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Antibacterial, antifungal and antimycobacterial activities of some pyrazoline, hydrazone and chalcone derivatives

DOI 10.1515/znc-2014-4195
Received November 17, 2014; revised July 29, 2015; accepted July 30, 2015

Abstract: Twenty-seven previously reported chalcones and their pyrazoline and hydrazone derivatives as well as two further chalcones have been screened for their antimicrobial, antifungal and antimycobacterial activities against standard microbial strains and drug resistant isolates. The minimum inhibitory concentration (MIC) value of each compound was determined by a two-fold serial microdilution technique. The compounds were found to possess a broad spectrum of antimicrobial activities with MIC values of 8–128 μg/mL. One compound [(*E*)-1-(4-hydroxyphenyl)-3-*p*-tolylprop-2-en-1-one] had equal activity with gentamycin (8 μg/mL) against *Enterococcus faecalis*. Chalcones were found to be more active than their hydrazone and 2-pyrazoline derivatives against *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212.

Keywords: chalcones; hydrazones; 2-pyrazolines.

1 Introduction

The need for new antimicrobial agents is ever-increasing because of antibiotic resistance. Infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) can be life threatening especially for immunocompromised patients due to HIV, surgery or any other illness [1, 2]. The human immunodeficiency virus (HIV) pandemic has increased the incidence of tuberculosis (TB) too [3]. Tuberculosis is a serious health problem and it has been estimated that approximately one-third of the world's population is infected with *Mycobacterium tuberculosis* [4].

Although current first-line anti-TB drugs treat the illness, drug resistance often causes problems [5]. TB treatment requires at least 6 months to decrease the risk of reactivation of TB bacilli. Long-term tuberculosis treatment causes patient non-adherence, and thereby the number of multidrug resistant *M. tuberculosis* (MDR-MTB) strains increases rapidly [6]. Invasive fungal infections cause morbidity and mortality [7]. Antifungal therapies can be limited because of toxicity, drug resistance and low efficacy rates [8]. New antifungal drugs are necessary to improve the efficacy of therapy. Therefore, new antitubercular, antimicrobial and antifungal agents are needed.

Chalcones have different pharmacological activities such as antibacterial [9–13], antifungal [14, 15], antimycobacterial [3, 16], antiinflammatory [17, 18] and antiviral [19, 20]. Pyrazoline and hydrazone derivatives are of interest because of their similarity to isoniazid (isonicotinic acid hydrazide). It is also known that pyrazoline and hydrazone derivatives have antibacterial [21–24], antifungal [25, 26] and antimycobacterial [27, 28] activities.

In this study, twenty-nine compounds having a chalcone, 2-pyrazoline or hydrazone structure were screened for their antibacterial, antifungal and anti-mycobacterial activity.

2 Results and discussion

Chemical formulas of the synthesized compounds are shown in Scheme 1. The synthetic route used for the preparation of the target compounds (**3a**-**3h**, **5a**-**5j**, **6a**-**6i**) and data on the compounds (**3a**-**3h**, **5a**-**5j**, **6a**-**6i**) have been published earlier [29].

Iproniazid, a non-selective, irreversible monoamine oxidase inhibitor (MAOI), was originally developed for the treatment of tuberculosis [30]. Linezolid, which is a

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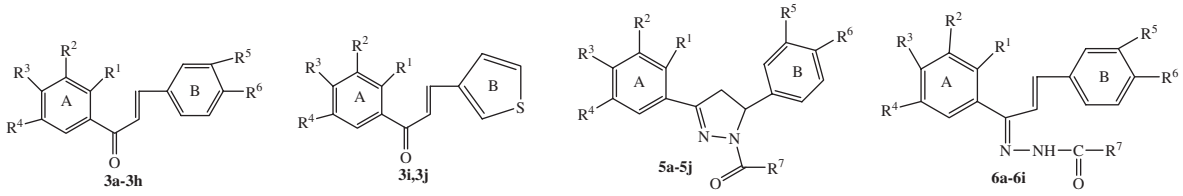

Compound	R^1	R^2	R^3	R^4	R^5	R^6	R^7
3a	-OH	-H	-H	-Cl	-H	$-CH_3$	-
3b	-OH	-H	-H	-Br	-H	$-CH_3$	-
3c	-H	-H	-Br	-H	-H	$-OCH_3$	-
3d	-H	-H	-Cl	-H	-H	$-CH_3$	-
3e	-H	-H	-Cl	-H	$-CH_3$	-H	-
3f	-H	-H	-Cl	-H	-H	$-OCH_3$	-
3g	-H	-H	-OH	-H	-H	$-CH_3$	-
3h	-H	-H	$-OCH_3$	-H	-H	$-CH_3$	-
3i	-OH	-H	$-OCH_3$	-H	-	-	-
3j	$-OCH_3$	-H	$-OCH_3$	-H	-	-	-
5a	-OH	-H	-H	-Cl	-H	$-CH_3$	Pyridine-4-yl
5b	-OH	-H	-H	-Cl	-H	$-CH_3$	Furan-2-yl
5c	-OH	-H	-H	-Cl	-H	$-CH_3$	Phenyl
5d	-OH	-H	-H	-Cl	-H	$-CH_3$	4-Methoxy-1-Phenyl
5e	-OH	-H	-H	-Cl	-H	$-CH_3$	4-Methyl-1,2,3-thiadiazole-5-yl
5f	-OH	-H	-H	-Br	-H	$-CH_3$	Pyridine-4-yl
5g	-OH	-H	-H	-Br	-H	$-CH_3$	Furan-2-yl
5h	-OH	-H	-H	-Br	-H	$-CH_3$	Phenyl
5i	-OH	-H	-H	-Br	-H	$-CH_3$	4-Methoxy-1-Phenyl
5j	-OH	-H	-H	-Br	-H	$-CH_3$	Thiophene-2-yl
6a	-H	-H	-Br	-H	-H	$-OCH_3$	4-Methoxy-1-Phenyl
6b	-H	-H	-Br	-H	-H	$-OCH_3$	Furan-2-yl
6c	-H	-H	-Cl	-H	-H	$-CH_3$	Thiophene-2-yl
6d	-H	-H	-Cl	-H	-H	$-CH_3$	4-Methyl-1,2,3-thiadiazole-5-yl
6e	-H	-H	-Cl	-H	$-CH_3$	-H	4-Methyl-1,2,3-thiadiazole-5-yl
6f	-H	-H	-Cl	-H	-H	$-OCH_3$	4-Methyl-1,2,3-thiadiazole-5-yl
6g	-H	-H	-Cl	-H	-H	$-OCH_3$	4-Methoxy-1-Phenyl
6h	-H	-H	-OH	-H	-H	$-CH_3$	4-Methyl-1,2,3-thiadiazole-5-yl
6i	-H	-H	$-OCH_3$	-H	-H	$-CH_3$	4-Methyl-1,2,3-thiadiazole-5-yl

Scheme 1: Structures of compounds **3a-3j**, **5a-5j** and **6a-6i**.

synthetic antibacterial agent, is a reversible and weak MAOI at the same time [31]. Isoniazid and tedizolid are also monoamine oxidase inhibitors and are used for antimicrobial treatment [32]. According to this data, we investigated the antimicrobial effects of our compounds which we originally synthesized as inhibitors of monoamine oxidase.

Amine oxidases (AOs), a widespread class of enzymes, are present in all living systems, where they control the level of physiologically very active compounds, i.e. mono-, di-, and polyamines. Amine oxidases have been divided into two main categories, depending on the nature of the cofactor involved. One class is characterized by the presence of flavin adenine dinucleotide (FAD) as the redox cofactor. The enzymes belonging to this class are further subdivided into monoamine oxidases (MAOA and MAOB) and polyamine oxidases (PAOs). The second class is represented by enzymes having a tightly bound Cu^{II} ion and 2,4,5-trihydroxyphenethylamine quinone (TPQ) at the active site [33]. Monoamine oxidase catalyses the oxidation of tyramine, tryptamine, norepinephrine and some other monoamines of natural origin [34]. This enzyme is also present in bacteria and fungi. This enzyme is defined for *Escherichia coli* as a copper containing amine oxidase enzyme (CuAOs) [35, 36]. No membrane-bound monoamine oxidase activity was detected in *Pseudomonas aeruginosa* IFO 3456 [37] *P. aeruginosa* converts substrates with a membrane bound, uncharacterized amine dehydrogenase (quite different types of oxidoreductases) instead of monoamine oxidase [38]. Human monoamine oxidases contain FAD (flavin adenine dinucleotide) but usually bacterial amine oxidases contain copper and 2,4,5-trihydroxyphenethylamine quinone (TPQ) at their active site [39, 40].

In bacteria, the CuAOs have a well-defined role in the metabolism of primary amines as alternative sources of

carbon and nitrogen to support growth [41]. Murooka et al., declared that monoamine oxidases were not essential for growth of bacterial cells [40]. The antimicrobial activity of the compounds can be related to the inhibition of mono amine oxidase enzyme. Similarities of microbial MAOs and hMAO are important for inhibition of the bacterial enzymes. Sheepard et al., tested some amine derivatives as monoamine oxidase inhibitors against mammalian, plant, bacterial, and fungal copper-containing amine oxidases. Distinctions among the active sites were found to be responsible for differentiating the chemical interactions between the inhibitors and enzymes selected [36]. According to these data, more investigations are necessary for understanding the properties of amine oxidase enzymes of bacteria and fungi.

As seen in Table 1, 2-pyrazolines (**5a-5j**) and hydrazones (**6a-6i**) had similar but low antibacterial activity against *S. aureus* with a minimum inhibitory concentration (MIC) value of 128 μg/mL. Chalcones (**3a-3j**) had variable MIC values of 16–256 μg/mL against *S. aureus*. Compound **3g** is the most active one against the resistant *S. aureus* isolate (MRSA), having a MIC value of 32 μg/mL. All derivatives had low activity against standard strains of *E. coli* in comparison with the standard drugs. All compounds showed low activity against *M. tuberculosis*. Compounds **5b-5g** had moderate activity against *Enterococcus faecalis* with MIC values of 32 μg/mL. Compound **3g** has equal activity with gentamicin (8 μg/mL) against *E. faecalis*. Compound **6e** is the most active one against a resistant *E. faecalis* isolate (VRE), with a MIC value of 32 μg/mL. Compounds **5d-5g** had moderate activity against *Candida albicans* with a MIC value of 32 μg/mL MIC. Compound **3g** is the most active one against *C. albicans* (MIC 16 μg/mL). Compound **3g** (4′-hydroxy-4-methyl chalcone) had the lowest MIC values against *S. aureus*, *E. faecalis* and *C. albicans* (16, 8 and 16 μg/mL, respectively). All compounds had MIC values of 32–128 μg/mL against *Candida krusei*. Compounds **5g**, **5h**, **6f** and **3j** had equal activity with fluconazole against *C. krusei* with a MIC value of 32 μg/mL.

Enterococcus faecalis is an opportunistic pathogen and a major cause of both community-acquired and nosocomial infections, including pelvic infections, endocarditis, neonatal infections, respiratory infections and urinary tract infections. The rise in prevalence of antibiotic-resistant enterococci, including vancomycin-resistant enterococci (VRE), linezolid (LZD)-resistant enterococci has gained much attention in the clinical setting [42].

Increasing resistance against *E. faecalis* calls for the development of new drugs. Compound **3g**, having an activity against *E. faecalis* equal to that of gentamycin, is promising, but as it is not equally active against resistant *E. faecalis*, its applicability in clinical practice is restricted. Obviously, the comparative evaluation of active compounds requires further studies; the data reported in this article may be a helpful guide for medicinal chemists working in this area.

3 Experimental

3.1 Antibacterial and antifungal activity

Standard strains of *E. coli* (ATCC 25922 and ATCC 35218), *P. aeruginosa* (ATCC 27853), *S. aureus* (ATCC 29213), *E. faecalis* (ATCC 29212), *C. albicans* (ATCC 10231) and *C. krusei* (ATCC 6258) were included in the study. *Candida krusei* was used because of the natural resistance of the strain to fluconazole. An *E. coli* isolate (extended spectrum β-lactamases [ESBL), a *S. aureus* isolate (methicilline resistant *S. aureus* [MRSA]), resistant to all β-lactam antibiotics, a *P. aeruginosa* isolate (resistant to gentamicin), β-lactam antibiotics, and an *E. faecalis* isolate (resistant to vancomycin) were also used. Bacterial and fungal susceptibility tests were performed according to Clinical Laboratory Standards Institute (CLSI) guidelines M100-S16 [43] and M27-A3 [44], respectively.

Mueller Hinton Agar (MHA) (Merck, Darmstadt, Germany), Mueller Hinton Broth (MHB) (Merck), Sabouraud Dextrose Agar (SDA) (Merck), Sabouraud Liquid Medium (SLM) (Merck) and RPMI-1640 medium with L-glutamine (Sigma-Aldrich, St. Louis, MO, USA) buffered to pH 7 with MOPS (Sigma-Aldrich) were used in the study for subcultures and microdilution tests.

Standard preparations of ampicillin (Mustafa Nevzat Pharmaceuticals, Istanbul, Turkey), gentamicin (Paninkret Chem.-Pharm., Westerhorn, Germany), ofloxacin (Zhejiang Huangyan East Asia Chemical Co. Ltd., Huangyan, Zhejiang, China), meropenem (Astra Zeneca, Istanbul, Turkey), vancomycin (Mayne Pharma, Melbourne, Australia), ampicillin/sulbactam (1:1) (Mustafa Nevzat Pharmaceuticals), amoxicillin/clavulanic acid (2:1) (Deva, Istanbul, Turkey), fluconazole (Pfizer, Istanbul, Turkey), and amphotericin B (Bristol Myers Squibb, Istanbul, Turkey) were obtained from the manufacturers. Stock solutions of the tested compounds were made in dimethyl sulfoxide (DMSO) (Sigma-Aldrich). Standard antibiotic solutions were made in appropriate solvents recommended by CLSI guidelines [43, 44].

Stock solutions of the test compounds and standard drugs were diluted two-fold in the wells of microplates. Solutions of the synthesized compounds were prepared at 1024–0.5 μg/mL concentrations and standard drugs were prepared at 512–0.001 μg/mL concentrations.

For antibacterial susceptibility testing, 100 μL of Mueller Hinton Broth (MHB) was added to each well of the microplates. The bacterial suspensions used for inoculation were prepared at 10^5 CFU/mL by diluting fresh cultures at McFarland 0.5 density (10^7 CFU/mL). Suspensions of the bacteria at 10^5 CFU/mL concentration were inoculated into the solutions of the compounds. A 10 μL bacterial inoculum was added to each well of the microplates. Microplates were incubated at 37°C overnight. After incubation, the lowest concentrations of the compounds that completely inhibited macroscopic growth were determined and reported as minimum inhibitory concentrations (MICs).

Table 1: In vitro antimicrobial activities of 2-pyrazoline (**5a-5i**), hydrazone (**6a-6i**) and chalcone (**3a-3j**) derivatives in comparison with reference drugs.

MIC (μg/mL)												
Compound	**Gram-negative bacteria**					**Gram-positive bacteria**				**Fungi**		
	E.c.	**E.c.***	**E.c.****	**P.a.**	**P.a ***	**S.a.**	**S.a.***	**E.f.**	**E.f.***	**C.a.**	**C.k.**	**M.t.**
5a	128	128	128	64	64	128	64	64	64	64	64	128
5b	64	128	128	64	128	128	64	32	64	64	64	128
5c	128	128	128	64	128	128	64	32	64	64	128	64
5d	64	128	64	128	64	128	64	32	64	32	64	64
5e	64	128	128	64	128	128	64	32	64	32	64	64
5f	64	64	128	64	64	128	64	32	64	32	64	64
5g	64	128	128	64	64	128	64	32	128	32	32	64
5h	64	128	128	128	128	128	64	64	128	64	32	64
5i	64	128	128	64	64	128	64	64	128	64	64	64
5j	64	128	128	128	64	128	64	64	128	64	64	64
6a	64	128	128	128	64	128	64	128	128	128	64	64
6b	128	128	128	128	128	128	64	128	64	128	64	64
6c	64	128	128	128	128	128	64	64	128	128	64	64
6d	128	128	128	128	128	128	64	128	128	128	64	64
6e	128	128	128	128	128	128	64	128	32	64	64	64
6f	64	128	128	128	64	128	32	64	128	64	32	64
6g	64	128	128	64	64	128	64	64	128	64	64	64
6h	128	128	128	64	128	128	64	128	128	128	64	64
6i	128	128	128	64	64	128	64	128	128	128	64	64
3a	128	128	128	64	64	64	64	128	128	64	64	64
3b	128	128	128	128	128	64	64	128	128	128	64	64
3c	128	128	128	64	128	256	64	128	128	128	64	64
3d	128	128	128	64	64	256	64	128	128	128	64	64
3e	128	128	128	64	128	128	64	128	128	128	64	64
3f	128	128	128	128	128	128	64	128	128	128	64	64
3g	128	128	128	128	128	16	64	8	128	16	64	64
3h	64	128	128	128	128	64	64	128	128	128	64	64
3i	64	128	128	128	64	64	64	128	128	64	64	64
3j	64	128	128	64	64	128	32	128	128	64	32	64
Ampicillin	2	n.d.	>1024	n.d.	n.d.	0.5	n.d.	0.5	0.5	n.d.	n.d.	n.d.
Gentamicin	0.25	n.d.	256	1	64	0.5	128	8	8	n.d.	n.d.	n.d.
Ofloxacin	0.015	n.d.	16	1	1	0.125	0.5	1	4	n.d.	n.d.	n.d.
Meropenem	0.008	n.d.	0.015	0.25	0.015	0.03	n.d.	4	8	n.d.	n.d.	n.d.
Vancomycin	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	1	1	8	n.d.	n.d.	n.d.
Ampicillin/sulbactam (1/1)	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Amoxicillin/clavulanic acid (2/1)	n.d.	16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d
Fluconazol	n.d	n.d	n.d.	n.d	n.d	n.d.	n.d.	n.d.	n.d.	0.0625	32	n.d.
Amphothericin B	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<0.03	0.5	n.d.
Ethambuthol												4
Isoniazid												0.125

n.d., not determined (microbiological assays were not performed due to following reasons: *P. aeruginosa* is naturally resistant to ampicillin; *E. coli* ATCC 35218 has ESBL; Gram-negative bacteria employed in the study are naturally resistant to vancomycin; antibacterial drugs were not assayed against fungi; antifungal drugs were not assayed against bacteria). E.c., *E. coli* ATCC 25922; E.c.*, *E. coli* ATCC 35218; E.c.**, *E. coli* isolate (ESBL); P.a., *P. aeruginosa* ATCC 25853; P.a.*, *P. aeruginosa* isolate (resistant to gentamicin); S.a., *S. aureus* ATCC 29213; S.a.*, *S. aureus* isolate (MRSA); E.f., *E. faecalis* ATCC 29212; E.f.*, *E. faecalis* isolate (VRE); C.a., *C. albicans* ATCC 10231; C.k., *C. krusei* ATCC 6258; M.t. *M. tuberculosis* H37RV ATCC 27294.

For fungal susceptibility testing, RPMI-1640 medium with L-glutamine buffered to pH 7 with MOPS was added to each well of the microplates. The yeast suspensions used for inoculation were prepared at 10^4 CFU/mL by diluting fresh cultures at McFarland 0.5 density (10^6 CFU/mL). Suspensions of the yeast at 10^4 CFU/mL concentrations were inoculated into the solutions of the compounds. A 10 μL yeast inoculum was added to each well of the microplates. Microplates were incubated at 37°C for 24–48 h. After incubation, the lowest concentrations of the compounds that completely inhibited macroscopic growth were determined and reported as minimum inhibitory concentrations (MICs).

3.2 Antitubercular activity

Mycobacterium tuberculosis H37RV (ATCC 27294) was grown on Middlebrook 7H11 agar (BD, Becton Dickinson, NJ, USA). Culture suspensions were prepared in 0.04% (v/v) Tween 80–0.2% bovine serum albumin (Sigma-Aldrich) at MacFarland 1 density. Suspensions were then diluted 1:25 in 7H9GC broth containing 4.7 g of Middlebrook 7H9 broth base (Difco, Detroit, MI, USA), 20 mL of 10% (v/v) glycerol (Sigma-Aldrich), 1 g of Bacto Casitone (Difco), 880 mL of distilled water, 100 mL of OADC (oleic acid, albumin, dextrose, catalase) supplement (Sigma-Aldrich) that includes 5 g bovine albumin fraction, 2 g dextrose, 0.004 g catalase, 0.05 g oleic acid and 0.85 g sodium chloride.

Compounds were dissolved in dimethyl sulfoxide (DMSO) (Merck) at a final concentration of 4096 μg/mL and sterilized by filtration using 0.22 μm syringe filters (Merck-Millipore, Darmstadt, Germany) and used as the stock solutions. The stock solutions of the agents were diluted within liquid media. Isoniazid (INH) and ethambutol (EMB) were obtained from Sigma-Aldrich. Stock solutions of INH and EMB were prepared in deionized water. The solutions of the compounds and drugs were prepared and diluted at 4096-0.0625 μg/mL concentrations in the wells of microplates in the liquid media.

Two hundred microliters of sterile deionized water were added to the outer-perimeter wells to minimize evaporation of the medium in the test wells during incubation. One hundred microliters of 7H9GC broth was added to the wells in rows B to G in columns 3 to 11. One hundred microliters of stock solutions were added to the wells in rows B to G in columns 2 and 3 by using a multichannel pipette. One hundred microliters was transferred from column 3 to column 4, and the contents of the wells were mixed. Serial two-fold dilutions were made through column 10.

One hundred microliters of *M. tuberculosis* inoculum was added to the wells in rows B to G in columns 2 to 11 by using a multichannel pipette. The wells in column 11 were used for growth controls. The plates were sealed with parafilm and were incubated at 37°C for 5 days. Fifty microliters of a freshly prepared 1:1 mixture of 10X Alamar Blue (AbD Serotec) reagent and 10% Tween 80 were added to well B11. The plates were incubated at 37°C for 24 h. B11 turned pink and 50 μL of the reagent mixture were added to all wells in the microplate. The microplates were resealed with parafilm and were incubated for 24 h at 37°C, and the colors of all wells were recorded. A blue color in the well was recorded as no growth, and a pink color was scored as growth. The MIC was defined as the lowest drug concentration which prevented a color change from blue to pink [45].

All organisms were tested in triplicate in each run of the experiments. Before the inoculum preparation, the microorganisms passaged at least twice to ensure purity and viability. The quality control strains (ATCC strains) demonstrated the accuracy of the experiment because all the results were within the MIC values mentioned in CLSI standards. Non-inoculated and antimicrobial free medium was used as the sterility control. Inoculated and sterile medium was used as microbial growth control.

3.3 Chemistry

Chalcone derivatives were prepared by the reaction of acetophenone and benzaldehyde derivatives, **1** and **2**, in KOH/MeOH. The ensuing chalcone derivatives **3a-3j** were then reacted with hydrazide compounds to furnish hydrazone and 2-pyrazoline derivatives, **5a**-**5j** and **6a**-**6i** (Scheme 1). Synthesis details, physicochemical and spectral characterization of the synthesized compounds have been reported earlier for all compounds except **3i**, **3j** [29]. The last-mentioned compounds were prepared analogously from acetophenone derivatives and 3-thiophenecarboxaldehyde.

3.3.1 General procedure for the preparation of chalcone derivatives (3i, 3j): To a stirred solution of KOH (50% w/v) in water (5 mL) cooled in an ice bath, a solution of the acetophenone **1** (4.99 mmol) and the 3-thiophenecarboxaldehyde **2** (6.018 mmol) in ethanol (20 mL) was added dropwise. The reaction mixture was stirred at room temperature overnight. Then the mixture was poured into ice, adjusted to a pH of 3–4 with 1 M HCl, and then filtered. The precipitate was crystallized from ethanol [46].

3.3.2 (*E*)-1-(2-hydroxy-4-methoxyphenyl)-3-(thiophen-3-yl)prop-2-en-1-one (3i): Brown colored solid (ethanol).-Yield:72.7%. M.p. 103.4°C. – IR (KBr): ν = 3429 (OH), 1680 (C=O). ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 3.82 (s, 3H, -OCH_3), 6.49–6.56 (2H, aromatic-H), 7.64-8.23 (6H, aromatic-H, α-ethylenic-H, β-ethylenic-H), 13.52 (s, 1H, -OH). – MS (ESI): m/z = 261 $[M + H]^+$ (99%), 262 $[M + H + 1]^+$ (33%). $C_{14}H_{12}O_3S$: C 64.27, H 4.76, S 12.25; calcd. C 64.60, H 4.65, S 12.32.

3.3.3 (*E*)-1-(2,4-dimethoxyphenyl)-3-(thiophen-3-yl)prop-2-en-1-one (3j): Black colored solid (ethanol).-Yield:79.6%. M.p. 81°C. – IR (KBr): ν = 1692 (C=O). – ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 3.82 (s, 3H, -OCH_3), 3.85 (s, 3H, -OCH_3), 6.59–6.65 (2H, aromatic-H), 7.32 (d, 1H, J = 16 Hz, α-ethylenic-H), 7.49-7.96 (5H, aromatic-H, β-ethylenic-H). – MS (ESI): m/z = 275 $[M + H]^+$ (100%), 297 $[M + Na]^+$ (97%). $C_{15}H_{14}O_3S$: C 65.58, H 5.33, S 11.74; calcd. C 65.67, H 5.14, S 11.69 [21–24].

4 Conclusion

In conclusion, we have designed and synthesized several chalcone, hydrazone and 2-pyrazoline compounds and evaluated their antibacterial, antimycobacterial and antifungal activities. The 2-pyrazoline and hydrazone functions did not increase the antimicrobial activity. Chalcones had better activity against *S. aureus* and *E. faecalis*, as compared to hydrazone and 2-pyrazoline derivatives. A hydroxy group at the fourth position of the A ring increased the activity against *S. aureus*, *E. faecalis* and *C. albicans* for chalcones. If the same scaffold on A ring was on a hydrazone structure, the activity was decreased.

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