Saudi Pharmaceutical Journal (2015) **23**, 202–209

King Saud University

Saudi Pharmaceutical Journal

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Design, synthesis and antibacterial potential of 5-(benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-1-substituted-4,5-dihydropyrazoles

Mohammed F. El-Behairy [a], Tarek E. Mazeed [b,1], Aida A. El-Azzouny [a], Mohamed N. Aboul-Enein [a,*]

[a] *Medicinal and Pharmaceutical Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, 12622 Dokki, Giza, Egypt*
[b] *Chemistry of Natural and Microbial Products Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, 12622 Dokki, Giza, Egypt*

Received 1 June 2014; accepted 18 July 2014
Available online 28 July 2014

KEYWORDS

1,3-Benzodioxole;
2-Pyrazoline;
Antibacterial

Abstract A series of 5-(benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-1-substituted-4,5-dihydropyrazole derivatives **4a–e** and **6a–g** have been synthesized and spectrally characterized. The antibacterial activity of the novel candidates has been screened using the agar diffusion test. These compounds were endowed with high antibacterial activity against different Gram +ve and Gram −ve bacteria when compared with standard antibacterial drugs. In the light of zone of inhibition and MIC results, *Sarcina* and *Staphylococcus aureus* are the most sensitive bacteria where pyrrolidinomethanone derivative **4e** showed MICs at 80 and 110 nM, respectively. While hydroxypiperidinoethanone derivative **6c** showed MIC at 90 nM for *Sarcina*.

1. Introduction

Recently, after years of misuse and overuse of antibiotics, potential global health crisis has become very apparent due to remarkable increase in bacterial resistance (Kathiravan et al., 2012) which has been firmly correlated to higher rates of morbidity and mortality (Ozdemir et al., 2007). In order to overcome such resistance new antibacterial candidates have to be introduced that consist of chemical features which differ from those of the present drugs. Thus, these leads have to be novel nevertheless resemble known biologically active molecules by the presence of critical pharmacophoric structural moieties. This impetus led to innovative heterocyclic permutations with unique action

* Corresponding author. Tel.: +20 233335454.
E-mail addresses: mohabeha@gmail.com (M.F. El-Behairy), tarek_mazeed@yahoo.co.uk (T.E. Mazeed), elazzounyaida@yahoo.com (A.A. El-Azzouny), mnaboulenein@yahoo.com (M.N. Aboul-Enein).
[1] Present address: William G. Lowrie Department of Chemical and Bimolecular Engineering, The Ohio State University, Columbus, OH 43210, USA.

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2014.07.009

or diverse function. Also, it could be achievable *via* using small heterocyclic molecules which are highly functionalized scaffolds and are well known pharmacophores incorporated in a great number of molecules characterized by antibacterial activity.

During discovery of novel antibacterial agents, powerful activity has been displayed by natural molecules containing the 1,3-benzodioxole system for example protopine (Carradori et al., 2012) and egonol (Emirdag-Ozturk et al., 2011) and also by several chemically synthesized antimicrobial candidates such as compounds **I** and **II** (Chimenti et al., 2011; Wani et al., 2011; Secci et al., 2012) (Fig. 1).

2-Pyrazoline is a ubiquitous small heterocyclic pharmacophore in medicinal chemistry. Many pharmacological activities have been reported for various 2-pyrazolines such as hypoglycemic (Cottineau et al., 2002), analgesic (Carradori et al., 2012), anti-inflammatory (Chakrabarthi et al., 1987), antitumor (Rostom et al., 2003), antibacterial (Akbas and Berber, 2005), and antifungal activities (Akbas and Berber, 2005). In particular pyrazoline-methanones (e.g. compound **III**) and pyrazoline-ethanones (e.g. compound **IV**) have shown an intense antimicrobial potency against TB and several candida species (Mamolo et al., 2001, 2003) (Fig. 2).

On the other hand, alicyclic amines occur widely in drugs (Gorrod and Aislaitner, 1994). Many of these amines such as piperidine and morpholine fostered the antimicrobial activity of chemically synthesized candidates (Qing et al., 2010; Sangshetti et al., 2011). These amines are susceptible to metabolism giving rise to diverse end products. Noteworthy, one of the most common metabolic pathways of alicyclic amines is α-carbonyl formation leading to lactam structures (Gorrod and Aislaitner, 1994) which are well known pharmacophores in antibacterial agents (Fig. 3).

Thus it was of interest to implement the symbiotic approach in drug design (Baldwin et al., 1979; Christiaans and Timmerman, 1996) to synthesize novel candidates by joining the benzodioxolpyrazoline moiety with different alicyclic amines *via* methanone or ethanone linker in order to screen their antibacterial potentials.

2. Materials and methods

2.1. Chemistry

All melting points were determined using an Electrothermal Capillary melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded as thin film (for oils) in NaCl discs or as KBr pellets (for solids) with a JASCO

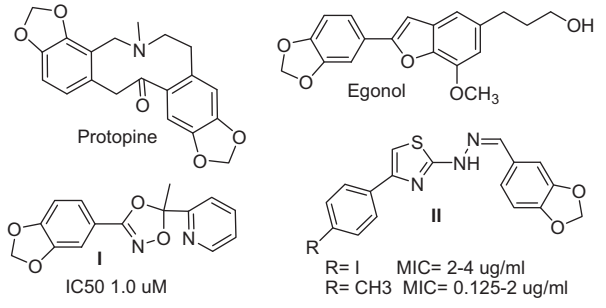


Figure 1 Antimicrobial candidates containing 1,3-benzodioxol system.

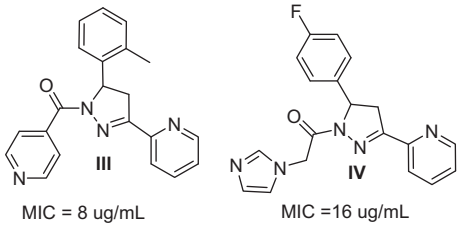


Figure 2 Antimicrobial candidates containing pyrazoline-methanone and ethanone scaffolds.

FT/IR-6100 Spectrometer (Japan) and values are represented in cm^{-1}. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Jeol ECA 500 MHz spectrometer (Japan) using TMS as internal standard and chemical shift values were recorded in ppm on the δ scale. Silica gel TLC (thin layer chromatography) cards from Merck (silica gel precoated aluminium cards with fluorescent indicator at 254 nm) were used for thin layer chromatography. Visualization was performed by illumination with UV light source (254 nm). Column chromatography was carried out on silica gel 60 (0.063–0.200 mm) obtained from Merck. The mobile phase consisted of chloroform or chloroform/ethyl acetate 1/1 v/v. Tetracycline, gentamicin and ofloxacin standard antibiotic discs were purchased from Bioanalyse®, Turkey.

2.1.1. Synthesis of 1,3-benzodioxole-5-carbaldehyde (*1*, Piperonal) (Aboul-Enein et al., 2012)

The Aboul-Enein method has been followed to afford light brown solid mp 37 °C.

2.1.2. Synthesis of (1E)-1-(1,3-benzodioxol-5-yl)-4,4-dimethylpent-1-en-3-one (*2*) (Aboul-Enein et al., 2012; Vallet, 1975)

The Aboul-Enein method has been followed to afford 13 g (78%) of **2** as pale yellow crystals mp 92 °C.

2.1.3. Synthesis of 5-(1,3-benzodioxol-5-yl)-3-tert-butyl-4,5-dihydro-1H-pyrazole (*3*) (Aboul-Enein et al., 2012)

Hydrazine hydrate (0.62 mL, 0.64 g, 0.012 mol) was added to a solution of **2** (1.0 g, 0.0043 mol) in absolute ethanol (30 mL). The reaction mixture was stirred under reflux for 2 h, and evaporated under vacuum to afford 1.0 g (90%) of **3** as yellowish oil.

^1H-NMR ($CDCl_3$): 1.0 (s, 9H, *t*-butyl), 2.3 (dd, J = 10.7, 16.1 Hz, 1H, $CHCH_2$), 2.9 (dd, J = 9.95, 16.1 Hz, 1H, $CHCH_2$), 4.5 (t, J = 9.9, 10.7 Hz, 1H, $CHCH_2$), 5.9 (s, 2H, O–CH_2–O), 6.7 (s, 1H, NH), 6.75 (dd, J = 1.5, 7.6 Hz, 1H, H-6), 6.7 (d, J = 8.4 Hz, 1H, H-7), 6.8 (s, 1H, H-4) ppm. ^{13}C NMR ($CDCl_3$) of **3** (base): 27.9 ($C(CH_3)_3$), 35.5 ($C(CH_3)_3$), 40.0 ($CHCH_2$), 60.0 ($CHCH_2$), 101 (OCH_2O), 107, 108, 120 (CH_{ar}), 137 (C_{ar}), 146, 147 (C_{ar}), 160 (C=N) ppm.

2.1.4. General procedures for synthesis of (5-(benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(heteroalicyclic)methanones (*4a-e*)

To a stirred solution of **3** (0.5 gm, 2 mmol) in 20 ml $CHCl_3$, phosgene (12.5% W/V solution in toluene, 1.6 ml, 2 mmol)

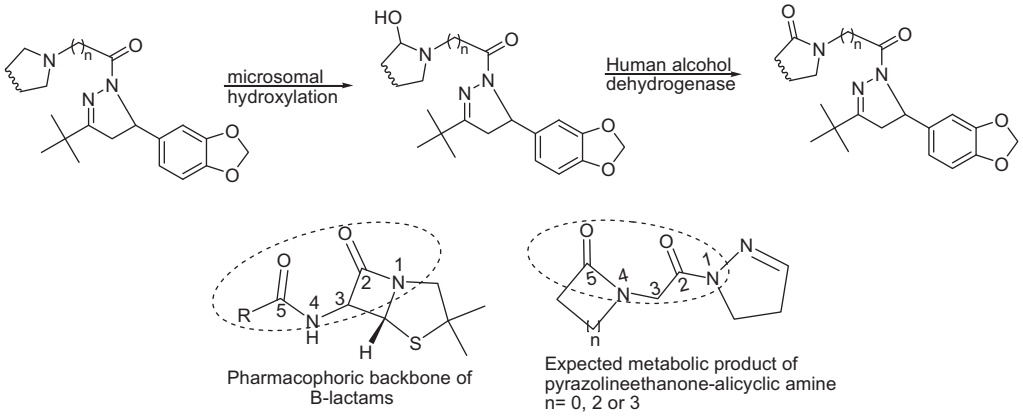


Figure 3 Pharmacophoric similarity between β-lactams and the expected metabolic products.

was added. The mixture was stirred at room temperature for 10 min followed by the addition of the appropriate amine (6 mmol, 3 mol equivalents). The reaction mixture was kept under stirring at room temperature overnight then washed with $NaHCO_3$ (10%, 20 ml), water and the organic layer was separated, dried (Na_2SO_4), and evaporated under vacuum to afford the corresponding products **4a**–**e**. Solid products were further purified by recrystallization from ethanol while oils by column chromatography.

*2.1.4.1. (5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(morpholino) methanone (**4a**).* Brown solid, mp 138–140 °C; yield 77%. IR (KBr, cm^{-1}): 1658 (C═O), 1493 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.1 (s, 9H, *t*-butyl), 2.55 (dd, J = 9.2, 17.6 Hz, 1H, CHCH_2), 3.15 (dd, J = 6.1, 17.55 Hz, 1H, CHCH_2), 3.65 (m, 8H, H for morpholine), 5.2 (t, J = 11.45, 2.4 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH$_2$–O), 6.67–6.7 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 28 (C(CH_3)$_3$), 34 (C(CH$_3$)$_3$), 41 (CHCH$_2$), 46 (N-(CH$_2$)$_2$ for morpholine), 62 (CHCH$_2$), 67 (O-(CH$_2$)$_2$ for morpholine), 101 (O–CH$_2$–O), 106, 108, 119, 136, 146, 148 (CH_{ar}, C_{ar}), 157 (C═O), 162 (C═N) ppm. MS: for $C_{19}H_{25}N_3O_4$, calcd. 359.42 ($M^+ + 1$), found 360.24.

*2.1.4.2. (5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(piperidin-1-yl)methanone (**4b**).* Yellow oil, yield 80%, IR (thin film, cm^{-1}): 1647 (C═O), 1443 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (s, 9H, *t*-butyl), 1.5 (m, 6H, H for piperidine), 2.52 (dd, J = 9.95, 17.55 Hz, 1H, CHCH_2), 3.13 (dd, J = 10.7, 16.8 Hz, 1H, CHCH_2), 3.45 (m, 4H, N-(CH$_2$)$_2$ for piperidine), 5.2 (t, J = 9.95, 10.7 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH$_2$–O), 6.67–6.7 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 24.8, 26, 28 (C(CH_3)$_3$), 33 (C(CH$_3$)$_3$), 41 (CHCH$_2$), 47 (N-(CH$_2$)$_2$ for piperidine), 62 (CHCH$_2$), 101 (O–CH$_2$–O), 106, 108, 119, 137, 146, 148 (CH_{ar}, C_{ar}), 157 (C═O), 161 (C═N) ppm. MS: for $C_{20}H_{27}N_3O_3$, calcd. 357.45 ($M^+ + 1$), found 358.20.

*2.1.4.3. 5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(4-hydroxy piperidin-1-yl)methanone (**4c**).* Pale yellow solid, mp 156–158 °C; yield 95%. IR (KBr, cm^{-1}): 3399 (OH), 1637 (C═O), 1447 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.1 (s, 9H, *t*-butyl), 1.5 (m, 2H, H for piperidine), 1.8 (m, 2H, H for piperidine), 2.55 (dd, J = 9.2, 17.6 Hz, 1H, CHCH_2), 2.9 (s, 1H, OH), 3.0 (m, 1H, H for piperidine), 3.15 (m, 1H, CHCH_2), 3.6 (m, 1H, N-(CH$_2$)$_2$ for piperidine), 3.75 (m, 1H, N-(CH$_2$)$_2$ for piperidine), 3.95 (m, 1H, N-(CH$_2$)$_2$ for piperidine), 4.0 (m, 1H, N-(CH$_2$)$_2$ for piperidine), 5.2 (t, J = 9.2, 1.5 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH$_2$–O), 6.67–6.72 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 28 (C(CH_3)$_3$), 33, 34, 41 (C(CH$_3$)$_3$), 47 (N-(CH$_2$)$_2$ for piperidine), 62, 67 (CHOH), 101 (O–CH$_2$–O), 106, 108, 119, 136, 146, 148 (CH_{ar}, C_{ar}), 157 (C═O), 162 (C═N) ppm. MS: for $C_{20}H_{27}N_3O_4$, calcd. 373.45 ($M^+ + 1$), found 374.26.

*2.1.4.4. (5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(4-ethyl piperazin-1-yl)methanone (**4d**).* Yellow oil, yield 90%; IR (thin film, cm^{-1}): 1648 (C═O), 1455 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (t, J = 6.9, 6.85 Hz, 3H, CH_3CH$_2$) 1.1 (s, 9H, *t*-butyl), 2.32–2.45 (m, 6H, CH$_3$CH_2 & H for piperazine), 2.55 (dd, J = 5.4, 17.6 Hz, 1H, CHCH_2), 3.15 (dd, J = 5.35, 16.8 Hz, 1H, CHCH_2), 3.7 (t, J = 6.9, 5.53 Hz, 4H, H for piperazine), 5.2 (t, J = 11.45, 9.2 Hz, 1H, CHCH$_2$), 5.9 (s, 2H, O–CH$_2$–O), 6.7–6.8 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 12 (CH$_3$), 28 (C(CH$_3$)$_3$), 34 (C(CH$_3$)$_3$), 41 (CHCH$_2$), 45 (CH$_2$), 52 (C for piperazine), 53 (C for piperazine), 62 (CHCH$_2$), 101 (O–CH$_2$–O), 106, 108, 120, 137, 147, 148 (CH_{ar}, C_{ar}), 157 (C═O), 162 (C═N) ppm. MS: for $C_{21}H_{30}N_4O_3$, calcd. 386.49 ($M^+ + 1$), found 387.24.

*2.1.4.5. (5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(pyrrolidin-1-yl)methanone (**4e**).* Yellowish white solid, mp 160–162 °C; yield 95%. IR (KBr, cm^{-1}): 1641 (amide, C═O), 1503 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (s, 9H, *t*-butyl), 1.7 (m, 4H, H for pyrrolidine), 2.4 (m, 1H, CHCH_2), 3.1 (m, 1H, CHCH_2), 3.4 (m, 4H, H for pyrrolidine), 5.1 (m, 1H, CHCH$_2$), 5.7 (s, 2H, O–CH$_2$–O), 6.6 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 24, 28 (C(CH$_3$)$_3$), 33 (CHCH$_2$), 40 (C(CH$_3$)$_3$), 48 (N-(CH$_2$)$_2$) for pyrrolidine, 61 (CHCH$_2$), 101 (O–CH$_2$–O), 106, 108, 119, 137, 146, 147 (CH_{ar}, C_{ar}), 156 (C═O, amide), 161 (C═N) ppm. MS: for $C_{19}H_{25}N_3O_3$, calcd. 343.42 ($M^+ + 1$), found 344.29.

*2.1.5. Synthesis of 1-(5-(benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-chloroethanone (**5**)*

To a stirred solution of **3** (0.5 g, 2 mmol) in 20 ml $CHCl_3$, chloroacetyl chloride (0.16 ml, 0.23gm, 2 mmol) was added. The

mixture was stirred at room temperature for 30 min then the reaction mixture was washed with $NaHCO_3$ (10%, 20 ml) and the organic layer was separated, dried (Na_2SO_4), and evaporated under vacuum to afford crude **5**. Crystallization from ethanol afforded 0.6 g (100%) of yellowish white solid mp 172 °C.

^{1}H NMR ($CDCl_3$): δ 1.1 (s, 9H, *t*-butyl), 2.7 (dd, J = 4, 17.75 Hz, 1H, CHCH_2), 3.3 (dd, J = 11.45, 18.35 Hz, 1H, CHCH_2),4.4 (s, 2H, CH_2Cl), 5.3 (dd, J = 4.6, 16.05 Hz, 1H, CHCH_2), 5.8 (s, 2H, O–CH_2–O), 6.5–6.8 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 28 (C(CH_3)$_3$), 34 (CHCH_2), 41 (C(CH_3)$_3$), 42 (CH_2Cl), 60 ($CHCH_2$), 101 (O–CH_2–O), 105, 108, 119, 135, 147, 148 (CH_{ar}, C_{ar}), 163 (C═O, amide), 167 (C═N) ppm.

*2.1.6. General procedures for synthesis of (5-(benzo[d][1,3] dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(heteroalicyclic)ethanones (**6a–c** and **6g**)*

To a stirred solution of **5** (0.5 gm, 1.5 mmol) in 30 ml ethanol, the appropriate alicyclic amine (4.5 mmol) was added and the mixture was stirred under reflux overnight. The solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate, washed with $NaHCO_3$ (10%, 20 ml) then water, dried (Na_2SO_4), and evaporated under vacuum to afford the corresponding products **6a–g**. Solid products were further purified by recrystallization from ethanol while oils by column chromatography (silica gel 60 (0.063–0.200 mm) mobile phase: chloroform 100% or chloroform/ethyl acetate 1/1 v/v).

*2.1.6.1. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-morpholinoethanone (**6a**).* Yellowish white solid, mp 136 °C; yield 87%; IR (KBr, cm^{-1}): 1658 (C═O), 1492 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.1 (s, 9H, *t*-butyl), 2.5 (m, 4H, N-(CH_2)$_2$ for morpholine), 2.7 (m, 1H, CHCH_2), 3.27 (m, 1H, CHCH_2), 3.5 (m, 2H, COCH_2N), 3.7 (m, 4H, O-(CH_2)$_2$ for morpholine), 5.2 (dd, J = 3.85, 11.5 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH_2–O), 6.5–6.7 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 28 (C(CH_3)$_3$), 34 (CHCH_2), 41 (C(CH_3)$_3$), 43 (COCH_2N), 54 (N-(CH_2)$_2$ for morpholine), 59 ($CHCH_2$), 66 (O-(CH_2)$_2$ for morpholine), 101 (O–CH_2–O), 105, 108, 119, 136, 146, 148 (CH_{ar}, C_{ar}), 166 (C═O), 167 (C═N) ppm. MS: for $C_{20}H_{27}N_3O_4$, calcd. 373.2 (M + 1), found 374.3.

*2.1.6.2. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-(piperidin-1-yl)ethanone (**6b**).* Yellowish white solid, mp 108–110 °C; yield 79%; IR (KBr, cm^{-1}): 1668 (C═O), 1502 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.1 (s, 9H, *t*-butyl), 1.3–1.5 (m, 6H, H for piperidine), 2.5 (m, 4H, N-(CH_2)$_2$ for piperidine), 2.66 (m, 1H, CHCH_2), 3.25 (m, 1H, CHCH_2), 3.5 (m, 2H, COCH_2N), 5.2 (m, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH_2–O), 6.4–6.7 (m, 3H, H aromatic) ppm.^{13}C NMR ($CDCl_3$): δ 24, 26, 28 (C(CH_3)$_3$), 33 (CHCH_2), 41 (C(CH_3)$_3$), 54 (N-(CH_2)$_2$ for piperidine), 59 (COCH_2N), 60 ($CHCH_2$), 101 (O–CH_2–O), 105, 108, 118, 136, 146, 147 (CH_{ar}, C_{ar}), 165 (C═O), 167 (C═N) ppm. MS: for $C_{20}H_{28}N_4O_3$, calcd. 372.46 (M^+ + 1), found 373.24.

*2.1.6.3. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-(4-hydroxypiperidin-1-yl)ethanone (**6c**).* Yellowish liquid; yield 80%; IR (thin film, cm^{-1}): 3406 (OH), 1651 (C═O), 1453 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (s, 9H, *t*-butyl), 1.45 (d, J = 9.2 Hz, 2H, H for piperidine), 1.6 (s, 2H, H for piperidine), 2.1 (d, J = 9.9 Hz, 2H, N-(CH_2)$_2$ for piperidine), 2.55 (d, J = 18.35 Hz, 1H, CHCH_2), 2.7 (d, J = 12.25 Hz, 2H, N-(CH_2)$_2$ for piperidine), 3.1 (m, 1H, CHCH_2), 3.4–3.6 (m, 4H,CHOH, COCH_2N), 5.1 (d, J = 11.45 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH_2–O), 6.4–6.6 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 28 (C(CH_3)$_3$), 34 ((CH_2)$_2$CHOH for piperidine), 34.1 (CHCH_2), 41 (C(CH_3)$_3$), 51 (N-(CH_2)$_2$ for piperidine), 58 (COCH_2N),59 ($CHCH_2$), 67 (CHOH), 101 (O–CH_2–O), 105, 108, 118, 136, 146, 147 (CH_{ar}, C_{ar}), 166 (C═O), 167 (C═N) ppm. MS: for $C_{21}H_{29}N_3O_4$, calcd. 387.22, (M + 1), found 388.32.

*2.1.6.4. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-(piperazin-1-yl)ethanone (**6g**).* Yellowish oil; yield 87%; IR (thin film, cm^{-1}): 3310 (NH), 1661 (C═O), 1484 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (s, 9H, *t*-butyl), 2.3–2.9 (m, 10H, 1H for CHCH_2 and 8H for piperazine, NH), 3.1 (m, 1H, CHCH_2), 3.4 (m, 2H, COCH_2N), 5.2 (m, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH_2–O), 6.4–6.6 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 28 (C(CH_3)$_3$), 34 (CHCH_2), 41 (C(CH_3)$_3$), 45 (C for piperazine), 54 (C for piperazine), 59.5 (COCH_2N), 59.6 ($CHCH_2$), 101 (O–CH_2–O), 105, 108, 118, 136, 146, 148 (CH_{ar}, C_{ar}), 165 (C═O), 167 (C═N) ppm. MS: for $C_{20}H_{28}N_4O_3$, calcd. 372.22 (M + 1), found 373.25.

*2.1.7. General procedures for synthesis of (5-(benzo[d][1,3] dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)(heteroalicyclic)ethanones (**6d–f**)*

To a stirred solution of **5** (0.5 g, 1.5 mmol) in 30 ml ethanol, the appropriate alicyclic amine (4.5 mmol) was added followed by NaOH (0.06 g, 1.5 mmol). The mixture was stirred under reflux overnight then the solvent was evaporated under reduced pressure and the residue was dissolved in ethyl acetate, washed with $NaHCO_3$ (10%, 20 ml) then water, dried (Na_2SO_4), and evaporated under vacuum to afford the corresponding products **6d–f** which were further purified by recrystallization from ethanol.

*2.1.7.1. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-(4-ethyl piperazin-1-yl)ethanone (**6d**).* Yellow solid, mp 118–120 °C; yield 65%, IR (KBr, cm^{-1}): 1675 (C═O), 1485 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (m, 12H, CH_3-CH_2, *t*-butyl), 2.3–3.6 (m, 11H, CHCH_2, CH_3CH_2 & 8H for piperazine), 4.3 (m, 2H, COCH_2N), 5.3 (dd, J = 3.8, 11.45 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH_2–O), 6.5–6.7 (m, 3H, H aromatic) ppm.^{13}C NMR ($CDCl_3$): δ 15 (CH_3), 28 (C(CH_3)$_3$), 34 (CHCH_2), 41 (C(CH_3)$_3$), 52 (CH_2), 53 (C for piperazine), 59 (C for piperazine), 67 (COCH_2N), 68 ($CHCH_2$), 101 (O–CH_2–O), 105, 108, 119, 135, 147, 148 (CH_{ar}, C_{ar}), 166 (C═O), 167 (C═N) ppm. MS: for $C_{22}H_{32}N_4O_3$, calcd. 400.25 (M^+), found 400.25.

*2.1.7.2. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-(pyrrolidin-1-yl)ethanone (**6e**).* Yellowish white solid, mp 134–136 °C; yield 81%. IR (KBr, cm^{-1}): 1670 (amide, C═O), 1485 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.0 (s, 9H, *t*-butyl), 1.7 (m, 4H, H for pyrrolidine), 2.65 (m, 5H, CHCH_2, (N-(CH_2)$_2$) for pyrrolidine), 3.2 (dd, J = 11.45, 17.55 Hz, 1H, CHCH_2), 3.6 (dd, J = 16.8, 16.8 Hz, 2H, COCH_2N), 5.3 (dd, J = 3.85, 11.5 Hz, 1H, CHCH$_2$), 5.9 (s,

2H, O–CH_2–O), 6.5–6.7 (m, 3H, H aromatic) ppm.^{13}C NMR ($CDCl_3$): δ 23, 28 (C(CH_3)$_3$), 34 (CHCH_2), 41 (C(CH_3)$_3$), 54 (N-(CH_2)$_2$) for pyrrolidine), 57 (COCH_2N), 59 ($CHCH_2$), 101 (O–CH_2–O), 105, 108, 119, 136, 146, 148 (CH_{ar}, C_{ar}), 165 (C═O), 168 (C═N) ppm. MS: for $C_{20}H_{27}N_3O_3$, calcd. 357.21 (M^+), found 357.24.

*2.1.7.3. 1-(5-(Benzo[d][1,3]dioxol-5-yl)-3-tert-butyl-4,5-dihydropyrazol-1-yl)-2-(pyrrolidin-2-one-1-yl)ethanone (**6f**).* Yellowish white solid, mp 114 °C; yield 65%; IR (KBr, cm^{-1}): 1681 (ketone C═O), 1617 (amide, C═O), 1501 (C═N). ^{1}H NMR ($CDCl_3$): δ 1.1 (s, 9H, *t*-butyl), 2.1–2.3 (m, 2H, CHCH_2), 2.7 (m, 2H, H for pyrrolidin-2-one), 3.25–3.37 (m, 2H, H for pyrrolidin-2-one), 3.5 (m, 2H, H for pyrrolidin-2-one), 4.4 (dd, J = 16.05, 16.05 Hz, 2H, COCH_2N), 5.3 (dd, J = 3.8, 11.45 Hz, 1H, CHCH$_2$), 5.8 (s, 2H, O–CH_2–O), 6.5–6.7 (m, 3H, H aromatic) ppm. ^{13}C NMR ($CDCl_3$): δ 15, 28 (C(CH_3)$_3$), 30 (CH_2CO for pyrrolidin-2-one), 34 (CHCH_2), 41 (C(CH_3)$_3$), 59 (CH_2N for pyrrolidin-2-one), 67 (COCH_2N), 68 ($CHCH_2$), 101 (O–CH_2–O), 105, 108, 119, 135, 147, 148 (CH_{ar}, C_{ar}), 166 (C═O, amide), 167 (C═N), 179 (C═O, ketone) ppm. MS: for $C_{20}H_{25}N_3O_4$, calcd. 371.18 (M^+), found 371.25.

2.2. Antibacterial assay

Antibacterial activities of compounds **4a–e** and **6a–g** were tested using the agar well diffusion method. The concentration of the microbial suspensions was adjusted to 0.5 McFarland standards. The bacterial suspensions were seeded on nutrient agar plates. In each of these plates many wells were cut using a sterilize cork borer. Using a micropipette, 200 µL of sample was added into different wells. A positive control antibiotic disc was placed in the plate. Bacterial plates were incubated for 24 h at 37 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition.

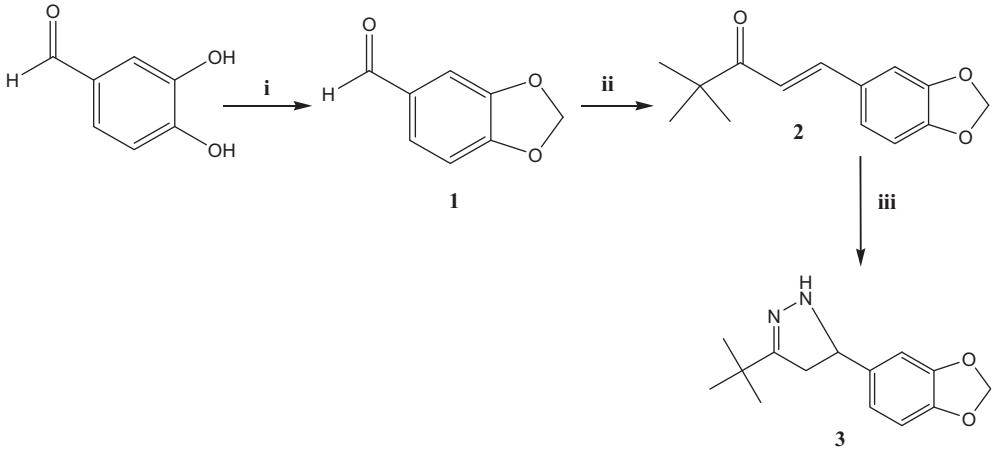
3. Results and discussion

3.1. Chemistry

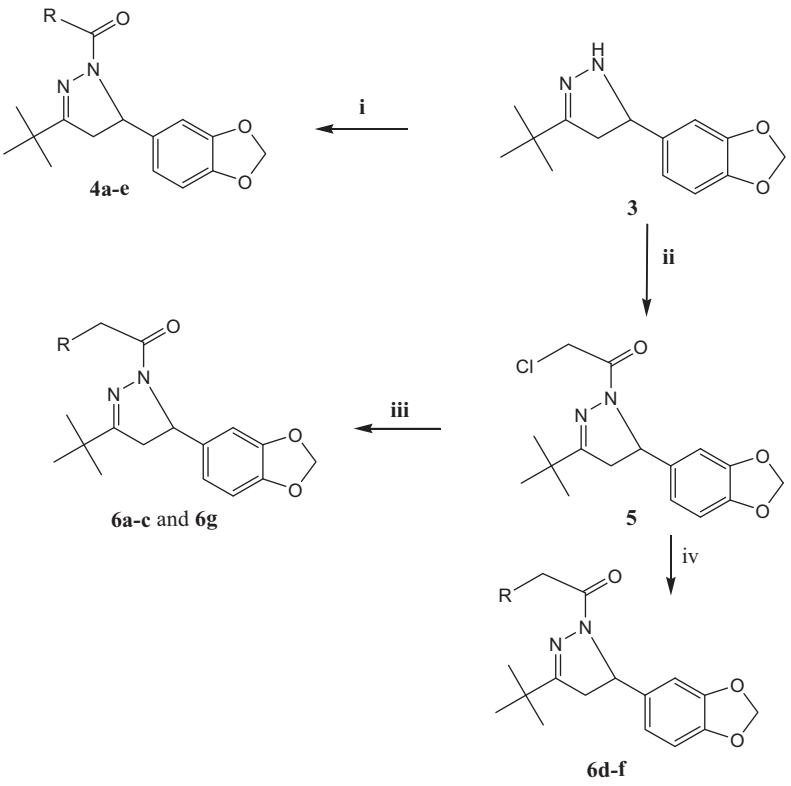
The synthesis of the target compounds **4a–e** and **6a–g**, and their intermediates **1–3** and **5** is depicted in Schemes 1 and 2. Piperonal (**1**) (Aboul-Enein et al., 2012; Nakatani et al., 1977) was obtained by reacting 3,4-dihydroxybenzaldehyde with CH_2Cl_2 in the presence of K_2CO_3 under reflux conditions. Subsequent Claisen–Schmidt condensation with pinacolone afforded α,β-unsaturated ketone **2** in good yield. Chalcone **2** was reacted with hydrazine hydrate in ethanol under reflux conditions to yield pyrazoline derivative **3** (Aboul-Enein et al., 2012) (Scheme 1).

Key pyrazoline **3** was further reacted with phosgene at room temperature to afford the carbamoyl chloride intermediate which was – in the same pot – reacted with the appropriate amine to afford pyrazolinemethanones **4a–e** (Scheme 2). On the contrary, the intermediate pyrazoline chloroethanone **5** was stable enough to be isolated after the reaction of pyrazoline **3** with chloroacetyl chloride in $CHCl_3$ at room temperature. Further reaction of **5** with different alicyclic amines in ethanol under reflux gave the target compounds **6a–c** and **6g**. For compounds **6d–f** the reaction was performed in the presence of 1 mol equivalent of NaOH (Scheme 2).

The spectral data of the synthesized compounds were investigated. The ^{1}H NMR spectra of α,β-unsaturated ketone **2** showed the olefinic protons as doublet of doublet at δ 6.1 and doublet at δ 6.5 with coupling constant J = 15.3 which indicates trans geometric isomerism and it was in accordance with the assigned structure and the reported data (Aboul-Enein et al., 2012). For pyrazoline **3**, ^{1}H NMR spectra showed two doublet of doublet signals at 2.3 and 2.9 ppm corresponding to the prochiral CH_2 protons while CH proton responded as triplet signal at 4.5 ppm. One singlet signal at 6.7 ppm was found corresponding to the NH proton at position 1 of the pyrazoline ring. In addition, singlet signals were found at 1.0 and 5.9 ppm for *t*-butyl and the ethereal methylene bridge protons, respectively. The aromatic protons were elucidated at 6.4–6.8 ppm. ^{1}H NMR spectra of pyrazolinechloroethanone **5** revealed the characteristic signals of **3** in addition to the singlet signal corresponding to the methylene protons at δ 4.4. However in the final products **6f** and **6e**, methylene protons were magnetically un-equivalent and displayed as doublet of doublet signals at δ 4.4 for **6f** and δ 3.6 for **6e** with identical coupling constant. For ^{13}C NMR, the urea carbonyl of **4a–e** was elucidated at 156–157 ppm while the amidic carbonyl of **6a–g** was elucidated at 165–167 ppm.



Scheme 1 Reagents and conditions: (i) CH_2Cl_2, K_2CO_3, DMF, reflux, 4 h; (ii) pinacolone 50% KOH, CH_3OH, 70 °C, 5 h; (iii) H_2N–NH_2·H_2O, ethanol, reflux, 2 h;



i) ClCOCl/ $CHCl_3$/ 10 min/rt then appropriate amine; ii) $ClCOCH_2Cl$, $CHCl_3$, rt, 30 min; iii) appropriate amine, absolute ethanol, reflux, 18 h; iv) appropriate amine, absolute ethanol, NaOH, reflux, 18 h

	R
a	morpholine
b	piperidine
c	4-hydroxypiperidine
d	4-ethylpiperazine
e	pyrrolidine
f	pyrrolidin-2-one
g	piperazine

Scheme 2

Table 1 Antibacterial activity of compounds **4a**–**e** and **6a**–**g**.

Microorganism	Zone of inhibition (mm)						
	Gram +ve				Gram −ve		
	Bacillus subtilis	*Staphylococcus aureus*	*Enterococcus faecalis*	*Sarcina*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Enterobacter cloacae*
Test compounds (μM)							
4a 2.7	24	25	18	14	20	18	20
4b 2.8	25	17	20	16	19	21	20
4c 2.6	16	19	17	+/−	17	14	13
4d 2.5	15	24	17	15	12	16	13
4e 2.9	29	25	28	20	15	25	28
6a 2.6	15	19	14	16	18	20	15
6b 2.6	15	23	21	23	14	+/−	20
6c 2.5	17	12	11	17	20	+/−	17
6d 2.5	18	18	17	14	15	18	21
6e 2.8	15	19	19	22	20	15	19
6f 2.6	20	17	19	15	17	12	21
6g 2.6	16	19	17	17	17	15	20
Gentamicin 0.021	20	25	20	22	11	18	28
Tetracycline 0.067	30	25	25	28	9	35	18
Ofloxacin 0.013	16	30	16	19	10	25	30
DMSO	–	–	–	–	–	–	–

(+/−) = unclear zone of inhibition.

Table 2 MICs* for the most potent compounds (**4a**, **4b**, **4e**, **6c** and **6e**) against certain pathogenic bacteria.

Compound	*Staphylococcus aureus* (μM)	*Klebsiella pneumonia* (μM)	*Pseudomonas aeruginosa* (μM)	*Sarcina* (μM)
4a	0.7	0.55	0.9	0.11
4b	0.14	0.35	0.35	0.14
4e	0.11	0.48	0.96	0.09
6c	0.12	0.51	0.64	0.08
6e	0.18	0.46	0.92	0.11

* The minimum concentration of a compound that inhibits the growth of tested microorganisms.

3.2. Antibacterial activity

Antibacterial activities of compounds **4a–e** and **6a–g** were tested using the agar well diffusion method (Tagg et al., 1976). Tested microorganism strains are: *Bacillus subtilis* (ATCC 8037), *Staphylococcus aureus* (ATCC 29213), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27953), *Enterobacter cloacae* (ATCC), *Enterococcus faecalis* (ATCC 29212), and *Sarcina* sp. (NRC isolate). Tetracycline (0.067 μM) (Aghatabay et al., 2009), gentamicin (0.021 μM) (Al-Abbas, 2012), and ofloxacin (0.013 μM) (Aghatabay et al., 2009) were used as positive control and DMSO as negative control. The observed data on the antibacterial activity of the compounds and control drugs are given in Tables 1 and 2.

Broad spectrum activities of the newly synthesized candidates (**4a–e** and **6a–g**) have been revealed being active against all tested human bacteria (*B. subtilis*, *S. aureus*, *K. pneumonia*, *P. aeruginosa*, *E. cloacae*, *E. faecalis*, and *Sarcina* sp.) in micromolar concentrations. In the light of zone of inhibition (Table 1) particular candidates have been selected for the estimation of MICs against pathogenic bacteria. In general, MICs were in the micromolar range while the most active candidates showed MICs in nanomolar range (80 and 90 nM by compounds **6c** and **4e** against *Sarcina*, respectively) which reflect the ingenuity of the novel candidates (Table 2).

It was found that *Sarcina* sp. is the most susceptible bacteria to our candidates which showed MIC range 0.08–0.14 μM. The ethanone derivative **6c** with R as the hydroxypiperidine is the most potent inhibitor for *Sarcina* at 0.08 μM followed by **4e** (pyrrolidinomethanone) at 0.09 μM and **4a** (morpholinomethanone), **6e** (pyrrolidinoethanone) at 0.11 μM. As well, very good activity against *S. aureus* has been discovered. The maximum activity against *S. aureus* was displayed by **4e** (pyrrolidinomethanone) at 0.11 μM and **6c** (hydroxypiperidinoethanone) at 0.12 μM. For both *K. pneumonia* and *P. aeruginosa*, compound **4b** (piperidinomethanone) was the most active candidate with MIC at 0.35 μM.

3.3. Conclusion

Broad spectrum antibacterial derivatives of 5-(1,3-benzodioxol)-4,5-dihydropyrazol-1-yl methanones **4a–e** and ethanones **6a–g** have been revealed. The novel candidates have shown activity against 7 Gram +ve and Gram −ve bacteria. Particular potency has been discovered against *Sarcina* sp. and *S. aureus* by compounds **4e** and **6c** at nanomolar concentrations.

Conflict of Interest

None.

Acknowledgements

The authors would like to thank the National Research Centre, Dokki, Cairo, Egypt for the support and financing the research through project No. 10010302.

References

Aboul-Enein, M.N., El-Azzouny, A.A., Attia, M.I., Maklad, Y.A., Amin, K.M., Abdel-Rehim, M., El-Behairy, M.F., 2012. Design and synthesis of novel stiripentol analogues as potential anticonvulsants. Eur. J. Med. Chem. 47, 360–369.

Aghatabay, N.M., Mahmiani, Y., Cevik, H., Dulger, B., 2009. Synthesis, Raman, FT-IR, NMR spectroscopic data and antimicrobial activity of mixed aza-oxo-thia macrocyclic compounds. Eur. J. Med. Chem. 44, 365–372.

Akbas, E., Berber, I., 2005. Antibacterial and antifungal activities of new pyrazolo[3,4-d]pyridazin derivatives. Eur. J. Med. Chem. 40, 401–405.

Al-Abbas, M.J.A., 2012. Antimicrobial susceptibility of *Enterococcus faecalis* and a novel *Planomicrobium* isolate of bacteremia. Int. J. Med. Med. Sci. 4, 19–27.

Baldwin, J.J., Lumma, W.C., Lundell, G.F., Ponticello, G.S., Raab, A.W., Engelhardt, E.L., Hirschmann, R., Sweet, C.S., Scriabine, A., 1979. Symbiotic approach to drug design: antihypertensive beta-adrenergic blocking agents. J. Med. Chem. 22, 1284–1290.

Carradori, S., Secci, D., Bolasco, A., De Monte, C., Yáñez, M., 2012. Synthesis and selective inhibitory activity against human COX-1 of novel 1-(4-substituted-thiazol-2-yl)-3,5-di(hetero)aryl-pyrazoline derivatives. Arch Pharm (Weinheim) 345, 973–979.

Chakrabarthi, K.J.E., Richard, J.G., Peter, T., Janette, H., Terrence, H.A., 1987. 5-Acyl-3-substituted benzofuran-2(3H)-ones as potential antiinflammatory agents. J. Med. Chem. 30, 1663–1668.

Chimenti, F., Bizzarri, B., Bolasco, A., Secci, D., Chimenti, P., Granese, A., Carradori, S., D'Ascenzio, M., Lilli, D., Rivanera, D., 2011. Synthesis and biological evaluation of novel 2,4-disubstituted-1,3-thiazoles as anti-Candida spp. agents. Eur. J. Med. Chem. 46, 378–382.

Christiaans, J.A.M., Timmerman, H., 1996. Cardiovascular hybrid drug: combination of more than one pharmacological property in one single molecule. Eur. J. Pharm. Sci. 4, 1–22.

Cottineau, B., Toto, P., Marot, C., Pipaud, A., Chenault, J., 2002. Synthesis and hypoglycemic evaluation of substituted pyrazole-4-carboxylic acids. Bioorg. Med. Chem. Lett. 12, 2105–2108.

Emirdag-Ozturk, S., Karayildirim, T., Anil, H., 2011. Synthesis of egonol derivatives and their antimicrobial activities. Bioorg. Med. Chem. 19, 1179–1188.

Gorrod, l.W., Aislaitner, G., 1994. The metabolism of alicyclic amines to reactive iminium ion intermediates. Eur. J. Drug Metab. Pharmacokinet. 19, 209–217.

Kathiravan, M.K., Salake, A.B., Chothe, A.S., Dudhe, P.B., Watode, R.P., Mukta, M.S., Gadhwe, S., 2012. The biology and chemistry of antifungal agents. Bioorg. Med. Chem. 20, 5678–5698.

Mamolo, M.G., Zampieri, D., Falagiani, V., Vio, L., Banfi, E., 2001. Synthesis and antimycobacterial activity of 5-aryl-1-isonicotinoyl-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazole derivatives. Il Farmaco 56, 593–599.

Mamolo, M.G., Zampieri, D., Falagiani, V., Vio, L., Banfi, E., 2003. Synthesis and antifungal activity of (9/)-1-(5-aryl-3-pyridin-2-yl-4,5-dihydro-pyrazol-1-yl)-2-imidazol-1-yl-ethanone derivatives. Il Farmaco 58, 315–322.

Nakatani, K., Inoue, T., Nishizawa, T., Numata, S., Ishii, T., 1977. Process for preparing piperonal. US 4157333.

Ozdemir, A., Turan-Zitouni, G., Kaplancıklı, Z.A., Revial, G., Güven, K., 2007. Synthesis and antimicrobial activity of 1-(4-aryl-2-thiazolyl)-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives. Eur. J. Med. Chem. 42, 403–409.

Qing, Z., Kui, C., Xiao, Z., Kai, L., Qing, J., Hai, Z., 2010. Synthesis of some N-alkyl substituted urea derivatives as antibacterial and antifungal agents. Eur. J. Med. Chem. 45, 3207–3212.

Rostom, S.A.F., Shalaby, M.A., El-Demellawy, M.A., 2003. Polysubstituted pyrazoles, part 5. Synthesis of new 1-(4-chlorophenyl)-4-hydroxy-1H-pyrazole-3-carboxylic acid hydrazide analogs and some derived ring systems. A novel class of potential antitumor and anti-HCV agents. Eur. J. Med. Chem. 38, 959–974.

Sangshetti, J.N., Chabukswar, A.R., Shinde, D.B., 2011. Microwave assisted one pot synthesis of some novel 2,5-disubstituted 1,3,4-oxadiazoles as antifungal agents. Bioorg. Med. Chem. Lett. 21, 444–448.

Secci, D., Bizzarri, B., Bolasco, A., Carradori, S., D'Ascenzio, M., Rivanera, D., Mari, E., Polletta, L., Zicari, A., 2012. Synthesis, anti-Candida activity, and cytotoxicity of new (4-(4-iodophenyl)thiazol-2-yl)hydrazine derivatives. Eur. J. Med. Chem. 53, 246–253.

Tagg, T.R., Dajani, A.S., Wannamaker, L.W., 1976. Bacteriocin of Gram positive bacteria. Bacteriol. Rev. 40, 722–756.

Vallet, F.M.J., 1975. 1-[3,4(methylenedioxy)-phenyl]-4,4-dimethyl-pent-1-en-3-ol. US 3,910,959 A.

Wani, M.Y., Athar, F., Salauddin, A., Agarwal, S.M., Azam, A., Choi, H., Bhat, A., 2011. Novel terpene based 1,4,2-dioxazoles: synthesis, characterization, molecular properties and screening against *Entamoeba histolytica*. Eur. J. Med. Chem. 46, 4742–4752.