European Journal of Chemistry

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Synthesis and biological evaluation of triphenyl-imidazoles as a new class of antimicrobial agents

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RESEARCH ARTICLE

10.5155/eurjchem.9.4.369-374.1785

Received: 01 September 2018
Received in revised form: 13 October 2018
Accepted: 15 October 2018
Published online: 31 December 2018
Printed: 31 December 2018

KEYWORDS

Synthesis
Imidazole
Hepatic toxicity
Antifungal activity
Enzyme estimation
Antimicrobial activity

ABSTRACT

Newer triphenyl-imidazole derivatives (4a-h) were synthesized in good yields by the reaction of benzil and substituted benzaldehydes in equimolar quantities and refluxing the product with acetyl chloride thereafter. Structures were confirmed by using FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods. All the synthesized compounds were tested for their antimicrobial activity using agar diffusion technique against Gram positive (*Staphhylococcus aureus* and *Bacillus subtilis*), Gram negative (*Escherichia coli* and *Pseudomonas aureginosa*) as well as Fungal strain (*Candida albicans*). Interestingly compounds 4a, 4b, 4f and 4h showed significant antibacterial activity, whereas compound 4b was found to have remarkable activity against the fungal strain. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of most active compounds were determined by broth dilution method and compound 4b emerged to have potent activities against most of the strains having MIC in the range of 25-200 µg/mL. To check the possible toxicities of the most active compounds, they were orally administered in rats and the concentration of liver enzymes serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALKP) were determined. Compound 4h showed significant increase in the enzymes level depicting the hepatotoxicity. The structure-activity relationship studies showed the importance of electron withdrawing groups at the distant phenyl ring at ortho and para positions as the compounds having chloro or nitro at these positions tend to be more active than the compounds with electron releasing groups such as methoxy. These compounds may act as lead compounds for further studies and appropriate modification in their structure may lead to agents having high efficacy with lesser toxicity.

Cite this: *Eur. J. Chem.* **2018**, *9(4)*, 369-374 **Journal website**: www.eurjchem.com

1. Introduction

In the current world, health system is very much affected by serious systemic bacterial and fungal infections. It is reported that fungal infections are exaggerated very much due to excessive use of broad spectrum antibiotics, anticancer drugs and immunosuppressive agents [1]. Most of the existing medicines to combat these deadly microbes are getting microbial resistance and became an important concern in antimicrobial therapy [2]. Although, many compounds have been synthesized to control these infections but their clinical use are restricted due to high toxicities and microbial resistance [3].

In clinical medicine, imidazole derivatives possess extensive medicinal applications and this stimulated the researchers to develop a big number of newer therapeutic agents. There is evidence confirming that numerous pharmacologically active derivatives hold nitrogen containing five membered rings [4]. In the last few decades, imidazole rings having active hydrogen atom have been emerged as potential pharmacophore possessing numerous biological activities [5] and proved to be remarkable significance in the field of medical science. Azole antifungal drugs with imidazole ring have been found to destroy the lipid bilayer membranes of fungi by preventing the accumulation of methylated sterols. Cell wall of fungus is mainly composed of erogesterol, which plays crucial role in membrane permeability, enzyme activity as well as cell cycle functioning [6]. At high concentrations, imidazole drugs directly exert inhibitory effects on the membranes without intervention with the sterols [7,8].

http://dx.doi.org/10.5155/eurjchem.9.4.369-374.1785

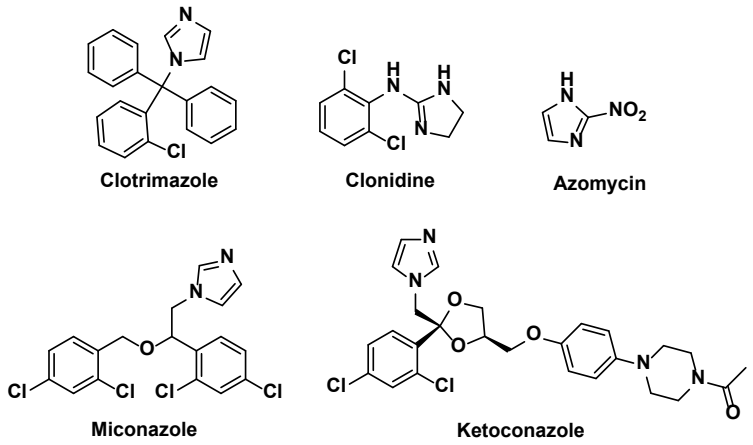


Figure 1. Structures of marketed drugs having imidazole ring.

In last few years, a great attention has been given to imidazole heterocyclics due to significant pharmacological activities such as antitubercular, antifungal, antibacterial, anti-inflammatory and antitumor [9]. Numerous well reported drugs in the market with remarkable therapeutic activities like clotrimazole, clonidine, azomycine, miconazole, moxonidine, ketoconazole, cimetidine and etomidate have potential imidazole ring (Figure 1) [10,11]. These numerous advantages of imidazole ring motivated us to discover novel derivatives of imidazole and study against well-known Gram (+ve) and Gram (-ve) bacteria as well as fungus *C. albicans*.

In our present work, newer imidazole derivatives have been designed and screened for antibacterial activity against Gram negative bacteria (*E. coli* and *P. aureginosa*), Gram positive bacteria (*S. aureus* and *B. subtilis*) and fungus *C. albicans* using reference drugs ampicillin and griseofulvin. Furthermore, toxicity evaluation by liver enzyme estimation was performed for the selected most potent compounds to evaluate any hepatotoxicity caused by them. Promising results of the present study would form the basis of further preclinical and clinical investigation to develop newer imidazole derivatives as potential antimicrobial agents.

2. Experimental

2.1. Instrumentations and materials

The melting points of the synthesized derivatives were estimated by the open capillary method and were uncorrected. FT-IR Spectra (KBr) was taken on Jasco FT/IR 410 spectrometer. NMR spectra of triphenyl-imidazoles were recorded on Bruker 400 Ultra shield NMR spectrometer operating at 400 MHz to record ^{1}H NMR and 100 MHz for ^{13}C NMR in $CDCl_3$ solvent with tetramethylsilane (TMS) as internal standard [12,13]. Log P was determined by octanol: phosphate buffer method. Retention factor (R_f) was calculated through thin layer Chromatography (TLC) using solvent system of benzene: acetone (60:40, *v*:*v*). Elemental analyses were performed on a Perkin-Elmer model 240c analyzer (Perkin Elmer, USA).

2.2. Synthesis of 2-(3-substituted phenyl)- 4,5-diphenyl -1H-imidazoles (3a-h)

Benzil (1 mol) (**1**), ammonia solution (5 mL) and substituted benzaldehdyes (1 mol) (**2**) were mixed with 50 mL glacial acetic acid in a 100 mL round bottom flask (RBF) and refluxed for 2-3 h on heating mantle. Completion of the reaction was monitored by using Thin Layer Chromatography (TLC). After completion of reaction, the reaction mixture was poured onto 300 mL cold water and neutralized with 5% ammonium hydroxide solution. The mixture was then kept in fridge overnight. The precipitated product was filtered and recrystallized with absolute ethanol to obtain colorless or pale yellow crystalline solid (Scheme 1) [14-16].

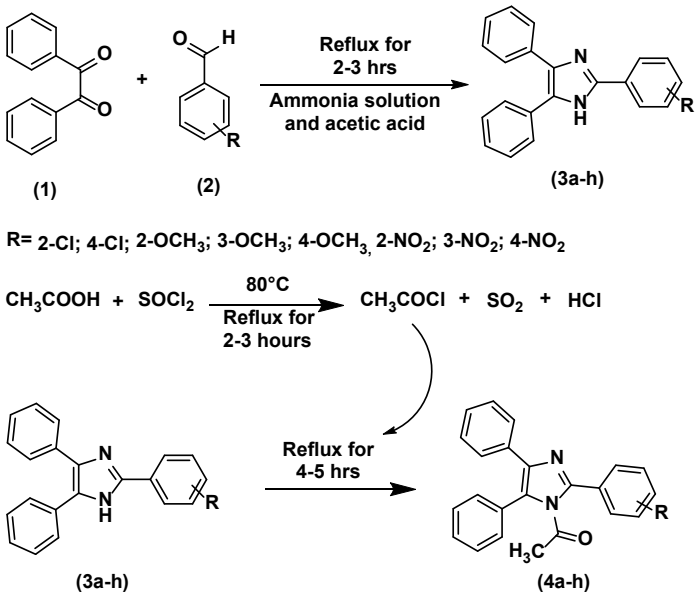
2.3. Synthesis of 1-(2-(2-substituted phenyl)-4, 5-diphenyl -1H-imidazol-1-yl) ethanones (4a-h)

0.5 g of substituted imidazole derivatives (**3a**-**h**) and freshly prepared 2.5 mL acid chloride solution were taken in round bottom flask with benzene (30 mL) as a solvent, pyridine as a catalyst and was refluxed for 4-5 h. TLC with solvent system of benzene: acetone (60:40, *v*:*v*) was utilized to check the reaction status. Mixture was cooled at room temperature, poured into ice and kept overnight in refrigerator. The compounds were filtered and recrystallized by ethanol (Scheme 1).

1-(2-(2-Chlorophenyl)-4, 5-diphenyl-1H-imidazol-1-yl)ethanone (**4a**): Color: Colorless. Yield: 48 %. M.p.: 248-250 °C. FT-IR (KBr, ν, cm^{-1}): 3362 (Ar-CH), 1635 (C=O), 1568 (C=C), 1438 (C=N), 758 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.20 (s, 3H, CH_3), 7.22-7.37 (m, 10H, CH, benzil), 7.38-7.48 (m, 4H, CH, chlorobenzene). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25 (CH_3), 122 (C=C, Imidazole), 167.8 (C=O), 127.4, 127.5, 128.8, 128.9, 129.3, 129.4, 130.2, 132.3, 133.3, 138.5 (C, Ar), 143.5 (C=N, Imidazole). R_f value: 0.71 (Benzene: acetone, 60:40). Log P: 5.72.

1-(2-(4-Chlorophenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4b**): Color: Colorless. Yield: 45 %. M.p.: 246-248 °C. FT-IR (KBr, ν, cm^{-1}): 3300 (Ar- CH), 1630 (C=O), 1530 (C=C), 1420 (C=N), 740 (C-Cl). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.21 (s, 3H, CH_3), 7.22-7.40 (m, 10H, CH, benzil), 7.41-7.48 (m, 4H, CH, chlorobenzene). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.5 (CH_3), 122.4 (C=C, Imidazole), 166.6 (C=O), 128.3, 127.3, 128.5, 128.7, 129.4, 129.2, 130.6, 132.5, 133.7, 138.2 (C, Ar), 144.5 (C=N, Imidazole). R_f value: 0.77 (Benzene: acetone, 60:40). Log P :5.70.

1-(2-(2-Methoxyphenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4c**): Color: Colorless. Yield: 35 %. M.p.: 165-167 °C. FT-IR (KBr, ν, cm^{-1}): 3100 (Ar- CH), 1540 (C=O), 1510 (C=C), 1410 (C=N), 1022(C-O-C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.26 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.83- 7.37 (m, 4H, CH, methoxybenzene), 7.38-7.48 (m, 10H, CH, benzil). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.1 (CH_3), 56.2 (OCH_3), 121.5 (C=C, Imidazole), 167.2 (C=O), 114.3, 127.2, 127.4, 128.5, 128.7, 129.6, 129.7, 130.3, 132.4, 133.2, 138.3 (C, Ar), 143.2 (C=N, Imidazole). R_f value: 0.83 (Benzene: acetone, 60:40). Log P: 5.04.



Scheme 1. Synthetic route to the titled compounds **4a**-**h**.

1-(2-(3-Methoxyphenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4d**): Color: Colorless. Yield: 34 %. M.p.: 166-168 °C. FT-IR (KBr, ν, cm^{-1}): 3450 (Ar-CH), 1650 (C=O), 1530 (C=C), 1360 (C=N), 1023 (C-O-C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.14 (s, 3H, CH_3), 3.43 (s, 3H, OCH_3), 6.46-7.11 (m, 4H, CH, methoxybenzene), 7.43-7.79 (m, 10H, CH, benzil). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 25.4 (CH_3), 55.8 (OCH_3), 122.4 (C=C, Imidazole), 167.2 (C=O), 114.3, 127.2, 127.4, 128.6, 128.7, 129.4, 129.5, 130.5, 132.6, 133.7, 138.3 (C, Ar), 143.4 (C=N, Imidazole). R_f value: 0.80 (Benzene: acetone, 60:40). Log P: 5.02.

1-(2-(4-Methoxyphenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4e**): Color: Colorless. Yield: 32 %. M.p.: 167-169 °C. FT-IR (KBr, ν, cm^{-1}): 3230 (Ar- CH), 1610 (C=O), 1570 (C=C), 1390 (C=N), 1043 (C-O-C). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.28 (s, 3H, CH_3), 3.52 (s, 3H, OCH_3), 6.56- 7.10 (m, 4H, CH, methoxybenzene), 7.15-7.46 (m, 10H, CH, benzil). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.6 (CH_3), 55.2 (OCH_3), 122.3 (C=C, Imidazole), 167.1 (C=O), 114.2, 127.1, 127.4, 128.4, 128.8, 129.4, 129.5, 130.6, 132.2, 133.5, 138.6 (C, Ar), 142.5 (C=N, Imidazole). R_f value: 0.79 (Benzene: acetone, 60:40). Log P: 5.0.

1-(2-(2-Nitrophenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4f**): Color: Pale yellow. Yield: 71 %. M.p.: 168-170 °C. FT-IR (KBr, ν, cm^{-1}): 3120 (Ar- CH), 1670 (C=O), 1540 (C=C), 1480(-NO_2), 1260 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.20 (s, 3H, CH_3), 7.22-7.48 (m, 10H, CH, benzil), 7.71- 8.25 (m, 4H, CH, nitrobenzene). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.7 (CH_3), 55.2 (OCH_3), 122.3 (C=C, Imidazole), 167.3 (C=O), 114.4, 127.2, 127.5, 128.5, 128.7, 129.3, 129.6, 130.5, 132.3, 133.4, 138.3 (C, Ar), 142.3 (C=N, Imidazole). R_f value: 0.68 (Benzene: acetone, 60:40). Log P: 4.98.

1-(2-(3-Nitrophenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4g**): Color: Pale yellow. Yield: 70.58 %. M.p.: 166-168 °C. FT-IR (KBr, ν, cm^{-1}): 3110 (Ar- CH), 1650 (C=O), 1510 (C=C), 1450(-NO2), 1250 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.10 (s, 3H, CH_3), 7.10-7.88 (m, 10H, CH, benzil), 7.91- 8.25 (m, 4H, CH, nitrobenzene). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.6 (CH_3), 55.8 (OCH_3), 121.3 (C=C, Imidazole), 168.3 (C=O), 113.4, 127.4, 127.6, 128.1, 128.6, 129.4, 129.7, 130.2, 132.7, 133.3, 138.5 (C, Ar), 141.3 (C=N, Imidazole). R_f value: 0.68 (Benzene: acetone, 60:40). Log P: 4.96.

1-(2-(4-Nitrophenyl)-4, 5-diphenyl-1H-imidazol-1-yl) ethanone (**4h**): Color: Pale yellow. Yield: 72 %. M.p.: 168-170 °C. FT-IR (KBr, ν, cm^{-1}): 3510 (Ar- CH), 1610 (C=O), 1560 (C=C), 1410(-NO2), 1230 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 2.60 (s, 3H, CH_3), 7.11-7.56 (m, 10H, CH, benzil), 7.60- 8.15 (m, 4H, CH, nitrobenzene). ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 24.3 (CH_3), 55.7 (OCH_3), 121.1 (C=C, Imidazole), 167.3 (C=O), 112.4, 126.4, 127.3, 128.3, 128.5, 129.6, 129.8, 130.6, 132.4, 133.6, 138.4 (C, Ar), 140.3 (C=N, Imidazole). R_f value: 0.65 (Benzene: acetone, 60:40). Log P: 4.90.

2.4. Antimicrobial activity

2.4.1. Determination of zone of inhibition

All the newly synthesized imidazole derivatives (**4a**-**h**) were evaluated for antibacterial and antifungal activities on different strains of microbes (Table 1 and 2). Different cultures of bacteria such as *P. aeruginosa* (NCIM 2242), *B. subtilis* (NCIM 2708), *S. aureus* (NCIM 2079), *E. coli* (NCIM 2685) and one fungal strain of *Candida albicans* were brought from Pune, India. These microorganisms were conserved by sub-culturing in nutrient agar medium. Antimicrobial assay of these compounds were studied through agar diffusion technique by determining zone of inhibition [17-19]. Prior to activity, petri dishes were sterilized at 160 °C for 1.5 h in hot air oven. 1% of inoculums were introduced in sterilized agar media. Three bores of 6 mm diameter was made in agar nutrient medium and 1×10 CFU/mL concentration of bacterial culture was used. For antimicrobial studies, 500 and 600 µg/mL concentrations of synthesized compounds were used and zone of inhibition was calculated and compared with the results obtained by 500 µg/mL solution of standard drugs ampicillin and griseofulvin in DMF.

2.4.2. Determination of minimum inhibitory concentration and minimum bactericidal/fungicidal concentration

MIC is the minimum concentration that inhibits the significant growth of the microorganisms. DMSO was used as diluent to prepare a concentration of 20 mg/mL of the synthesized compounds.

Table 1. Antimicrobial activity of synthesized compounds **4a-h**.

Compound	Concentration (µg/mL)	Zone of inhibition (mm), (Mean±SEM) [a]				
		Gram negative bacteria		Gram positive bacteria		Fungus
		E. coli (NCIM 2685)	*P. aureginosa* (NCIM 2242)	*S. aureus* (NCIM 2079)	*B. subtilis* (NCIM 2708)	*C. albicans* (ATCC 60193)
4a	500	18.3±0.27**	17.2±0.31**	17.8±0.27**	16.0±0.27***	13.7±0.13
	600	19.1±0.16**	18.5±0.431**	20.3±0.21***	17.0±0.19***	14.9±0.13**
4b	500	18.3±0.27**	17±0.31**	20.3±0.21***	16.0±0.27***	14.3±0 .13**
	600	19±0.16***	17.9±0.32**	21.3±0.31***	17.0±0.19***	15.7±0.21***
4c	500	13.4±0.28	15.6±0.21	13.5±0.31	14.4±0.33	13.2±0.31
	600	14.8±0.23	16.3±0.07**	14.7±0.19	14.8±0.13	13.7±0.21
4d	500	13.2±0.25	15.1±0.22	13.7±0.37	14.5±0.36	13.1±0.32
	600	14.6±0.22	16.1±0.02**	14.8±0.18	14.7±0.14	13.8±0.22
4e	500	17.0±0.31**	15.6±0.21	13.9±0.31	14.7±0.33	14.7±0.21**
	600	17.9±0.32**	15.9±0.07	14.3±0.19	15.3±0.13**	15.1±0.01**
4f	500	19.1±0.21**	15.6± 0.31	15.9±0.54**	14.2±0.33	13.6±0.22
	600	19.9 ±0.27***	15.67± 0.22	16.8±0.41**	14.3±0.32	14.1±0.32
4g	500	14.3±0.21	15.8± 0.25	15.1±0.71**	14.3±0.46	13.5±0.31
	600	15.0±0.11**	15.9±0.13	16.5±0.32**	14.4±0.36	14.5±0.22
4h	500	17.7±0.21**	15.5±0.45	18.3±0.32**	14.2± 0.26	13.6±0.35
	600	18.9 ±0.27***	16.5±0.45	19.9±0.21***	14.4±0.16	14.1±0.52
Ampicillin	500	20.0±.27***	19.3±0.26***	23.0±0.21***	17.1±0.19***	- [b]
Griseofulvin	500	NT [c]	NT	NT	NT	16±0.27***
DMSO	-	-	-	-	-	-

[a] Data are presented with mean±SEM using one way ANOVA. Extremely significant and significant values are represented as ***p < 0.001 and **p < 0.01 respectively.
[b] No significant inhibitory activity (< 5 mm).
[c] Not Tested.

Table 2. Minimum inhibitory concentration and minimum bactericidal concentration of selected compounds.

Organism	Compound	MIC (µg/mL)	MBC (µg/mL)
E. coli	4b	25	50
NCIM 2685	4f	50	50
	4h	25	50
	Ampicillin	6.25	12.5
P. aureginosa	4b	100	200
NCIM 2242	4f	100	200
	4h	300	400
	Ampicillin	> 500	> 500
S. aureus	4b	50	50
NCIM 2079	4f	100	200
	4h	100	200
	Ampicillin	6.25	12.5
B. subtilis	4b	200	300
NCIM 2708	4f	300	300
	4h	300	400
	Ampicillin	6.25	6.25
C. albicans	4b	100	300
ATCC 60193	4f	200	300
	4h	200	300
	Griseofulvin	6.25	6.25 MFC [a]

[a] MIC: Minimum inhibitory concentration, MFC: Minimum fungicidal concentration.

On the basis of the antimicrobial activity shown in agar diffusion technique, three most active compounds were selected for MIC determination. Broth dilution method was employed in this study using 96 wells micro-titre plate [20-22]. Dilutions were made to obtain different concentrations of 600, 500, 400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL. Culture for bacterial and fungal strains were prepared by adding equal volumes and incubating at 37 °C for 24 h and 30 °C for 48 h, respectively.

Bacterial growth was indicated by appearance of turbidity and ELISA reader at 590 nm was used to measure the absorbance. The well with zero absorbance was measured to be the MIC. DMSO (1-16%) was used as control. All examinations were implemented in triplicates.

Minimum Bactericidal concentration (MBC) was examined by sub culturing the solutions, which did not show any indication of growth, in neat as well as in dilutions of 1:10 and 1:100. MBC was indicated by 99.9% decline in the growth of original inoculum.

2.5. Toxicity evaluation/liver enzyme estimation

Experimental design for biochemical estimation of most potent synthesized compounds on albino rat of either sex (150g-200 g) had been approved by Institutional animal ethical committee (IAEC) of Translam Institute of Pharmaceutical Education & Research, Meerut, Uttar Pradesh, INDIA (Registration number 1207/PO/c/2008/CPCSEA).

To assess the liver toxicity, biochemical parameters such as serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALKP) enzyme levels were measured from the serum of treated rats using Roche assay kits [23-26]. The experiment was conducted as per NIH guidelines. These enzymes catalyze the reversible reaction of α-ketoglutaric acid and amino acids. GPT enzyme is mainly present in hepatocytes and its elevated level in blood is used to diagnose any liver toxicity whereas GOT enzyme is present in kidney, heart and liver tissues therefore, is helpful in diagnosis of toxicities of these organs. Furthermore, alkaline phosphatase is another liver enzyme that hydrolyzes para nitrophenyl phosphate to yellow color para nitro phenol during estimation and is used to estimate alkaline phosphatase level in blood sample.

2.6. Statistical analysis

The investigations were done in triplicate. Results were statistically presented as mean±standard error of mean (SEM).

Table 3. Toxicity evaluation of selected compounds [a].

Compounds	Serum glutamate pyruvate transaminase (SGPT), IU/L	Serum glutamate oxaloacetate transaminase (SGOT), IU/L	Alkaline phosphatase (ALKP), IU/L
Control	46.14±0.42	41.12±0.35	14.77±0.12
4b	49.24±0.32 *	40.45±0.45	15.76±0.24
4f	43.32±0.58 *	42.27±0.34	19.46±0.26 *
4h	54.52±0.49 **	55.26±0.27 **	28.37±0.13 **

[a] Mean±SEM values (n = 6). Significantly different from control: * $p < 0.05$, ** $p < 0.01$.

Statistical values p < 0.001 and p < 0.01 were considered as extremely significant and significant respectively using Graph prism software.

3. Results and discussion

3.1. Synthesis

In the present research work, newer imidazole derivatives (**4a**-**h**) were synthesized by the reaction of benzil and substituted benzaldehydes that afforded compounds **3a**-**h**. These imidazole derivatives (**3a**-**h**) were refluxed with freshly prepared acetyl chloride in presence of benzene or pyridine to undergo acetylation. Synthetic route to the titled compounds **4a**-**h** is shown as Scheme 1.

All the synthesized compounds were characterized through FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopic methods. Physicochemical properties and spectral characterization supported the structures of the synthesized compounds.

FT-IR spectra showed the characteristic bands for Ar-CH_{str}, C=O_{str}, C=C_{str}, C=N_{str}, NO_{2str}, C-O-C_{str} and C-Cl_{str} to be in the range of wavenumber 3510-3100, 1670-1610, 1570-1510, 1438-1230, 1480-1410, 1043-1022 and 758-740 cm^{-1}, respectively.

The ^{1}H NMR spectra was confirmed for -CH_3 and –OCH_3 groups by detecting singlets at δ 2.14-2.60 and 3.43-3.73 ppm, respectively. On the other hand, CH-methoxybenzene, CH-benzil, CH-nitrobenzene and CH-chlorobenzene groups were confirmed by showing multiplet at δ 6.46-7.46, 7.10-7.88, 7.60-8.25 and 7.38-7.48 ppm, respectively. The ^{13}C NMR spectra confirmed the presence of -CH_3, –OCH_3, C=C, imidazole and benzene ring.

3.2. Antimicrobial activity

All the synthesized compounds (**4a**-**h**) were tested for their antimicrobial activity using agar diffusion technique against Gram +ve (*Staphhylococcus aureus* and *Bacillus subtilis*), Gram –ve (*Escherichia coli* and *Pseudomonas aureginosa)* as well as Fungal strain (*Candida albicans*). The antimicrobial study revealed promising results for most of the compounds of the series. It was noticed that compounds **4a**, **4b** and **4c** were effective (**$p < 0.01$) against *P. aureginosa* at 600 µg/mL concentration, whereas, compounds **4b**, **4f** and **4h** were found to be significantly (***$p < 0.001$) active against Gram negative *E coli*. Interestingly, compounds **4a** and **4b** also displayed significant inhibition (***$p < 0.001$) against *S. aureus* and *B subtilis.* These results were comparable to that of standard drug ampicillin. In screening results for antifungal activity, compound **4b** was found to be significantly (***$p < 0.001$) active against *C. albicans* at 600 µg/mL concentration and was comparable to standard drug griseofulvin. The data for antimicrobial activities are shown in Table 1.

Minimum inhibitory concentration and minimum bactericidal concentration of most active compounds **4b**, **4f** and **4h** were examined using broth dilution method against bacterial strains *S. aureus* (NCIM 2079), *B. subtilis* (NCIM 2708), *E. coli* (NCIM 2685) and *P. aureginosa* (NCIM 2242) as well as fungal strain *C. albicans* (ATCC 60193). The results obtained were in close comparison to the standard antimicrobial drugs ampicillin and griseofulvin.

The results of MICs and MBCs for the selected compounds **4b**, **4f** and **4h** are summarized in Table 2 and were found to be in the range of 25-50 µg/mL for *E. coli* and were lesser than the standard drug ampicillin (6.25 µg/mL). When tested against *P. aureginosa*, MICs were obtained to be in the range of 100-300 µg/mL. Against other bacterial strains *S. aureus* and *B. subtilis*, the MICs for the selected compounds were found to be in higher range (50-300 µg/mL) as compared to ampicillin (6.25 µg/mL). Similar results were obtained when these compounds were tested against fungal species *C. albicans* as the MIC values were found to be in the range of 100-200 µg/mL as compared to standard drug griseofulvin (6.25 µg/mL). In the present study, compound **4b** emerged to have most potent activities against most of the strains having MIC in the range of 25-200 µg/mL.

3.3. Toxicity evaluation/liver enzyme estimation

Toxicity evaluation was performed for the most active compounds **4b**, **4f** and **4h** to check any toxicity on the liver by estimating the liver enzymes and the data are shown in Table 3. The results were found to be comparable to control and the compounds were devoid of hepatotoxic effects except compound **4h** that showed raised levels of SGOT, SGPT and ALP.

4. Conclusion

In the present study, newer triphenyl-imidazole derivatives (**4a**-**h**) were synthesized and tested for their antimicrobial activity using agar diffusion technique against Gram +ve, Gram –ve as well as fungal strains. From the antimicrobial activity results, it was observed that compounds **4a**, **4b**, **4f** and **4h** with electron withdrawing group at ortho or para positions (2-Cl, 4-Cl, 2-NO_2 and 4-NO_2) substituted at distant phenyl ring showed highest activities against Gram positive and Gram negative bacterial stains. Whereas, electron releasing group –OCH_3 at ortho, meta or para position at distal phenyl ring as in compounds **4c**, **4d** and 4e showed moderate results. Compound with electron withdrawing group chloro at para position to phenyl ring was found to be significantly effective against *C. albicans* fungal strain. These results indicate the influence of the type and position of electron withdrawing groups such as chloro and nitro on antimicrobial activities. Also, three hydrophobic phenyl rings attached to the heterocyclic imidazole in the synthesized compounds increased the lipophilicity and formed a large non-polar structure which could act as a substrate of lanosterol-α-demethylase enzyme and therefore could result into the inhibition of ergosterol synthesis and might be the mechanism behind the antifungal activity of the compounds. Moreover, the present study would form the basis of further preclinical and clinical investigations to develop newer imidazole derivatives as potential antimicrobial agents.

Acknowledgements

Authors are thankful to College of Pharmacy, Jazan University, Saudi Arabia and TIPER, Meerut, U.P. India for providing necessary facilities.

Disclosure statement

Conflict of interests: Authors have no any conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.

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