Antimicrobial profile of some novel keto esters: Synthesis, crystal structures and structure-activity relationship studies

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Abstract: Rapid increase in bacterial resistance has become a major public concern by escalating alongside a lack of development of new anti-infective drugs. Novel remedies in the battle against multidrug-resistant bacterial strains are urgently needed. So, in this context, the present work is towards the investigation of antimicrobial efficacy of some novel keto ester derivatives, which are prepared by the condensation of substituted benzoic acids with various substituted phenacyl bromides in dimethylformamide at room temperature using triethylamine as a catalyst. The structural build-up of the target compounds was accomplished by spectroscopic techniques including FTIR, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry. The purity of the synthesized compounds was ascertained by elemental analysis. The molecular structures of compounds (4b) and (4l) were established by X-ray crystallographic analysis. The prepared analogues were evaluated for their antimicrobial activity against Gram-positive (*Staphylococcus aureus, Micrococcus leuteus*) and Gram-negative (*Pseudomonas picketti, Salmonella setuball*) bacteria and two fungal pathogenic strains (*Aspergillus niger, Aspergillus flavus*), respectively. Among the screened derivatives, several compounds were found to possess significant activity but (4b) and (4l) turned out to be lead molecules with remarkable antimicrobial efficacy. The structure-activity relationship analysis of this study also revealed that structural modifications on the basic skeleton affected the antimicrobial activity of the synthesized compounds.

Keywords: Keto esters, Crystal structure, Antimicrobial activity, Structure-activity relationship.

INTRODUCTION

Pathogen infections have threatened human's health worldwide (Chopra *et al*., 2008). Excessive use of antibiotics, both for treatment of humans and in livestock production, together with declining reports from pharmaceutical companies in pursuing development of new anti-infective agents has led to the emergence of untreatable infections (Projan, 2003). During past decades, with the exploitation of antimicrobial agents, more and more drug-resistant pathogens have been found. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) is a prominent pathogen, which causes a public health concern worldwide and associates with a high mortality (Coates *et al*., 2002; Fernandes, 2006; MacLean *et al*., 2010). In addition, the treatment of infectious diseases is more complicated in immuno-suppressed patients, such as those infected with the HIV, undergoing anticancer therapy or transplants. Given the evidence for the rapid global spread of resistant clinical isolates and prevalence of multidrug resistance among clinically significant bacterial pathogens, underscore a critical need for the discovery and development of antimicrobial agents active against these resistant strains (Gouveia *et al*., 2009).

Keto esters, an important class of versatile intermediates, are extensively used in agrochemical, pharmaceutical and dyestuff industries. They are also indispensable organic building blocks for the synthesis of complex natural products and are frequently employed synthons in organic synthesis, especially in heterocyclic synthesis (Stanovnik and Svete, 2004; Sheibani *et al*., 2006, 2007; Pal *et al*., 2008). Keto esters and their derivatives have also been reported to show antitumor activities against Ehrlich cells and HeLa cells (Kinoshita and Umezawa, 1960), and they could also regulate flowering times of some plants (Kai *et al*., 2007). Recent studies have revealed that they have also been found to exhibit inhibitory activities against two isozymes of 11β-hydroxysteroid dehydrogenases (11β-HSD1 and 11 β-HSD2), which catalyze the interconversion of active cortisol and inactive cortisone (Zhang *et al*., 2009).

To the best of our knowledge, there is no report on the biological profile of keto ester scaffold as antimicrobial agents. So, in corollary of these intriguing findings and in an attempt to achieve compounds with curative antimicrobial potential, we have synthesized a series of some novel keto esters (4a-p) and the preliminary results are presented in this paper.

MATERIALS AND METHODS

Chemicals and instrumentation

All the chemicals and solvents were of analytical grade or chemically pure and purchased from Sigma-Aldrich and Alfa aesar companies. Melting points were determined on

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a Gallenkamp melting point apparatus in open capillaries and remain uncorrected. The IR spectra were recorded on Bruker Optics Alpha FT-IR Spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker AV-300 in $CDCl_3$ solution using residual solvent peak as the reference. Mass Spectra were recorded on a Finnigan MAT-311A (Germany) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis was performed on a Carlo Erba Strumentazion-Mod-1106 Italy. Thin layer chromatography was performed on pre-coated silica gel plates (Kieselgel 60, F254 Merck) and the chromatograms were visualized under UV at 254 and 365 nm.

Synthesis of ketoesters

General procedure for the synthesis of bromoacetophenones (2a-h)

Substituted acetophenones (1a-h) were brominated to afford (2a-h) using the literature procedure (Tümer *et al*., 2004). The bromoacetophenones were obtained in 70-83% yield.

General procedure for the synthesis of keto ester derivatives (4a-p)

To a stirred solution of corresponding benzoic acid 3 (1.0 mmol) in *N*,*N*-dimethylformamide (4 mL) was added triethylamine (3-4 drops) at room temperature. After stirring for 30 min, the corresponding phenacyl bromide 2 (1.0 mmol) was added and the reaction mixture was stirred at room temperature for 2 h (Soural and Krchňák, 2007). The reaction mass was poured into water and extracted with ethyl acetate (2×15 mL) and excess solvent was evaporated under reduced pressure on rotary evaporator to afford the corresponding keto ester (4). The title compounds were purified by recrystallization in ethanol.

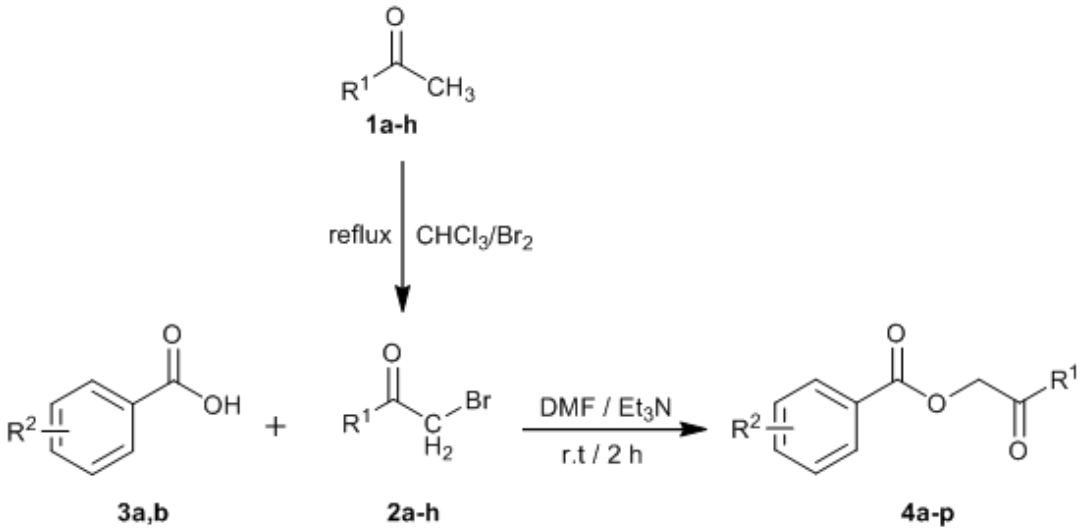
Pharmacological screening

Antibacterial assay

The antibacterial activities of synthesized compounds were determined using agar well diffusion method (Boakye *et al*., 1977). Gram-positive bacteria (*Staphylococcus aureus, Micrococcus leuteus*) and Gram-negative (*Pseudomonas picketti, Salmonella setuball*) were used as standard bacterial strains of human pathogens. The samples were prepared by dissolving compounds in μg/mL of organic solvent (10% DMSO). The inocula of the respective bacteria were streaked on to the nutrient agar plates using a sterile cotton swab in such a way to ensure thorough coverage of the plates. Wells were cut out on to nutrient agar plates using a sterile cork borer (6 mm) streaked with the respective bacterial strains and filled with 20 μL of test sample and the standard drug, ciprofloxacin (5 μg/mL) into separate well. The plates were allowed to stay for 1-2 h at room temperature. Finally, the plates were incubated at 37 °C for 24 h and the resulting diameters of zones (mm) of inhibition were measured.

Antifungal assay

Agar tube dilution assay (Rehman *et al*., 2001) was used for the determination of the antifungal activity. Two fungal pathogenic strains (*Aspergillus niger, Aspergillus flavus*) were used in the antifungal assay. Test sample was dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar (SDA) was prepared in the usual



Scheme 1: Synthetic protocol for the preparation of keto ester derivatives (4a-p).

manner and known amount of the media was dispensed into screw caped test tubes with final concentration 400 μg/mL. Test tubes containing the media were autoclaved at 121 °C for 15 minutes and allowed to cool to 50 °C. Test sample of the desired concentration was pipetted from the stock solution into the non-solidified SDA media and allowed to solidify in a slanting position at room temperature. For inoculation sterile wire was used to cut a small piece (4 mm) from the seven day old fungus culture. All cultures containing the cultures were then incubated at optimum temperature of 27 °C for growth for 7 days in case of molds and 3 days in case of yeast. Humidity (40-50%) was controlled by placing an open pan of water in the incubator. After the incubation period of 7 days the test tube with no visible fungal growth was taken to represent the represent the activity in terms of inhibition by the test sample which was expressed in μg/mL. Growth in the media containing the test sample was determined by measuring the growth (mm).

Crystal data and structure determination

Suitable single crystals of the target compounds (4b and 4l) were selected and flash cooled to 130K using an Oxford Cryostream cooling device. The reflection data for the title compounds were collected on an Oxford Super Nova CCD diffract ometer using Cu-Kα (l=1.54184 Å) X-radiation. The structures were solved by direct methods and refined by full-matrix least squares using *SHELX*-97 (Sheldrick, 2008).

RESULTS

Chemistry

Aromatic acids (3a,b) were reacted with substituted phenacyl bromides (2a-h), prepared by the bromination of various acetophenones (Tümer *et al*., 2004) in dimethyl-formamide under basic conditions to afford the title compounds (4a-p) in good yields as outlined in Scheme-1 (Soural and Krchňák, 2007). The physical data of the synthesized compounds (4a-p) is presented in table 1.

Table 1: Physical data of synthesized compounds (4a-p).

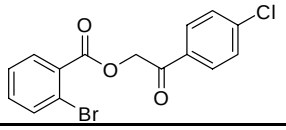
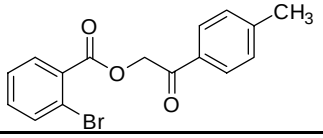
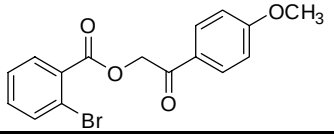
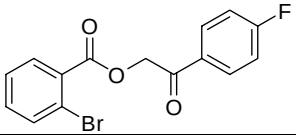
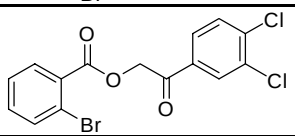
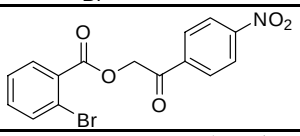
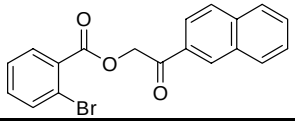
Entry	Product 4	Yield (%)	M.P (°C)	R_f*
4a		79	94-95	0.25
4b		75	91-92	0.27
4c		80	107-108	0.20
4d		78	98-99	0.35
4e		75	84-85	0.23
4f		72	92-93	0.14
4g		74	88-89	0.34

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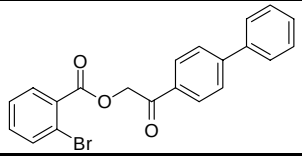
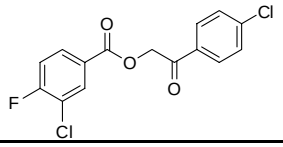
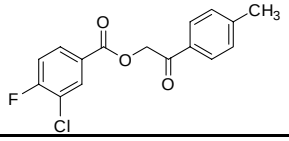
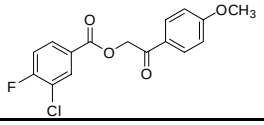
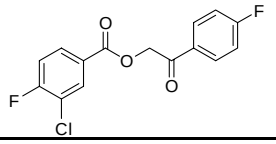
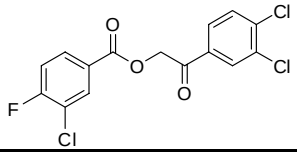
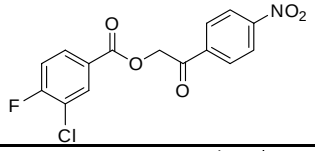
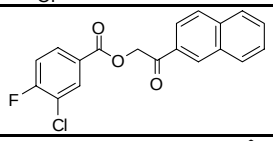
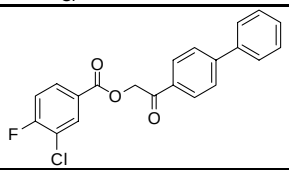
Table 1: Physical data of synthesized compounds (4a-p).

Entry	Product 4	Yield (%)	M.P (°C)	R_f*
4h		80	101-102	0.48
4i		77	121-122	0.61
4j		82	137-138	0.42
4k		78	134-135	0.35
4l		81	104-105	0.41
4m		75	110-111	0.53
4n		74	116-117	0.24
4o		76	126-127	0.40
4p		75	130-131	0.42

Spectroscopic characterization

2-(4-Chlorophenyl)-2-oxoethyl 2-bromobenzoate (4a)

The general experimental procedure described above afforded (4a) as an off-white solid. IR (ATR, cm^{-1}): 3085 (C_{sp2}-H), 2932, 2868 (C_{sp3}-H), 1724 ($C{=}O_{ester}$), 1698 ($C{=}O_{ketone}$), 1584, 1471 (C=C), 1224 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08-8.06 (m, 1H, Ar-H), 7.99-7.94 (m, 2H, Ar-H), 7.80-7.77 (m, 1H, Ar-H), 7.58-7.50 (m, 2H, Ar-H), 7.49-7.36 (m, 2H, Ar-H), 5.57 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.6, 165.4, 140.6, 134.6, 133.2, 132.5, 132.1, 130.9, 129.4, 129.4, 127.4, 122.6, 66.6. EIMS: m/z353 (M^+). Anal. Calcd. for $C_{15}H_{10}BrClO_3$: C, 50.95; H, 2.85. Found: C, 50.81; H, 2.72.

2-Oxo-2-p-tolylethyl 2-bromobenzoate (4b)

The general experimental procedure described above afforded (4b) as brown solid. IR (ATR, cm^{-1}): 3031 (C_{sp2}-H), 2924, 2844 (C_{sp3}-H), 1728 ($C{=}O_{ester}$), 1685 ($C{=}O_{ketone}$), 1603, 1481 (C=C), 1230 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06-8.02 (m, 1H, Ar-H), 7.98-7.91 (m, 2H, Ar-H), 7.78-7.73 (m, 1H, Ar-H), 7.56-7.52 (m, 2H, Ar-H), 7.46-7.36 (m, 2H, Ar-H), 5.57 (s, 2H, OCH_2),

2.44 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.7, 165.8, 140.4, 134.3, 133.2, 132.4, 132.0, 130.9, 129.4, 129.4, 127.3, 122.1, 66.6, 21.8. EIMS: m/z 333 (M^+). Anal. Calcd. for $C_{16}H_{13}BrO_3$: C, 57.68; H, 3.93. Found: C, 57.49; H, 3.80.

2-(4-Methoxyphenyl)-2-oxoethyl 2-bromobenzoate (4c)

The general experimental procedure described above afforded (4c) as light brown solid. IR (ATR, cm^{-1}): 3011 (C_{sp2}-H), 2946, 2842 (C_{sp3}-H), 1728 (C=O_{ester}), 1682 (C=O_{ketone}), 1598, 1571 (C=C), 1233 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.08-8.04 (m, 1H, Ar-H), 7.99-7.94 (m, 2H, Ar-H), 7.72-7.69 (m, 1H, Ar-H), 7.45-7.35 (m, 2H, Ar-H), 7.02-6.97 (m, 2H, Ar-H), 5.57 (s, 2H, OCH_2), 3.90 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.2, 165.5, 164.1, 134.4, 133.0, 132.0, 131.3, 130.2, 127.3, 127.1, 122.0, 114.1, 66.4, 55.6. EIMS: m/z 349 (M^+). Anal. Calcd. for $C_{16}H_{13}BrO_4$: C, 55.04; H, 3.75. Found: C, 54.91; H, 3.59.

2-(4-Fluorophenyl)-2-oxoethyl 2-bromobenzoate (4d)

The general experimental procedure described above afforded (4d) as light brown solid. IR (ATR, cm^{-1}): 3015 (C_{sp2}-H), 2950, 2840 (C_{sp3}-H), 1732 (C=O_{ester}), 1697 (C=O_{ketone}), 1594, 1506 (C=C), 1223 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.06-7.99 (m, 3H, Ar-H), 7.73-7.70 (m, 1H, Ar-H), 7.46-7.36 (m, 2H, Ar-H), 7.24-7.18 (m, 2H, Ar-H), 5.57 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.2, 167.9, 165.3, 134.5, 133.1, 132.0, 131.0, 130.6, 130.5, 130.5, 127.3, 122.1, 116.4, 116.1, 66.5. EIMS: m/z 337 (M^+). Anal. Calcd. for $C_{15}H_{10}BrFO_3$: C, 53.44; H, 2.99. Found: C, 53.30; H, 2.81.

2-(3,4-Dichlorophenyl)-2-oxoethyl 2-bromobenzoate (4e)

The general experimental procedure described above afforded (4e) as brown solid. IR (ATR, cm^{-1}): 3101 (C_{sp2}-H), 2926, 2844 (C_{sp3}-H), 1728 (C=O_{ester}), 1701 (C=O_{ketone}), 1584, 1489 (C=C), 1213 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.07 (d, 1H, *J*=2.1 Hz, Ar-H), 8.04-8.01 (m, 1H, Ar-H), 7.80 (dd, 1H, *J*=8.4, 1.8 Hz, Ar-H), 7.73-7.70 (m, 1H, Ar-H), 7.60 (s, 1H, Ar-H), 7.46-7.37 (m, 2H, Ar-H), 5.54 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.9, 165.3, 138.7, 134.5, 133.8, 133.6, 133.2, 132.0, 131.1, 130.8, 129.9, 127.3, 126.8, 122.2, 66.5. EIMS: m/z 388 (M^+). Anal. Calcd. for $C_{15}H_9BrCl_2O_3$: C, 46.43; H, 2.34. Found: C, 46.34; H, 2.47.

2-(4-Nitrophenyl)-2-oxoethyl 2-bromobenzoate (4f)

The general experimental procedure described above afforded (4f) as brown solid. IR (ATR, cm^{-1}): 3045 (C_{sp2}-H), 2946, 2837 (C_{sp3}-H), 1725 (C=O_{ester}), 1698 (C=O_{ketone}), 1574, 1491 (C=C), 1227 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.37-8.32 (m, 2H, Ar-H), 8.29-8.24 (m, 2H, Ar-H), 8.07-8.03 (m, 1H, Ar-H), 7.97-7.93 (m, 2H, Ar-H), 7.70-7.66 (m, 1H, Ar-H), 5.57 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.1, 165.3, 148.8, 146.5, 138.7, 134.4, 133.3, 132.6, 132.1, 131.0, 129.9, 127.3, 66.4. EIMS: m/z 364 (M^+). Anal. Calcd. for $C_{15}H_{10}BrNO_5$: C, 49.47; H, 2.77; N, 3.85. Found: C, 49.33; H, 2.64; N, 3.68.

2-(Naphthalen-2-yl)-2-oxoethyl 2-bromobenzoate (4g)

The general experimental procedure described above afforded (4g) as brown solid. IR (ATR, cm^{-1}): 3054 (C_{sp2}-H), 2925, 2847 (C_{sp3}-H), 1732 (C=O_{ester}), 1687 (C=O_{ketone}), 1625, 1590 (C=C), 1246 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.50 (s, 1H, Ar-H), 8.10-7.90 (m, 5H, Ar-H), 7.73-7.70 (m, 1H, Ar-H), 7.69-7.58 (m, 2H, Ar-H), 7.47-7.36 (m, 2H, Ar-H), 5.75 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.7, 165.5, 136.0, 134.4, 133.0, 132.4, 132.0, 131.5, 129.7, 129.6, 129.0, 128.9, 127.9, 127.3, 127.1, 123.3, 122.1, 66.8. EIMS: m/z 369 (M^+). Anal. Calcd. for $C_{19}H_{13}BrO_3$: C, 61.81; H, 3.55. Found: C, 61.61; H, 3.34.

2-(Biphenyl-4-yl)-2-oxoethyl 2-bromobenzoate (4h)

The general experimental procedure described above afforded (4h) as brown solid. IR (ATR, cm^{-1}): 3037 (C_{sp2}-H), 2933, 2842 (C_{sp3}-H), 1732 (C=O_{ester}), 1692 (C=O_{ketone}), 1600, 1560 (C=C), 1226 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.10-8.04 (m, 3H, Ar-H), 7.77-7.73 (m, 2H, Ar-H), 7.68-7.64 (m, 3H, Ar-H), 7.54-7.39 (m, 5H, Ar-H), 5.65 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.3, 165.4, 146.7, 139.6, 134.5, 133.1, 132.8, 132.0, 131.2, 130.4, 129.1, 128.5, 128.5, 127.6, 127.3, 122.1, 66.7. EIMS: m/z 395 (M^+). Anal. Calcd. for $C_{21}H_{15}BrO_3$: C, 63.81; H, 3.83. Found: C, 63.67; H, 3.70.

2-(4-Chlorophenyl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4i)

The general experimental procedure described above afforded (4i) as light brown solid. IR (ATR, cm^{-1}): 3052 (C_{sp2}-H), 2939, 2845 (C_{sp3}-H), 1717 (C=O_{ester}), 1698 (C=O_{ketone}), 1588, 1494 (C=C), 1217 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.23 (dd, 1H, *J* = 2.1, 2.1 Hz, Ar-H), 8.08-8.02 (m, 1H, Ar-H), 7.93-7.90 (m, 2H, Ar-H), 7.53-7.49 (m, 2H, Ar-H), 7.28-7.22 (m, 1H, Ar-H), 5.56 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.6, 164.1, 163.1, 159.7, 140.7, 132.3, 129.4, 126.5, 121.8, 121.6, 117.0, 116.7, 66.6. EIMS: m/z 327 (M^+). Anal. Calcd. for $C_{15}H_9C_{l2}FO_3$: C, 55.07; H, 2.77. Found: C, 55.17; H, 2.62.

2-Oxo-2-p-tolylethyl 3-chloro-4-fluorobenzoate (4j)

The general experimental procedure described above afforded (**4j**) as off white solid. IR (ATR, cm^{-1}): 3054 (C_{sp2}-H), 2936, 2841 (C_{sp3}-H), 1722 (C=O_{ester}), 1694 (C=O_{ketone}), 1593, 1494 (C=C), 1223 (C-O); ^1H NMR (300 MHz, $CDCl_3$): δ 8.24 (dd, 1H, *J*=2.1, 2.1 Hz, Ar-H), 8.09-8.04 (m, 1H, Ar-H), 7.87 (d, 2H, *J*=8.1 Hz, Ar-H), 7.34-7.22 (m, 3H, Ar-H), 5.58 (s, 2H, OCH_2), 2.45 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.2, 164.2, 163.0, 159.6, 145.1, 132.8, 130.5, 126.7, 121.7, 121.5, 116.9, 116.6, 66.7, 21.8. EIMS: m/z 306 (M^+). Anal. Calcd. for $C_{16}H_{12}ClFO_3$: C, 62.65; H, 3.94. Found: C, 62.51; H, 3.99.

Table 2: Crystal data and structure refinement parameters for (4b) and (4l).

Structural parameters	Compound 4b	Compound 4l
Empirical formula	$C_{16}H_{13}BrO_3$	$C_{15}H_9ClF_2O_3$
Formula weight	333.17	310.67
Temperature (K)	130.0(1)	130.0(1)
Wavelength (Å)	1.5418	1.5418
Crystal system	Monoclinic	Monoclinic
Space group	P21	P 1 21/n 1
Unit cell dimensions (Å)	a = 8.0295(18)	a = 14.671(2)
	b = 4.9979(6)	b = 5.0698(3)
	c = 17.450(2)	c = 18.038(3)
	β = 98.21(1)°	β = 104.734(16)°
Volume (Å^3)	693.10(19)	1297.5(3)
Z	4	2
Density (calculated) (mg/m^3)	1.596	1.590
Absorption coefficient (mm^{-1})	4.086	2.929
F(000)	336	632
Crystal size (mm^3)	0.35 × 0.28 × 0.08	0.58 × 0.11 × 0.03
Theta range for data collection (°)	5.12 to 67.44	3.48 to 67.49
Reflections collected	2275	4344
Independent reflections	1660 [R(int) = 0.0160]	2313 [R(int) = 0.0363]
Completeness to theta = 67.50∞ (%)	99.6	99.3
Absorption correction	Gaussian	Analytical
Max. and min. transmission	0.747 and 0.372	0.907 and 0.486
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	1660 / 1 / 182	2313 / 0 / 190
Goodness-of-fit on F^2	1.073	1.014
Final R indices [I>2σ(I)]	R_1 = 0.0228, wR_2 = 0.0615	R_1 = 0.042, wR_2 = 0.10
R indices (all data)	R_1 = 0.0228, wR_2 = 0.0615	R_1 = 0.055, wR_2 = 0.111
Largest diff. peak and hole (e.Å^{-3})	0.407 and -0.453	0.475 and -0.293
CCDC	931629	931630

2-(4-Methoxyphenyl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4k)

The general experimental procedure described above afforded (4k) as white solid. IR (ATR, cm^{-1}): 3048 (C_{sp2}-H), 2941, 2844 (C_{sp3}-H), 1720 (C=O_{ester}), 1687 (C=O_{ketone}), 1594, 1573 (C=C), 1217 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (dd, 1H, J=2.1, 2.1 Hz, Ar-H), 8.09-8.04 (m, 1H, Ar-H), 7.98-7.93 (m, 2H, Ar-H), 7.26 (d, 1H, J=8.7 Hz, Ar-H), 7.02-6.97 (m, 2H, Ar-H), 5.56 (s, 2H, OCH_2), 3.91 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.0, 164.2, 163.0, 159.6, 132.6, 130.5, 126.7, 121.7, 121.5, 116.9, 116.6, 114.2, 66.5, 55.6. EIMS: m/z 322 (M^+). Anal. Calcd. for $C_{16}H_{12}ClFO_4$: C, 59.55; H, 3.75. Found: C, 59.41; H, 3.60.

2-(4-Fluorophenyl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4l)

The general experimental procedure described above afforded (4l) as an off-white solid. IR (ATR, cm^{-1}): 3054 (C_{sp2}-H), 2955, 2834 (C_{sp3}-H), 1723 (C=O_{ester}), 1702 (C=O_{ketone}), 1592, 1496 (C=C), 1218 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.23 (dd, 1H, J=8.7, 2.1 Hz, Ar-H), 8.07-7.99 (m, 3H, Ar-H), 7.29-7.18 (m, 3H, Ar-H), 5.57 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.1, 167.9, 164.5, 164.1, 163.1, 159.7, 130.6, 126.5, 121.8, 121.6, 116.7, 116.4, 66.5. EIMS: m/z 310 (M^+). Anal. Calcd. for $C_{15}H_9ClF_2O_3$: C, 57.99; H, 2.92. Found: C, 57.81; H, 2.74.

2-(3,4-Dichlorophenyl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4m)

The general experimental procedure described above afforded (4m) as brown solid. IR (ATR, cm^{-1}): 3064 (C_{sp2}-H), 2973, 2938 (C_{sp3}-H), 1718 (C=O_{ester}), 1699 (C=O_{ketone}), 1582, 1494 (C=C), 1216 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24-8.17 (m, 1H, Ar-H), 8.08-8.02 (m, 2H, Ar-H), 7.81-7.78 (dd, 1H, J = 2.1, 2.1 Hz, Ar-H), 7.63 (d, 1H, J=8.4 Hz, Ar-H), 7.29-7.23 (m, 1H, Ar-H), 5.53 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 189.8, 164.0, 163.2, 138.8, 133.8, 133.5, 132.8, 130.5, 130.4, 129.8, 126.3, 121.9, 117.0, 116.7, 66.5. EIMS: m/z 361 (M^+). Anal. Calcd. for $C_{15}H_8Cl_3FO_3$: C, 49.83; H, 2.23. Found: C, 49.70; H, 2.11.

Table 3: Selected bond distances (Å), angles and dihedral angles (°) for (4b) and (4l).

	Compound 4b	Compound 4l
Bond distances (Å)		
C (1)-C (7)	1.493(4)	1.483(3)
C (7)-C (8)	1.520(4)	1.518(3)
C (8)-O (3)	1.433(3)	1.427(3)
C (9)-O (3)	1.348(4)	1.348(3)
C (9)-O (2)	1.202(3)	1.202(3)
C (9)-C (10)	1.498(3)	1.478(3)
Bond angles (°)		
C (1)-C (7)-C (8)	116.8(3)	117.8(2)
O (1)-C (7)-C (8)	121.3(3)	120.7(2)
O (3)-C (8)-C (7)	111.0(2)	111.38(19)
C (9)-O (3)-C (8)	114.1(2)	114.79(18)
O (3)-C (9)-C (10)	111.4(2)	112.8(2)
O (2)-C (9)-O (3)	122.8(2)	122.9(2)
Dihedral angles (°)		
O (1)-C (7)-C (8)-O (3)	6.3(4)	1.1(3)
C (1)-C (7)-C (8)-O (3)	-174.9(2)	-178.42(18)
C (7)-C (8)-O (3)-C (9)	73.7(3)	73.3(2)
C (10)-C (9)-O (3)-C (8)	-179.8(2)	-179.84(18)
O (2)-C (9)-O (3)-C (8)	-0.8(4)	2.1(3)

2-(4-Nitrophenyl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4n)

The general experimental procedure described above afforded (4n) as brown solid. IR (ATR, cm^{-1}): 3052 (C_{sp2}-H), 2951, 2844 (C_{sp3}-H), 1724 ($C{=}O_{ester}$), 1697 ($C{=}O_{ketone}$), 1582, 1490 (C=C), 1224 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.37-8.33 (m, 2H, Ar-H), 8.30-8.26 (m, 2H, Ar-H), 8.24 (dd, 1H, *J*=2.1, 2.1 Hz, Ar-H), 8.07-7.99 (m, 1H, Ar-H), 7.33-7.26 (m, 1H, Ar-H), 5.58 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.2, 167.9, 164.2, 163.0, 159.6, 148.8, 132.8, 130.6, 126.7, 121.6, 117.0, 116.6, 66.5. EIMS: m/z 337 (M^+). Anal. Calcd. for $C_{15}H_9ClFNO_5$: C, 53.35; H, 2.69; N, 4.15. Found: C, 53.18; H, 2.77; N, 4.00.

2-(Naphthalen-2-yl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4o)

The general experimental procedure described above afforded (4o) as light brown solid. IR (ATR, cm^{-1}): 3056 (C_{sp2}-H), 2948, 2854 (C_{sp3}-H), 1721 ($C{=}O_{ester}$), 1692 ($C{=}O_{ketone}$), 1590, 1496 (C=C), 1227 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.50 (s, 1H, Ar-H), 8.23 (dd, 1H, *J* = 2.1, 2.1 Hz, Ar-H), 8.10-7.90 (m, 5H, Ar-H), 7.73-7.70 (m, 3H, Ar-H), 5.74 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.5, 165.5, 164.4, 164.1, 136.0, 133.0, 132.4, 131.5, 130.6, 130.5, 129.7, 129.6, 126.6, 126.5, 121.8, 121.5, 117.0, 116.7, 66.7. EIMS: m/z 342 (M^+). Anal. Calcd. for $C_{19}H_{12}ClFO_3$: C, 66.58; H, 3.53. Found: C, 66.46; H, 3.34.

2-(Biphenyl-4-yl)-2-oxoethyl 3-chloro-4-fluorobenzoate (4p)

The general experimental procedure described above afforded (4p) as brown solid. IR (ATR, cm^{-1}): 3049 (C_{sp2}-H), 2942, 2858 (C_{sp2}-H), 1720 ($C{=}O_{ester}$), 1701 ($C{=}O_{ketone}$), 1578, 1482 (C=C), 1223 (C-O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.24 (dd, 1H, *J*=2.1, 2.1 Hz, Ar-H), 8.10-7.99 (m, 6H, Ar-H), 7.77-7.73 (m, 2H, Ar-H), 7.68-7.64 (m, 3H, Ar-H), 5.65 (s, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.3, 167.9, 165.4, 164.4, 146.7, 139.6, 134.5, 133.1, 132.9, 131.2, 130.6, 128.5, 126.5, 121.8, 117.0, 116.6, 66.5. EIMS: m/z 368 (M^+). Anal. Calcd. for $C_{21}H_{14}ClFO_3$: C, 68.39; H, 3.83. Found: C, 68.29; H, 3.71.

X-ray crystallography

In parallel, for a full structural elucidation, structures of compounds (4b) and (4l) were established by X-ray crystallography (Sheldrick, 2008). fig. 1 demonstrates the molecular structures of compounds (4b) and (4l) with displacement ellipsoids at the 50% probability level, respectively. The crystal and instrumental parameters used in the unit cell determination, the data collection, and structure refinement parameters are presented in table 2 whereas the selected bond distances, angles and dihedral angles are listed in table 3.

Antimicrobial activity

In vitro antimicrobial screening of the newly synthesized compounds was carried out by agar well diffusion method (Boakye *et al*., 1977) and agar tube dilution assay (Rehman *et al*., 2001) at Minimum Inhibitory Concentrations (MICs) against different strains. Ciprofloxacin and amphotericin were used as positive controls for bacteria and fungi, respectively. The results of this study are presented in tables 4 and 5.

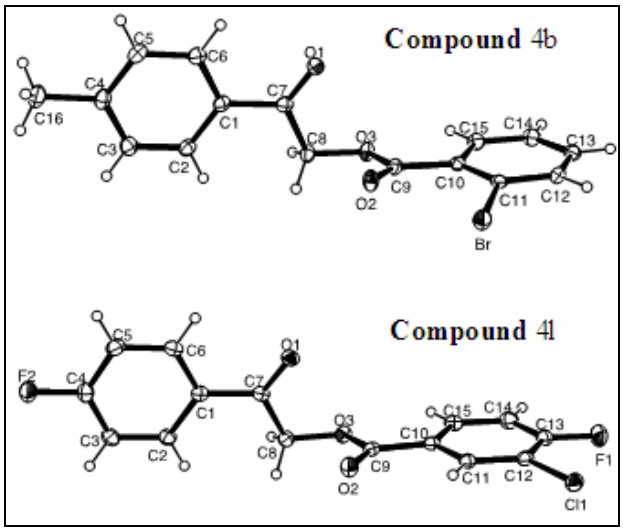


Fig. 1: Molecular structures of (4b and 4l) with displacement ellipsoids at the 50% probability level

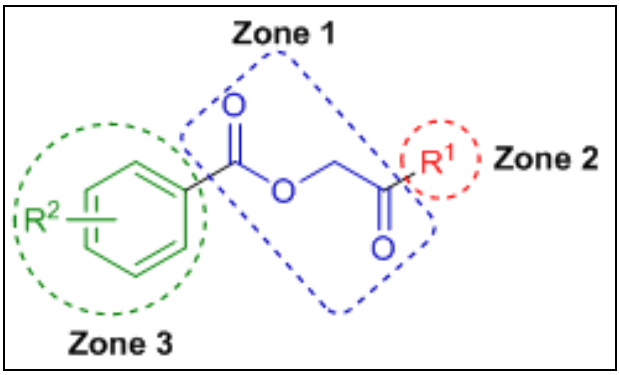


Fig. 2: Three-zone approach model for structure-activity relationship analysis of keto esters (4a-p).

DISCUSSION

Chemistry

The formation of keto esters (4a-p) was indicated by the appearance of typical stretching vibrations $\nu C{=}O_{ester}$ (1732-1717) and $\nu C{=}O_{Keto}$ (1702-1687) cm^{-1}, and the disappearance of characteristic IR stretching absorptions ascribable to carboxylic acid group in the region of 3400-2400 cm^{-1}, respectively. In ^{1}H NMR spectra, the disappearance of characteristic signal for COOH proton also confirmed the formation of title compounds (4a-p).

The appearance of characteristic singlet at 5.75-5.53 ppm assigned to methylene group also confirmed the formation of target compounds. The ^{13}C NMR spectra displayed two characteristic signals for keto and ester carbonyls in the range of 191.7-189.8 and 168.0-164.0 ppm, respectively, along with the expected number of signals for aromatic and methylene carbons at appropriate chemical shift values, confirming the formation of compounds (4a-p). The synthesized compounds were further confirmed by mass spectrometry and the purity was ascertained by elemental analysis.

X-ray crystallography

The structures of compounds (4b) and (4l) were unambiguously established by X-ray crystallography. The conformation about the O3-C8 bond is identical in both structures and compares with many of the acyloxybenzoate derivatives in the literature which adopt either a gauche conformation about this bond an antiperiplanar conformation, or crystallize with Z'>2 with both conformations represented (Fun *et al*., 2011; Jin *et al*., 2008).

Pharmacological studies

Antimicrobial activity

In vitro antimicrobial screening of the newly synthesized compounds was carried out by agar well diffusion method (Boakye *et al*., 1977) and agar tube dilution assay (Rehman *et al*., 2001) at Minimum Inhibitory Concentrations (MICs) against different strains. Ciprofloxacin and amphotericin were used as positive controls for bacteria and fungi, respectively. The preliminary results revealed that the synthesized compounds exhibited moderate to good activity against the tested bacterial and fungal strains.

Structure-activity relationship analysis

On the basis of antimicrobial screening results obtained for the prepared structures, we have used a three-zone approach to analyze the results and to guide our initial round of medicinal chemistry structure-activity relationship studies (fig. 2). In particular, zone 1, the core scaffold has been found to be non-influential on the activity results. The zone 2 possessed a broad variation of substituents on the aryl ring and in certain cases aryl ring itself. The investigation of these exclusive modifications combined with zone 3 least alterations indicated the generation of lead structures with improved activity.

The results of antimicrobial studies are presented in table 4 along with MIC values in table 5. Among the tested compounds, several derivatives like (4b-c), (4h-i), (4l), (4n), and (4p) exhibited significant antibacterial activity at variable concentrations against different bacterial strains. But, among them, compounds (4b) and (4l) turned out to be the lead molecules with highest antibacterial activity (comparable to ciprofloxacin, used as a positive control) against *Micrococcus leuteus* at MIC values of 1.5625 and 3.125 (μg/mL), respectively. This highest activity may be attributed to the presence of methyl and fluoro groups at *para*-position on aryl ring in zone 2 indicating that both electron-donating and electron-withdrawing groups are equally potent. If we observe first set of compounds (4a-h), where only variants are in the zone 2, the maximum antibacterial activity was shown by compound (4b), and (4g) exhibited least activity against *Salmonella setuball* at MIC value of 1.5625 (μg/mL), which might be due to the presence of bulkier naphthyl group instead of substituted aryl ring. On moving from methyl substituted aryl ring to biphenyl group (4h), a slight decrease in activity was

Table 4: Inhibitory zone (diameter, mm) of compounds (4a-p) against bacterial and fungal strains.

Compounds	Mean zone inhibition[a]					
	P.p	*S.s*	*S.a*	*M.l*	*A.n*	*A.f*
4a	10	13	11	12	11	13
4b	7	12	14	18	12	12
4c	8	12	19	9	17	16
4d	9	14	12	8	15	17
4e	10	11	14	12	13	11
4f	13	10	8	14	14	15
4g	8	1	9	11	16	19
4h	10	9	13	15	14	11
4i	8	4	11	15	12	8
4j	5	12	13	12	16	13
4k	10	18	8	9	11	14
4l	9	12	13	18	12	12
4m	8	9	8	11	22	18
4n	11	13	19	17	15	17
4o	9	7	8	12	13	12
4p	12	8	14	16	16	10
Ciprofloxacin	20	24	22	18	-	-
Amphotericin	-	-	-	-	28	32

Ciprofloxacin (5μg/mL) and amphotericin (5μg/mL) were used as positive controls. Compounds (4a-p) (25μg/mL); *P.p*., *Pseudomonas picketti*; *S.s*., *Salmonella setuball*; *S.a*., *Staphylococcus aureus*; *M.l*., *Micrococcus leuteus*; *A.n*., *Aspergillus niger*; *A.f*., *Aspergillus flavus*. [a]Values are mean (n=3).

Table 5: Inhibitory activity of compounds (4a-p) expressed as MIC (μg/mL).

Compounds	Microorganism					
	P.p	*S.s*	*S.a*	*M.l*	*A.n*	*A.f*
4a	1.5625	3.125	3.125	1.5625	6.25	6.25
4b	3.125	3.125	6.25	1.5625	6.25	3.125
4c	3.125	1.5625	6.25	3.125	3.125	6.25
4d	3.125	1.5625	1.5625	1.5625	3.125	6.25
4e	1.5625	1.5625	1.5625	12.5	6.25	3.125
4f	1.5625	3.125	6.25	1.5625	6.25	6.25
4g	6.25	1.5625	1.5625	12.5	1.5625	1.5625
4h	3.125	3.125	3.125	1.5625	1.5625	3.125
4i	6.25	6.25	3.125	3.125	6.25	6.25
4j	12.5	1.5625	1.5625	6.25	12.5	6.25
4k	6.25	1.5625	6.25	6.25	12.5	6.25
4l	3.125	6.25	3.125	3.125	6.25	3.125
4m	1.5625	1.5625	6.25	6.25	1.5625	1.5625
4n	1.5625	3.125	1.5625	1.5625	1.5625	1.5625
4o	3.125	6.25	6.25	3.125	6.25	3.125
4p	1.5625	6.25	3.125	1.5625	6.25	3.125
Ciprofloxacin	1.5625	3.125	1.5625	1.5625	-	-
Amphotericin	-	-	-	-	1.5625	3.125

observed. Compound (4c) with electron-donating methoxy group possessed moderate activity against *Staphylococcus aureus*. On the other hand, the second set of compounds (4i-p), where zone 2 variants are the same while zone 3 substituents are different. Among these compounds, (4l) exhibited maximum activity comparable to the standard, which may be attributed to the presence of chloro and fluoro (electronegative) groups on aryl ring in zone 3. The replacement of chloro group with fluoro on aryl ring in zone 2 leads to a decreased activity as shown by compound (4i), against *M. Leuteus*, and least activity against *S. aureus*. In addition, replacement of fluoro substituent with electron-withdrawing nitro group (4n) and phenyl group (4p) at *para*-position of aryl ring leads

to a small decrease in activity. Similarly, compound (4n) also found to possess highest activity against *S. aureus* at MIC value of 1.5625 (μg/mL). Moreover, compound (4k) with electron-rich methoxy group possessed highest activity within the tested series against *Salmonella setuball* at MIC value of 1.5625 (μg/mL).

Overall, the change of substituents on both aryl rings in zone 2 and 3 with bulkier groups like biphenyl and naphthyl leads to decreased activity. The electronegative substituents like chloro group on aryl ring in compounds (4a) and (4i) depicted moderate activity against most of the tested bacterial strains and in some cases showed poor activity.

Similarly, the synthesized compounds have also been evaluated for antifungal activity against *Aspergillus niger* and *Aspergillus flavus*, and the results revealed low to moderate inhibition. The compounds (4m) and (4g) comprising halo substituents like 3-chloro-4-fluoro and bromo in zone 3 and 3,4-dichloro on aryl ring and bulkier naphthyl group in zone 2 exhibited the highest antifungal inhibition against *A. niger* and *A. flavus* at MIC value of 1.5625 (μg/mL), respectively. Rest of the compounds including (4c-d), (4j) and (4n-p) showed moderate activity against both pathogenic fungal strains at variable MIC values. Overall, the synthesized structures were found to possess better antibacterial efficacy as compared to the antifungal activity.

CONCLUSION

In summary, the present study reports an efficient and convenient synthesis of various substituted keto ester derivatives, which were characterized by analytical and spectroscopic data. Good to excellent yields were obtained for a wide range of aryl partners with electron-rich and electron-poor substituents. The structures of (4b) and (4l) were explicitly confirmed by X-ray diffraction analysis. The activity results revealed that the structures incorporating methyl and fluoro substituents on the *para*-position of aryl rings (4b) and (4l), respectively, exhibited potent antibacterial activity against *Micrococcus leuteus*, comparable to the ciprofloxacin, used as a positive control. This methodology allows rapid access to a diverse variety of keto ester products and could open the way to the design of new biologically active compounds with appropriate structural requirements. The development of mechanism of action and application of keto esters as viable functional handles in heterocyclic chemistry is the subject of ongoing efforts in our group.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY MATERIAL

Crystallographic data for the structures (4b and 4l) reported in this paper have been deposited with the Cambridge Crystallographic Center. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

REFERENCES

Boakye YK, Fiagbe N and Ayim J (1977). Antimicrobial properties of some west African medicinal plants. *J. Nat. Prod*., **40**: 543-545.

Chopra I, Schofield C, Everett M, O'Neill A, Miller K, Wilcox M, Frere JM, Dawson M, Czaplewski L, Urleb U and Courvalin P (2008). Treatment of health-care-associated infections caused by Gram-negative bacteria: A consensus statement. *Lancet Infect Dis*., **8**: 133-139.

Coates A, Hu YM, Bax R and Page C (2002). The future challenges facing the development of new antimicrobial drugs. *Nat. Rev. Drug Discov*., **1**: 895-910.

Fernandes P (2006). Antibacterial discovery and development the failure of success? *Nat. Biotechnol*., **24**: 1497-1503.

Fun HK, Loh WS, Garudachari B, Isloor AM and Satyganarayan MN (2011). 2-(4-Chlorophenyl)-2-oxoethyl 3-(trifluoromethyl) benzoate. *Acta. Cryst*., **E67**: o1597-o1598.

Fun HK, Quah CK, Garudachari B, Isloor AM and Satyanarayan MN (2011). 2-(4-Bromophenyl)-2-oxoethyl 4-methylbenzoate. *Acta. Cryst*., **E67**: o3154.

Fun HK, Shahani T, Garudachari B, Isloor AM, Satyganarayan MN (2011). 2-(4-Chloro-phenyl)-2-oxoethyl benzoate. *Acta. Cryst*., **E67**: o1802.

Gouveia FL, de Oliveira RM, de Oliveira TB, da Silva IM, do Nascimento SC, de Sena KX and de Albuquerque JF (2009). Synthesis, antimicrobial and cytotoxic activities of some 5-arylidene-4-thioxo-thiazolidine-2-ones. *Eur. J. Med. Chem*., **44**: 2038-2043.

Jin Y, Guo JN, Lin K, Tang G and Zhao YF (2008). Benzoyl-methyl 4-chloro-benzoate. *Acta. Cryst*., **E64**: o507.

Kai K, Yano F, Suzuki F, Hideo K, Masayuki S, Mineyuki Y and Naoharu W (2007). Metabolism of α-ketol derivative of linolenic acid (KODA), a flowering-related compound, in *Pharbitis nil*. *Tetrahedron,* **63**: 10630-10636.

Kinoshita M and Umezawa S (1960). Studies on antibiotics and related substances. syntheses of some unsaturated ketocarboxylic acids, anti-tumor substances. *Bull. Chem. Soc. Jpn*., **33**: 1075-1080.

MacLean RC, Hall AR, Perron GG and Buckling A (2010). The population genetics of antibiotic

resistance: integrating molecular mechanisms and treatment contexts. *Nat. Genet*., **11**: 405-414.

Pal S, Mareddy J and Devi NS (2008). High-speed synthesis of pyrazolones using microwave-assisted ATR reaction technology. *J. Braz. Chem. Soc*., **19**: 1207-1214.

Projan SJ (2003). Why is big pharma getting out of antibacterial drug discovery? Curr. *Opin. Microbiol*., **6**: 427-430.

Rehman AU, Choudhary MI and Thomsen WJ (2001). Bioassay techniques for drug development. Harward academic publishers, p.20.

Sheibani H, Islami MR, Hassanpour A and Hosseininasab FA (2006). Nucleophilic substitution of 2,2'-disulfanediyldianiline by *β*-keto esters and 1,3-diketones in the presence of triethylamine. *ARKIVOC* **xv**: 68-75.

Sheibani H, Islami MR, Hassanpur A and Saidi K (2007). One-pot multi-component approach to the synthesis of 1,4-benzothiazines in aqueous media. *Phosphorus Sulfur Silicon Relat. Elem*., **183**: 13-20.

Sheibani H, Mosslemin MH, Behzadi S, Islami MR and Saidi K (2006). A convenient one-pot synthesis of pyrano [3,2-*c*] quinolin-2,5 (6*H*)-dione and 2*H*,5*H*-pyrano [3,2-*c*] chromene-2,5-dione derivatives. *Synthesis,* **3**: 435-439.

Sheldrick GM (2008). A short history of *SHELX*. *Acta. Cryst*., **A64**: 112-122.

Soural M and Krchňák V (2007). Efficient solid-phase synthesis of 2-substituted-3-hydroxy-4(1*H*)-quinolinone-7-carboxamides with two diversity positions. *J. Comb. Chem*., **9**: 793-796.

Stanovnik B and Svete J (2004). Synthesis of heterocycles from alkyl 3-(dimethylamino) propenoates and related enaminones. *Chem. Rev*., **104**: 2433-2480.

Tümer F, Ekinci D, Zilbeyaz K and Demir Ü (2004). An efficient synthesis of substituted 4-aryl-3-cyano-2-amino thiophenes by a stepwise Gewald reaction. *Turk. J. Chem*., **28**: 395-403.

Zhang L, Shen Y and Zhu HJ (2009). Pentanol derivatives from basidiomycete *Catathelasma imperiale* and their 11 *β*-hydroxysteroid dehydrogenases inhibitory activity. *J. Antibiot*., **62**: 239-242.