European Journal of Medicinal Chemistry 62 (2013) 270–279

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of some new 2-(3-pyridyl)-4,5-disubstituted thiazoles as potent antimicrobial agents

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ARTICLE INFO

Article history:
Received 15 November 2012
Received in revised form
26 December 2012
Accepted 27 December 2012
Available online 5 January 2013

Keywords:
Thiazole
Pyridine
Pyrazoline
Chalcone
Antimicrobial activity

ABSTRACT

As a part of ongoing studies in developing new potent antimicrobial agents, a series of novel 2-(3-pyridyl)-4,5-disubstituted thiazoles was efficiently synthesized and characterized by spectral and elemental analyses. The newly synthesized compounds were evaluated for their *in vitro* antimicrobial activity against ten bacterial and five fungal human pathogenic strains using the disc diffusion assay. Among the synthesized compounds, 5-acetyl-4-methyl-2-(3-pyridyl)thiazole (**5**) exhibited twofold antibacterial activity of ampicillin in inhibiting the growth of *Staphylococcus epidermidis* (MIC 0.24 μg/mL) and also showed equipotent antifungal activity with amphotricin B against *Geotricum candidum* (MIC 0.48 μg/mL). From structure–activity relationship (SAR) point of view, increasing the size of the substitutions either at position 4 or 5 on the thiazole nucleus decreased the antimicrobial activity.

1. Introduction

The treatment of infectious diseases remains an important issue because of a combination of factors including emerging newer infectious diseases and increasing number of multi-drug resistant microbial pathogens. This problem is particularly pronounced for the Gram-positive bacteria [1–4]. The therapeutic problem is an important part of hospitalized patients, immunosuppressed patients with AIDS and those undergoing anticancer therapy or organ transplants. Despite a large number of antibiotics and chemotherapeutics available for medical use, the emerging resistance to old and new antibiotics has created a substantial need for new classes of antimicrobial agents. A potential approach to overcome the problem of antibiotic resistance is to design innovative agents with different modes of action so that no cross resistance with present drugs can occur [5].

Thiazoles are a familiar group of heterocyclic compounds possessing a wide variety of biological activities, and their usefulness as medicines are well established. Thiazole nucleus is also an integral part of all the available penicillins, which have revolutionized the therapy of bacterial diseases [6]. Thiazoles have attracted continuing interest because of their varied biological activities [7], which have found applications in the treatment of allergies [8], hypertension [9], inflammation [10], schizophrenia [11], microbial infections [12,13], HIV infections [14], hypnotics [15] and for the treatment of pain [16]. They have been also used as fibrinogen receptor antagonists with antithrombotic activity [17] and as new inhibitors of bacterial DNA gyrase B [18].

On the other hand, chalcone, a biosynthetic product of the shikimate pathway, is a separate class of compounds, those have a wide range of biological properties. Chalcones are useful synthons in the synthesis of a large number of bioactive molecules such as pyrazolines that are well known nitrogen containing heterocyclic compounds. Considerable interest has been focused on the pyrazoline structure, which possesses a broad spectrum of biological activities such as antiamoebic [19,20], antimicrobial [21], monoamine oxidase inhibitors [22], antimycobacterial [23,24], antidepressant [25,26], anticonvulsant [27] and anti-inflammatory [28,29].

Moreover, the pyridine nucleus is prevalent in numerous natural products and is extremely important in the chemistry of biological systems. Pyridine derivatives have been used as bactericides [30], fungicides [31], and anticancer agents [32–35].

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http://dx.doi.org/10.1016/j.ejmech.2012.12.050

In view of the above-mentioned findings, and as a continuation of our effort to identify new candidates that may be value in designing new, potent, selective, and less toxic antimicrobial agents [36–39], we report herein the synthesis and antimicrobial evaluation of some novel structure hybrids incorporating the pyridine, thiazole and pyrazole ring systems. This combination was suggested to investigate the influence of such hybridization and structure variation on the anticipated biological activities, hoping to add some synergistic biological significance to the target molecules. The target compounds were rationalized so as to comprise some pharmacophores that are believed to be responsible for the biological activity of some relevant chemotherapeutic agents such as the acetyl and chalcone functionalities. The substitution pattern of the thiazole and pyrazole rings was carefully selected in order to confer different electronic environment to the molecules.

2. Results and discussion

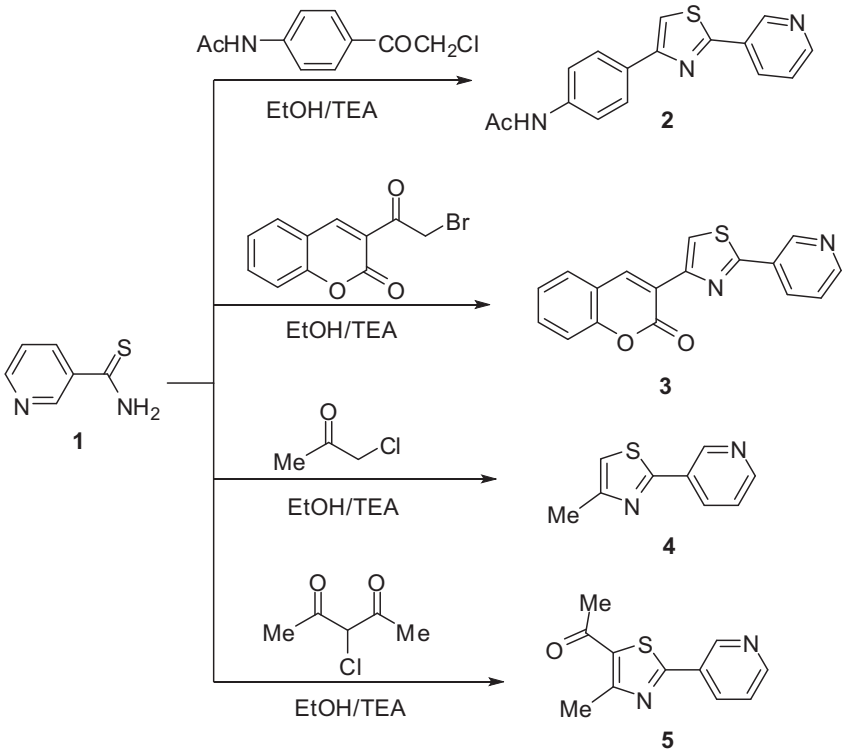
2.1. Chemistry

The synthetic strategies adopted for the synthesis of the target compounds are depicted in Schemes 1 and 2. In Scheme 1, the Hantzsch reaction of thionicotinamide (**1**) with α-haloketones, namely *p*-chloroacetylacetanilide [40], 3-bromoacetylcoumarin [41], chloroacetone and 3-chloroacetylacetone in refluxing ethanol containing a catalytic amount of triethylamine afforded, in each case, a single product, that was identified as 2-(3-pyridyl)thiazole derivatives **2**–**5**. It has been found that elemental analysis and spectral data were in consistent with the proposed 2-(3-pyridyl) thiazole structures. The IR spectrum of 1-(4-methyl-2-(3-pyridyl) thiazol-5-yl)ethanone (**5**), as a representative example, showed a strong absorption band at 1695 cm^{-1} that is due to a carbonyl group. Its ^1H NMR spectrum showed besides the expected aromatic signals, two new singlets at δ 2.65 and 2.75 ppm assigned to the methyl and acetyl protons. The ^{13}C NMR spectrum displayed eleven carbon signals, the most important signals appeared at δ 17.9, 30.3, 190.7 ppm characteristic for two methyl and carbonyl carbons, respectively. The mass spectrum revealed a molecular ion peak at $m/z = 218$ corresponding to a molecular formula $C_{11}H_{10}N_2OS$.

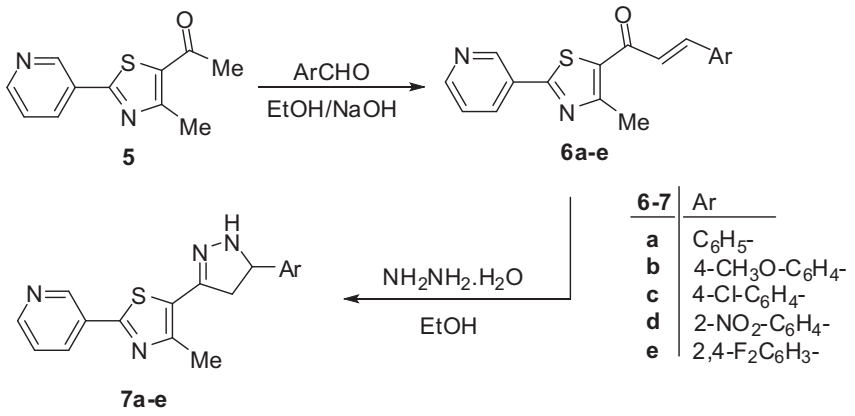
The presence of the acetyl group in the thiazole **5** makes it versatile precursor for the synthesis of chalcones and pyrazoline derivatives. Thus, the Claisen–Schmidt condensation of 5-acetylthiazole **5** with aromatic aldehydes in ethanolic sodium hydroxide furnished the chalcone derivatives **6a**–**e**. The structure of the latter products were confirmed by IR spectra which showed the characteristic band for conjugated C=O at 1652–1660 cm^{-1} and their ^1H NMR spectra displayed a pair of doublets at δ 7.35–7.49 and 7.71–7.75 ppm, with coupling constant value (J = 15.0–15.5 *Hz*), due to the *trans*-olefinic protons. Cyclocondensation of chalcones **6a**–**e** with hydrazine hydrate in boiling ethanol furnished the respective pyrazolines **7a**–**e** (Scheme 2). The structures of the pyrazolines were established by IR spectra which showed disappearance of C=O band of chalcones. A strong band appeared at 1590–1605 cm^{-1} assigned to C=N of pyrazoline ring. Pyrazolines **7a**–**e** showed an additional sharp band in the region 3200–3235 cm^{-1} due to their NH stretching. Their ^1H NMR spectra revealed the signals of CH_2 protons of the pyrazoline ring in the region 2.79–2.98 and 3.25–3.62 ppm as a pair of doublets. The CH proton appeared as double of doublets at 4.90–4.98 ppm. The ^{13}C NMR spectrum of compound **7a**, as a representative example, displayed, besides the expected methyl and aromatic signals, three characteristic signals at δ 42.4, 64.3 and 160.2 ppm due to the carbons of CH_2, CH and C=N of pyrazoline ring, respectively.

2.2. Antimicrobial evaluation

The antimicrobial screening and minimal inhibitory concentrations of the tested compounds were carried out at the Regional



Scheme 1. Synthesis of 2-(3-pyridyl)thiazoles **2**–**5**.



Scheme 2. Synthesis of chalcones **6a**–**e** and pyrazolines **7a**–**e**.

Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. Thirteen of the newly synthesized target compounds were evaluated for their *in vitro* antimicrobial activities against the human pathogens *Staphylococcus aureus* (RCMB 010027), *Staphylococcus epidermidis* (RCMB 010024), *Streptococcus pyogenes* (RCMB 010015), *Bacillis subtilis* (RCMB 010063) and *Enterococcus faecalis* (RCMB 010068) as examples of Gram-positive bacteria and *Neisseria gonorrhoeae* (RCMB 010076), *Proteous vulgaris* (RCMB 010085), *Klebsiella pneumonia* (RCMB 010093), *Shigella flexneri* (RCMB 0100542) and *Pseudomonas aeruginosa* (RCMB 010043) as examples of Gram-negative bacteria. They were also evaluated for their potential antifungal activities against the following fungal strains; *Aspergillus fumigates* (RCMB 02564), *Aspergillus clavatus* (RCMB 02593), *Candida albicans* (RCMB 05035), *Geotricum candidum* (RCMB 05096), and *Penicillium marneffei* (RCMB 01267).

Agar-diffusion method was used for the determination of the preliminary screening of antibacterial and antifungal activities. Ampicillin, gentamycin and amphotricin B were used as reference drugs. The results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm. The minimal inhibitory concentrations (MICs) for compounds that showed significant growth inhibition zones (>10 mm) were determined using twofold serial dilution method [42]. The inhibition zone diameters, attributed to the tested original concentration (5 mg/mL) as a preliminary test, are shown in Figs. 1–6 and the MICs (μg/mL) are shown in Tables 1 and 2.

2.2.1. Antibacterial activity

Preliminary antibacterial screening was carried out for the substituted 2-(3-pyridyl)thiazoles **2**–**5**. Among this series, compounds **3**–**5** showed good inhibitory activity against all the screened Gram-positive bacteria (Fig. 1). Compounds **4** and **5** showed better results when compared with ampicillin as revealed from their MIC values (0.97–31.25 μg/mL) and (0.06–7.81 μg/mL), respectively, with superior activity for compound **5** (Table 1). The 5-acetylthiazole **5** showed twofold potency of ampicillin in inhibiting the growth of *S. epidermidis* (MIC 0.24 μg/mL) and 25% of the potency of ampicillin against *S. aureus* and *E. faecalis* with MIC values (0.24 μg/mL) and (7.81 μg/mL), respectively. 4-Methyl-2-(3-pyridyl)thiazole **4** displayed 50% less activity compared to ampicillin against *S. epidermidis* (MIC 0.97 μg/mL). The results depicted in Fig. 1 revealed that the coumarinylthiazole **3** showed moderate to weak activity against the growth of the different Gram-positive bacteria (MIC 3.9–62.5 μg/mL), and its best result was observed against *S. epidermidis* (MIC 3.9 μg/mL). On the other hand, compound **2** showed narrow spectrum of activity in inhibiting the growth of Gram-positive bacteria as it was inactive against *S. pyogenes* and *E. faecalis* and showed 25% the activity of ampicillin against *S. epidermidis* (MIC 1.95 μg/mL).

By investigating the biological activity of the substituted 2-(3-pyridyl)thiazoles **2**–**5** as anti-Gram negative bacterial agents (Fig. 2), it was observed that compounds **3**–**5** were only active with broad spectrum activity against all the screened microbes. Unfortunately, compound **2** was completely inactive.

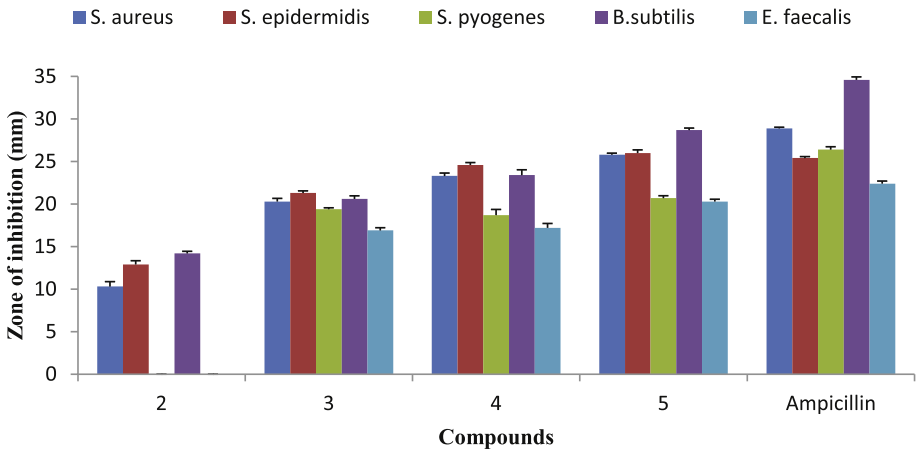


Fig. 1. Preliminary antibacterial activity of thiazoles **2**–**5** against Gram-positive bacteria.

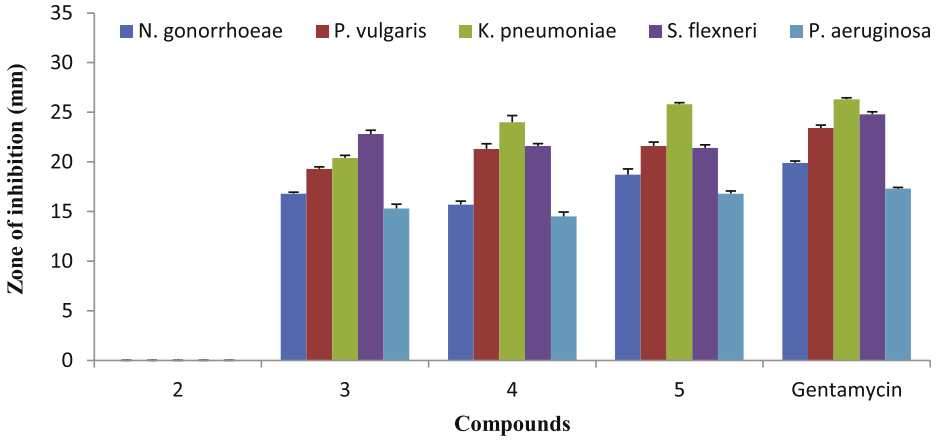


Fig. 2. Preliminary antibacterial activity of thiazoles **2**–**5** against Gram-negative bacteria.

Interestingly, 5-acetylthiazole **5** was one-fold less active than gentamycin against *N. gonorrhoeae*, *P. vulgaris* and *K. pneumonia* with MIC values (15.63 μg/mL), (3.9 μg/mL) and (0.48 μg/mL), respectively, and showed weak effect in inhibiting the growth of *S. flexneri* (MIC 3.9 μg/mL). Thiazole **4** was 50% less active than gentamycin against *P. vulgaris* (MIC 3.9 μg/mL) and 75% less active than gentamycin against the growth of *P. aeruginosa* (MIC 125 μg/mL). The coumarinylthiazole **3** was 50% less active than gentamycin against *P. aeruginosa* (MIC 62.5 μg/mL) and 75% less active than gentamycin against *S. flexneri* (MIC 1.95 μg/mL); while its effect against *N. gonorrhoeae* and *P. vulgaris* was much weaker than gentamycin with MIC values (62.5 μg/mL) and (15.63 μg/mL), respectively.

The above results revealed that the 5-aceylthiazole **5** showed the best antibacterial activity. For that reason, compound **5** was chemically transformed to the respective chalcone **6a**–**e** and pyrazoline **7a**–**e** derivatives hoping to get more potent antibacterial agents. From the chalcone series **6a**–**e**, compounds **6b** and **6d** only showed broad spectrum activity against all the screened Gram-positive bacteria with weak to moderate potency (Fig. 3). In contrast to the activity of the precursor 5-acetylthiazole **5**, chalcone **6d** showed 50% potency of ampicillin against *S. epidermidis* (MIC 0.97 μg/mL); while chalcone **6b** showed three fold weaker potency against the same bacteria (MIC 3.9 μg/mL) (Table 1). The chalcones **6a** and **6e** were active against all the screened Gram-positive bacteria except *S. pyogenes,* while compound **6c** was active against *S. aureus*, *S. epidermidis* and *B. subtilis* only.

Concerning the antibacterial activity of pyrazoline derivatives, the results displayed that compounds **7a**–**c** and **7e** were active against all screened Gram-positive bacteria except *S. pyogenes* and their potencies were weaker than that of their corresponding chalcones **6a**–**e** (Fig. 3). Moreover, pyrazoline **7b** showed narrow spectrum of activity on contrast to its corresponding chalcone **6b**.

Similarly, the effect of chalcones **6a**–**e**, pyrazolines **7a**–**c** and **7e** on Gram-negative bacteria was also tested. The results are illustrated in Fig. 4. Surprisingly, chalcones **6b** and **6d** were only showed weak to moderate broad spectrum activity against all the screened Gram-negative bacteria. Chalcones **6b** and **6d** recorded twofold lower potency than gentamycin against *P. vulgaris* (MIC 7.81 μg/mL); chalcone **6d** also showed twofold lower potency than gentamycin in inhibiting the growth of *K. pneumonia* (MIC 0.97 μg/mL) and *P. aeruginosa* (MIC 125 μg/mL). On the other hand, chalcone **6a** was active against *P. vulgaris*, *K. pneumonia* and *S. flexneri*; while chalcones **6c** and **6e** were active against *P. vulgaris* and *K. pneumonia* only and their potencies were weaker than chalcones **6b** and **6d**. Unexpectedly, among the pyrazolines **7a**–**c** and **7e**, only pyrazoline **7b** was the active one as it showed activity against all the screened Gram-negative bacteria except *P. aeruginosa* with weaker potency than their corresponding chalcone **6b**.

The results of antibacterial screening revealed that most of the tested thiazoles displayed variable inhibitory effects on the growth of tested Gram-positive and Gram-negative bacterial strains. In general, most of the studied thiazoles revealed better activity against the Gram-positive rather than Gram-negative bacterial

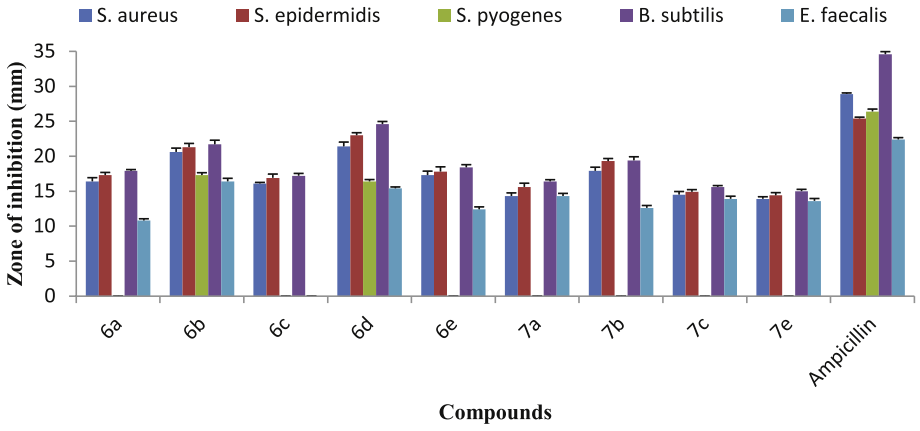


Fig. 3. Preliminary antibacterial activity of the chalcones **6a**–**e** and pyrazolines **7a**–**c** and **7e** against Gram-positive bacteria.

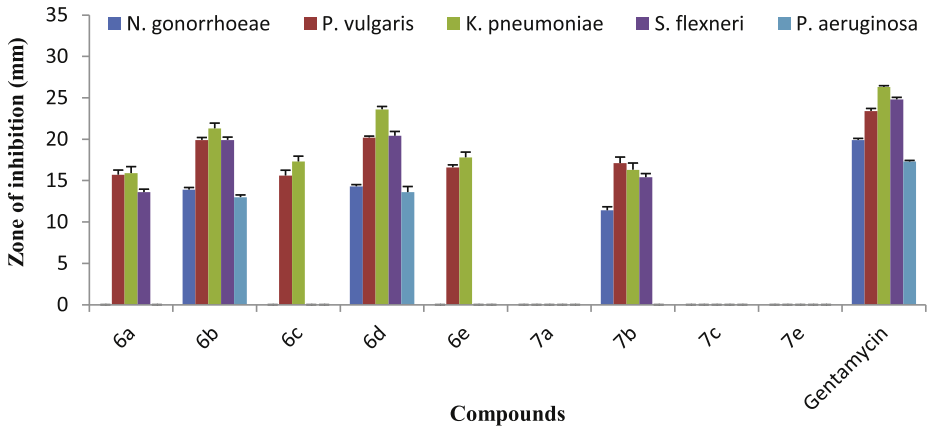


Fig. 4. Preliminary antibacterial activity of the chalcones **6a**–**e** and pyrazolines **7a**–**c** and **7e** against Gram-negative bacteria.

strains. It was also observed that transformation of 5-acetylthiazole **5** to the respective chalcone and pyrazoline derivatives reduced the antibacterial activity.

2.2.2. Antifungal activity

Regarding the activity of the substituted thiazoles **2**–**5** against fungal strains, results in this study revealed that compounds **3**–**5** displayed broad spectrum antifungal activity against all the screened fungi (Fig. 5). Interestingly, the 5-acetylthiazole **5** was equipotent to amphotricin B against *G. candidum* (MIC 0.48 μg/mL) and it showed 50% less potent effect than amphotricin B against *A. fumigates* (MIC 1.95 μg/mL), *A. clavatus* (MIC 3.9 μg/mL) and *P. marneffei* (MIC 3.9 μg/mL). Moreover, 4-methyl-2-(3-pyridyl) thiazole **4** displayed, one-fold lower activity than that of amphotricin B against *P. marneffei* (MIC 3.9 μg/mL), twofold lower activity against *G. candidum* (MIC 1.95 μg/mL), and weak to moderate

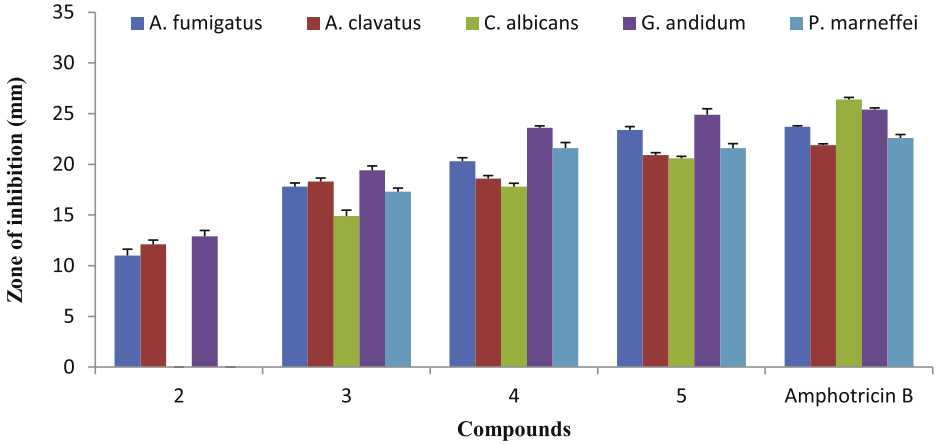


Fig. 5. Preliminary antifungal activity of thiazoles **2**–**5**.

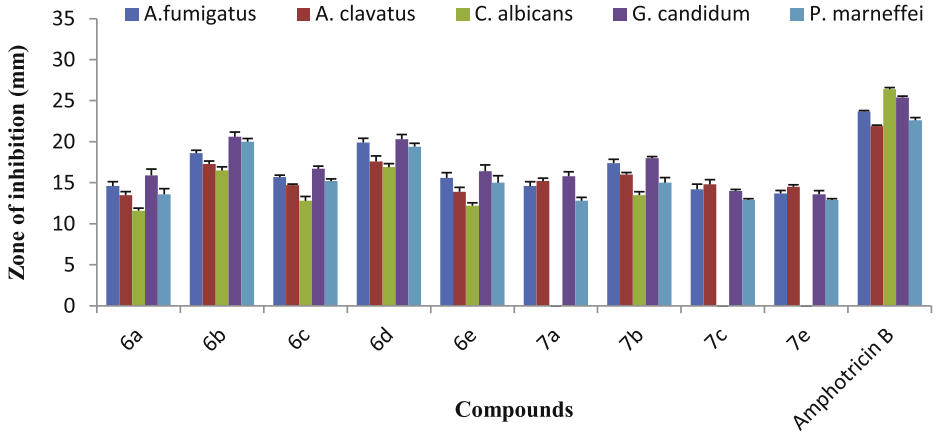


Fig. 6. Preliminary antifungal activity of the chalcones **6a**–**e** and pyrazolines **7a**–**c** and **7e**.

Table 1
Antibacterial minimal inhibitory concentrations (MIC, μg/mL) of some new synthesized 2-(3-pyridyl)-4,5-disubstituted thiazoles.

Compounds	2	3	4	5	6a	6b	6c	6d	6e	7a	7b	7c	7e	AMPI	GEN
Gram-positive bacteria															
S. aureus	3.9	7.81	0.97	0.24	62.5	3.9	62.5	3.9	31.25	125	15.63	125	125	0.06	NT[a]
S. epidermidis	1.95	3.9	0.97	0.24	31.25	3.9	31.25	0.97	31.25	62.5	15.63	62.5	125	0.48	NT
S. pyogenes	–[b]	7.81	15.63	3.9	–	31.25	–	62.5	–	–	–	–	–	0.24	NT
B. subtilis	0.97	3.9	1.95	0.06	31.25	1.95	31.25	0.97	15.63	62.5	15.63	62.5	62.5	0.007	NT
E. faecalis	–	62.5	31.25	7.81	500	62.5	–	62.5	250	125	250	125	125	1.95	NT
Gram-negative bacteria															
N. gonorrhoeae	–	62.5	62.5	15.63	–	125	–	125	–	–	500	–	–	NT	7.81
P. vulgaris	–	15.63	3.9	3.9	62.5	7.81	62.5	7.81	62.5	–	31.25	–	–	NT	1.95
K. pneumonia	–	7.81	1.95	0.48	62.5	3.9	31.25	0.97	31.25	–	62.5	–	–	NT	0.24
S. flexneri	–	1.95	3.9	3.9	125	7.81	–	7.81	–	–	62.5	–	–	NT	0.48
P. aeruginosa	–	62.5	125	500	–	250	–	125	–	–	–	–	–	NT	31.25

AMPI, Ampicillin; GEN, Gentamycin.
[a] NT, Not tested.
[b] No activity.

potency against the rest of the tested fungi when compared with the reference drug (MIC 7.81–31.25 μg/mL). On the other hand, compound **2** showed narrow spectrum of activity which is three to four fold lesser than amphoticin B (MIC 7.81–15.63 μg/mL).

Finally, the antifungal activity was tested for the chalcones **6a**–**e** and pyrazolines **7a**–**c** and **7e** (Fig. 6). The chalcones **6a**–**e** showed broad spectrum antifungal activity with lower potency than 5-acetylthiazole **5**. Chalcone **6b** revealed twofold lower potency than amphotricin B against *P. marneffei* (MIC 7.81 μg/mL); while chalcone **6d** showed half potency against *P. marneffei* with MIC values (7.81 μg/mL) and (15.83 μg/mL), respectively. Chalcones **6a**, **6c** and **6e** are equipotent and their potencies are weaker than chalcones **6b** and **6d**. Among the pyrazolines, only **7b** showed broad spectrum antifungal activity with MIC values (31.25–250 μg/mL); while pyrazolines **7a**, **7c** and **7e** were active against all the screened fungi except *C. albicans*. In general, the pyrazoline derivatives exhibited lower antifungal activity in comparison to their corresponding chalcones and precursor thiazole **5**.

2.3. Structure activity relationship (SAR)

The results of the antimicrobial screening demonstrated the following assumptions about the structural activity relationship (SAR) (Fig. 7):

- It is interesting to point out that substituted 2-(3-pyridyl) thiazole nucleus showed good antimicrobial activity.
- The highest antimicrobial activity observed when the substitutions at positions 4 and 5 of the thiazole nucleus are smaller in size as the case of thiazoles **4** and **5**.
- The presence of the electron withdrawing acetyl group at position 5 and small electron donating methyl group at position 4 of the thiazole **5** resulted in the highest antimicrobial activity among all the compounds investigated in this study.
- Replacement of the methyl group at position 4 of the thiazole ring by hydrogen atom decreased the antimicrobial activity.
- Introduction of 3-coumarinyl moiety at position 4 of the thiazole ring resulted in biologically active compound **3** which was quite less potent than thiazoles **4** and **5** and this may be due to the huge size of the coumarin moiety. Its observed biological activity could be attributed to the synergistic effect of both the coumarin ring and 2-(3-pyridyl)thiazole nucleus as antimicrobial moieties.
- Replacement of the small size acetyl group at position 5 of the thiazole ring by bigger cinnamoyl group (Ar–CH=CH–CO) resulted in decreasing the antimicrobial activity from one to three folds.
- The presence of pyrazoloaryl moiety at position 5 of the thiazole nucleus resulted in the lowest antimicrobial activity among all the tested compounds.
- The type of the substitutions on the benzene ring of the chalcone moiety is also important. It was noticed that the presence of electron withdrawing groups such as chloride and fluoride at the para and/or the ortho position of the benzene ring in chalcones **6c** and **6e** displayed no effect on the antimicrobial activity.
- The highest antimicrobial activities of the chalcone series were observed in case of compounds **6b** and **6d**, those have the electron donating methoxy group at the para position of the benzene ring and electron withdrawing nitro group on the ortho position of the benzene ring, respectively. This indicates that the type of the electronic effect of the benzene ring substitutions have no effect; and it is suggested that the bigger size of the substitutions on the benzene ring is responsible for the observed high effect of these derivatives among their series as it may cause the anchoring of these compounds to their target receptor.
- The observed antimicrobial activity of compounds **6a**–**e** could be attributed to the synergistic effect of both the 2-(3-pyridyl) thiazole and chalcone moieties.

Table 2
Antifungal minimal inhibitory concentrations (MIC, μg/mL) of some new synthesized 2-(3-pyridyl)-4,5-disubstituted thiazoles.

Compounds	2	3	4	5	6a	6b	6c	6d	6e	7a	7b	7c	7e	AMPH
Fungi														
A. fumigates	7.81	15.63	7.81	1.95	125	15.63	62.5	7.81	62.5	125	31.25	125	125	0.97
A. clavatus	15.63	15.63	15.63	3.9	125	31.25	125	31.25	125	62.5	62.5	125	125	1.95
C. albicans	–	125	31.25	7.81	500	62.5	250	31.25	500	–	250	–	–	0.24
G. candidum	7.81	15.63	1.95	0.48	62.5	7.81	31.25	7.81	62.5	62.5	31.25	125	125	0.48
P. marneffei	–	31.25	3.9	3.9	125	7.81	62.5	15.83	62.5	250	62.5	250	250	1.95

AMPH, Amphotricin B; (–), No activity.

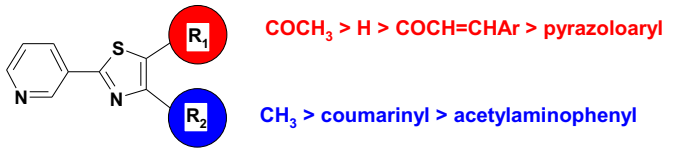


Fig. 7. Order of antimicrobial activity of 2-(3-pyridyl)-4,5-disubstituted thiazoles.

3. Conclusion

In conclusion, the objective of the present study was to synthesize and investigate the antimicrobial activities of some new 2-(3-pyridyl)-4,5-disubstituted thiazoles with the hope of discovering new structures that could be used as potent antimicrobial agents. Our aim has been verified by the synthesis of five different groups of structural hybrids comprising basically the 2-(3-pyridyl) thiazole moiety attached to either acetyl, 3-coumarinyl, 4-acetylaminophenyl, 3-aryl-propenone-1-yl, and/or 5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl. Our results clearly revealed that the substituted 2-(3-pyridyl)thiazoles exhibited good antimicrobial activity. The best antimicrobial activity was observed for 5-acetyl-4-methyl-2-(3-pyridyl)thiazole **5** followed by 4-methyl-2-(3-pyridyl)thiazole **4**. Increasing the size of the substitutions either at position 4 or 5 on the thiazole nucleus decreased the antimicrobial activity.

4. Experimental

Melting points were determined on digital Gallen-Kamp MFB-595 instrument using open capillary tubes and are uncorrected. IR spectra were recorded on Schimadzu FTIR 440 spectrometer using KBr pellets. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker model 500 MHz Ultra Shield NMR spectrometer in DMSO-d$_6$ using tetramethylsilane (TMS) as an internal standard; chemical shifts are reported as δ_{ppm} units. Mass spectra were recorded on Shimadzu Qp-2010 plus mass spectrometer at 70 eV. The elemental analyses were carried out at the Microanalytical Center, Cairo University, Cairo, Egypt. TLC was carried out on Fluka silica gel/TLC-cards 91835. All the chemicals and solvents used were obtained from Merck.

4.1. General procedure for the synthesis of compounds (**2**–**5**)

To a hot solution of thionicotinamide **1** (0.69 g, 0.005 mol) in absolute ethanol (20 mL), triethylamine (0.5 mL) was added. To this mixture, each of, *p*-chloroacetylacetanilide (1.05 g, 0.005 mol), 3-bromoacetylcoumarin (1.33 g, 0.005 mol), chloroacetone (0.46 g, 0.005 mol) and 3-chloroacetylacetone (0.67 g, 0.005 mol) was added and the reaction mixture was refluxed for 8 h. The progress of the reaction was monitored by TLC. The reaction mixture was allowed to cool, poured onto ice water (50 mL) and neutralized with diluted HCl to afford a precipitate that was filtered, washed with water (2 × 30 mL), air dried and then recrystallized from the ethanol.

4.1.1. *N*-[4-(2-Pyridin-3-yl-thiazol-4-yl)-phenyl]-acetamide (**2**)

Yellow powder, Yield (73%), mp 251–252 °C; IR (KBr) νmax/cm^{-1}: 3190 (NH), 3055 (CH–Ar), 2950, 2925 (CH-sp^3), 1680 (C=O), 1595 (C=N); ^{1}H NMR (DMSO-d$_6$): δ_{ppm} = 2.21 (s, 3H, CH$_3$), 7.26 (d, *J* = 7.8 Hz, 2H, 2CH$_{Ar}$), 7.42 (d, *J* = 7.8 Hz, 2H, 2CH$_{Ar}$), 7.56 (s, 1H, Thiazole-H$_5$), 7.91 (dd, *J* = 8 Hz, 5.5 Hz, 1H, Pyridine-H$_5$), 8.41 (s, 1H, NH), 8.72 (d, *J* = 5.5 Hz, 1H, Pyridine-H$_4$), 8.81 (d, *J* = 5.0 Hz, 1H, Pyridine-H$_6$), 9.27 (s, 1H, Pyridine-H$_2$); MS *m*/*z* (%): 295 (M$^+$, 2.7), 253 (M$^+$ − COCH$_3$, 2.8), 222 (2.9), 177 (20.5), 162 (60.8), 149 (1.8), 135 (17.5), 121 (17.5), 120 (100), 106 (7.3), 104 (4.2), 92 (19.2), 79 (47.5), 65 (14.3), 63 (43.4); Anal. Calcd. for C$_{16}$H$_{13}$N$_3$OS (295.37): C, 65.06; H, 4.44; N, 14.23%, Found: C, 65.02; H, 4.41; N, 14.22%.

4.1.2. 3-(2-Pyridin-3-yl-thiazol-4-yl)-chromen-2-one (**3**)

Brown powder, Yield (55%), mp 246–247 °C; IR (KBr) νmax/cm^{-1}: 3055 (CH–Ar), 1725 (C=O), 1620 (C=N); ^{1}H NMR (DMSO-d$_6$): δ_{ppm} = 7.21–7.56 (m, 4H, CH$_{Ar}$), 7.61 (s, 1H, Thiazole-H$_5$), 7.89 (dd, *J* = 8 Hz, 5 Hz, 1H, Pyridine-H$_5$), 8.75 (d, *J* = 5 Hz, 1H, Pyridine-H$_4$), 8.84 (d, *J* = 5.0 Hz, 1H, Pyridine-H$_6$), 8.91 (s, 1H, Coumarin-H$_4$), 9.33 (s, 1H, Pyridine-H$_2$); MS *m*/*z* (%): 306 (M$^+$, 3.5), 286 (28.1), 272 (14.8), 244 (33.0), 226 (87.7), 223 (28.0), 200 (30.3), 178 (14.3), 159 (39.9), 146 (37.2), 131.1 (34.6), 121 (32.2), 120 (37.3), 106 (10.6), 90 (44.9), 86 (100), 78 (14.0), 76 (21.1); Anal. Calcd. for C$_{17}$H$_{10}$N$_2$O$_2$S (306.35): C, 66.65; H, 3.29; N, 9.14%, Found: C, 66.69; H, 3.27; N, 9.11%.

4.1.3. 3-(4-Methylthiazol-2-yl)-pyridine (**4**)

Yellow powder, Yield (60%), mp 200–201 °C; IR (KBr) νmax/cm^{-1}: 3130–3025 (CH–Ar), 2990–2945 (CH-sp^3); ^{1}H NMR (DMSO-d$_6$): δ_{ppm} = 2.48 (s, 3H, CH$_3$), 7.59 (s, 1H, Thiazole-H$_5$), 7.95 (dd, *J* = 8 Hz, 5 Hz, 1H, Pyridine-H$_5$), 8.77 (d, *J* = 5 Hz, 1H, Pyridine-H$_4$), 8.87 (d, *J* = 5.0 Hz, 1H, Pyridine-H$_6$), 9.27 (s, 1H, Pyridine-H$_2$); ^{13}C NMR (DMSO-d$_6$): δ_{ppm} = 16.7 (CH$_3$), 117.5 (Thiazole-C$_5$), 126.6 (Pyridine-C$_5$), 130.9 (Pyridine-C$_3$), 139.1 (Pyridine-C$_4$), 141.5 (Pyridine-C$_2$), 144.9 (Thiazole-C$_4$), 154.0 (Pyridine-C$_6$), 160.8 (Thiazole-C$_2$); MS *m*/*z* (%): 176 (M$^+$, 100), 161 (M$^+$ − CH$_3$, 1.5), 150 (3.3), 122 (1.3), 105 (18.8), 88 (8.7), 72 (97.8), 69 (4.4), 51 (7.3); Anal. Calcd. for C$_9$H$_8$N$_2$S (176.24): C, 61.34; H, 4.58; N, 15.89%, Found: C, 61.32; H, 4.55; N, 15.81%.

4.1.4. 1-(4-Methyl-2-pyridin-3-yl-thiazol-5-yl)-ethanone (**5**)

Yellow powder, Yield (75%), mp 247–248 °C; IR (KBr) νmax/cm^{-1}: 3125–3015 (CH–Ar), 2998–2956 (CH-sp^3), 1695 (C=O); ^{1}H NMR (DMSO-d$_6$): δ_{ppm} = 2.62 (s, 3H, CH$_3$), 2.75 (s, 3H, CH$_3$CO) 7.91 (dd, *J* = 8 Hz, 5 Hz, 1H, Pyridine-H$_5$), 8.75 (d, *J* = 5 Hz, 1H, Pyridine-H$_4$), 8.90 (d, *J* = 5.0 Hz, 1H, Pyridine-H$_6$), 9.31 (s, 1H, Pyridine-H$_2$); ^{13}C NMR (DMSO-d$_6$): δ_{ppm} = 17.9 (CH$_3$), 30.3 (CH$_3$), 126.7 (Thiazole-C$_5$), 129.5 (Pyridine-C$_5$), 133.9 (Pyridine-C$_3$), 138.3 (Pyridine-C$_4$), 143.2 (Pyridine-C$_2$), 150.3 (Thiazole-C$_4$), 158.0 (Pyridine-C$_6$), 163.5 (Thiazole-C$_2$), 190.7 (C=O); MS *m*/*z* (%): 218 (M$^+$, 96.5), 203 (M$^+$ − CH$_3$, 100), 192 (4.9), 175 (30.9), 160 (6.2), 134 (5.1), 105 (25.5), 87 (2.8), 71 (46.9), 64 (50.2), 51 (5.4); Anal. Calcd. for C$_{11}$H$_{10}$N$_2$OS (218.28): C, 60.53; H, 4.62; N, 12.83%, Found: C, 60.51; H, 4.64; N, 12.86%.

4.2. General procedure for the synthesis of chalcones **6a**–**e**

A mixture of 1-(4-methyl-2-pyridin-3-yl-thiazol-5-yl)-ethanone (**5**) (0.654 g, 0.003 mol), appropriate aromatic aldehyde (0.003 mol) and 10% aqueous sodium hydroxide (15 mL) in methanol (25 mL) was stirred at room temperature for about 3 h. The resulting solid was filtered off, rinsed with water, dried, and crystallized from ethanol.

4.2.1. 1-(4-Methyl-2-pyridin-3-yl-thiazol-5-yl)-3-phenyl-propenone (**6a**)

Yellow powder, Yield (78%), mp 150–151 °C; IR (KBr) νmax/cm^{-1}: 3032 (CH–Ar), 2936, 2887 (CH-sp^3), 1654 (C=O), 1605 (C=C); ^{1}H NMR (DMSO-d$_6$): δ_{ppm} = 2.80 (s, 3H, CH$_3$), 7.37 (d, *J* = 15.0 Hz, 1H, CH=), 7.47–7.60 (m, 5H, CH$_{Ar}$), 7.75 (d, *J* = 15.0 Hz, 1H, CH=), 7.85 (dd, *J* = 7.8 Hz, 5 Hz, 1H, Pyridine-H$_5$), 8.39 (d, *J* = 5 Hz, 1H, Pyridine-H$_4$), 8.74 (d, *J* = 5.0 Hz, 1H, Pyridine-H$_6$), 9.20 (s, 1H, Pyridine-H$_6$); ^{13}C NMR (DMSO-d$_6$): δ_{ppm} = 18.2 (CH$_3$), 124.3 (Thiazole-C$_5$), 124.4 (2CH$_{Ar}$), 128.1 (CH=), 128.9 (2CH$_{Ar}$), 129.0 (Pyridine-C$_5$), 130.9

(CH_{Ar}), 132.3 (C_{Ar}), 133.9 (Pyridine-C_3), 134.0 (Pyridine-C_4), 144.0 (CH═), 147.1 (Pyridine-C_2), 151.8 (Thiazole-C_4), 158.9 (Pyridine-C_6), 165.5 (Thiazole-C_2), 182.2 (C═O); MS *m/z* (%): 306 (M^+, 100), 305 ($M^+ - H$, 44.5), 289 (4.1), 273 (6.6), 263 (3.9), 229 (41.5), 215 (10.3), 202 (22.0), 175 (4.9), 159 (1.8), 131 (26.0), 115 (4.3), 103 (41.0), 91 (5.3), 77 (31.7), 51 (8.9); Anal. Calcd. for $C_{18}H_{14}N_2OS$ (306.39): C,70.56; H, 4.61; N, 9.14%, Found: C, 70.51; H, 4.57; N, 9.11%.

4.2.2. 3-(4-Methoxyphenyl)-1-(4-methyl-2-pyridin-3-yl-thiazol-5-yl)-propenone (**6b**)

Yellow powder, Yield (91%), mp 155–156 °C; IR (KBr) νmax/cm^{-1}: 3040 (CH–Ar), 2915, 2887 (CH-sp^3), 1655 (C═O), 1600 (C═C); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.79 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 7.04 (d, *J* = 6.8 Hz, 2H, $2CH_{Ar}$), 7.35 (d, *J* = 15.5 Hz, 1H, CH═), 7.58 (m, 1H, Pyridine-H_5), 7.72 (d, *J* = 15.5 Hz, 1H, CH═), 7.82 (d, *J* = 6.8 Hz, 2H, $2CH_{Ar}$), 8.38 (d, *J* = 5 Hz, 1H, Pyridine-H_4), 8.73 (d, *J* = 4.9 Hz, 1H, Pyridine-H_6), 9.19 (s, 1H, Pyridine-H_2); ^{13}C NMR (DMSO-d_6): δ_{ppm} = 18.2 (CH_3), 55.4 (OCH_3), 114.6 (CH═), 122.2 (Pyridine-C_4), 124.3 (Thiazole-C_5), 126.6 ($2CH_{Ar}$), 128.2 ($2CH_{Ar}$), 130.9 (C_{Ar}), 132.6 (CH_{Ar}), 133.9 (C_{Ar}), 144.2 (Pyridine-C_5), 147.1 (CH═), 151.8 (Pyridine-C_2), 158.6 (Thiazole-C_4), 161.7 (Pyridine-C_6), 165.2 (Thiazole-C_2), 182.1 (C═O); MS *m/z* (%): 336 (M^+, 100), 335 (36.6), 321 ($M^+ - CH_3$, 8.1), 305 ($M^+ - OCH_3$, 21.0), 293 (6.1), 278 (1.3), 232 (47.1), 217 (21.1), 202 (8.3), 189 (3.7), 161 (25.1), 145 (2.9), 133 (28.0), 121 (85.2), 108 (14.9), 90 (12.5), 80 (42.1), 64 (20.7); Anal. Calcd. for $C_{19}H_{16}N_2O_2S$ (336.42): C, 67.84; H, 4.79; N, 8.33%, Found: C, 67.77; H, 4.75; N, 8.29%.

4.2.3. 3-(4-Chlorophenyl)-1-(4-methyl-2-pyridin-3-yl-thiazol-5-yl)-propenone (**6c**)

Yellowish white powder, Yield (85%), mp 155–156 °C; IR (KBr) νmax/cm^{-1}: 3045 (CH–Ar), 2965, 2915 (CH-sp^3), 1658 (C═O), 1598 (C═C); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.72 (s, 3H, CH_3), 7.29 (d, *J* = 7.0 Hz, 2H, $2CH_{Ar}$), 7.39 (d, *J* = 15 Hz, 1H, CH═), 7.56 (dd, *J* = 7.8, 5 Hz, 1H, Pyridine-H_5), 7.75 (d, *J* = 15 Hz, 1H, CH═), 7.86 (d, *J* = 7.0 Hz, 2H, $2CH_{Ar}$), 8.43 (d, *J* = 5 Hz, 1H, Pyridine-H_4), 8.78 (d, *J* = 5 Hz, 1H, Pyridine-H_6), 9.23 (s, 1H, Pyridine-H_2); MS *m/z* (%): 340 (M^+, 100), 399 ($M^+ - H$, 36.3), 323 (4.9), 305 (21.3), 277 (3.3), 229 (45.0), 215 (20.9), 203 (24.3), 175 (16.2), 165 (20.4), 139 (36.7), 122 (12.4), 102 (37.9), 78 (15.2), 71 (49.2), 64 (33.2), 51 (16.4); Anal. Calcd. for $C_{18}H_{13}ClN_2OS$ (340.83): C, 63.43; H, 3.84; N, 8.22%, Found: C, 63.40; H, 3.79; N, 8.21%.

4.2.4. 1-(4-Methyl-2-pyridin-3-yl-thiazol-5-yl)-3-(2-nitrophenyl)-propenone (**6d**)

Buff flaks, Yield (55%), mp 132–133 °C; IR (KBr) νmax/cm-^{1}: 3005 (CH–Ar), 2979, 2885 (CH-sp^3), 1660 (C═O), 1600 (C═C), 1520, 1350 (NO_2); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.75 (s, 3H, CH_3), 7.16–7.43 (m, 4H, CH_{Ar}), 7.49 (d, *J* = 15.2 Hz, 1H, CH═), 7.59 (dd, *J* = 8, 4.8 Hz, 1H, Pyridine-H_5), 7.81 (d, *J* = 15.2 Hz, 1H, CH═), 8.49 (d, *J* = 4.8 Hz, 1H, Pyridine-H_4), 8.89 (d, *J* = 5 Hz, 1H, Pyridine-H_6), 9.18 (s, 1H, Pyridine-H_2); Anal. Calcd. for $C_{18}H_{13}N_3O_3S$ (351.39): C, 61.53; H, 3.73; N, 11.96%, Found: C, 61.51; H, 3.76; N, 11.98%.

4.2.5. 3-(2,4-Difluorophenyl)-1-(4-methyl-2-pyridin-3-yl-thiazol-5-yl)-propenone (**6e**)

Yellowish white powder, Yield (72%), mp 162–163 °C; IR (KBr) νmax/cm^{-1}: 3100, 3060 (CH–Ar), 2987, 2877 (CH-sp^3), 1652 (C═O), 1598 (C═C); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.78 (s, 3H, CH_3), 7.22–7.41 (m, 2H, CH_{Ar}), 7.49 (d, *J* = 15.5 Hz, 1H, CH═), 7.58 (s, 1H, CH_{Ar}), 7.71 (d, *J* = 15.5 Hz, 1H, CH═), 8.07 (m, 1H, Pyridine-H_5), 8.37 (d, *J* = 5 Hz, 1H, Pyridine-H_4), 8.73 (d, *J* = 4.8 Hz, 1H, Pyridine-H_6), 9.17 (s, 1H, Pyridine-H_2); ^{13}C NMR (DMSO-d_6): δ_{ppm} = 18.2 (CH_3), 104.5 (CH_{Ar}), 112.5 (CH_{Ar}), 118.7 (C_{Ar}), 124.3 (Thiazole-C_5), 126.3 (Pyridine-C_5), 128.1 (CH═), 131.5 (Pyridine-C_3), 131.7 (CH_{Ar}), 132.2 (Pyridine-C_4), 134.0 (Pyridine-C_2), 134.7 (CH═), 134.8 (Thiazole-C_4), 147.2 (Pyridine-C_6), 151.9 (CF_{Ar}), 159.4 (Thiazole-C_2), 165.8 (CF_{Ar}), 181.8 (C═O); MS *m/z* (%): 342 (M^+, 100), 341 ($M^+ - H$, 26.3), 325 (4.8), 306 (4.9), 238 (19.9), 229 (28.3), 218 (27.6), 203 (34.6), 175 (16.2), 167 (26.5), 139 (39.5), 119 (46.1), 105 (36.9), 97 (22.6), 71 (75.5); Anal. Calcd. for $C_{18}H_{12}F_2N_2OS$ (342.37): C, 63.15; H, 3.53; N, 8.18%, Found: C, 63.11; H, 3.50; N, 8.15%.

4.3. General procedure for the synthesis of 5-aryl-4,5-dihydro-1H-pyrazoles **7a**–**e**

A solution of the appropriate chalcones **6a**–**e** (0.3 mmol) and hydrazine hydrate (0.6 mmol) in ethanol (15 mL) was refluxed for 3 h. The reaction mixture was cooled and kept at 0 °C overnight. The resulting solid was collected by filtration and recrystallized from ethanol to give compounds **7a**–**e**.

4.3.1. 3-[4-Methyl-5-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-thiazol-2-yl]-pyridine (**7a**)

Canary yellow powder, Yield (68%), mp 163–164 °C; IR (KBr) νmax/cm^{-1}: 3215 (NH), 3037 (CH–Ar), 2976 (CH-sp^3), 1592 (C═N); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.58 (s, 3H, CH_3), 2.98–3.00 (m, 1H, Pyrazoline-H_4), 3.56–3.62 (m, 1H, Pyrazoline-H_4), 4.90 (dd, *J* = 12.0, 5.6 Hz, 1H, Pyrazoline-H_5), 7.21–7.41 (m, 5H, CH_{Ar}), 7.53 (s, 1H, NH), 7.82 (m, 1H, Pyridine-H_5), 8.27 (d, *J* = 5 Hz, 1H, Pyridine-H_4), 8.65 (d, *J* = 4.9 Hz, 1H, Pyridine-H_6), 9.09 (s, 1H, Pyridine-H_2); ^{13}C NMR (DMSO-d_6): δ_{ppm} = 16.9 (CH_3), 42.4 (Pyrazoline-C_4), 64.3 (Pyrazoline-C_5), 124.2 (Thiazole-C_5), 126.6 ($2CH_{Ar}$), 126.9 (Pyridine-C_5), 127.5 ($2CH_{Ar}$), 128.4 (Pyridine-C_3), 128.8 (CH_{Ar}), 133.2 (Pyridine-C4), 141.3 (C_{Ar}), 142.3 (Pyrazoline-C_3), 146.6 (Thiazole-C_4), 150.1 (Pyridine-C_2), 150.7 (Pyridine-C_6), 160.2 (Thiazole-C_2); Anal. Calcd. for $C_{18}H_{16}N_4S$ (320.42): C, 67.47; H, 5.03; N, 17.49%, Found: C, 67.45; H, 5.01; N, 17.52%.

4.3.2. 3-{5-[5-(4-Methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-methyl-thiazol-2-yl}-pyridine (**7b**)

Canary yellow powder, Yield (72%), mp 153–154 °C; IR (KBr) νmax/cm^{-1}: 3220 (NH), 3014 (CH–Ar), 2981 (CH-sp^3), 1589 (C═N); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.58 (s, 3H, CH_3), 3.04 (dd, *J* = 11.8, 5.0 Hz, 1H, Pyrazoline-H_4), 3.61 (dd, *J* = 7.5, 5.0 Hz, 1H, Pyrazoline-H_4), 3.92 (s, 3H, OCH_3), 4.96 (dd, *J* = 11.8, 5 Hz, 1H, Pyrazoline-H_5), 7.18 (d, *J* = 6.8 Hz, 2H, $2CH_{Ar}$), 7.56 (s, 1H, NH), 7.69 (d, *J* = 6.8 Hz, 2H, $2CH_{Ar}$), 7.86 (m, 1H, Pyridine-H_5), 8.32 (d, *J* = 4.9 Hz, 1H, Pyridine-H_4), 8.73 (d, *J* = 4.9 Hz, 1H, Pyridine-H_6), 9.21 (s, 1H, Pyridine-H_2); MS *m/z* (%): 350 (M^+, 20.7), 312 (62.0), 279 (72.4), 250 (100), 224 (59.2), 219 (64.9), 204 (60.9), 193 (90.8), 162 (60.9), 146 (64.9), 121 (14.9), 90 (72.9), 79 (70.1); Anal. Calcd. for $C_{19}H_{18}N_4OS$ (350.44): C, 65.12; H, 5.18; N, 15.99%, Found: C, 65.09; H, 5.14; N, 16.03%.

4.3.3. 3-{5-[5-(4-Chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-methyl-thiazol-2-yl}-pyridine (**7c**)

Canary yellow powder, Yield (62%), mp 192–193 °C; IR (KBr) νmax/cm^{-1}: 3225 (NH), 3045, 3030 (CH–Ar), 2920 (CH-sp^3), 1590 (C═N), 1565 (C═C); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.51 (s, 3H, CH_3), 2.96 (dd, *J* = 11, 5.4 Hz, 1H, Pyrazoline-H_4), 3.59 (dd, *J* = 11, 7.6 Hz, 1H, Pyrazoline-H_4), 4.93 (dd, *J* = 11, 5.4 Hz, 1H, Pyrazoline-H_5), 7.35–7.45 (m, 4H, CH_{Ar}), 7.50 (s, 1H, NH), 7.84 (d, *J* = 3.0 Hz, 1H, Pyridine-H_4), 8.25 (dd, *J* = 5, 3 Hz, 1H, Pyridine-H_5), 8.64 (d, *J* = 5 Hz, 1H, Pyridine-H_6), 9.09 (s, 1H, Pyridine-H_2); ^{13}C NMR (DMSO-d_6): δ_{ppm} = 16.9 (CH_3), 42.3 (Pyrazoline-C_4), 63.5 (Pyrazoline-C_5), 124.2 (Thiazole-C_5), 127.3 ($2CH_{Ar}$), 128.5 (Pyridine-C_5), 128.7 ($2CH_{Ar}$), 128.9 (C_{Ar}), 131.7 (Pyridine-C_3), 133.2 (Pyridine-C_4), 141.4 (C_{Ar}), 142.5 (Pyrazoline-C_3), 146.6 (Thiazole-C_4), 150.2 (Pyridine-C_2), 150.7 (Pyridine-C_6), 160.3 (Thiazole-C_2); MS *m/z* (%): 354 (M^+, 100), 339 (2.6), 323 (4.1), 319 (2.5), 290 (2.1), 243 (20.5), 226 (2.2), 215

(14.5), 202 (3.0), 177 (2.5), 138 (6.9), 105 (8.7), 89 (2.4), 71 (6.5), 59 (2.6); Anal. Calcd. for $C_{18}H_{15}ClN_4S$ (354.86): C, 60.92; H, 4.26; N, 15.79%, Found: C, 60.96; H, 4.28; N, 15.83%.

4.3.4. 3-{4-Methyl-5-[5-(2-nitrophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-thiazol-2-yl}-pyridine (**7d**)

Yellowish brown powder, Yield (60%), mp 123–124 °C; IR (KBr) νmax/cm^{-1}: 3218 (NH), 3040, 3015 (CH–Ar), 2976 (CH-sp^3), 1595 (C=N), 1528, 1357 (NO_2); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.62 (s, 3H, CH_3), 2.90 (dd, J = 11.4, 5.0 Hz, 1H, Pyrazoline-H_4), 3.52 (dd, J = 11.4, 7.2 Hz, 1H, Pyrazoline-H_4), 4.98 (dd, J = 11.4, 5.0 Hz, 1H, Pyrazoline-H_5), 7.28–7.49 (m, 4H, CH_{Ar}), 7.54 (s, 1H, NH), 7.81 (d, J = 4.8 Hz, 1H, Pyridine-H_4), 8.31 (dd, J = 4.8, 4.1 Hz, 1H, Pyridine-H_5), 8.69 (d, J = 4.8 Hz, 1H, Pyridine-H_6), 9.23 (s, 1H, Pyridine-H_2); Anal. Calcd. for $C_{18}H_{15}N_5O_2S$ (365.42): C, 59.17; H, 4.14; N, 19.17%, Found: C, 59.13; H, 4.12; N, 19.14%.

4.3.5. 3-{5-[5-(2,4-Difluorophenyl)-4,5-dihydro-1H-pyrazol-3-yl]-4-methyl-thiazol-2-yl}-pyridine (**7e**)

Canary yellow powder, Yield (65%), mp 125–126 °C; IR (KBr) νmax/cm^{-1}: 3200 (NH), 3050 (CH–Ar), 1590 (C=N); ^{1}H NMR (DMSO-d_6): δ_{ppm} = 2.51 (s, 3H, CH_3), 2.79 (dd, J = 12.1, 5.8 Hz, 1H, Pyrazoline-H_4), 3.25 (dd, J = 7.8, 5.8 Hz, 1H, Pyrazoline-H_4), 5.40 (dd, J = 12.1, 7.8 Hz, 1H, Pyrazoline-H_5), 6.90–7.60 (m, 4H, $3CH_{Ar}$ + NH), 8.30–8.45 (m, 1H, Pyridine-H_5), 8.27 (d, J = 5.8 Hz, 1H, Pyridine-H_4), 8.74 (d, J = 5.0 Hz, 1H, Pyridine-H_6), 9.14 (s, 1H, Pyridine-H_2); ^{13}C NMR (DMSO-d_6): δ_{ppm} = 18.3 (CH_3), 50.6 (Pyrazoline-C_4), 62.7 (Pyrazoline-C_5), 124.4 (Thiazole-C_5), 128.2 (CH_{Ar}), 129.4 (CH_{Ar}), 131.7 (CH_{Ar}), 132.2 (Pyridine-C_5), 133.8 (Pyridine-C_3), 134.2 (Pyridine-C_4), 134.9 (C_{Ar}), 146.2 (Pyrazoline-C_3), 146.9 (C_{Ar}), 147.1 (C_{Ar}), 150.2 (Thiazole-C_4), 151.5 (Pyridine-C_2), 151.7 (Pyridine-C_6), 159.4 (Thiazole-C_2); MS m/z (%): 356 (M^+, 16.0), 355 (M^+ – H, 25.6), 354 (100), 342 (68.8), 325 (4.8), 250 (1.5), 229 (17.6), 220 (17.7), 203 (17.8), 189 (4.2), 141 (18.6), 119 (28.2), 105 (26.9), 71 (30.7), 69 (12.2), 51 (8.2); Anal. Calcd. for $C_{18}H_{14}F_2N_4S$ (356.40): C, 60.66; H, 3.96; N, 15.72%, Found: C, 60.69; H, 3.98; N, 15.75%.

5. Antimicrobial evaluation

The discs of Whatman filter paper were prepared with standard size (6.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. These bottles are kept into hot air oven at a temperature of 150 °C. Then, the standard sterilized filter paper discs impregnated with a solution of the test compound in DMF (100 μl, 5 mg/mL) were placed on nutrient agar plate seeded with the appropriate test organism in triplicates. Standard concentrations of 10^6 CFU/mL (Colony Forming Units/mL) and 10^4 CFU/mL were used for antibacterial and antifungal assay, respectively. Pyrex glass Petri dishes (9 cm in diameter) were used and two discs of filter paper were inoculated in each plate. The utilized test organisms were *S. aureus*, *S. epidermidis*, *S. pyogenes*, *B. subtilis* and *E. faecalis* as examples of Gram-positive bacteria and *N. gonorrhoeae*, *P. vulgaris*, *K. pneumonia*, *S. flexneri* and *P. aeruginosa* as examples of Gram-negative bacteria. They were also evaluated for their *in vitro* antifungal potential against *A. fumigates*, *A. clavatus*, *C. albicans*, *G. candidum* and *P. marneffei* fungal strains. Ampicillin and gentamycin were used as standard antibacterial agents; while amphotricin B was used as standard antifungal agent. DMF alone was used as control at the same above-mentioned concentration and due this there was no visible change in bacterial growth. The plates were incubated at 37 °C for 24 h for bacteria and for 48 h at 25 °C for fungi. The mean zone of inhibition were measured in mm ± standard deviation on a range of environmental and clinically pathogenic microorganisms. Compounds that showed growth inhibition zones (>10 mm) using the twofold serial dilution technique, were further evaluated for their minimal inhibitory concentrations (MICs).

5.1. Minimal inhibitory concentration (MIC) measurement

The microdilution susceptibility test in Müller–Hinton Broth (Oxoid) and Subouraud Liquid Medium (Oxoid) was used for the determination of antibacterial and antifungal activity, respectively. Stock solutions of the tested compounds, ampicillin, gentamycin and amphotricin B were prepared in DMF at concentrations 1000 μg/mL. Each stock solution was diluted with standard method broth (Difco) to prepare serial twofold dilutions in the range of 500–0.007 μg/mL 10 mL of the broth containing about 10^6 CFU/mL of test bacteria was added to each well of 96-well microtiter plate. The sealed microplates were incubated at 37 °C for 24 h for antibacterial activity and at 25 °C for 48 h for antifungal activity in a humid chamber. At the end of the incubation period, the minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that inhibited the growth of the tested organisms judged by the absence of visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

Acknowledgments

The authors are thankful to the Deanship of Scientific Research at King Khalid University for the financial support of the project no. (KKU-SCII-12-092).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.12.050.

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