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An Efficient Catalyst for the Fabrication of Substituted Pyrroles with Specific Nanomorphology as Potential Antimicrobial and Antioxidant Agents

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Abstract

Highly typical tetra-substituted pyrroles**4a-j** were prepared by the condensation of 1,3-dicarbonyl compounds, benzoin, and ammonium acetate *via* a one-pot hydrothermal synthesis procedure in the presence of sulfamic acid (SA), as a solid-phase organic catalyst which has recently become quite popular. The efficiency of SA as a new catalyst and $ZnCl_2$ astraditional catalystin the synthesis of 1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethanone**4a** was performed as an example for the present comparison. Scanning electron microscopy (SEM) was used to compare the morphologies of the 1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethanonefilms **4a** that prepared by those catalysts. An appropriate ratio of sulfamic acid was enough to start the reaction with a higher yield and a higher surface area to volume ratio. Besides, it provided nanoscale-interpenetrating networks with homogenous structures. The chemical structure of the target compounds was characterized by NMR and FTIR spectroscopy. In addition, the prepared compounds were evaluated to study their antimicrobial and antioxidant activities.

***Keywords*:** nano structures, multicomponent reaction, pyrrole, sulfamic acid, solid acids, antimicrobial activity, antioxidant activity

1. INTRODUCTION

Currently, Nano structuring of pharmaceutical materials has proved efficient for various promising applications, such as particular control of solubility and release kinetics and improving activity [1,2] In this study, we demonstrate a successful solvent-free synthesis of micro-nanostructured pharmaceutical molecules using a simple and efficient catalyst. A variety of organic catalysts have been used in heterocyclic synthesis, among them $ZnCl_2$, tetrachlorosilane, trifloroacetic acid, and silica sulfuric acid. However, such catalysts fail to meet the current ecological and economicrequirements to a well-designed synthetic route and, in this respect, sulfamic acid NH_2SO_3H as a catalyst offers a lot of advantages from the green chemistry perspective. An ideal route in synthesis is atom-economic, uses renewable starting materials, delivers high yields, is environmentally safe and cost effective, and involves as few synthetic steps as possible. In addition, Sulfamic acid is derived from natural resources, it is ecofriendly, commercially available, inexpensive, and recyclable catalyst. Furthermore, Sulfamic acid is a nonvolatile, noncorrosive, and non-hygroscopic

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crystalline substance. The specific catalytic properties of his solid acid are due to its zwitterionic nature, and SA proved to be a highly efficient catalyst for a great number of organic reactions [3–8]. Moreover, Sulfamic acid in solvent-free conditions was found quite efficient catalytic system for the synthesis of aminonitriles and pyrroles, because the catalyst could be recovered by simple filtration and further recycled [9]. Based on these findings, we decide to employ SA as a catalyst for the preparation of pyrrole derivatives. As known, pyrroles are an essential class of compounds due to their wide range of biological activities. Pyrroles possess a large variety of therapeutic effects and are active materials in various applications. Furthermore, pyrroles are the most important class among nitrogen-containing heterocyclic compounds. The pyrrole core is present in such essential natural compounds as vitamins, myoglobin, cytochromes, chlorophyll and haemoglobin. A wide range of medicines contain the pyrrole moiety in their fundamental structures [10]. Additionally, a large number of pyrrole derivatives, such as 2-methyl-4, 5-diphenyl-1H-pyrrol-3-yl, have exhibited diverse pharmacological and biochemical properties [11-15], including antioxidant, analgesic, anti-inflammatory etc. Pyrrole compounds are also incorporated in conductive electrode materials which can be used in super- capacitors. In this regard, polypyrroles and poly- or hetero-arylpyrroles play a vital role in providing electronic delocalization [16]. In view of the aforesaid, we decided to screen the pyrrole derivatives synthesized in the present work for antimicrobial and antioxidant activity. Despite incredible efforts focused over the past on developing operative procedures for creating the pyrrole ring, such procedures still remain an important challenge, because conventional synthesis requires costly and hazardous chemicals, as well as harsh reaction conditions. One-pot multicomponent reactions provide a promising alternative synthetic approach to pyrrole derivatives. Therefore, in this work we employed the approach for exploring the potential of SA as a catalyst for the synthesis of pyrrole derivatives.

2. EXPERIMENTAL

2.1 Chemicals and Supplies

The chemicals were purchased from Merck and Aldrich. The products were characterized by comparison of their physicochemical and spectral characteristics with those reported in the literature. The IR spectra were measured on a Shimadzu FTIR 8300 spectrophotometer in KBr pellets. The ^{1}H NMR spectra were recorded on a BrukerAvance Ultrashield400 spectrometer in DMSO-$d_6$or $CDCl_3$ with TMS as internal standard. The mass spectra were re1corded on a Finnigan MAT 8430 instrument at ionization energy of 70 eV. The melting points were determined in open capillary tubes on Barnstead Electrothermal 9100 BZ circulating oil melting point apparatus and are uncorrected. The reaction monitoring was accomplished by TLC on PolyGram SILG/UV254 plates. The morphology of compound **4a** was determined by scanning electron microscopy on a Carl ZeissLEO 1550VP Gemini SEM.

2.2.The general procedure for the synthesis of 2,3,4,5-tetrasubstituted pyrroles4a–4j using sulfamic acid

Sulfamic acid (2mmol) was added to a mixture of benzoin derivative (2 mmol), 1,3-dicarbonyl compound (2 mmol), and NH_4OAc (3 mmol). The resulting mixture was stirred at 70–80 °C for the given times (Table 1). After completion of the reaction (by TLC), the mixture was cooled to room temperature, diluted with water (10 mL), The catalyst was simply recovered by evaporation of the aqueous phase, and then stored before use in a subsequent run under the same conditions without any treatment. The precipitate of crude pyrrole that was filtered off was recrystallized from MeOH–H_2O (70:30) in order to obtain the pure product.

2.3.Synthesis of 21-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethanone(4a) using $ZnCl_2$ (Nano-synthesize)

$ZnCl_2$(2mmol) was added to a mixture of 2-hydroxy-1,2-diphenylethan-1-one (**1a**, 2 mmol), pentane-2,4-dione (2a, 2 mmol), and NH_4OAc (3 mmol). The resulting mixture was stirred at 70–80 °C for 7 hours. The mixture was cooled to room temperature, diluted with water (15 mL), The precipitate of crude pyrrole that was filtered off was recrystallized from MeOH–H_2O (70:30) in order to obtain the pure product.

2.4. 1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethanone(4a)

Pale yellow solid, mp 175-177°C (reported mp 170–172°C [17]. FT-IR spectrum, ν, nm: 3170 [ν(NH)], 1638 [ν(CO)]. ^{1}H NMR spectrum (DMSO-d_6), δ,

ppm: 1.75 s (3H, CH_3), 2.50 s (3H, CH_3), 7.15–7.41 m (10H, Ar), and 11.66br s (1H, NH). Mass spectrum: *m/z:* 275 (I_{rel}52.2 %) $[M]^+$.Anal.Calcd for $C_{19}H_{17}NO$ (275): C, 82.88; H, 6.22; N, 5.09. Found, %: C, 80.99; H, 6.01; N, 6.31.

2.5.(2-Methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)(phenyl)methanone(4b)

Yellow solid, m.p.: 216-218 °C (reported mp 218-219 [17]. FT-IR: υ, nm: 3282 [(ν NH stretching)] and 1608 [ν (CO)] cm^{-1}.^{1}H NMR (DMSO-d6): δ (ppm) 2.24 s (3*H*, CH_3), 7.00-7.85 m (15*H*, Ar), 11.55 s (NH). Mass spectrum: *m/z*: 337 (44.2%) $[M]^+$. Anal.Calcd for $C_{24}H_{19}NO$ (337): C, 85.43; H, 5.68; N, 4.15; Found: C, 84.99; H, 6.01; N, 4.31%.

2.6.(4-Fluorophenyl)(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)methanone(4c).

Yellow solid, m.p.: 212-214 °C. FT-IR: υ. nm: 3258 [ν (NH stretching)] and 1610[ν (CO)] cm^{-1}.^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 2.29 s (3*H*, CH_3), 6.92-7.55 m (14*H*, Ar), 11.66 s (NH). Mass spectrum: *m/z*: 355 (M^+; 50.2). Anal.Calcd for $C_{24}H_{18}FNO$ (355): C, 81.11; H,5.10; N, 3.94; Found: C, 80.99; H, 5.41; N, 4.31%.

2.7.(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)(thiophen-2-yl)methanone (4d)

Yellow solid, m.p.: 212-214 °C. FT-IR: υ 3342 [(ν NH stretching)] and 1660 [ν (CO)] cm^{-1}. ^{1}H NMR (DMSO-d_6): δ (ppm) 2.3 s (3*H*, CH_3), 6.89-7.74 m (13*H*, Ar), 11.60 s (NH).^{13}C NMR (DMSO-d_6): δ (ppm) 12.2(CH_3), 120.4, 125.7, 126.8, 127.6,128.6, 129.9, 132.4, 133.1, 133.7, 135.5, 137.9, 145,4, 148.2 (20C, aromatic carbon), 184.7(C=O). Mass spectrum: *m/z*: 343 (M^+; 61.4). Anal.Calcd for $C_{22}H_{17}NOS$ (343): C, 79.94; H, 4.99; N, 4.08; S, 9.34; Found: C, 80.51; H, 5.41; N, 4.31; S, 8.98%.

2.8.Phenyl(2,4,5-triphenyl-1H-pyrrol-3-yl)methanone (4e)

Yellow solid, m.p.: 194-196 °C. FT-IR: υ 3038 [(ν (NH stretching)] and 1622 ν (CO) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ (ppm) 6.59-8.10 m (21*H*, Ar and NH). Mass spectrum: *m/z*: 399 (M^+; 57.8). Anal.Calcd for $C_{29}H_{21}NO$ (399): C, 87.19; H, 5.30; N, 3.51; Found: C, 86.99; H, 5.41; N, 4.55%.

2.9-2,3-Diphenylindeno[1,2-b]pyrrol-4(1H)-one (4f)

Violet solid, m.p.: 216-218 °C. FT-IR: υ 3167 (ν NH stretching), 1625 [ν (CO)]cm^{-1}.^{1}H NMR (DMSO-d6): δ (ppm) 7.1-9.2 m (15H, Ar and NH). Mass spectrum: *m/z*: 321 (M^+; 66.28). Anal.Calcd for $C_{23}H_{15}NO$ (321): C, 85.96; H, 4.70; N, 4.36; Found: C, 86.09; H, 3.98; N, 4.55%.

2.10 [4,5-Bis(4-methoxyphenyl)-2-methyl-1H-pyrrol-3-yl](phenyl)methanone (4g)

Yellow solid, m.p.: 223-225°C. FT-IR: υ 3288 [υ (NH stretching)] and 1617 ν (CO) cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6): δ (ppm) 2.12 s (3*H*, CH_3), 3.77 s (6*H*, 2OCH_3), 6.59-7.50 m (13*H*, Ar), 11.43 s (NH); ^{13}C NMR (100 MHz, DMSO-d_6): δ (ppm) 13.2(CH_3), 55.4 (2 OCH_3), 113.6,114.2, 120.9, 126.8, 127.6, 128.2, 128.8, 129.4, 131.7, 133.5, 140.5, 137.9, 157.6, 158.2 (22C, aromatic carbon), 193.0 (C=O). Mass spectrum: *m/z*: 397 (M^+; 89). Anal.Calcd for $C_{26}H_{23}NO_3$ (397): C, 78.57; H, 5.83; N, 3.52; Found: C, 79.09; H, 5.98; N, 3.55%.

2.11.[4-(Fluorophenyl)(5-(3-methoxyphenyl)-4-(4-methoxyphenyl)-2-methyl-1H-pyrrol-3-yl] methanone (4h)

Yellow solid, m.p.: 223-225 °C. FT-IR: υ 3291 [ν (NH stretching)], 1613 (ν (C O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ (ppm) 2.26 s (3*H*, CH_3), 3.77 s (6*H*, 2OCH_3), 6.60-7.50 m(12*H*, Ar), 11.45 s (NH). Mass spectrum: *m/z*: 415 (M+; 70.28). Anal.Calcd for $C_{26}H_{22}FNO_3$ (415): C, 75.17; H, 5.34; F, 4.57; N, 3.37; Found: C, 76.09; H, 4.98; F, 5.01; N, 3.55%.

2.12.[5-(3-Methoxyphenyl)-4-(4-methoxyphenyl)-2-phenyl-1H-pyrrol-3-yl](phenyl) methanone (4i)

Yellow solid, m.p.: 194-196 °C. FT-IR: υ 3227 (ν NH stretching) and 1613 ν (CO)cm^{-1}. ^{1}H NMR (DMSO-d_6): δ (ppm) 3.77 s (6*H*, 2OCH_3), 6.09-8.17 m (19H, Ar and NH). Mass spectrum: *m/z*: 459 (M^+; 46.28). Anal.Calcd for $C_{31}H_{25}NO_3$(459): C, 81.02; H, 5.48; N, 3.05; Found: C, 81.39; H, 5.98; N, 2.99%.

2.13.2,3-Bis(methoxyphenyl)indeno[1,2-b]pyrrol-4(1H)-one(4j)

Violet solid, m.p.: 216-218 °C FT-IR: υ 3160 [υ (NH stretching)], 1622 ν (CO) cm−1.^{1}H NMR (DMSO-d6): δ (ppm) 3.81 s (6H, 2OCH3), 6.91-9.2 m (13H, Ar and NH); ^{13}C NMR (100 MHz, DMSO-d6): δ (ppm) 53.4 (2C, 2OCH3), 120.7, 122.0, 125.7, 126.8, 127.6, 127.8, 128.9,129.6, 130.2, 131.3, 132.2, 134.0, 135.6, 139.7,163.2 (22C, aromatic carbon) 190.8 (C=O). Mass spectrum: *m/z*: 381 (M^+; 35.28).

Anal.Calcd for $C_{25}H_{19}NO_3$ (381): C, 78.72; H, 5.02; N, 3.67; Found: C, 77.9; H, 5.34; N, 3.12%.

2.14.Antimicrobial activity assay

The antimicrobial activity of target compounds were evaluated to be in vitro against a variety of pathogenic local and standard microorganisms, namely *Staphylococcus aureus(ATCC2913), Streptococcus pneumonia (Gram-positive bacteria), Escherichia coli(ATCC27953), Pseudomonas aeruginosa(ATCC27953), Klebsiella pneumonia(ATCC13883), Proteus mirabilis (Gram-negative bacteria), Candida albicans(NRRY-477) (fungus) using by well diffusion method* (Shalaby et al. 2018 &Pepeljnjak, et al. 2005) [18,19]. The test samples were prepared by dissolved each tested compound in 1 mL of DMSO, and sterilized by filtration through a 0.22 µm membrane filter using the Milliopore membrane filter apparatus. 150 µl of each sample was added separately to the wells in the petri dishes, well diffusion method, using the test microbes [19].

Synthesized compounds were evaluated for their antimicrobial activity via well diffusion method by placing pre-sterilized filter paper disks (6mm diameter) impregnated with 50µg/dick DMSO solvent, with no inhibition zones. The inhibition zones (IZ) of the tested compounds (mm) were measured after 24 h for bacteria at 37°C and after 48 h for fungi at 28°C. The experiment was carried out twice and mean of reading was recorded. Forcetex and Dermofix were used as positive control for bacterial and fungal test microbes at the same concentrations.

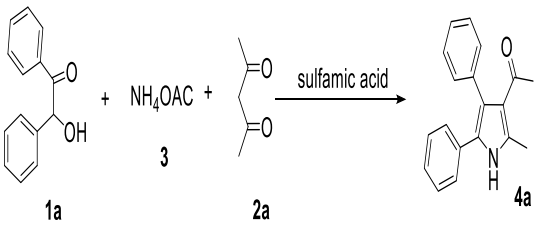
2.15.Antioxidant activity assay

The experiment was performed according to literature [20, 21]. The stock solutions of synthesized compounds were prepared by dissolving 10 mg of the samples in 1 mL DMSO, and then diluted serially (specify concentrations). A triplicate of 10 µl from each concentration was prepared after that 90 µg /mL of DPPH was added on an Eliza plate, and then the plate was stored in dark and cover with an aluminum foil for 30 min then measured at 520 nm on an Eliza reader [22].

3. RESULTS AND DISCUSSION

As part of our ongoing effort to develop green synthetic solvent-free methodologies for organic synthesis [23], we describe here the preparation of tetrasubstitutedpyrroles using 1,3-dicarbonyl compounds, benzoin derivatives and ammonium acetate through solvent-free reaction condition. The SA catalyst was used *via a* one-pot multi-component reaction as shown in (Scheme 1). The preferred product was recrystallized from a mixture of water and ethanol with high yields without time consuming [24, 25].

At the beginning of the study, we tested various potential catalysts for the direct synthesis of 1-(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethanone (**4a**) by the model reaction of benzoin (2 equiv), acetylacetone (2equiv), and ammonium acetate (3 equiv) under different conditions (Scheme 1), with the results listed in Table 1.As shown in (Table 1), SA was found to be a good catalyst and the best one for increasing the yield of the pyrrole derivatives. The experiments were performed at 70-80°C under solvent-free conditions with catalyst and without catalyst. In the second set of experiments, the reaction failed to provide any products without catalyst at 70-80°C for 24 h. Therefore, a solvent-free catalyst is a significant element for MCR. As shown in Table 2, the aimed products **4a–j**were obtained in 75–95 % yield.

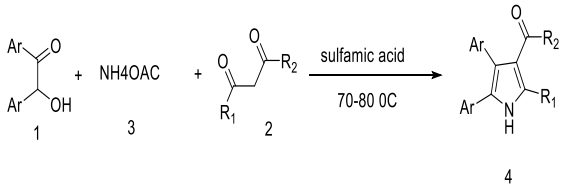


Scheme 1*. One-pot three-component synthesis of 1-(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethanone (4a)*

Reagents and conditions: benzoin (2 equiv), acetylacetone (2 equiv), ammonium acetate (3 equiv), sulfamic acid (2 equiv), 70-80°C, 27min, stirring, 95% yield. Further on we reacted benzoin derivatives **1**, 1,3-dicarbonyls **2**, and ammonium acetate (**3**) under optimized conditions, specifically in the presence of 5 mol% of SA, in the absence of solvent, under heating at 70°C, to obtain tetrasubstitutedpyrroles**4** in moderate-to-excellent yields (Table 1). This could be due to the density of

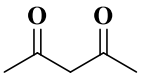
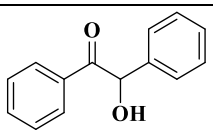
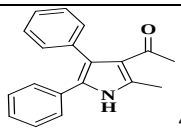
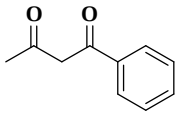
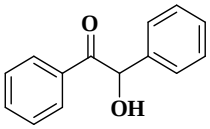
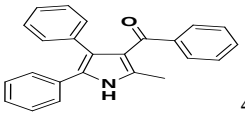
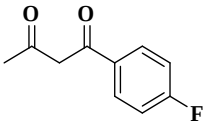
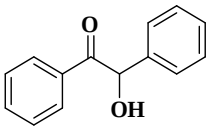
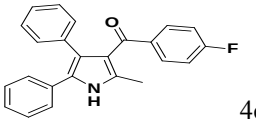
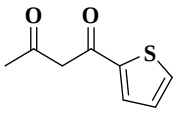
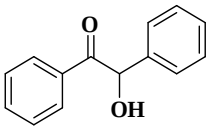
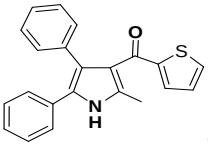
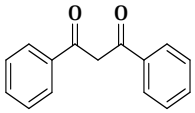
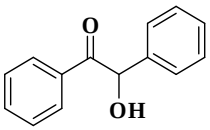
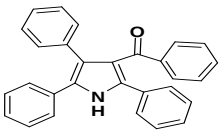
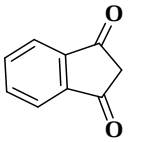
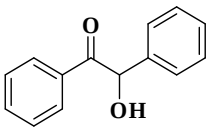
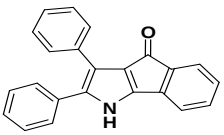
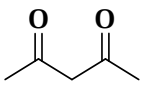
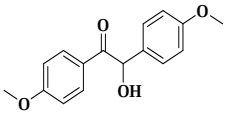
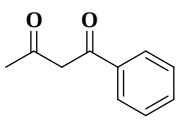
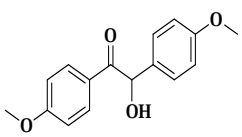
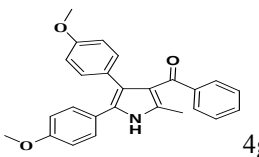
positive charge on the carbon attached to the hydroxyl group. However, the reaction with 4-methoxy-substituted benzoin with acetyl acetone and ammonium acetate under the optimized conditions gave no target pyrrole (Table 1, entry 7). The reaction occurred smoothly with both symmetrical and unsymmetrical 1,3-dicarbonyl compounds. At the same time, the reactions with unsymmetrical 1,3-dicarbonyls resulted in single regioisomers (**4b**, **4c**, and **4d**) in high yields. But the reaction with symmetrical 1,3-dicarbonylsmixtures of regioisomeric products are possible. This may be attributed to the difference in reactivity of carbonyl groups influenced by the nature of substitution on them. The identification tools for these regioisomers were ^{1}HNMR, ^{13}C NMR, FT-IR spectroscopy, and microanalysis.

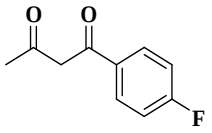
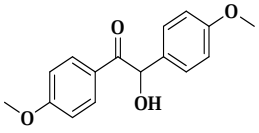
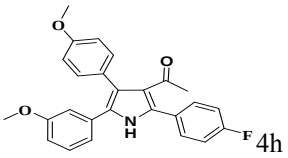
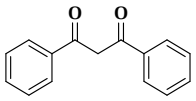
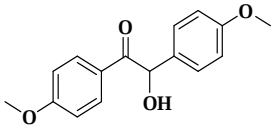
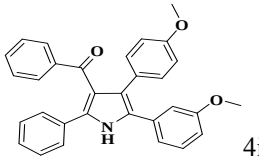
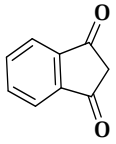
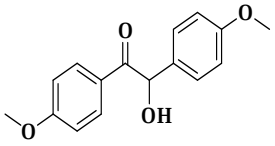
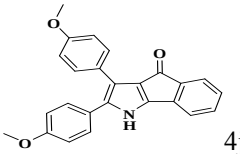
As a result, a series of polysubstitutedpyrroles were prepared in one-step with moderate-to-excellent yields (Scheme 2, Table1).



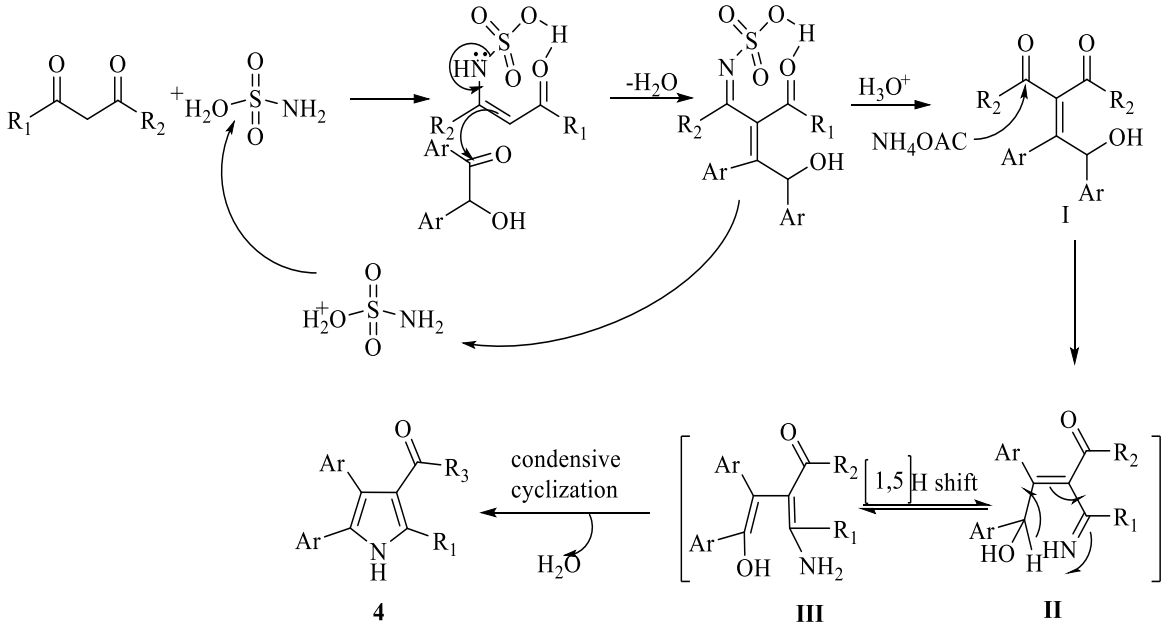
Scheme 2. *A series of polysubstitutedpyrroles*

Table 1: *Three-component SA-catalyzed synthesis of tetrasubstituted pyrroles under solvent-free conditions.*

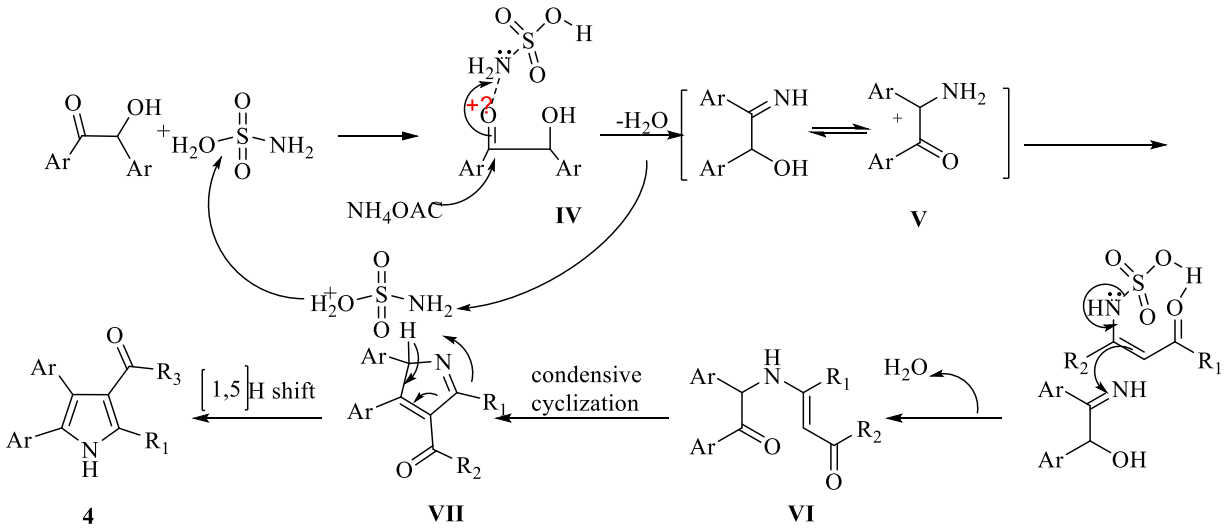
Entry	1,3-Dicarbonyl reagent	Benzoin reagent	Product	Yield %	Reaction time minutes
1.			 4a	95	27
2			 4b	80	30
3			 4c	85	32
4			 4d	90	28
5			 4e	70	34
6			 4f	50	28
7			No reaction	-	-
8			 4g	90	26

9			 4h	85	33
10			 4i	88	29
11			 4j	60	26

There are two possible mechanisms for the present three-component protocol based on electronic effects and region selectivity of the reaction as illustrated in Scheme **3, 4**. First, as shown in the scheme **3** the reaction activates the carbonyl group in 1,3-dicarbonyls by SA as a Brønsted acid to give an active electrophilic center. Next, the intermediate **I** is formed by the condensation of 1,3-dicarbonyls with the carbonyl group of benzoin in the presence of sulfamic acid after dehydration. Simultaneously, the intermediate **II** is resulted from condensation of more reactive carbonyl groups of the intermediate **I** with ammonium acetate. Then, the intermediate **III** is easily rearranged and cyclized to the more stable product **4** (Path A). Alternatively, the benzoin carbonyl group was activated by sulfamic acid giving an active electrophilic center. Then, ammonium acetate attacked the activated benzoin to form a-hydroxyimine **IV** with elimination of H_2O, followed by isomerization to achieve the intermediate **V** after keto-enol tautomerism. The reaction of the more reactive carbonyl moiety of 1,3-dicarbonyls with the amino group of the intermediate **V** can form the enaminone intermediate **VI**. In the last step, the desired pyrrole **4** is obtained by cyclization of the intermediate **VII** followed by [1,5] proton shift (Path B, Scheme 4).



Scheme 3. *The path A for the proposed mechanism of the synthesis of tetrasubstituted pyrroles.*



Scheme 4. *The path A for the proposed mechanism of the synthesis of tetrasubstituted pyrroles.*

Thus, we developed simple, solvent free, less time consuming and ecologically benign methods for the synthesis of the pyrrole derivatives. In addition, the use of solid acids as heterogeneous catalysts has received great attention in several organic syntheses. As preliminary experiments have been done in order to compare pyrrole films of the 1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethanone (**4a)** which are fabricated by sulfamic acid and $ZnCl_2$. Scanning electron microscopy is used to compare the morphologies of pyrrole films.

The preliminary result of SEM images of the pyrrole film prepared by using $ZnCl_2$ (Figure 1a) show crystalline like structures as large as up to several micrometers. On the other hand, SEM images of the pyrrole film prepared by using sulfamic acid (Figure 1b) show a hierarchical assembly of the pyrrole structures down to the nanoscale. Such structures with a huge surface area to volume ratio might increase the solubility of pharmaceutical molecules.

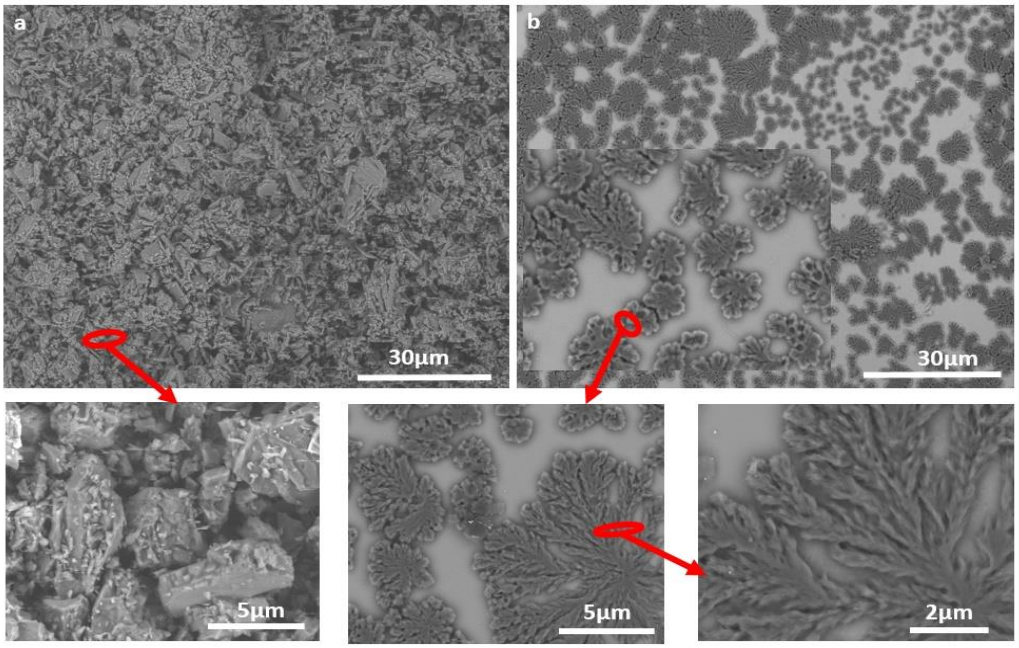


Figure 1. *The morphology of the synthesized pyrrole derivative 4a: The SEM images demonstrate the morphology of compound* **4a** *prepared by $ZnCl_2$ (a), and sulfamic acid (b) catalysts at low and high magnifications.*

4. BIOLOGY

4.1. *Antimicrobial activity evaluation*

All fabricated compounds were preliminary screened in vitro as antimicrobial activity against a variety of pathogenic local and standard microorganisms, namely Staphylococcus aureus(ATCC2913), Streptococcus pneumonia (Gram-positive bacteria), Escherichia coli(ATCC27953), Pseudomonas aeruginosa(ATCC27953), Klebsiella pneumonia(ATCC13883), Proteus mirabilis (Gram-negative bacteria), Candida albicansNRRY-477 (fungus) using well diffusion method. The results are demonstrated in (Table 1) as growth inhibition zone (mm). The data illustrates that compounds 4a, 4b and 4f exhibited comparatively high antimicrobial activities ranging from 15-20 mm against most tested microbes. On the other hand, most of tested compounds showed potent inhibition values in order of 4d (24mm)> 4e, 4i (22mm)> 4a, 4f, 4j (20mm) against P. mirabilis. Additionally, compound 4f showed potent antibacterial activity with inhibition zone of 20, 18, 20, 20, and 20 against S. aureus, S. pneumonia, E. coli, P. aeruginosa and P. mirabilis.

For C. albicans, three compounds showed potent inhibition in order of 4d (25mm)> 4i (20mm)> 4c (19mm) in comparison with the reference dermofix (20mm). Generally, from above result Compound 4f considered bactericidal agent according to structure and activity relationship which have diphenylindeno substitution at C-2,3-position enhanced antibacterial activity, whereas, Compound 4d considered fungacidal agent against C. albicans showed significant inhibition zone 25 mm due to its structure phenyl group enhanced activity.

4.2. Assessment of MIC value

Minimum inhibitory concentration value (MIC as shown in Table 3) is achieved according to Andrews et al. [26] and expressed as the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism after incubation 24 hr for bacteria at 37°C and 48 hr for fungi at 28°C. Well-cut diffusion technique was used to determine the minimal inhibitory effect of the final concentrations of antibacterial and antifungal agents were 25, 50, 100, 200 µg /mL DMSO against all studied pathogens.

Table 2. *Invitro* antimicrobial activity of the new synthesized compounds **4a–4j** against a variety of pathogenic microorganisms [a.]

Compd. No[b]	**Mean diameter of inhibition zone (mm)**						
	Gram positive		**Gram negative**				**fungi**
	S. aureus (ATCC29213)	*S. Peneumoniae*	*E. coli(ATCC5922)*	*P. aeruginosa (ATCC27953)*	*K. peneumoniae(ATCC13883)*	*Proteus mirabilis*	*C.Albicas* (Y477)
4a	15±0.05	15±0.2	20±0.5	19±0.2	20±0.1	20±0.2	17±0.5
4b	15±0.1	15±0.2	15±0.02	17±0.2	20±0.1	18±0.2	18±0.2
4c	20±0.2	NA	NA	15±0.2	20±0.1	18±0.1	19±0.1
4d	NA	NA	15±0.2	15±0.2	18±0.1	24±0.1	25±0.5
4e	18±0.2	15±0.01	15±0.1	NA	15±0.2	22±0.2	18±0.1
4f	20±0.1	18±0.5	20±0.2	15±0.5	20±0.2	20±0.2	15±0.1
4g	15±0.005	NA	NA	NA	18±0.2	18±0.2	18±0.2
4h	NA	NA	NA	NA	15±0.02	18±0.1	15±0.5
4i	NA	15±0.1	20±0.2	20±0.2	20±0.1	22±0.1	20±0.2
4j	15±0.2	NA	15±0.1	18±0.1	18±0.1	20±0.1	15±0.1
Forcetex	22	20	22	20	20	25	NA
Dermofx	NA	NA	NA	NA	NA	NA	20

a: well diffusion method; b: concentration at 150 µl; NA: not active

Table 3*.Minimum inhibition concentration of analogous pyrrol compounds*

Compd.	**MIC value** (μg/mL)						
	Gram+ve bacteria		**Gram-ve bacteria**				**Fungi**
	Staph. Aureus (ATCC29213)	*Strept. Peneumoniae*	*E. coli (ATCC 5922)*	*P. aeruginosa (ATCC27953)*	*K.* peneumoniae *(ATCC13883)*	*Proteus*	*C. albicans* Y477
4b	100	100	100	100	100	100	100
4a	100	100	100	100	100	100	100
4c	150	N.A.	N.A.	150	150	150	150
4d	N.A.	N.A.	150	150	150	150	150
4e	150	150	150	N.A.	150	150	150
4g	200	N.A.	N.A.	N.A.	200	200	200
4h	N.A.	N.A.	N.A.	N.A.	150	150	150
4i	N.A.	100	100	100	100	100	100
4j	150	N.A.	150	150	150	150	150
4f	100	100	100	100	100	100	100
Forcetex	100	100	100	100	20	100	N.A.
Dermofix	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	100

N.A: not active

4.3. Antioxidant activity evaluation

The antioxidant activity of the test compounds 4a-4j was calculated in terms of hydrogen-donating or radical scavenging ability utilizing the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) according to Brand-Williams et al. [20]. The scavenging activity of each investigated compound and vitamin C was evaluated by comparing the DPPH signals in the antioxidant-radical reaction mixture and the control reaction at the same time, and by expressing the percentage of the DPPH remaining.

As shown from the Table 4 and Figure 2, 2,3-diphenylindeno[1,2-b]pyrrol-4(1H)-one (4f) displayed the highest scavenging activity of 50.5% at a concentration of 0.2 mg/mL, compared to the other test compounds, followed by the Pyrroleformimidate 4a, and 4b ranked second (28 and 27%, respectively). The other tested pyrrole derivatives exhibited a weak scavenging activity ranging of (23.4-11.7%). Apparently, the high scavenging activity of tetrasubstitutedpyrrole 4f can be attributed to the presence and ability of the free electrons in the aromatic rings to reduce free radical formation and scavenge free radicals. Obviously, any substitution for this group decreases the antioxidant activity (Table 4).

Table 4: *The antioxidant activity of the tested compounds 4a-4j*

Compd. No.	*C*(mg/mLEtOH or DMF)	Scavenging activity (%)
DPPH	10^{-3} M	-
4a	0.2	28
4b	0.2	27
4c	0.2	11.8
4d	0.2	13.8
4e	0.2	23.4
4f	0.2	50.5
4g	0.2	11.7
4h	0.2	16
4i	0.2	23.4
4j	0.2	19.2
Ascorbic acid (1 mg/ml)	10^{-3} M	90

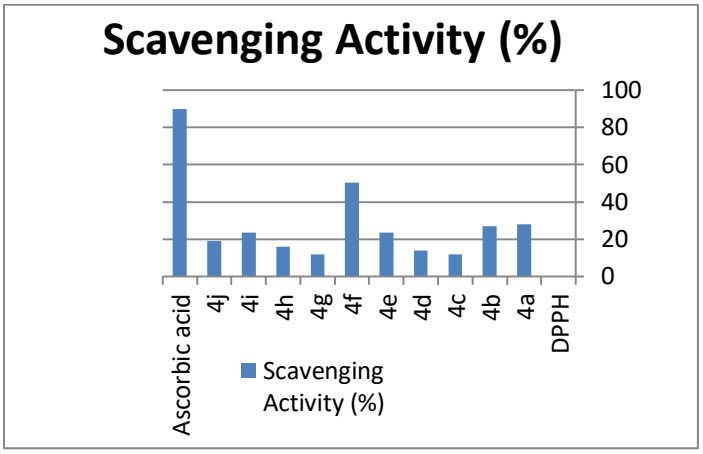


Figure 2. The antioxidant activity of tetrasubstitutedpyrroles 4a-4j

5. CONCLUSIONS

In this research study, we constructed an efficient selective synthesis method for polysubstituted pyrrole via the condensation reaction of benzoin derivatives, ammonium acetate and 1,3-diketones. The reaction proceeded in the existence of sulfamic acid that provided a high yield and a huge surface area to volume ratio through a solvent free method. The heterogeneous solid acids are superior to the conventional homogeneous acid catalysts as they can be simply recycled from the reaction mixture through a simple filtration process. The synthesized pyrrole derivatives were evaluated with respect to different biomedical activities as bactericidal, fungicidal and antioxidant agents.

It was found that compounds 4a, 4b and 4f exhibited relatively high antimicrobial activities ranging from 15-20 mm against most tested microbes. On the other hand, most of tested compounds showed potent inhibition values in order of 4d (24mm)> 4e, 4i (22mm)> 4a, 4f, 4j (20mm) against P. mirabilis. Additionally, compound 4f showed potent antibacterial activity with inhibition zone of 20, 18, 20, 20, and 20 against S. aureus, S. pneumonia, E. coli, P. aeruginosa and P. mirabilis.

For C. albicans, three compounds showed potent inhibition in order of 4d (25mm)> 4i (20mm)> 4c (19mm) in comparison with the reference dermofix (20mm).

Conflicts of interest

The authors declare no conflict of interest.

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