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Short communication

Synthesis and antibacterial evaluation of novel 4-alkyl substituted phenyl β-aldehyde ketone derivatives

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Abstract

A series of novel 4-alkylphenyl β-aldehyde ketones and their derivatives were designed and synthesized on the basis of the chemical structures of Houttuynin and β-lactam antibiotics. Antibacterial activities of these compounds were investigated. The results demonstrated that most of the compounds tested had moderate antibacterial activities against Gram-positive pathogen *Staphylococcus aureus* (ATTC-25923) than Houttuynin, and Gram-positive bacteria were more susceptible to the compounds than Gram-negative bacteria. Compound **23** was found to be the most potent compound with MIC of 1.0 μg/mL against *S. aureus*. Particularly, compounds **16**, **22** and **23** showed more active antibacterial activities against the clinically important pathogenic bacteria, methicillin-resistant *S. aureus* (MRSA) than Houttuynin and levofloxacin. The preliminary structure–activity relationship (SAR) analysis suggested that (1) the introduction of appropriate alkyl substituents into position 4 of phenyl ring enhanced antibacterial activities of these compounds, and isopropyl substituent might be more favorable; (2) the presence of ketone carbonyl moiety might play a vital role in determining significant antibacterial activities of these compounds.

Keywords: Houttuynin; β-Ketone aldehyde; Synthesis; Antibacterial activity

1. Introduction

The increasing incidence of bacterial resistance to currently available antibacterial agents represents one of the major growing global health problems [1–6]. Obviously, there is an urgent need for the discovery and development of novel and effective antibacterial drugs, which can inhibit or kill bacteria by novel mechanisms, rather than analogues of existing antibiotics [7–12]. Houttuynin (Fig. 1), *n*-nonyl-β-oxo-aldehyde, was first isolated from *Houttuynia cordata Thunb* as the major active constituent, and its structure was characterized and shown to have a β-aldehyde ketone unit as an important pharmacological moiety. Previous researches showed that Houttuynin possesses broad spectrum pharmacological properties including antibacterial, antivirus, anti-inflammation and enhanced immunity [13]. Sodium houttuyfonate (Fig. 1), derived from Houttuynin, has been clinically used in antimicrobial medications for many years in China [13–16]. Sodium houttuyfonate was mainly dependent on the hydrophobic interaction with membrane proteins or lipid of bacteria to inhibit bacteria growth [17]. *In vitro* experiments suggested that modifying the structure of cell membrane would be one of the important factors of sodium houttuyfonate exerting the pharmaceutical effects [14]. On the other hand, most of the β-lactam antibiotics (Fig. 2), such as cefuroxime axetil and cefotaxime sodium, contained *O*-alkyl oxime unit which played an important role in enhancing their antibacterial activities. Stimulated by the above knowledge of

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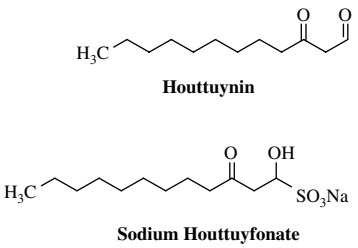


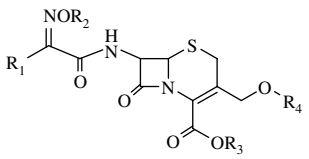
Fig. 1. The chemical structure of Houttuynin and sodium houttuyfonate.

sodium houttuyfonate and β-lactam antibiotics in the present investigation, we attempted to combine the *O*-alkyl oxime unit of β-lactam antibiotics with β-aldehyde ketone of Houttuynin and sodium houttuyfonate to design and synthesize novel promising antibacterial drugs. The present investigation aimed at (1) elucidating the preliminary structure–activity relationships; (2) probing structural requirements for the potent antibacterial activities of these compounds; (3) discovering and developing novel and potent antibacterial drugs with novel action mechanism. To the best of our knowledge, all 4-alkylphenyl β-aldehyde ketones and their derivatives except compounds **5**, **6** and **16** are novel.

2. Results and discussion

2.1. Chemistry

The general procedure for the synthesis of 3-(4-alkylphenyl)-3-oxopropanal and their derivatives are described in Scheme 1. In brief, the reaction of 4′- alkylacetophenone with 2.0 equiv of ethyl formate and 1.5 equiv of sodium ethoxide or metal sodium in dry toluene at room temperature gave the corresponding intermediate, sodium salt of phenyl-3-hydroxyprop-2-en-1-one (**1**) and 4-alkylphenyl-3-hydroxyprop-2-en-1-one (**2**–**4**). Compounds **1** and **2** were acidified with 5% hydrochloric acid to give the 3-phenyl-3-oxopropanal (**5**) and 3-(4-alkylphenyl)-3-oxopropanal (**6**) in 65–76% overall yields. Compounds **3** and **4** were treated with saturated sodium bisulfite at room temperature to give sodium 1-hydroxy-3-(4-alkylphenyl)-3-oxopropane-1-sulfonate (**7**–**8**). Reaction of 3-phenyl-3-oxopropanal (**5**) and 3-(4-alkylphenyl)-3-oxopropanal (**6**) with semicarbazide, and thiosemicarbazide in EtOH, respectively, gave the corresponding Schiff base (**9**–**12**) in 52–91% overall yield. Compounds **13**–**29** were obtained by the treatment of sodium salts of 3-(4-alkylphenyl)-3-oxopropanal with hydroxylamine hydrochloride, methoxyamine hydrochloride, ethoxyamine hydrochloride, respectively.



Cefuroxime axetil, R^1 = 2-furanyl, R^2 = CH_3, R^3 = 1-acetoxyethyl, R^4 = aminoformyl
Cefotaxime sodium, R^1 = 4-(2-aminothiazolyl), R^2= CH_3, R^3 = Na, R^4 = acetyl

Fig. 2. The chemical structure of β-lactam antibiotics.

2.2. Antibacterial activity in vitro

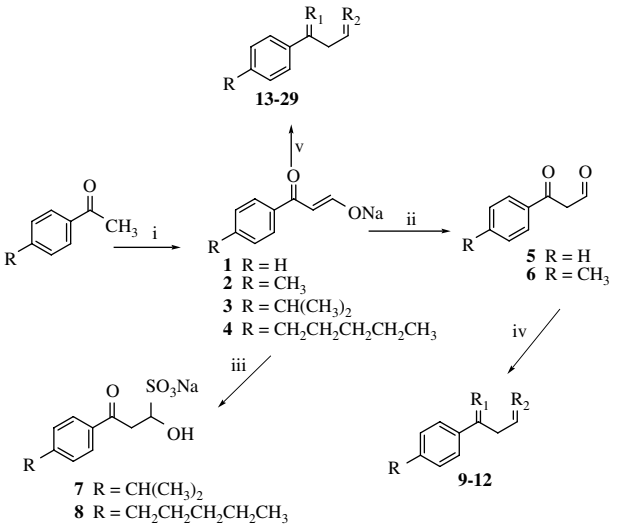
All the synthesized compounds were tested for their *in vitro* antibacterial activities against both Gram-positive bacteria *Staphylococcus aureus* (ATTC-25923) and Gram-negative bacteria *Escherichia coli* (ATTC-25922) and *Pseudomonas aeruginosa* (ATTC-27853). The antibacterial activity was reported as the minimum inhibitory concentration (MIC) in μg/mL that was determined by the agar dilution method as recommended by the NCCLS (National Committee for Clinical Laboratory Standards) [18]. The MICs are summarized in Table 1 along with the data of levofloxacin, clarithromycin and Houttuynin for comparison.

As shown in Table 1, most of the compounds had moderate antibacterial activities against Gram-positive pathogen *S. aureus*, but compounds **16**, **17** and **26** only showed weak activity against the Gram-negative pathogens *E. coli* and *P. aeruginosa*. Particularly, compound **23** was found to be the most potent compound with MIC of 1.0 μg/mL against *S. aureus*. These results indicated that Gram-positive bacteria were more susceptible to the newly synthesized compounds than Gram-negative bacteria.

Among all the compounds investigated, the oxime (**13** and **15**) and oxime-alkylether derivatives (**14** and **16**–**29**) exhibited more active antibacterial activity than other compounds, which implicated that oxime and *O*-alkyl oxime moiety might play an important role in enhancing antibacterial activities of these compounds. Unfortunately, 3-oxo-3-phenylpropanal (**5**), 3-(4-methylphenyl) -3-oxopropanal (**6**) and sodium 1-hydroxy-3-(4-alkylphenyl)-3-oxopropane-1-sulfonate (**7**–**8**) failed to exhibit the antibacterial activity against the three strains investigated at the concentration of 64 μg/mL. Similarly, the semicarbazones and thiosemicarbazone derivatives (**9**–**12**) were also inactive.

In order to examine the effect of alkyl substituents attached to the phenyl ring on the antibacterial activities of the compounds, a series of 3-phenyl β-aldehyde ketone derivatives bearing different alkyl substituents at position 4 of phenyl ring were synthesized. As shown in Table 1, compound **14** having no substituent at position 4 of phenyl ring showed weak antibacterial activity. Introducing a short chain alkyl substituent into position 4 of phenyl ring led to compounds **16** (methyl), **18** (ethyl), **22** (isopropyl) and **26** (*n*-pentyl), which all exhibited better antibacterial activities against the Gram-positive pathogen *S. aureus* than their parent compound **14**. Similarly, compounds **17** (methyl), **19** (ethyl), **23** (isopropyl) and **27** (n-pentyl) also displayed more potent antibacterial activities than compound **14**. Interestingly, compounds **22** and **23** which bearing the same isopropyl substituents at position 4 of phenyl ring demonstrated more prominent antibacterial activities than other 4-alkylphenyl derivatives, and compound **23** was found to be the best compound with MIC of 1.0 μg/mL against *S. aureus*. These results indicated that the introduction of appropriate alkyl substituents into position 4 of phenyl ring enhanced antibacterial activities of these compounds, and isopropyl substituent might be more favorable.

To explore the influence of the ketone carbonyl on antibacterial activities of the 4-alkylphenyl β-aldehyde ketone



9 R = H, R^1 = O, R^2 = $NNHCONH_2$
10 R = H, R^1 = O, R^2 = $NNHCSNH_2$
11 R = CH_3, R^1 = O, R^2 = $NNHCONH_2$
12 R = CH_3, R^1 = O, R^2 = $NNHCSNH_2$
13 R = H, R^1 = O, R^2= NOH
14 R = H, R^1 = O, R^2 = $NOCH_3$
15 R = CH_3, R^1 = O, R^2= NOH;
16 R = CH_3, R^1 = O, R^2 = $NOCH_3$
17 R = CH_3, R^1 = O, R^2 = $NOCH_2CH_3$
18 R = CH_2CH_3, R^1 = O, R^2 = $NOCH_3$
19 R = CH_2CH_3, R^1 = O, R^2 = $NOCH_2CH_3$
20 R = CH_2CH_3, R^1 = R^2 = $NOCH_3$
21 R = CH_2CH_3, R^1 = R^2 = $NOCH_2CH_3$
22 R = $(CH_3)_2CH$, R^1 = O, R^2 = $NOCH_3$
23 R = $(CH_3)_2CH$, R^1 = O, R^2 = $NOCH_2CH_3$
24 R = $(CH_3)_2CH$, R^1 = R^2 = $NOCH_3$
25 R = $(CH_3)_2CH$, R^1 = R^2 = NO CH_2CH_3
26 R = $CH_3(CH_2)_4$, R^1 = O, R^2 = $NOCH_3$
27 R = $CH_3(CH_2)_4$, R^1 = O, R^2 = NO CH_2CH_3
28 R = $CH_3(CH_2)_4$, R^1 = R^2 = $NOCH_3$
29 R = $CH_3(CH_2)_4$, R^1 = R^2 = $NOCH_2CH_3$

Scheme 1. Synthesis of 4-alkylphenyl β-aldehyde ketones and their derivatives. *Reagents*: (i) ethyl formate/Na/toluene, (ii) 5% HCl, (iii) saturated sodium bisulfite solution, (iv) $NH_2NHCSNH_2$ or $NH_2NHCSNH_2$/EtOH/reflux, (v) $NH_2OH{\cdot}HCl$ or $NH_2OR{\cdot}HCl$/EtOH/RT.

derivatives, the dioxime-alkylethers were designed and synthesized, and their antibacterial activities *in vitro* against Gram-positive bacteria and Gram-negative bacteria were also investigated. As shown in Table 1, the 4-alkylphenyl β-aldehyde ketone monooxime-alkylether derivatives (**18**–**19**, **22**–**23** and **26**–**27**) displayed 8–32 times more active against *S. aureus* than the corresponding dioxime-alkylether derivatives (**20**–**21**, **24**–**25** and **28**–**29**). These results indicated that the presence of ketone carbonyl moiety might play a vital role in determining significant antibacterial activities of these compounds.

With the purpose of further corroborating the prominent antibacterial activities, compound **23** was selected to investigate the antibacterial activities against 10 strains of clinical isolates of *S. aureus* and the results are summarized in Table 2. As shown in Table 2, compound **23** exhibited prominent antibacterial activities.

Furthermore, three compounds (**16**, **22** and **23**) were selected to test the antibacterial activities *in vitro* against important pathogenic methicillin-resistant *S. aureus* (MRSA) and the results are summarized in Table 3. As shown in Table 3, compounds **16**, **22** and **23** displayed moderate antibacterial activities against MRSA, which are slightly superior to the Houttuynin and levofloxacin.

3. Conclusions

The present investigation reported, for the first time, that 4-alkylphenyl β-aldehyde ketone derivatives had potent antibacterial activities, and Gram-positive pathogens were more susceptible to these compounds. Of all the investigated compounds, compounds **22** and **23** bearing the isopropyl substituents at position 4 of phenyl ring demonstrated more prominent antibacterial activities than other 4-alkylphenyl substituted derivatives. Interestingly, compounds **16**, **22** and **23** exhibited more potent antibacterial activities against clinically important pathogenic bacteria MRSA than Houttuynin and levofloxacin. The preliminary structure–activity relationship (SAR) analysis suggested that (1) the introduction of appropriate alkyl substituents into position 4 of phenyl ring enhanced antibacterial activities of these compounds, and isopropyl substituent might be more favorable; (2) the presence of ketone carbonyl moiety might play a vital role in determining significant antibacterial activities of these compounds.

We have explored the influence of substituents at position 4 of phenyl ring on the ability of these compounds against Gram-positive pathogens. To acquire more information about the structural requirements for the possible improvement of

Table 1
In vitro antibacterial activities of 4-alkylphenyl β-aldehyde ketones and their derivatives

Compounds	MIC (μg/mL)		
	Staphylococcus aureus (ATTC-25923)	*Escherichia coli* (ATTC-25922)	*Pseudomonas aeruginosa* (ATTC-27853)
5	>64	>64	>64
6	>64	>64	>64
7	>64	>64	>64
8	>64	>64	>64
9	>64	>64	>64
10	>64	>64	>64
11	>64	>64	>64
12	>64	>64	>64
13	64	>64	>64
14	64	>64	>64
15	64	>64	>64
16	4	64	64
17	4	64	64
18	4	>64	>64
19	4	>64	>64
20	32	>64	>64
21	32	>64	>64
22	2	>64	>64
23	1	>64	>64
24	32	>64	>64
25	32	>64	>64
26	4	64	64
27	4	>64	>64
28	64	>64	>64
29	64	>64	>64
Clarithromycin	0.12	32	>64
Levofloxacin	0.25	0.025	0.5
Houttuynin	32	–	–

the antibacterial properties, synthesis of additional new β-aldehyde ketone derivatives is desirable. Further biological evaluation required to confirm these 4-alkyl substituted phenyl β-aldehyde ketone derivatives in animal models are in progress in our laboratories. Moreover, the molecular mechanisms of these compounds are ongoing to further design and develop more potent compounds.

Table 2
MIC of compound **23**, sodium houttuyfonate, and levofloxacin against strains of *Staphylococcus aureus*

Strains	MIC (μg/mL)		
	Compound **23**	Levofloxacin	Houttuynin
S. aureus 1	4	0.25	64
S. aureus 2	16	0.5	64
S. aureus 3	4	0.25	64
S. aureus 4	2	0.25	64
S. aureus 5	1	0.125	32
S. aureus 6	4	0.5	64
S. aureus 7	8	0.25	64
S. aureus 8	8	0.25	64
S. aureus 9	4	0.25	64
S. aureus 10	4	0.5	32
MIC_{50}	4	0.25	64
MIC_{90}	8	0.5	64

4. Experimental

4.1. Reagents and general methods

Most chemical reagents were purchased from Darui Chemical Co. (Shanghai, PR China) and 1-(4-alkylphenyl) ethanones were prepared by the F–C acylation reaction according to the methods described by Wang [19]. Toluene was dried over molecular sieve. The other commercially available reagents and solvents were used without further purification. All reactions were monitored by TLC (Merck Kieselgel 60, F254). Melting points (mp) were determined with WRS-1B melting point apparatus and are uncorrected. NMR spectra were recorded on Mercury-Plus300 spectrometers at 25 °C using $CDCl_3$ or DMSO-d_6 as a solvent. All chemical shifts (δ) were quoted in parts per million downfield from TMS and coupling constants (J) are given in hertz. Abbreviations used in the splitting pattern were as follows: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Infrared (IR) spectra were recorded on a Perkin–Elmer Infracord 221 spectrometer and reported in cm^{-1}. LC–MS spectra were recorded using the LCMS-2010A. Elemental analyses were performed on a Vario EL instrument.

4.2. General procedures for 4-alkylphenyl β-aldehyde ketone (**5**, **6**) [20]

1-(4-Alkylphenyl) ethanone (0.05 mol) was dissolved in dry toluene and the mixture was stirred in the ice bath. Sodium (0.075 mol) was added to the solution and the mixture was stirred for 30 min in the ice bath. Ethyl formate was then added dropwise to the reaction mixture, which was maintained at <5 °C, after the addition, the reaction mixture was stirred for another 2 h and allowed to warm to room temperature, then stirred overnight (about 15 h). Water (150 mL) was added to the slurry mixture, and the reaction was stirred for an additional 30 min and then partitioned between organic layer and water. Water layer was extracted with dichloromethane (2 × 50 mL). These extracts were discarded and pooled. The aqueous phase was acidified with 5% hydrochloric acid and extracted with dichloromethane (3 × 50 mL). This extract was washed with water and brine, dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure to give yellow oil. 3-Oxo-3-phenylpropanal (**5**) was purified further by recrystallization from hexane to afford the solid product.

Table 3
MIC of compounds **16**, **22** and **23** against strains of methicillin-resistant *Staphylococcus aureus* (MRSA)

Compounds	MIC (μg/mL)					MIC_{50}	MIC_{90}
	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5		
16	8	8	8	8	8	8	8
22	8	8	8	8	4	8	8
23	16	8	8	16	8	8	16
Levofloxacin	8	8	16	16	16	16	16
Houttuynin	16	16	16	16	16	16	16

4.2.1. 3-Oxo-3-phenylpropanal (**5**)

Yield 76%, yellow solid; mp 109–110 °C. IR (KBr): 3059, 2923, 1657, 1591, 1249, 1005, 701 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.27 (d, $J = 4.2$ Hz, 1H, =CH), 7.81 (d, $J = 8.4$ Hz, 2H, PhH), 7.63 (t, $J = 7.5$ Hz, 1H, PhH), 7.51 (t, $J = 8.1$ Hz, 2H, PhH), 6.19 (d, 1H, $J = 4.2$ Hz, =CH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.6, 172.9, 138.6, 134.0, 130.5, 129.4, 97.1. ESI-MS *m/z*: 149 (M + 1).

4.2.2. 3-(4-Methylphenyl)-3-oxopropanal (**6**)

Yield 65%, yellow oil. IR (KBr): 2963, 1683, 1607, 1417, 1056, 843 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.35 (d, $J = 5.1$ Hz, 1H, =CH), 7.80 (d, $J = 8.1$ Hz, 2H, PhH), 7.07 (d, $J = 8.1$ Hz, 2H, PhH), 6.16 (d, $J = 5.1$ Hz, H, =CH), 2.36 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.8, 172.5, 152.6, 134.4, 129.1, 126.7, 97.8, 23.8. ESI-MS *m/z*: 163 (M + 1).

4.3. General procedure for the preparation of sodium 1-hydroxy-3-(4-alkylphenyl)-3-oxopropane-1-sulfonate (**7**, **8**)

1-(4-Alkylphenyl) ethanone (0.05 mol) was dissolved in dry toluene and stirred in the ice bath. Sodium (0.075 mol) was added to the solution and the mixture was stirred for 30 min in the ice bath. Ethyl formate was then added dropwise to the reaction mixture, which was maintained at <5 °C, after the addition, the mixture was stirred for another 2 h. Then the reaction mixture was warmed to RT and stirred overnight (about 15 h). The precipitates were separated by filtration and dried to afford the corresponding sodium salt of 4-alkylphenyl-3-hydroxyprop-2-en-1-one. The treatment of sodium salt of 4-alkylphenyl-3-hydroxyprop-2-en-1-one with saturated solution of sodium bisulfite at RT for 2 h, the precipitates were separated by filtration and crystallized from 50% ethanol to afford sodium 1-hydroxy-3-(4-alkylphenyl)-3-oxopropane-1-sulfonate.

4.3.1. Sodium 1-hydroxy-3-(4-isopropylphenyl)-3-oxopropane-1-sulfonate (**7**)

Yield 63%, white solid; mp 192–194 °C. IR (KBr): 3449, 2960, 1683, 1606, 1411, 1361, 1060, 827 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 7.80 (d, $J = 6.6$ Hz, 2H, PhH), 7.33 (d, $J = 6.6$ Hz, 2H, PhH), 5.28 (t, 1H, CH), 4.88 (s, 1H, OH), 3.35 (d, 2H, CH_2), 2.86–2.89 (m, 1H, CH), 1.12 (d, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.5, 152.8, 136.7, 129.5, 126.1, 81.4, 56.8, 36.7, 23.2. ESI-MS *m/z*: 271 (M − 23).

4.3.2. Sodium 1-hydroxy-3-oxo-3-(4-pentylphenyl) propane-1-sulfonate (**8**)

Yield 46%, white solid; mp 179–181 °C. IR (KBr): 3424, 3238, 2925, 1677, 1599, 1412, 1053, 829 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 7.84 (d, $J = 8.1$ Hz, 2H, PhH), 7.31 (d, $J = 8.1$ Hz, 2H, PhH), 4.91 (t, 1H, CH), 4.72 (s, 1H, OH), 3.18 (d, 2H, CH_2), 2.59 (t, 2H, CH_2), 1.87–1.89 (m, 4H, $2CH_2$), 1.53–1.55 (m, 2H, CH_2), 0.75 (t, $J = 6.3$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.4, 143.5, 134.7, 129.1, 128.6, 82.8, 54.7, 36.1, 31.9, 31.1, 23.0, 14.6. ESI-MS *m/z*: 299 (M − 23).

4.4. General procedure for the preparation of semicarbazone and thiosemicarbazone of 4-alkylphenyl β-aldehyde ketone (**9–12**) [21–23]

The corresponding 4-alkylphenyl β-keto aldehyde (5 mmol) was dissolved in anhydrous ethanol (10 mL). Semicarbazide or thiosemicarbazide (5 mmol) was added to the solution. The mixture was stirred for 5–8 h under refluxing conditions. The reaction mixture was cooled to RT and the precipitates were filtered.

4.4.1. 3-Oxo-3-phenylpropanal semicarbazone (**9**)

Yield 52%, yellow solid; mp 170–172 °C. IR (KBr): 3342, 3336, 1685, 1593, 1451, 1068, 745 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.31 (br s, 1H, NH), 7.92 (d, $J = 7.9$ Hz, 2H, PhH), 7.80 (br s, 1H, NH), 7.62 (t, $J = 5.4$ Hz, 1H, =CH), 7.59 (br s, 1H, NH), 7.51 (t, $J = 7.5$ Hz, 1H, PhH), 7.32 (t, $J = 8.0$ Hz, 2H, PhH), 4.02 (d, $J = 5.4$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.8, 158.2, 154.8, 137.6, 134.3, 129.5, 128.4, 36.2. ESI-MS *m/z*: 206 (M + 1).

4.4.2. 3-Oxo-3-phenylpropanal thiosemicarbazone (**10**)

Yield 91%, yellow solid; mp 180–181 °C. IR (KBr): 3369, 3265, 1650, 1593, 1065, 748 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.62 (br s, 1H, NH), 8.28 (br s, 1H, NH), 7.86 (d, $J = 8.7$ Hz, 2H, PhH), 7.70 (t, $J = 5.7$ Hz, 1H, =CH), 7.56 (br s, 1H, NH), 7.48 (t, $J = 8.1$ Hz, 1H, PhH), 7.28 (t, $J = 7.6$ Hz, 2H, PhH), 4.46 (d, $J = 5.7$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.4, 183.1, 154.7, 130.8, 129.6, 129.2, 127.6, 35.8. ESI-MS *m/z*: 222 (M + 1).

4.4.3. 3-Oxo-3-p-tolylpropanal semicarbazone (**11**)

Yield 72%, yellow solid; mp 183–185 °C. IR (KBr): 3241, 3178, 2920, 1713, 1599, 1438, 807 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 10.01 (br s, 1H, NH), 8.22 (br s, 1H, NH_2), 7.87 (d, $J = 8.4$ Hz, 2H, PhH), 7.51 (br s, 1H, NH_2), 7.34 (d, $J = 8.4$ Hz, 2H, PhH), 7.25 (t, $J = 5.4$ Hz, 1H, CH), 6.18 (br s, 1H, NH_2), 3.96 (d, $J = 5.4$ Hz, 2H, CH_2), 2.37 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.6, 159.1, 154.7, 139.9, 133.3, 129.3, 128.4, 35.2, 23.4. ESI-MS *m/z*: 220 (M + 1).

4.4.4. 3-Oxo-3-p-tolylpropanal thiosemicarbazone (**12**)

Yield 58%, yellow solid; mp 158–159 °C. IR (KBr): 3250, 3171, 1664, 1594, 1167, 827 cm^{-1}. ^1H NMR (300 MHz, DMSO-d_6): δ 11.26 (br s, 1H, NH), 8.16 (br s, 1H, NH_2), 7.87 (d, $J = 8.1$ Hz, 2H, PhH), 7.63 (br s, 1H, NH_2), 7.59 (t, $J = 5.7$ Hz, 1H, CH), 7.34 (d, $J = 8.1$ Hz, 2H, PhH), 4.01 (d, $J = 5.7$ Hz, 2H, CH_2), 2.38 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.6, 183.1, 154.9, 138.5, 130.8, 129.6, 128.0, 34.3, 24.3. ESI-MS *m/z*: 236 (M + 1).

4.5. General procedure for the preparation of oxime and oxime-ether of 4-alkyl-phenyl β-aldehyde ketone (**13–29**)

The sodium salt of 4-alkylphenyl-3-hydroxyprop-2-en-1-one (5 mmol) was added to 20 mL anhydrous ethanol and the

mixture was stirred at RT. The corresponding hydroxylamine hydrochloride, methoxyamine hydrochloride or ethoxyamine hydrochloride (5 mmol or 10 mmol) were added to the reaction mixture. The mixture was stirred at RT for 6–9 h. After completion of the reaction as indicated by TLC, the solution was concentrated in vacuo. The residue was dissolved in methylene chloride and washed with water. The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated. The resulting oil obtained was purified by silica gel column chromatography to give first the dioxime and dioxime-ether derivatives followed by the oxime and oxime-ether derivatives.

*4.5.1. 3-Oxo-3-phenylpropanal oxime (**13**)*

Yield 58%, yellow solid; mp 135–136 °C. IR (KBr): 3427, 1640, 1605, 1150 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.86 (d, $J = 7.9$ Hz, 2H, PhH), 7.77 (t, $J = 5.4$ Hz, 1H, CH), 7.49 (t, $J = 7.1$ Hz, 1H, PhH), 7.31 (t, $J = 7.5$ Hz, 2H, PhH), 3.45 (d, $J = 5.4$ Hz, 2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.8, 154.2, 137.8, 127.0, 124.4, 124.3, 34.6. ESI-MS *m/z*: 164 (M + 1).

*4.5.2. 3-Oxo-3-phenylpropanal oxime-methyl ether (**14**)*

Yield 62%, yellow solid; mp 73–75 °C. IR (KBr): 2947, 1657, 1595, 1149, 1005, 768 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.83 (d, $J = 8.3$ Hz, 2H, PhH), 7.63 (t, $J = 6.0$ Hz, 1H, CH), 7.51 (t, $J = 8.1$ Hz, 1H, PhH), 7.35 (t, $J = 7.5$ Hz, 2H, PhH), 3.92, 3.75 (dd, $J = 6.0$, 6.9 Hz, 2H, CH_2), 3.71 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.7, 142.3, 133.8, 131.0, 128.7, 127.9, 61.0, 34.1. ESI-MS *m/z*: 178 (M + 1). Anal. Calcd for $C_{10}H_{11}NO_2$: C, 67.78; H, 6.26; N, 7.90. Found: C, 68.12; H, 6.39; N, 7.78.

*4.5.3. 3-Oxo-3-p-tolylpropanal oxime (**15**)*

Yield 46%, yellow solid; mp 176–178 °C. IR (KBr): 3592, 3083, 1642, 1607, 1458, 829 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.71 (t, $J = 5.1$ Hz, 1H, CH), 7.53 (d, $J = 8.1$ Hz, 2H, PhH), 7.25 (d, $J = 8.1$ Hz, 2H, PhH), 3.45 (d, $J = 5.1$ Hz, 2H, CH_2), 2.32 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 192.7, 154.3, 137.8, 132.6, 127.3, 124.1, 34.6, 19.3. ESI-MS *m/z*: 178 (M + 1).

*4.5.4. 3-Oxo-3-p-tolylpropanal oxime-methyl ether (**16**)*

Yield 68%, yellow oil. IR (KBr): 2939, 1648, 1574, 1419, 1047, 819 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.68 (t, $J = 5.1$ Hz, 1H, CH), 7.58 (d, $J = 8.1$ Hz, 2H, PhH), 7.21 (d, $J = 8.1$ Hz, 2H, PhH), 4.01 (s, 3H, CH_3), 4.00, 3.85 (dd, $J = 5.1$, 8.1 Hz, 2H, CH_2), 2.31 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.3, 145.3, 144.1, 133.9, 129.6, 128.5, 62.1, 39.3, 22.0. ESI-MS *m/z*: 192 (M + 1). Anal. Calcd for $C_{11}H_{13}NO_2$: C, 69.09; H, 6.85; N, 7.32. Found: C, 69.34; H, 6.67; N, 7.71.

*4.5.5. 3-Oxo-3-p-tolylpropanal oxime-ethyl ether (**17**)*

Yield 57%, yellow oil. IR (KBr): 2977, 1614, 1515, 1382, 1046, 820 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.89 (d, $J = 7.8$ Hz, 2H, PhH), 7.66 (t, $J = 4.8$ Hz, 1H, CH), 7.28 (d, $J = 7.8$ Hz, 2H, PhH), 4.10–4.19 (m, 2H, CH_2), 4.02, 3.63 (dd, $J = 5.1$, 8.1 Hz, 2H, CH_2), 2.42 (s, 3H, CH_3), 1.29 (t, $J = 6.9$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 195.4, 145.0, 143.8, 133.9, 129.6, 128.5, 69.9, 39.5, 22.0, 15.0. ESI-MS *m/z*: 206 (M + 1). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.54; H, 7.22; N, 6.47.

*4.5.6. 3-(4-Ethylphenyl)-3-oxopropanal oxime-methyl ether (**18**)*

Yield 64%, yellow oil. IR (KBr): 2967, 2937, 1684, 1607, 1413, 1346, 1042, 821 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, $J = 7.2$ Hz, 2H, PhH), 7.67 (t, $J = 4.8$ Hz, 1H, CH), 7.30 (d, $J = 7.2$ Hz, 2H, PhH), 4.00, 3.86 (dd, $J = 4.8$, 8.1 Hz, 2H, CH_2), 3.93 (s, 3H, CH_3), 2.67–2.75 (m, 2H, CH_2), 1.29 (t, $J = 7.5$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.6, 150.8, 145.3, 134.1, 128.6, 128.4, 62.1, 39.3, 29.3, 15.5. ESI-MS *m/z*: 206 (M + 1). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 69.85; H, 7.10; N, 6.75.

*4.5.7. 3-(4-Ethylphenyl)-3-oxopropanal oxime-ethyl ether (**19**)*

Yield 68%, yellow oil. IR (KBr): 2971, 2932, 1684, 1602, 1411, 1333, 1046, 824 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, $J = 8.0$ Hz, 2H, PhH), 7.64 (t, $J = 3.9$ Hz, 1H, CH), 7.28 (d, $J = 8.0$ Hz, 2H, PhH), 4.09–4.18 (m, 2H, CH_2), 4.00, 3.86 (dd, $J = 3.9$, 6.3 Hz, 2H, CH_2), 2.66–2.73 (m, 2H, CH_2), 1.23–1.29 (m, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.9, 149.1, 144.2, 130.6, 129.6, 125.4, 69.9, 38.4, 29.2, 16.3, 14.8. ESI-MS *m/z*: 220 (M + 1). Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.51; H, 7.90; N, 6.59.

*4.5.8. 3-(4-Ethylphenyl)-3-oxopropanal dioxime-methyl ether (**20**)*

Yield 31%, yellow oil. IR (KBr): 2965, 2820, 1684, 1571, 1425, 1335, 1048, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, $J = 8.4$ Hz, 2H, PhH), 7.42 (t, $J = 5.4$ Hz, 1H, CH), 7.21 (d, $J = 8.4$ Hz, 2H, PhH), 3.92–4.00 (m, 6H, $2CH_3$), 3.77, 3.63 (dd, $J = 5.4$, 6.3 Hz, 2H, CH_2), 2.64–2.70 (m, 2H, CH_2), 1.27 (t, $J = 7.5$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 146.1, 132.7, 128.7, 128.1, 126.5, 62.5, 62.1, 29.0, 25.2, 15.8. ESI-MS *m/z*: 235 (M + 1). Anal. Calcd for $C_{13}H_{18}N_2O_2$: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.87; H, 7.93; N, 11.91.

*4.5.9. 3-(4-Ethylphenyl)-3-oxopropanal dioxime-ethyl ether (**21**)*

Yield 28%, yellow oil. IR (KBr): 2972, 2933, 2820, 1682, 1577, 1382, 1337, 1048, 832 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.61 (d, $J = 8.4$ Hz, 2H, PhH), 7.49 (t, $J = 4.5$ Hz, 1H, CH), 7.21 (d, $J = 8.4$ Hz, 2H, PhH), 4.08–4.30 (m, 4H, $2CH_2$), 3.81, 3.67 (dd, $J = 4.5$, 5.7 Hz, 2H, CH_2), 2.63–2.70 (m, 2H, CH_2), 1.23–1.37 (m, 9H, $3CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.3, 145.9, 133.1, 128.6, 128.2, 126.4, 70.2, 69.4, 29.0, 25.4, 15.9, 15.2, 14.9. ESI-MS *m/z*: 263 (M + 1). Anal. Calcd for $C_{15}H_{22}N_2O_2$: C, 68.67; H, 8.45; N, 10.68. Found: C, 68.87; H, 8.27; N, 10.47.

*4.5.10. 3-(4-Isopropylphenyl)-3-oxopropanal oxime-methyl ether (**22**)*

Yield 77%, yellow oil. IR (KBr): 2963, 2821, 1684, 1606, 1417, 1364, 1054, 853 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.92 (d, $J = 7.3$ Hz, 2H, PhH), 7.66 (t, $J = 4.8$ Hz, 1H, CH), 7.34 (d, $J = 7.3$ Hz, 2H, PhH), 4.01, 3.87 (dd, $J = 4.8$, 7.5 Hz, 2H, CH_2), 3.94 (s, 3H, CH_3), 2.96–3.00 (m, 1H, CH), 1.30 (d, $J = 6.6$ Hz, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.3, 154.6, 148.2, 134.5, 128.7, 127.8, 61.7, 39.0, 31.3, 19.5. ESI-MS *m/z*: 220 (M + 1). Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.21; H, 7.81; N, 6.39. Found: C, 71.52; H, 7.97; N, 6.22.

*4.5.11. 3-(4-Isopropylphenyl)-3-oxopropanal oxime-ethyl ether (**23**)*

Yield 81%, yellow oil. IR (KBr): 2966, 2870, 1683, 1605, 1413, 1387, 1047, 822 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.93 (d, $J = 8.4$ Hz, 2H, PhH), 7.68 (t, $J = 4.8$ Hz, 1H, CH), 7.34 (d, $J = 8.4$ Hz, 2H, PhH), 4.15–4.22 (m, 2H, CH_2), 4.11, 3.89 (dd, $J = 4.8$, 6.3 Hz, 2H, CH_2), 2.91–3.03 (m, 1H, CH), 1.30 (d, 9H, $3CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.7, 149.8, 143.9, 131.2, 128.6, 126.1, 69.7, 38.4, 29.1, 16.8, 14.5. ESI-MS *m/z*: 234 (M + 1). Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.37; H, 8.39; N, 5.86.

*4.5.12. 3-(4-Isopropylphenyl)-3-oxopropanal dioxime-methyl ether (**24**)*

Yield 22%, yellow oil. IR (KBr): 2961, 2820, 1574, 1417, 1339, 1051, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.61 (d, $J = 8.4$ Hz, 2H, PhH), 7.43 (t, $J = 4.8$ Hz, 1H, CH), 7.24 (d, $J = 8.4$ Hz, 2H, PhH), 3.83–4.00 (m, 6H, $2CH_3$), 3.77, 3.63 (dd, $J = 4.8$, 6.3 Hz, 2H, CH_2), 2.87–2.94 (m, 1H, CH), 1.27 (d, $J = 6.6$ Hz, 6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.4, 146.0, 133.1, 128.9, 128.5, 126.2, 62.9, 62.2, 29.3, 24.9, 15.2. ESI-MS *m/z*: 249 (M + 1). Anal. Calcd for $C_{14}H_{20}N_2O_2$: C, 67.71; H, 8.12; N, 11.28. Found: C, 68.12; H, 8.25; N, 11.21.

*4.5.13. 3-(4-Isopropylphenyl)-3-oxopropanal dioxime-ethyl ether (**25**)*

Yield 18%, yellow oil. IR (KBr): 2968, 2933, 2880, 1570, 1417, 1384, 1046, 837 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, $J = 7.3$ Hz, 2H, PhH), 7.48 (t, $J = 5.1$ Hz, 1H, CH), 7.23 (d, $J = 7.3$ Hz, 2H, PhH), 4.08–4.27 (m, 4H, $2CH_2$), 3.80, 3.64 (dd, $J = 5.1$, 6.3 Hz, 2H, CH_2), 2.88–2.94 (m, 1H, CH), 1.25–1.37 (m, 12H, $4CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 145.8, 132.8, 128.6, 128.1, 126.6, 70.5, 69.7, 29.2, 25.0, 15.9, 15.1, 14.7. ESI-MS *m/z*: 277 (M + 1). Anal. Calcd for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14. Found: C, 69.28; H, 8.79; N, 10.13.

*4.5.14. 3-Oxo-3-(4-pentylphenyl)propanal oxime-methyl ether (**26**)*

Yield 69%, yellow oil. IR (KBr): 2957, 2932, 2858, 1684, 1606, 1570, 1414, 1042, 863 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.90 (d, $J = 8.1$ Hz, 2H, PhH), 7.67 (t, $J = 4.8$ Hz, 1H, CH), 7.29 (d, $J = 8.1$ Hz, 2H, PhH), 4.01, 3.87 (dd, $J = 4.8$, 6.0 Hz, 2H, CH_2), 3.93 (s, 3H, CH_3), 2.69 (t, $J = 8.1$ Hz, 2H, CH_2), 1.60–1.67 (m, 2H, CH_2), 1.32–1.35 (m, 4H, $2CH_2$), 0.92 (t, $J = 6.3$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 193.9, 149.7, 145.3, 134.1, 129.0, 128.6, 62.1, 39.4, 36.3, 31.8, 31.1, 22.9, 14.4. ESI-MS *m/z*: 248 (M + 1). Anal. Calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; N, 5.66. Found: C, 72.64; H, 8.46; N, 5.49.

*4.5.15. 3-Oxo-3-(4-pentylphenyl)propanal oxime-ethyl ether (**27**)*

Yield 64%, yellow oil. IR (KBr): 2957, 2931, 2858, 1683, 1606, 1573, 1415, 1045, 875 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.91 (d, $J = 8.1$ Hz, 2H, PhH), 7.67 (t, $J = 4.8$ Hz, 1H, CH), 7.28 (d, $J = 8.1$ Hz, 2H, PhH), 4.22 (m, 2H, CH_2), 4.02, 3.87 (dd, $J = 4.8$, 6.0 Hz, 2H, CH_2), 2.69 (t, $J = 7.8$ Hz, 2H, CH_2), 1.62–1.69 (m, 2H, CH_2), 1.24–1.35 (m, 7H, $2CH_2$, CH_3), 0.92 (t, $J = 6.6$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 194.8, 149.6, 145.0, 134.1, 129.2, 128.6, 69.6, 39.6, 36.3, 31.8, 31.1, 22.9, 15.0, 14.4. ESI-MS *m/z*: 262 (M + 1). Anal. Calcd for $C_{16}H_{23}NO_2$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.21; H, 8.46; N, 5.40.

*4.5.16. 3-Oxo-3-(4-pentylphenyl)propanal dioxime-methyl ether (**28**)*

Yield 32%, yellow oil. IR (KBr): 2932, 2862, 1576, 1427, 1336, 1048, 852 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.59 (d, $J = 8.1$ Hz, 2H, PhH), 7.45 (t, $J = 5.1$ Hz, 1H, CH), 7.19 (d, $J = 8.1$ Hz, 2H, PhH), 4.00 (s, 3H, CH_3), 3.93 (s, 3H, CH_3), 3.77, 3.63 (dd, $J = 5.1$, 6.0 Hz, 2H, CH_2), 2.64 (t, 2H, CH_2), 1.59–1.64 (m, 2H, CH_2), 1.31–1.33 (m, 4H, $2CH_2$), 0.92 (t, $J = 6.6$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.6, 146.1, 144.7, 132.6, 128.8, 126.5, 62.4, 61.8, 36.0, 31.8, 31.4, 28.5, 22.9, 14.4. ESI-MS *m/z*: 277 (M + 1). Anal. Calcd for $C_{16}H_{24}N_2O_2$: C, 69.53; H, 8.75; N, 10.14. Found: C, 69.88; H, 8.35; N, 9.82.

*4.5.17. 3-Oxo-3-(4-pentylphenyl)propanal dioxime-ethyl ether (**29**)*

Yield 35%, yellow oil. IR (KBr): 2931, 1514, 1381, 1336, 1045, 865 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.60 (d, $J = 8.1$ Hz, 2H, PhH), 7.47 (t, $J = 5.1$ Hz, 1H, CH), 7.20 (d, $J = 8.1$ Hz, 2H, PhH), 4.04–4.29 (m, 4H, $2CH_2$), 3.79, 3.67 (dd, $J = 5.1$, 6.0 Hz, 2H, CH_2), 2.64 (t, $J = 7.5$ Hz, 2H, CH_2), 1.57–1.66 (m, 2H, CH_2), 1.31–1.35 (m, 6H, $2CH_3$), 1.21–1.31 (m, 4H, $2CH_2$), 0.92 (t, $J = 6.6$ Hz, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 153.5, 145.9, 144.1, 132.6, 128.2, 126.4, 70.2, 69.4, 36.1, 31.7, 31.0, 29.0, 22.4, 15.9, 15.7, 14.5. ESI-MS *m/z*: 305 (M + 1). Anal. Calcd for $C_{18}H_{28}N_2O_2$: C, 71.02; H, 9.27; N, 9.20. Found: C, 71.08; H, 9.35; N, 9.25.

4.6. In vitro antibacterial activity assays

All the synthesized compounds were tested for their antibacterial activity against both Gram-positive (*S. aureus*) and Gram-negative (*E. coli* and *P. aeruginosa*) bacteria strains. The test was performed by the agar dilution method according to the Clinical and Laboratory Standard Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) [18]. Twofold serial dilutions of the compounds and reference drugs

were prepared in Mueller–Hinton agar (MHA). The overnight cultures (after 16–18 h of incubation at 37 °C) of all the bacteria were used for the assay and adjusted to the turbidity of a 0.5 McFarland Standard. The stock solution (1 mg/mL) of all the test chemicals was prepared by dissolving 1 mg of the test chemical in 1 mL of dimethylsulfoxide (DMSO), and DMSO was used as control for all the test compounds. Twenty milliliters of MHA and 500 μL of each test bacterial culture of 16–18 h incubation adjusted at 0.5 McFarland were mixed and poured into sterilized and labeled plates. The wells of 6 mm diameter were punched in the solidified agar plates. Test chemicals of 100 μL were added to individual wells. The loaded plates were incubated at 37 °C for 24 h. The diameter of zone of growth inhibition around each well was measured after incubation using a Vernier Caliper. The MIC value is the lowest concentration of the antimicrobial agent that prevents the development of viable growth after16–18 h incubation. MIC values (mg/mL) were determined on Mueller–Hinton (MH) agar with medium containing dilutions of antibacterial agents by the literature method [3,24]. Stock solution of 4 mg/mL was prepared in DMSO and was appropriately diluted to get final concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.12 μg/mL. Levofloxacin (purchased from Darui Chemical Co.), clarithromycin (purchased from Hisunpharm Co.) and Houttuynin (purchased from Guangzhou Qingping Group Corporation Limited) were used as positive control.

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