented by Clinical and Laboratory Standards Institute. Briefly, 2-fold serial dilutions of each compound were prepared in a 96-well microplate with 90 µL Mueller-Hinton Broth (MHB) medium. Each microbial strain was cultured in MHB to match the turbidity of 1.5×10^8 CFU/mL. To prepare bacterial suspensions for inoculation, this suspension was diluted 20 fold with fresh preincubated MHB medium and 10 µL of that was inoculated into the each well, then, incubated at 37°C overnight. The optical density of the wells was measured by a microplate reader apparatus (BioTek, Power Wave XS2) at wavelength 600. In this assay, untreated cultured media and pure uncultured were served as positive and negative controls respectively. The MIC value was defined as lowest concentration of antimicrobial agents that inhibited 90% of the bacterial growth after an overnight incubation compare to untreated cultures. These tests were performed in triplicate.

As for *C. albicans* microdilution test was used to determine the antifungal effect of chemical compounds by adding serially 2-fold dilutions of antimicrobial agents into wells of 96-well microplate containing 90 µL RPMI medium, and microplates were then inoculated with 90 µL of *Candida* suspension that was spectrophotometerically adjusted turbidity of 2×10^5 CFU (in OD630) and incubated at 31°C for 24 h. Amphotericin B (500-10 µg/ml) was used as standard control. The MIC were considered as the lowest concentration of each compound that showed no growth after 24 h of incubation by comparing with the control tube that containing 90 µL MHB and 100 µL of *Candida* suspension.

2.4.3 Bactericidal and fungicidal assessment of compounds

To measure the minimum bactericidal concentrations (MBCs), the media from wells, in which no bacteria had growth, was cultured on tryptic soy agar and incubated for 24 h at 37°C. The MBC values were the lowest concentration of compounds reduced the viability of the initial bacterial inoculum by ≥99.9%; so that less than 4

countable colonies can be detected after 24 h incubation at 37°C in agar plates.

Minimum fungicidal concentrations (MFCs) measurement was the same except in culture media that was sabouraud-dextrose agar in this experiment.

3. RESULTS AND DISCUSSION

Comparison of $TiCl_4$/nano-sawdust with nano-sawdust was achieved by FT-IR (ATR) spectra (Fig. 2). The FT-IR spectrum of nano-sawdust shows a broad band at 3337 cm^{-1} which corresponds to the stretching vibrations of OH groups. The absorption bands around 1055 and 1108 cm^{-1} display the stretching vibrations of the C-O bonds. For $TiCl_4$/nano-sawdust, cellulose absorptions appear in addition to the stretching vibrations of C-O-Ti at 828 cm^{-1}; indicating that titanium chloride is supported on nano-cellulose.

The morphology of $TiCl_4$/nano-sawdust surface and its particle size were investigated by transmission electron microscopy (TEM) and field emission scanning electron microscopy (FESEM (Fig. 3). The morphology of catalyst is amorphous and the dimension of its particles are below 50 nm.

Existence of Ti and Cl in catalyst was confirmed by EDS analysis data (Fig. 4). The percentages of Ti and Cl in TiCl4 are 25.24 and 74.76, respectively. Thus, the amounts of Ti and Cl in EDS data (Ti: 20.3 %, Cl: 14.9 %) revealed the absence of any unreacted TiCl4 in catalyst. Hence, XRF analysis of $TiCl_4$/nano-sawdust was performed to determine its elemental component (Table 1, Fig. 5).

To obtain the Ti:Cl ratio in $TiCl_4$/nano-sawdust using XRF analysis, Killo Counts Per Seconds (KCPS) of elements in catalyst were compared with KCPS of the same elements in pure samples, NaCl and TiO_2. By this comparison, the obtained amount of Ti and Cl were 12.02 g (0.25 mol) and 14.3 g (0.4 mol), respectively. Thus, the ratio of Ti:Cl in catalyst is approximately 1:2.

The X-ray diffraction (XRD) pattern of $TiCl_4$/nano-sawdust is depicted in Fig. 6. The values of 2θ and FWHM are shown in Table 2. According to XRD pattern, the three signals in 2Θ equal to 15.13, 16.74 and 22.93 with FWHM equal to 0.472, 0.944, and 0.472 respectively, show the existence of sawdust. Other signals in 2Θ equal to 20.55, 28.56, 34.63 and 41.84 prove the bonding of Ti to sawdust backbone.

According to the above-mentioned data, we have proposed a structure for $TiCl_4$/nano-sawdust (Fig. 7). This catalyst does not need special precautions for preparation, handling or storage, and it can be stored at an ambient temperature for months without losing its catalytic activity.

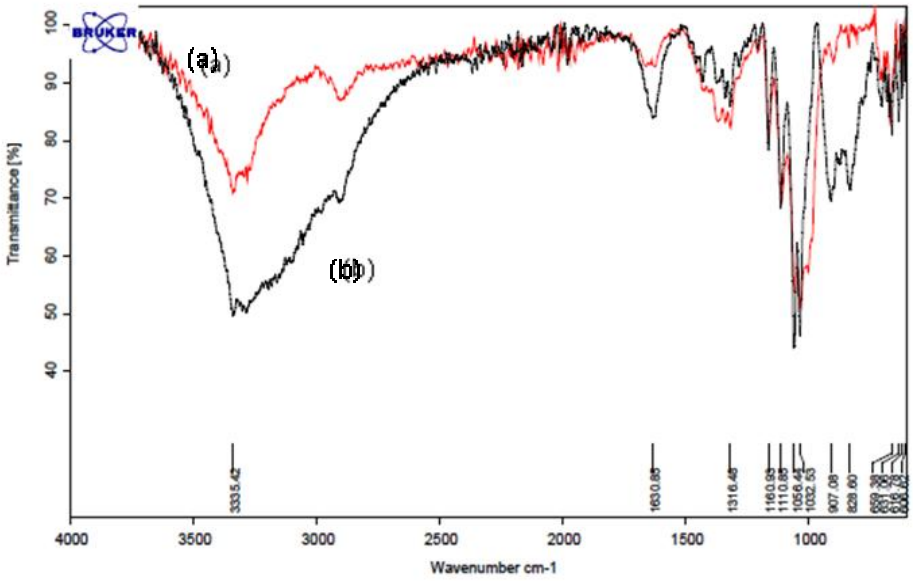


Fig. 2. FT-IR spectra of (a) nano-sawdust, (b) $TiCl_4$/nano-sawdust

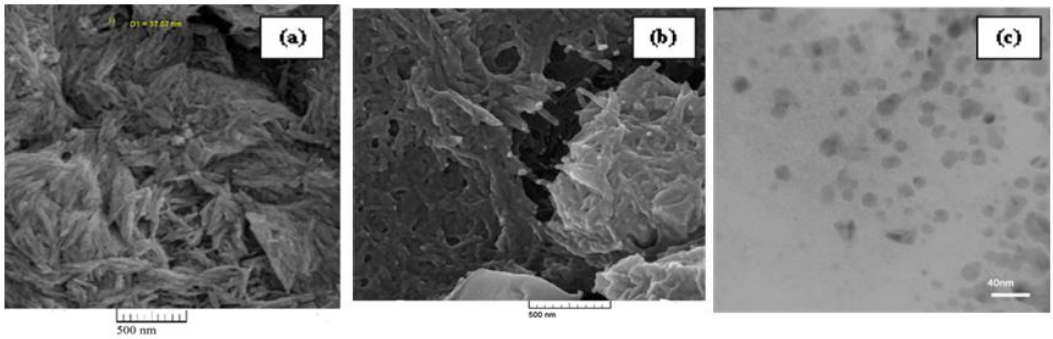


Fig. 3. (a) FESEM image of nano-sawdust, (b) FESEM of $TiCl_4$/nano-sawdust and (c) TEM of $TiCl_4$/nano-sawdust

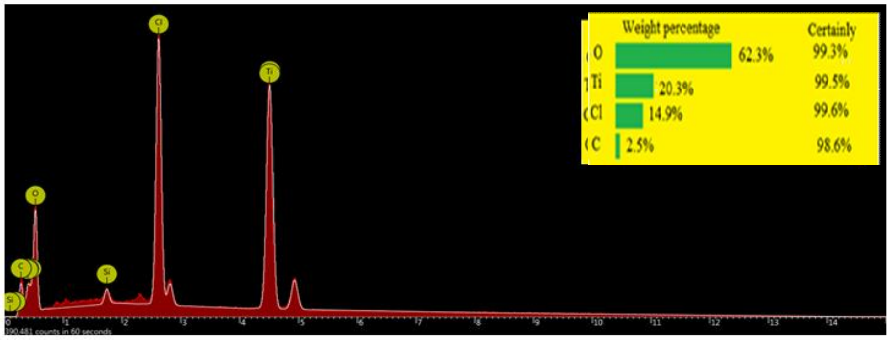


Fig. 4. EDS (EDX) spectra of $TiCl_4$/nano-sawdust

Table 1. Results of XRF analysis of $TiCl_4$/nano-sawdust and pure samples NaCl and TiO_2

Elemental component	$TiCl_4$/nano-sawdust		NaCl		TiO_2	
	KCPS	wt %	KCPS	wt %	KCPS	wt %
Cl	123.5	6.8	516.5	62.4		
TiO_2	464.3	22.2			2318.4	99.1
CO_2	1.6	67.1				
CaO	37.6	1.9				
SiO_2	7.1	1.43				
Al_2O_3	2.3	0.558				
SO_3	5.3	0.504				
MgO	1.9	0.374				
Total		100.86				

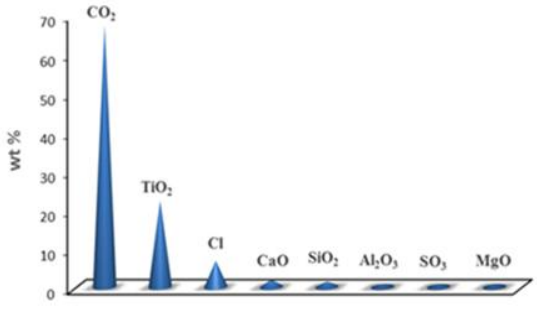


Fig. 5. XRF analysis of $TiCl_4$/nano-sawdust

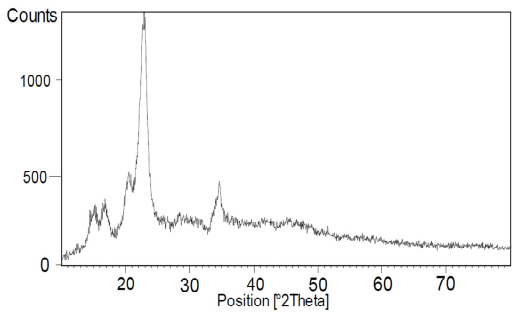


Fig. 6. XRD pattern of $TiCl_4$/nano-sawdust

Table 2. $TiCl_4$/nano-sawdust reflexes in XRD diffractogram

No	1	2	3	4	5	6	7
Pos. [°2Θ]	15.130	16.748	20.553	22.935	28.563	34.634	41.846
FWHM [°2Θ]	0.472	0.944	0.472	0.472	0.708	0.295	1.728

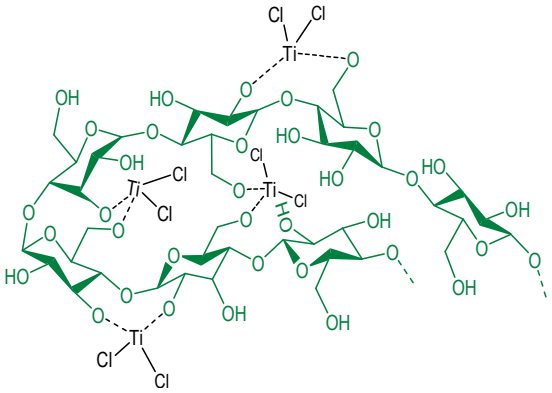
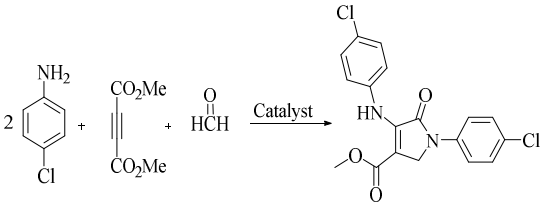


Fig. 7. Proposed structure of $TiCl_4$/nano-sawdust

In this study, we have investigated the catalytic activity of $TiCl_4$/nano-sawdust for the synthesis of dihydro-2-oxopyrroles via 4CRs of the dialkylacetylenedicarboxylates, amines and aldehydes. The synthesis of dihydro-2-oxopyrroles is an intermolecular nucleophilic addition reaction (Mannich reaction type) included several intermediates. Therefore, selection of appropriate conditions such as catalyst, solvent and temperature is required for the reaction. As a model reaction, synthesis of methyl-1-(4-chlorophenyl)-4-((4-chlorophenyl) amino)-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate (***S1***) (Scheme 1) was examined. Initially the reaction was performed in present of AcOH as homogeneous catalyst and refluxed in ethanol for 4 hours (Table 3, entry 1) [23]. After standard reaction workup, the desired compound was isolated. This homogeneous catalyst has some limitations such as inefficient separation of the catalyst from reaction mixtures, unrecyclable and environmentally limitations.

Therefore, different reaction conditions in the presence of $TiCl_4$/nano-sawdust was evaluated on the selected model reaction to develop a more robust and applicable protocol. As shown in Table 3, Most of the reaction efficiency was acquired using 3 mmol of formaldehyde in ethanol at 70 °C and in the presence of 0.07 g $TiCl_4$/nano-sawdust after 4 h (Table 3, Entry 10). Effect of different solvents on the reaction was investigated and revealed that ethanol gave the best results for this transformation. Reusability of the catalyst was investigated for three cycles (Table 3, Entries 15–17). For this purpose after each run the reaction mixture was diluted with acetone or ethanol and subsequently centrifuged to obtain the catalyst. The obtained catalyst was then washed with chloroform followed by drying in oven at 100°C for 4 h. The recovered catalyst was then used for the next run of the reactions. It was found that the reactivity of the catalyst decreases marginally for the next run (approximately, 4%).



Scheme 1. Synthesis of methyl-1(4-chlorophenyl)-4-((4-chlorophenyl)amino)-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate (*S1*)

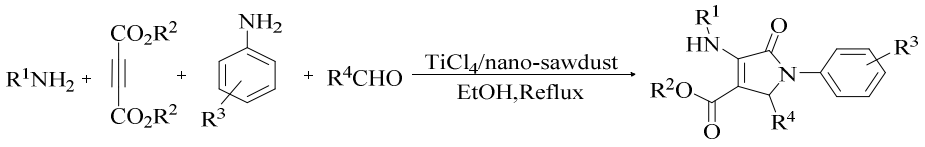
In comparison yield of synthesis of the compounds with $TiCl_4$/nano-sawdust as catalyst with another homogeneous catalysts for example, the yield if the reaction with catalyst AcOH [23] is about 90% and for *p*-TsOH [21] is between 52-70%), we should mention that in this project in addition of yield of the reaction, we tried to consider other parameters as well as an efficient catalyst for example short reaction times, scale up, readily available, inexpensive, biodegradable and environmentally and easy work-up are the advantages of this catalyst.

Finally, with optimized reaction conditions obtained for the synthesis of methyl-1(4-chlorophenyl)-4-((4-chlorophenyl)amino)-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate (Scheme 1), from the amounts of reactant, solvent, catalyst and reaction temperature point of view, the scope of this transformation was explored. Accordingly, for syntheses of different dihydro-2-oxopyrrole derivatives were examined and high yields were noticed in most cases (Scheme 2, Table 4).

Table 3. Preparation of methyl-1(4-chlorophenyl)-4-((4-chlorophenyl)amino)-5-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate (*S1*) under various conditions.[a]

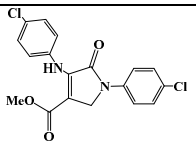
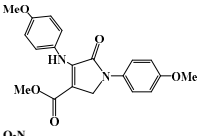
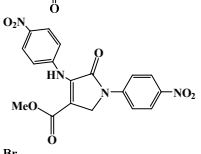
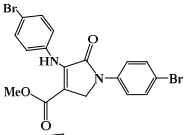
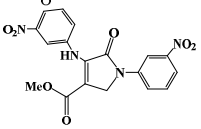
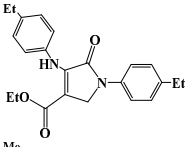
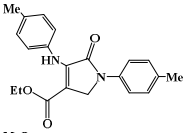
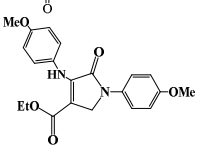
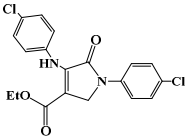
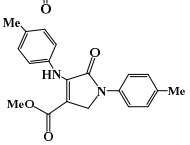
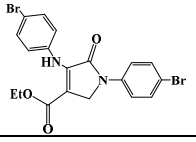
Entry	Solvent	Catalyst	Reactant I: II:III	Condition	Time (h)	Yield (%)[b]
1	EtOH	AcOH	3:1:1.5[c]	Reflux	4h	89% [23]
2	EtOH	$TiCl_4$/nano-sawdust (0.02)	2:1:1.5	R.T.	3h	14%
3	EtOH	$TiCl_4$/nano-sawdust (0.02)	2:1:1.5	Reflux	3h	30%
4	EtOH	$TiCl_4$/nano-sawdust (0.02)	2:1:2	Reflux	3h	36%
5	EtOH	$TiCl_4$/nano-sawdust (0.02)	2:1:2.5	Reflux	3h	44%
6	EtOH	$TiCl_4$/nano-sawdust (0.02)	2:1:3	Reflux	3h	49%
7	EtOH	$TiCl_4$/nano-sawdust (0.04)	2:1:3	Reflux	3h	61%
8	EtOH	$TiCl_4$/nano-sawdust (0.06)	2:1:3	Reflux	3h	73%
9	EtOH	$TiCl_4$/nano-sawdust (0.07)	2:1:3	Reflux	3h	78%
10	EtOH	$TiCl_4$/nano-sawdust (0.07)	2:1:3	Reflux	4h	91%
11	MeOH	$TiCl_4$/nano-sawdust (0.07)	2:1:3	Reflux	4h	63%
12	EtOH/MeOH	$TiCl_4$/nano-sawdust (0.07)	2:1:3	Reflux	4h	67%
13	$CHCl_3$	$TiCl_4$/nano-sawdust (0.07)	2:1:3	Reflux	4h	42%
14	*n*-Hexane	$TiCl_4$/nano-sawdust (0.07)	2:1:3	Reflux	4h	38%
15	EtOH	$TiCl_4$/nano-sawdust (0.07) ,2nd run	2:1:3	Reflux	4h	88%
16	EtOH	$TiCl_4$/nano-sawdust (0.07) ,3nd run	2:1:3	Reflux	4h	87%
17	EtOH	$TiCl_4$/nano-sawdust (0.07) ,4nd run	2:1:3	Reflux	4h	82%
18	MeOH	I_2 (10mol %)	2.1:1:1.2	R.T.	1h	81% [20]
19	MeOH	$Cu(OAc)_2 \cdot H_2O$ (0.4 eq)	2.6:1:1.4	R.T.	3h	86% [22]
20	MeOH	[*n*-Bu_4N][HSO_4] (10 mol %)	2:1:1	R.T.	4h	86% [26]
21	MeOH	$InCl_3$ (20 mol %)	2:1:1.5[d]	R.T.	3h	79% [25]

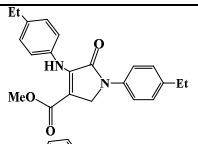
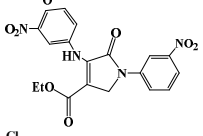
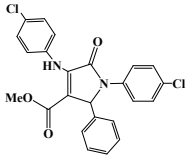
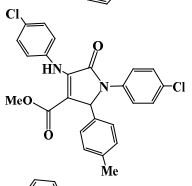
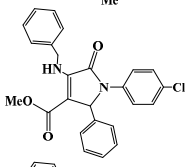
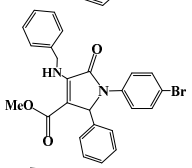
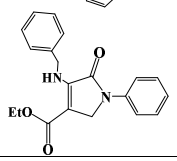
[a]Reactions were run with the following steps: In the first step, dimethylacetylendicarboxylate(1 mmol) and 4-chloroaniline (1 mmol) were added into 4 mL solvent and kept at room temperature for 15 min. Then, 4-chloroaniline(1 mmol), formaldehydeand catalyst were added to the above mixture respectively, and then stirred at rt or under reflux condition for desired time, [b]Isolated yield after recrystallization in ethanol, [c]4-bromoaniline instead of 4-chloroaniline was used, [d]Diethyl acetylenedicarboxylate instead of dimethyl acetylenedicarboxylate was used



Scheme 2. Synthesis of different dihydro-2-oxopyrrole derivatives under optimized reaction conditions

Table 4. Synthesis of dihydro-2-oxopyrrole derivatives in the presence of $TiCl_4$/nano-sawdust at 70°C.[a]

Entry	R^1	R^2	R^3	R^4CHO	Product	Time	Yield [b]	M.P. Ref
S1	4-Cl-C_6H_4	Me	4-Cl	HCHO		4h	91%	173-174 [22]
S2	4-OMe-C_6H_4	Me	4-OMe	HCHO		4h	83%	160-162 [20]
S3	4-NO_2-C_6H_4	Et	4-NO_2	HCHO		5h	75%	206-208
S4	4-Br-C_6H_4	Me	4-Br	HCHO		2h	90%	181-182 [22]
S5	3-NO_2-C_6H_4	Me	3-NO_2	HCHO		3h	79%	204-206
S6	4-Et-C_6H_4	Et	4-Et	HCHO		4h	80%	98-100
S7	4-Me-C_6H_4	Et	4-Me	HCHO		4h	88%	128-130 [23]
S8	4-OMe-C_6H_4	Et	4-OMe	HCHO		5h	85%	152-154 [25]
S9	4-Cl-C_6H_4	Et	4-Cl	HCHO		4h	95%	165-167 [26]
S10	4-Me-C_6H_4	Me	4-Me	HCHO		3h	84%	175-176 [22]
S11	4-Br-C_6H_4	Et	4-Br	HCHO		2.5h	91%	165-166 [22]

Entry	R^1	R^2	R^3	R^4CHO	Product	Time	Yield [b]	M.P. Ref
S12	4-Et-C_6H_4	Me	4-Et	HCHO		4h	81%	125-126 [20]
S13	3-NO_2-C_6H_4	Et	3-NO_2	HCHO		3h	85%	191-192
S14	4-Cl-C_6H_4	Me	4-Cl	C_6H_5CHO		3h	89%	175 – 177 [22]
S15	4-Cl-C_6H_4	Me	4-Cl	4-Me-C_6H_4CHO		4h	92%	148-150 [19]
S16	$PhCH_2$	Me	4-Cl	C_6H_5CHO		2h	94%	136-138 [22]
S17	$PhCH_2$	Me	4-Br	C_6H_5CHO		3h	91%	154-156 [22]
S18	$PhCH_2$	Et	H	HCHO		3h	95%	138-140 [23]

[a]For entries of 1-13 and 18, the amount of amine (mmol): dialkylacetylenedicarboxylate (mmol): formaldehyde (mmol): $TiCl_4$/nano-sawdust (g) are equal to 2:1:3:0.07. For entries of 14-17, the amount of amine (mmol): dialkylacetylenedicarboxylate (mmol): aldehyde (mmol): $TiCl_4$/nano-sawdust (g) are equal to 2:1:2:0.07.
[b]Isolated yields after recrystallization in ethanol

3.1 Antibacterial Activities of the Synthetic Compounds

Table 5 summarizes the inhibitory activities of the synthetic compounds against the tested bacteria. MIC values of the synthetic compounds showed ***S6***, ***S7*** and ***S8*** exhibited strong inhibitory activities against gram positive bacteria in comparison to the cefazolin and vancomycin as standard antibiotics.

Consideration on chemical structure of the ***S6***, ***S7*** and ***S8*** based on variation of substitutions on the 4-position of phenyl ring, 1 and 4-position of oxopyrrole ring, or various methyl or ethyl carboxylate substitutions on the 3-position of the oxopyrrole ring, be evidence for that all three active compounds, have an ethyl carboxylate substitution on the 3-position of the oxopyrrole ring but they have a different residue in 4-position of the phenyl rings at 1 and 4-position of oxopyrrole ring.

Ethyl residue in 4-position of phenyl ring of ***S6*** increased the activity of this compound in compare to ***S7*** with methyl residue and ***S8*** with methoxy group in the same position of the phenyl rings.

The biological behavior of these compounds seems too related to the ratio of their lipophilicity. The increase in lipophilicity, causes increase the permeability of the membrane. This mechanism can be explane as action on the cell wall or with more penetrate into the cells to exert their intracellular effects. It should be noted that higher lipophilicity can affect the solubility of compounds. Therefore a suitable hydrophilicty / lipophilicity balance in the compounds can cause the best final biological effect [31-32].

For the gram negative bacteria, compounds ***S6*** and ***S3*** were more effective. Compounds ***S2***, ***S1***, ***S12*** and ***S5*** also exhibit acceptable inhibitory activities against *E. coli* strains too.

The main difference between chemical structure of ***S6*** and ***S3*** related to various substitutions on the 4-position of the phenyl rings of the oxopyrrole scaffold. Compound ***S6*** with ethyl residue in the 4-position of phenyl ring exhibited a better antibacterial activity versus compound ***S3*** with nitro residue in the 4-position of phenyl ring. Electron donating or electron withdrawing properties of these substitutions could lead to variation of the antibacterial activity. This might be probably due to higher solubility of these compounds in aquatic media [31-32].

For *Pseudomonas aeroginosa* strains, compounds ***S6***, ***S9*** and ***S11*** had the best inhibitory effect. Compound ***S9*** and ***S11*** with Cl and Br residues in 4-positions of phenyl ring respectively exhibited lower antibacterial activities against the tested *Pseudomonas aeroginosa* strain than compound ***S6*** which had ethyl residue substitution in 4-positions of phenyl ring.

Table 5. Minimum inhibitory concentration (MIC in µg/mL), and minimum bactericidal concentration (MBC in µg/mL) mean values of different compounds

	S1		***S2***		***S3***		***S4***		***S5***		***S6***		***S7***	
	MIC_{90}	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**
E. coli	2.25	9	2.25	4.5	0.56	-	-	-	2.25	9	0.14	1.12	-	-
P. aeruginosa	-	-	-	-	-	-	2.25	-	-	-	0.14	0.28	-	-
S. aureus	9	-	-	-	-	-	-	-	-	-	0.14	0.56	0.56	4.5
E. faecalis	-	-	-	-	-	-	-	-	-	-	0.14	0.28	1.12	4.5

	S8		***S9***		***S10***		***S11***		***S12***		**Vancomycin**		**Cefazolin**	
	MIC_{90}	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**
E. coli	-	-	-	-	-	-	-	-	4.5	-	0.28	2.25	0.14	2.25
P. aeruginosa	1.12	-	0.28	2.25	-	-	0.14	0.28	-	-	0.14	2.25	0.28	4.5
S. aureus	2.25	4.5	-	-	-	-	-	-	-	-	0.56	4.5	0.14	2.25
E. faecalis	1.12	4.5	-	-	-	-	-	-	-	-	0.28	4.5	-	-

Table 6. Minimum inhibitory concentration (MIC in µg/mL), and minimum fungicidal concentration (MFC in µg/mL) mean values of different compounds

	S1		***S2***		***S3***		***S4***		***S5***		***S6***		***S7***	
	MIC_{90}	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**
C. albicans	0.28	4.5	0.28	4.5	0.28	4.5	0.28	4.5	1.12	9	0.28	4.5	0.28	4.5

	S8		***S9***		***S10***		***S11***		***S12***		***Amphotricin B***		***Fluconazole***	
	MIC_{90}	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**	**MIC_{90}**	**MBC**
C. albicans	0.28	4.5	0.28	4.5	0.56	4.5	0.56	4.5	0.28	4.5	0.14	4.5	0.14	4.5

As shown in the Table 6, most of the examined synthetic compounds were found to possess antifungal effect against *C. albicans*. The introduction of ethyl or nitro residue in the para position of phenyl ring increased antifungal activity. On the other hand, the lack of any substitutent caused a reduction in antifungal effect. Compounds ***S1***, ***S2***, ***S3***, ***S4***, ***S6***, ***S7***, ***S8*** and ***S12*** are nearly active as antifungal amphtricine-B and fluconazole with potency about 40–60%. However, none of the tested compounds showed superior activity than the reference standards.

4. CONCLUSION

In conclusion, an extremely efficient and green protocol has been developed for synthesis of various substituted dihydro-2-oxopyrrole derivatives by a simple and high efficient one-pot four-component procedure using $TiCl_4$/nano-sawdust. This protocol has many advantages including high conversions, low-cost and easy workup, which makes this method more attractive. Biological studies showed that dihydro-2-oxopyrrole derivatives had moderate antimicterial effect on pathogenic bacteria and fungi. Making a good hydrophilicty/lipophilicity balance in the 4-position of phenyl ring of dihydro-2-oxopyrroles increased their anti-bacterial activities.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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