Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Facile Regioselective Synthesis and Antimicrobial Activity of Novel 8-Isoxazolinyl Coumarins *via* Nitrile Oxide Cycloaddition Approach

Challa Krishna[1,✉], M. Vijaya Bhargavi[2] and G.L. David Krupadanam[1]

Asian Journal of Organic & Medicinal Chemistry

Volume: 2 Year: 2017
Issue: 4 Month: October–December
pp: 149–155
DOI: https://doi.org/10.14233/ajomc.2017.AJOMC-P80

Received: 20 July 2017
Accepted: 11 November 2017
Published: 29 December 2017

ABSTRACT

Ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**9a-e**) and ethyl-3-)7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**11a-e**) were prepared by 1,3-dipolar nitrile oxide cycloaddition reactions of 7-benzyloxy-4-methyl-coumarin aldehyde chloro oxime (**5**) and 7-benzyloxy-3-chloro-4-methyl-coumarin aldehyde chlorooxime (**6**) with ethyl *trans*-cinnamates (**8a-e**) at room temperature. The method is useful for the construction of several biologically active heterocycles. The structures of synthesized compounds are established based on IR, NMR (proton, NOESY) and mass spectrometry. The antimicrobial activity of synthesized compounds were evaluated.

KEYWORDS

Isoxazoline, Chlorooximes, Unsaturated esters, Nitrile oxide, 1,3-Dipolar cycloaddition, Regioselectivity, Antimicrobial activity.

INTRODUCTION

Coumarin and its derivatives are some of the most important oxygen heterocycles and are extensively found in various natural and synthetic products [1]. They are effective pharmacophores, widely used in the drug design and synthesis of novel biologically active compounds [2]. Accordingly, different biological activities such as anticoagulation and cardiovascular activities [3] and antimicrobial activities [4] have been reported. They also possess anticancer [5], anti-inflammatory and antioxidant [6], antiviral [7] and enzyme-inhibition effects [8]. At this juncture, fused coumarin derivatives have attracted attention because of their biological properties [9]. Their antiproliferative [10], anti-inflammatory [11], fluorescent probe imaging [12] have been reported in the literature.

Heterocycles display an array of significant bioactive properties [13,14] and heterocyclic scaffolds are present in a wide variety of drugs as well as drug like molecules of pharmaceutical relevance [15-19]. Among the family of heterocyclic compounds, isoxazoles and isoxazolines are an important class of heterocycles displaying a wide variety of biological properties including antiviral [20,21], antitubulin [22], as well as

Author affiliations:

[1]Department of Chemistry, Faculty of Science, Osmania University, Hyderabad-500 007, India
[2]Department of Pharmacy, Faculty of Technology, Osmania University, Hyderabad-500 007, India

✉To whom correspondence to be addressed:

E-mail: challakrishna76@gmail.com

Available online at: http://ajomc.asianpubs.org

anti-inflammatory activity [23]. The synthesis of this family of heterocycles continues to attract the attention of synthetic organic and medicinal chemists [24,25]. Isoxazolines are important pharmacophores in several compounds of pharmaceutical relevance [26-28]. They are also useful intermediates for synthesis of natural products and biologically active compounds [29,30]. A variety of synthetic methods has been developed for preparation of isoxazolines of which the most convenient and attractive route is probably the 1,3-dipolar cycloaddition of nitrile oxides (NOC) to alkenes [31-33]. Nitrile oxides are commonly generated from aldoximes *via* halogenation using different reagents such as NBS, NCS, NaOCl, *t*-BuOCl, *t*-BuOI *etc*.

In continuation of our research investigation towards the design and development of novel heterocycles, we developed a nitrile oxide cycloaddition (NOC) strategy to synthesize a variety of isoxazolines and isoxazoles. The hydroximoyl chlorides (**5** and **6**) for the 1,3-dipolar cycloaddition was conveniently synthesized in four steps. We envisioned the synthesis of isoxazolines (**9a-e** and **11a-e**) *via* 1,3-dipolar cycloaddition of *in situ* generated nitrile oxides with *trans*-cinnamates (**8a-e**). The required *trans*-cinnamates are synthesized from the corresponding acids *via* esterification method. The synthesized compounds were screened for their antibacterial and antifungal activities at various concentrations.

EXPERIMENTAL

All melting points were obtained on a Polmon instrument, India (model MP96) and are uncorrected. The IR spectra were measured on a Fourier transform infrared spectroscopy Perkin-Elmer 337 (Perkin Elmer instrument company, Massachusetts, USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz, Switzerland using TMS as an internal standard (Chemical shift in δ, ppm). *J*-Values are given in Hz. Mass spectral data were obtained with Agilent 6310 ion trap mass spectrometer, USA. All the materials and solvents were used directly unless otherwise stated. All the compounds synthesized were purified by recrystallization or column chromatography on silica gel (60-120 mesh, Spectrochem, Mumbai, India).

General procedure for the synthesis of hydroximoyl chlorides (5 and 6). To a stirred solution of 7-benzyloxy-4-methyl-coumarin aldehyde oxime (**4**) (1.2 g, 3.88 mmol) in DMF (18 mL) at 0 °C, was added NCS (0.62 g, 4.65 mmol). Upon warming to room temperature over 2 h, the reaction was quenched with H_2O/ice (90 mL), extracted with EtOAc (3 × 30 mL) and washed with brine (30 mL). The dried Na_2SO_4 extract was concentrated and purified by column chromatography to give (**5**). Yield = 1.1 g (syn:anti = 50:50), m.p. 142-143 °C and 3-chlorinated product (**6**). Yield = 0.2 g. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 2.41 (s, 4-CH_3), 5.37 (s, 7-OCH_2), 6.28 (s, H-3), 7.24-7.44 (m, Ar-H), 7.85 (d, *J* = 8.8 Hz, H-6), 7.94 (d, *J* = 9.2 Hz, H-5), 12.43 (s, OH), 12.49 (s, OH).

General procedure for synthesis of compounds (9a-e): To a stirred solution of chloro oxime (**5**) (0.5 g, 1.45 mmol) in chloroform (15 mL), Et_3N was added drop-wise (0.22 g, 2.17 mmol) continued stirring for 15 min. (*E*)-ethyl-3-phenyl-prop-2-enoate (**8a**) (0.3 g, 1.74 mmol) dissolved in chloroform (15 mL) was added and reaction mixture stirred for 24 h at room temperature. H_2O (30 mL) was added and extracted in to chloroform (30 mL), layers separated organic layer dried over Na_2SO_4 and concentrated to yield the crude isoxazoline which was purified by column chromatography using (60-120 silica gel ethylacetate:petroleum ether (6:4) to give ethyl-3-[7-(benzyloxy)-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (**9a**) as white solid.

Ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (9a): Yield: 75 %; white solid; m.p.: 118-120 °C; IR (KBr, ν_{max}, cm^{-1}): 1723 (-C=O), 1683 (-C=N), 1093 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (t, -$COOCH_2\underline{CH_3}$), 2.42 (s, 4-CH_3), 3.95 (qq, $COO\underline{CH_2}CH_3$), 4.68 (d, *J* = 8.8 Hz, H-4''), 5.21 (s, 7-OCH_2), 6.11 (d, *J* = 9.2 Hz, H-5''), 6.17 (s, H-3), 6.96 (d, *J* = 8.8 Hz, H-6), 7.33-7.39 (m, H-1''', H-3''' & H-5''', H-2' to H-6'), 7.43-7.46 (m, H-2'''& H-6'''), 7.6 (d, *J* = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-$COOCH_2\underline{CH_3}$), 18.7 (4-CH_3), 61.7 (C-4''), 63.1 (-$COO\underline{CH_2}CH_3$), 71.1 (7-OCH_2), 85.6 (C-5''), 106.8 (C-8), 108.5 (C-3), 112.6 (C-4a), 114.1 (C-6), 126.6 (C-4'''), 126.9 (C-2''' & C-6'''), 127.2 (C-4'), 128.3 (C-3' & C-5'), 128.6 (C-5), 128.7 (C-2' & C-6'), 128.8 (C-5''' & C-3'''), 135.4 (C-1'), 139.1 (C-1'''), 147.1 (C-4), 152.1 (C-8a), 153.1 (C-7), 159.5 (2-C=O), 159.8 (C-3''), 167.4 (ester C=O); ESI–MS: *m/z* 484 (M+1); Anal. calcd. (%) for $C_{29}H_{25}NO_6$: C, 72.04; H, 5.21; N, 2.90. Found: C, 72.02; H, 5.21; N, 2.91.

Ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-[3-(trifluoromethyl)phenyl]-4,5-dihydro-4-isoxazole carboxylate (9b): Yield: 60 %; white solid; m.p.: 146-147 °C; IR (KBr, ν_{max}, cm^{-1}): 1724 (-C=O), 1685 (-C=N), 1087 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.94 (t, -$COOCH_2\underline{CH_3}$), 2.39 (s, 4-CH_3), 3.95 (qq, -$COO\underline{CH_2}CH_3$), 4.6 (s, *J* = 8.4 Hz, H-4''), 5.16 (s, 7-OCH_2), 6.13 (d, *J* = 8.8 Hz, H-5''), 6.15 (s, H-3), 6.93 (d, *J* = 8.8 Hz, H-6), 7.3-7.31 (m, H-2' to H-6'), 7.4-7.62 (m, H-2''', H-4 ''' & H-5''', H-6'''), 7.67 (d, H-5, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-$COOCH_2\underline{CH_3}$), 18.7 (4-CH_3), 62.0 (C-4''), 63.0 (-$COO\underline{CH_2}CH_3$), 71.1 (7-OCH_2), 84.6 (C-5''), 106.5 (C-8), 108.5 (C-3), 112.7 (C-4a), 114.1 (C-6), 122.5 (C-4'''), 125.4 (C-6'''), 127.0 (C-2'''), 127.1 (C-4'), 128.3 (C-5'), 128.7 (C-3'), 129.6 (C-5), 129.6 (C-6'), 130.4 (C-2'), 130.8 (C-3'''), 131.4 (C-5'''), 135.3 (C-1'), 140.2 (C-1'''), 147.3 (C-4), 152.0 (C-8a), 153.0 (C-7), 159.4 (2-C=O), 159.7 (C-3''), 167.1 (-C=O); ESI–MS: *m/z* 552.5 (M+1); Anal. calcd. (%) for $C_{30}H_{24}NO_6F_3$: C, 72.04; H, 5.21; N, 2.90. Found: C, 72.02; H, 5.21; N, 2.91.

Ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-[4-(trifluoromethyl)phenyl]-4,5-dihydro-4-isoxazole carboxylate (9c): Yield: 55 %; white solid; m.p.: 141-142 °C; IR (KBr, ν_{max}, cm^{-1}): 1728 (-C=O), 1686 (-C=N), 1083 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (t, -$COOCH_2\underline{CH_3}$), 2.4 (s, 4-CH_3), 3.96 (qq, -$COO\underline{CH_2}CH_3$), 4.61 (d, *J* = 8.4 Hz, H-4''), 5.17 (s, 7-OCH_2), 6.14 (d, *J* = 8.4 Hz, H-5''), 6.16 (s, H-3), 6.94 (d, *J* = 9.2 Hz, H-6), 7.31-7.32 (m, C-2' to C-6'), 7.41-7.45 (m, H-2'''), 7.57-7.59 (m, C-6'''), 7.61-7.64 (m, C-3''' & C-5'''), 7.68 (d, H-5, *J* = 9.2 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-$COOCH_2\underline{CH_3}$), 18.7 (4-CH_3), 62.0 (C-4''), 63.0 (-$COO\underline{CH_2}CH_3$), 71.1 (7-OCH_2), 84.6 (C-5''), 106.5 (C-8), 108.5 (C-3), 112.7 (C-4a), 114.1 (C-6), 122.5 (C-4'''),

123.3 (C-2''' & C-6'''), 125.5 (C-4'), 127.0 (C-3' & C-5'), 127.1 (C-5), 128.7 (C-2' & C-6'), 129.6 (C-5''' & C-3'''), 135.3 (C-1'), 140.2 (C-1'''), 147.3 (C-4), 152.0 (C-8a), 153.0 (C-7), 159.4 (2-C=O), 159.7 (C-3''), 167.1 (ester C=O); ESI–MS: *m/z* 552.5 (M+1); Anal. calcd. (%) for $C_{30}H_{24}NO_6F_3$: C, 65.33; H, 4.39; N, 2.54. Found: C, 65.32; H, 4.39; N, 2.53.

Ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-(3,5-difluorophenyl)-4,5-dihydro-4-isoxazole carboxylate (9d): Yield: 60 %; white solid; m.p.: 167-168 °C; IR (KBr, ν_{max}, cm^{-1}): 1736 (-C=O), 1690 (-C=N), 1085 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (t, -COOCH$_2$CH$_3$), 2.41 (s, 4-CH$_3$), 3.98 (qq, -COOCH$_2$CH$_3$), 4.58 (d, *J* = 8.4 Hz, H-4''), 5.17 (s, 7-OCH$_2$), 6.05 (d, *J* = 8.0 Hz, H-5''), 6.16 (s, H-3), 6.72-6.75 (m, H-6), 6.95-6.97 (m, H-2''', H-6''' & H-4'''), 7.31-7.32 (m, H-2' to H-6'), 7.6 (d, *J* = 9.2 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-COOCH$_2$CH$_3$), 18.7 (4-CH$_3$), 62.0 (C-4''), 62.9 (-COOCH$_2$CH$_3$), 71.1 (7-OCH$_2$), 83.9 (C-5''), 106.3 (C-8), 108.5 (C-3), 108.9 (C-4a), 109.0 (C-6), 109.1 (C-4'''), 109.2 (C-6'''), 112.7 (C-2'''), 114.1 (C-4'), 127.1 (C-3' & C-5'), 128.4 (C-5), 128.7 (C-2' & C-6'), 135.2 (C-3'''), 143.1 (C-5'''), 143.2 (C-1'), 143.3 (C-1'''), 147.3 (C-4), 152.9 (C-8a), 159.7 (C-7), 161.9 (2-C=O), 164.4 (C-3''), 167.0 (ester C=O); ESI–MS: *m/z* 520 (M+1); Anal. calcd. (%) for $C_{29}H_{23}NO_6F_2$: C, 67.05; H, 4.46; N, 2.70. Found: C, 67.03; H, 4.46; N, 2.69.

Ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-(5–fluoro-1*H*-indolyl)-4,5-dihydro-4-isoxazole carboxylate (9e): Yield: 55 %; white solid; m.p.: 182-183 °C; IR (KBr, ν_{max}, cm^{-1}): 1741 (-C=O), 1691 (-C=N), 1085 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.93 (t, -COOCH$_2$CH$_3$), 2.4 (s, 4-CH$_3$), 3.93 (qq, -COOCH$_2$CH$_3$), 4.9 (d, *J* = 8.8 Hz, H-4''), 5.26 (s, 7-OCH$_2$), 6.17 (s, H-3), 6.4 (d, *J* = 8.8 Hz, H-5''), 6.92-6.97 (m, H-3''' & H-7'''), 7.29-7.33 (m, H-6, H-2' & H-6'), 7.37-7.4 (m, H-3' & H-5'), 7.44 (dd, *J* = 2.4 Hz, *J* = 2.4 Hz, H-6'''), 7.57 (d, *J* = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-COOCH$_2$CH$_3$), 18.7 (4-CH$_3$), 60.5 (C-4''), 61.7 (-COOCH$_2$CH$_3$), 70.9 (C-3''), 80.0 (7-OCH$_2$), 104.0 (C-3'''), 104.2 (C-7'''), 107.0 (C-4a), 108.7 (C-3), 110.7 (C-6), 110.9 (C-8), 112.3 (C-6'''), 125.6 (C-6'), 125.7 (C-2'), 125.8 (C-4'), 126.9 (C-5), 127.1 (C-5'), 128.2 (C-3'), 128.7 (C-3a'''), 133.1 (C-7a'''), 135.5 (C-1'), 147.3 (C-1'''), 152.4 (C-4a), 152.9 (C-5'''), 156.7 (C-1''), 159.1 (C-4), 159.4 (C-7), 160.1 (2-C=O), 167.8 (ester C=O); ESI–MS: *m/z* 540.9 (M+1); Anal. calcd. (%) for $C_{31}H_{25}N_2O_6F$: C, 68.88; H, 4.66; N, 5.18. Found: C, 68.86; H, 4.66; N, 5.17.

General procedure for the synthesis of compounds (11a-e): To a stirred solution of chloro oxime (**6**) (0.05 g, 0.145 mmol) in chloroform (5 mL), Et_3N was added drop-wise (0.02 g, 0.217 mmol) continued stirring for 15-20 min. (*E*)-ethyl-3-phenyl-prop-2-enoate (**8a**) (0.03 g, 0.174 mmol) dissolved in chloroform (5 mL) was added and reaction mixture stirred for 24 h at room temperature H_2O (15 mL) was added and extracted in to chloroform (15 mL), layers separated organic layer dried over Na_2SO_4 and concentrated to yield the crude isoxazoline which was purified by column chromatography using (60-120 silica gel ethylacetate:petroleum ether (8: 2) as eluent) to give the ethyl-3-[7-(benzyloxy)-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (**11a**).

Ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (11a): Yield: 60 %; white solid; m.p.: 118-120 °C; IR (KBr, ν_{max}, cm^{-1}): 1723 (-C=O), 1683 (-C=N), 1093 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.95 (t, -COOCH$_2$CH$_3$), 2.54 (s, 4-CH$_3$), 3.95 (qq, -COOCH$_2$CH$_3$), 4.62 (d, *J* = 8.8 Hz, H-4''), 5.21 (s, 7-OCH$_2$), 6.08 (d, *J* = 8.8 Hz, H-5''), 6.97 (d, *J* = 9.2 Hz, H-6), 7.31 (m, H-1''', H-3''' & H-5''', H-2' to H-6'), 7.4 (m, H-2''' & H-6'''), 7.6 (d, *J* = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-COOCH$_2$CH$_3$), 18.7 (4-CH$_3$), 61.7 (C-4''), 63.1 (-COOCH$_2$CH$_3$), 71.1 (7-OCH$_2$), 85.6 (C-5''), 106.8 (C-8), 108.5 (C-3), 112.6 (C-4a), 114.1 (C-6), 126.6 (C-4'''), 126.9 (C-2''' & C-6'''), 127.2 (C-4'), 128.3 (C-3' & C-5'), 128.6 (C-5), 128.7 (C-2' & C-6'), 128.8 (C-5''' & C-3'''), 135.4 (C-1'), 139.1 (C-1'''), 147.1(C-4), 152.1 (C-8a), 153.1 (C-7), 159.5 (2-C=O), 159.8 (C-3''), 167.4 (ester C=O); ESI–MS: *m/z* 518 (M+1), 520 (M+1+2); Anal. calcd. (%) for $C_{29}H_{24}NO_6Cl$: C, 67.25; H, 4.67; N, 2.70. Found: C, 67.24; H, 4.66; N, 2.70.

Ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-[3-(trifluoromethyl)phenyl]-4,5-dihydro-4-isoxazole carboxylate (11b): Yield: 55 %; white solid; m.p.: 146-147 °C; IR (KBr, ν_{max}, cm^{-1}): 1720 (-C=O), 1681 (-C=N), 1089 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.94 (t, -COOCH$_2$CH$_3$), 2.38 (s, 4-CH$_3$), 3.98 (qq, -COOCH$_2$CH$_3$), 4.61 (s, *J* = 8.4 Hz, H-4''), 5.18 (s, 7-OCH$_2$), 6.14 (d, *J* = 8.8 Hz, H-5''), 6.93 (d, *J* = 8.8 Hz, H-6), 7.31-7.33 (m, H-2' to H-6'), 7.42-7.62 (m, H-2''', H-4 ''' & H-5''', H-6'''), 7.67 (d, H-5, *J* = 8.8 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.8 (-COOCH$_2$CH$_3$), 18.9 (4-CH$_3$), 62.2 (C-4''), 63.3 (-COOCH$_2$CH$_3$), 71.2 (7-OCH$_2$), 84.6 (C-5''), 106.6 (C-8), 108.5 (C-3), 112.9 (C-4a), 114.1 (C-6), 122.6 (C-4'''), 125.4 (C-6'''), 127.2 (C-2'''), 127.1 (C-4'), 128.3 (C-5'), 128.7 (C-3'), 129.6 (C-5), 129.8 (C-6'), 130.4 (C-2'), 130.8 (C-3'''), 131.4 (C-5'''), 135.3 (C-1'), 140.2 (C-1'''), 147.3 (C-4), 152.2 (C-8a), 153.6 (C-7), 159.4 (2-C=O), 159.7 (C-3''), 167.1 (-C=O); ESI–MS: *m/z* 586.1 (M+1), 588.1 (M+1+2); Anal. calcd. (%) for $C_{30}H_{25}NO_6ClF_3$: C, 61.28; H, 4.29; N, 2.38. Found: C, 61.26; H, 4.29; N, 2.39.

Ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-[4-(trifluoromethyl)phenyl]-4,5-dihydro-4-isoxazole carboxylate (11c): Yield: 60 %; white solid; m.p.: 125-126 °C; IR (KBr, ν_{max}, cm^{-1}): 1738 (-C=O), 1696 (-C=N), 1085 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.97 (t, -COOCH$_2$CH$_3$), 2.4 (s, 4-CH$_3$), 3.96 (qq, -COOCH$_2$CH$_3$), 4.61 (d, *J* = 8.4 Hz, H-4''), 5.17 (s, 7-OCH$_2$), 6.13 (d, *J* = 8.4 Hz, H-5''), 6.97 (d, H-6, *J* = 8.8 Hz), 7.29-7.31 (m, H-2' to H-6'), 7.41-7.45 (m, H-2''' & H-6'''), 7.59-7.64 (m, H-3''' & H-5'''), 7.68 (d, *J* = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-COOCH$_2$CH$_3$), 18.7 (4-CH$_3$), 62.0 (C-4''), 63.0 (COOCH$_2$CH$_3$), 71.1 (7-OCH$_2$), 84.6 (C-5''), 106.5 (C-8), 108.5 (C-3), 112.7 (C-4a), 114.1 (C-6), 122.5 (C-4'''), 123.3 (C-2''' & C-6'''), 125.5 (C-4'), 127.0 (C-3' & C-5'), 127.1(C-5), 128.7 (C-2' & C-6'), 129.6 (C-5''' & C-3'''), 135.3 (C-1'), 140.2 (C-1'''), 147.3 (C-4), 152.0 (C-8a), 153.0 (C-7), 159.4 (2-C=O), 159.7 (C-3''), 167.1 (ester C=O); ESI–MS: *m/z* 586.1 (M+1), 588.1 (M+1+2); Anal. calcd. (%) for $C_{30}H_{25}NO_6ClF_3$: C, 61.49; H, 3.96; N, 2.39. Found: C, 61.49; H, 3.95; N, 2.38.

Ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-(3,5-difluorophenyl)-4,5-dihydro-4-isoxazolecarboxylate (11d): Yield: 50 %; white solid; m.p.: 142-143 °C; IR (KBr, ν_{max}, cm^{-1}): 1725 (-C=O), 1699 (-C=N), 1085 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.98 (t, -$COOCH_2\underline{CH_3}$), 2.57 (s, 4-CH_3), 3.98 (qq, -$COOCH_2\underline{CH_3}$), 4.55 (d, *J* = 8.0 Hz, H-4''), 5.17 (s, 7-OCH_2), 6.06 (d, *J* = 8.4 Hz, H-5''), 6.73 (d, *J* = 9.2 Hz, H-6), 6.94-7.02 (m, H-2''', H-4''' & H-6'''), 7.27-7.34 (m, H-2' to H-6'), 7.63 (d, *J* = 9.2 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.7 (-$COOCH_2\underline{CH_3}$), 18.7 (4-CH_3), 62.0 (C-4''), 62.9 (-$COO\underline{CH_2}CH_3$), 71.1 (7-OCH_2), 83.9 (C-5''), 106.3 (C-8), 108.5 (C-3), 108.9 (C-4a), 109.0 (C-6), 109.1 (C-4'''), 109.2 (C-6'''), 112.7 (C-2'''), 114.1 (C-4'), 127.1 (C-3' & C-5'), 128.4 (C-5), 128.7 (C-2' & C-6'), 135.2 (C-3'''), 143.1 (C-5'''), 143.2 (C-1'), 143.3 (C-1'''), 147.3 (C-4), 152.9 (C-8a), 159.7 (C-7), 161.9 (2-C=O), 164.4 (C-3''), 167.0 (ester C=O); ESI–MS: *m/z* 554 (M+1), 556 (M+1+2); Anal. calcd. (%) for $C_{29}H_{22}NO_6ClF_2$: C, 62.88; H, 4.00; N, 2.53. Found: C, 62.86; H, 3.99; N, 2.53.

Ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-(5-fluoro-1*H*-indolyl)-4,5-dihydro-4-isoxazole carboxylate (11e): Yield: 55 %; white solid; m.p.: 182-183 °C; IR (KBr, ν_{max}, cm^{-1}): 1749 (- C=O), 1693 (-C=N), 1088 (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 0.98 (t, -$COOCH_2\underline{CH_3}$), 2.41 (s, 4-CH_3), 3.96 (qq, -$COO\underline{CH_2}CH_3$), 4.91 (d, *J* = 8.8 Hz, H-4''), 5.24 (s, 7-OCH_2), 6.42 (d, *J* = 8.8 Hz, H-5''), 6.91-6.99 (m, H-3''' & H-7'''), 7.27-7.31 (m, H-6, H-2' & H-6'), 7.36-7.4 (m, H-3' & H-5'), 7.43 (dd, *J* = 2.4 Hz, *J* = 2.4 Hz, H-6'''), 7.56 (d, *J* = 8.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz) δ (ppm): 13.9 (-$COOCH_2\underline{CH_3}$), 18.6 (4-CH_3), 60.7 (C-4''), 61.9 (-$COO\underline{CH_2}CH_3$), 70.8 (C-3''), 80.2 (7-OCH_2), 104.3 (C-3'''), 104.4 (C-7'''), 107.2 (C-4a), 108.9 (C-3), 110.8 (C-6), 110.9 (C-8), 112.3 (C-6'''), 125.6 (C-6'), 125.7 (C-2'), 125.8 (C-4'), 126.9 (C-5), 127.1 (C-5'), 128.4 (C-3'), 128.7 (C-3a'''), 133.1 (C-7a'''), 135.5 (C-1'), 147.3 (C-1'''), 152.4 (C-4a), 152.9 (C-5'''), 156.8 (C-1''), 159.4 (C-4), 159.5 (C-7), 160.1 (2-C=O), 167.8 (ester C=O); ESI–MS: *m/z* 574.5 (M+1), 576.5 (M+1+2); Anal. calcd. (%) for $C_{31}H_{24}N_2O_6ClF$: C, 64.76; H, 4.21; N, 4.87. Found: C, 64.75; H, 4.22; N, 4.86.

Antimicrobial activity: The *in vitro* antimicrobial activity [34-36] of all the synthesized compounds were carried out using paper disk method, the compounds screened against *Bacillus subtilis* (Gram-positive) and *Pseudomonas aerogenosa* (Gram-negative) for antibacterial activity where as *Aspergillus niger* and *Rhizoctonia solani* for antifungal activity. The strains used for the activity procured from Institute of Microbial Technology (IMT), Chandigarah. Cultures of test organisms were maintained on nutrient agar (bacterial) and potato dextrose agar (fungal) media and subcultured in petri dishes prior to testing. The compounds are tested at concentrations 200 µg/mL, 100 µg/mL using DMSO as solvent. After solidification of media, petri plates inoculated with actively growing cultures of *B. subtilis* (Gram-positive), *P. aerogenosa* (Gram-negative) and *A. niger*, *R. solani* separately. Filter paper disks of 5 mm diameter dipped in the test solution of different concentrations. After drying the disk, kept on nutrient agar broth. Potato dextrose broth in petri plates seeded with 1 mL culture of *B. subtilis* (Gram-positive), *P. aerogenosa* (Gram-negative) and *A. niger*, *R. solani*, incubated for 24 h at 27 °C. Then the petri dishes were tested for growth of inhibition. The presence of clear zone of growth inhibition around the paper disk indicated the inhibition of growth of organisms. The diameter of zone of inhibition was calculated in millimeters, ampicillin is used as standard antibacterial drug, where as clotrimazole used as standard antifungal drug. The observed data of antimicrobial activity of compounds and the standard drugs are given in Table-1. From the obtained results it was evident that most of the compounds (*i.e.*, **9a**, **9c**, **9d**, **11a** and **11d**) showed good antibacterial activity and (*i.e.*, **9a**, **9b**, **9c**, **9d**, **11a** and **11d**) possess good antifungal activity in higher concentrations comparable with that of standard drugs tested. Although with respect to standard drugs, all the tested compounds were found to be moderately active. So, the result of all preliminary study indicated that the ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**9a-e**) and ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**11a-e**) moiety represent a new class of pharmacophore for broad spectrum of antibacterial and antifungal activity.

TABLE-1
ANTIMICROBIAL ACTIVITY OF COMPOUNDS **9(a-e)** AND **11(a-e)**

Compd. No.	Conc. (µg/mL)	Antibacterial activity		Antifungal activity	
		B. subtilis[a]	*P. aerogenosa*[a]	*A. niger*[a]	*R. solani*[a]
9a	200	31	25	26	25
	100	23	19	18	17
9b	200	16	32	27	11
	100	14	23	21	13
9c	200	28	27	24	29
	100	20	18	21	18
9d	200	30	30	32	22
	100	22	21	23	18
9e	200	13	13	14	12
	100	11	11	9	12
11a	200	27	26	28	26
	100	18	21	18	20
11b	200	14	12	13	10
	100	12	11	8	11
11c	200	12	18	15	11
	100	13	11	13	10
11d	200	21	36	31	23
	100	19	22	19	18
11e	200	8	16	12	9
	100	6	15	13	7
Ampicillin	20 µg/mL	20	18	NA	NA
Clotrimazole	20 µg/mL	NA	NA	21	19

[a]Zone of inhibition

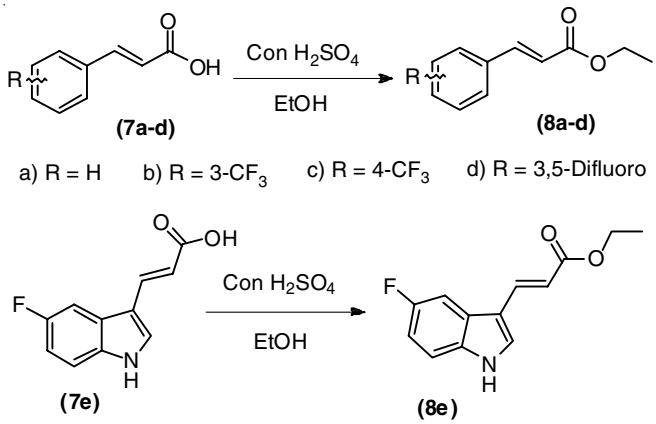
RESULTS AND DISCUSSION

In the present study, we have synthesized the novel series of ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**9a-e**) and ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-

5-aryl-4,5-dihydro-4-isoxazole carboxylates (**11a-e**) from 7-benzyloxy-4-methyl-coumarin aldehyde chloro oxime (**5**) and 7-benzyloxy-3-chloro-4-methyl-coumarin aldehyde chloro-oxime (**6**) on reaction with (*E*)-ethyl-3-arylprop-2-enoates (**8a-e**) in the presence of Et_3N in chloroform at room temperature for 24 h (**Scheme-III**).

Initially, 7-hydroxy-4-methyl coumarin (**1**) was synthesized from the resorcinol, ethylacetoacetate in the presence of conc. H_2SO_4 (Pechmann reaction) followed by Duffs formylation to give 8-formyl-7-hydroxy-4-methyl coumarin (**2**) [37]. Further by benzylation with benzyl bromide gave O-benzyl coumarin aldehyde (**3**) followed by oximation process with hydroxylamine hydrochloride to afford the corresponding O-benzyl coumarin oxime (**4**). 7-Benzyloxy-4-methyl-coumarin aldehyde chlorooxime (**5**) was synthesized from the chlorination of oxime (**4**) with NCS in chloroform at 0 °C and which was characterized from its spectral data. After purification by column chromatography 3-chlorinated product (**6**) was isolated as minor product (**Scheme-I**).

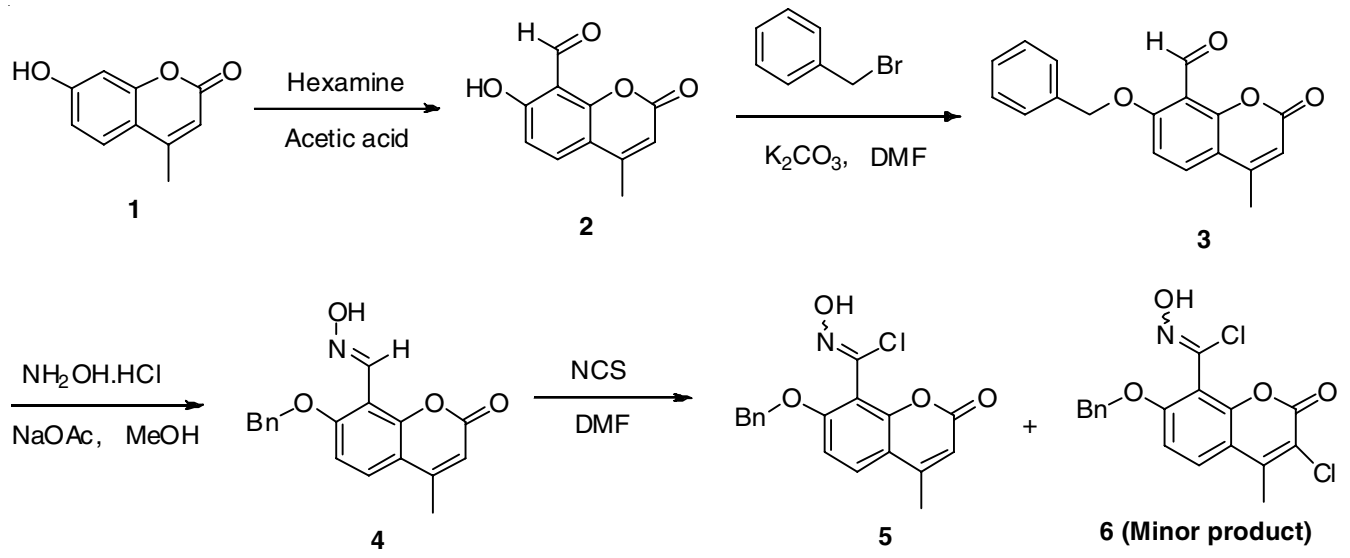
Esterification of substituted (*E*)-cinnamic acids **(8a-e)** in the presence of ethanol and catalytic amount of conc. H_2SO_4 gave (*E*)-ethyl-3-aryl-prop-2-enoates (**7a-e**) which are characterized from its spectral data (**Scheme-II**).



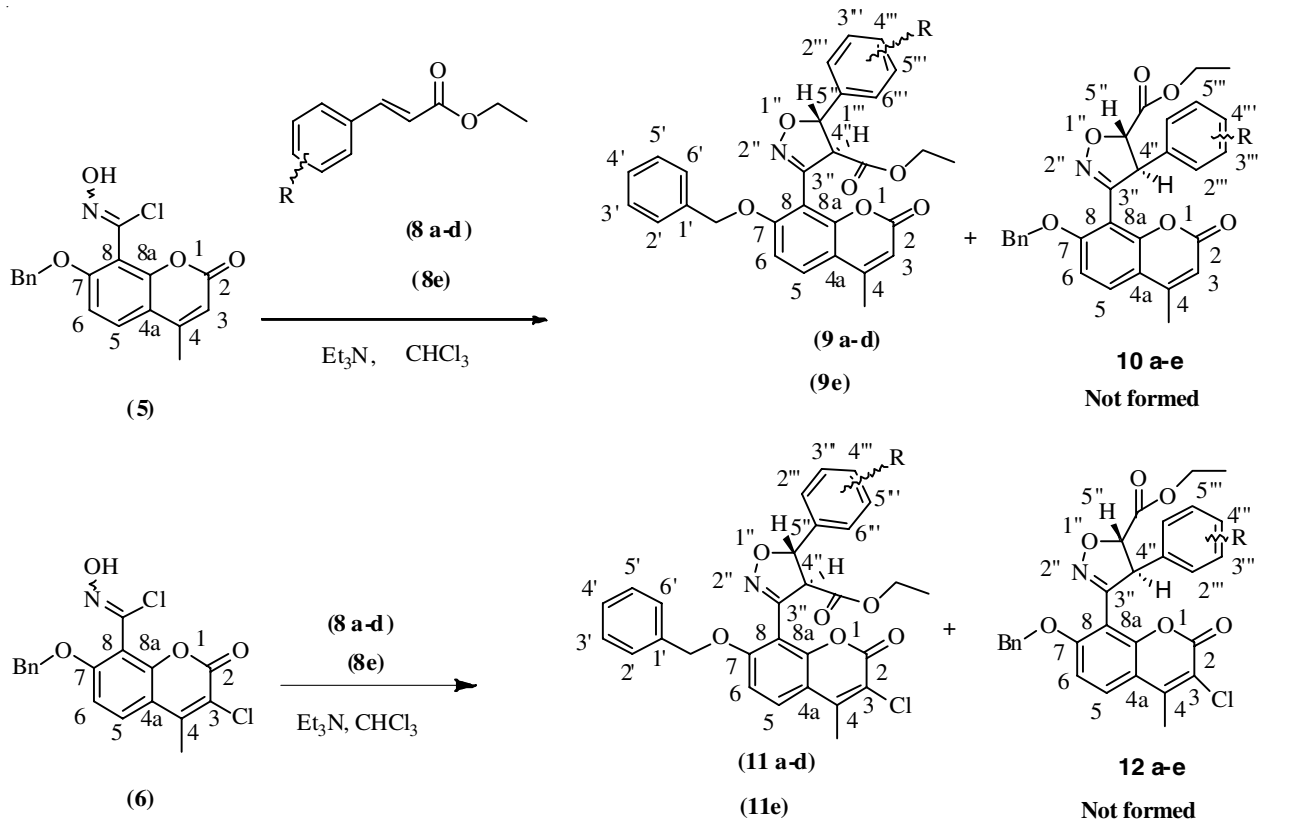
Scheme-II: Synthesis of (*E*)-ethyl-3-aryl-prop-2-enoates (**8a-e**)

7-Benzyloxy-4-methyl-coumarin aldehyde chlorooxime (**5**) on reaction with (*E*)-ethyl-3-arylprop-2-enoates (**8a-e**) in the presence of Et_3N in chloroform at room temperature for 24 h gave regioselectively ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**9a-e**). Upon similar conditions 7-benzyloxy-3-chloro-4-methyl-coumarin aldehyde chlorooxime (**6**) on reaction with (*E*)-ethyl-3-arylprop-2-enoates (**8a-e**) in the presence of Et_3N in chloroform at room temperature for 24 h gave regioselectively ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylates (**11a-e**) which are characterized by ^{1}H NMR, NOESY, ^{13}C NMR and mass spectrometry (**Scheme-III**).

Spectral analysis. The structural assignment of the title compounds (**9a-e** and **11a-e**) has been made on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies which were in full agreement with the proposed structures. The structure of **9a** is interpreted from spectroscopic data. In the ^{1}H NMR spectra of **9a**, the proton of the newly formed isoxazoline ring H-4" appeared at δ 4.68 (d, J = 8.8 Hz) and H-5" at δ 6.11 (d, J = 9.2 Hz). Ethyl ester protons appeared at δ 0.95 (t, -COOCH$_2$CH$_3$) and 3.95 (qq, -COOCH$_2$CH$_3$), phenyl protons attached to isoxazoline ring at δ 7.43-7.46 (m, H-2''' & H-6'''). H-3, H-5 and H-6 protons appeared at 6.17 (s), 7.6 (d, J = 8.8 Hz) and 6.96 (d, J = 8.8 Hz). 4-CH$_3$ and rest of aromatic protons appears in the region at 2.72 (s), 7.33-7.46 (m). Mass spectrum was consistent with assigned structure showing (M + 1) peak at 484. IR spectra of compound **9a** reveals absorption band in the region 1723 cm^{-1} corresponding to -C=O stretching and 1683 due to -COOCH$_3$. The proof for the regioselectivity of the 1,3-dipolar cycloaddition reaction product ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (**9a**) rather than other isomer ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl]-4-phenyl-4,5-dihydro-5-isoxazole carboxylate (**10a**) is from the analysis of NOESY spectrum. The isoxazoline proton H-5" at δ 6.11 (d, J = 9.2 Hz) showed a strong nOe with the H-4" of the isoxazoline ring at δ 4.68 (d, J = 8.8 Hz) and a medium



Scheme-I: Synthesis of 7-benzyloxy-4-methyl-coumarin aldehyde chlorooxime and 7-benzyloxy-3-chloro-4-methyl-coumarin aldehyde chloro oxime (**5** and **6**)



a) R = H b) R = 3-CF_3 c) R = 4-CF_3 d) R = 3,5-Difluoro e) R = Indolyl

Scheme-III: Synthesis of ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-phenyl-4,5-dihydro-4-isoxazole carboxylates (**9a-e**) and (**11a-e**)

nOe with the protons of the phenyl protons at δ 7.43-7.46 (m, H-2''' & H-6'''). The H-4'' at δ 4.68 (d, *J* = 8.8 Hz) showed a strong nOe with the H-5'' at δ 6.11 (d, *J* = 9.2 Hz). These nOe values are indicating the formation of the regioisomer ethyl-3-[7-(benzyloxy)-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (**9a**).

The structure of **11a** is interpreted from spectroscopic data. In the ^{1}H NMR spectra of **11a**, the characteristic H-3 proton absent at δ 6.17. The protons of the newly formed isoxazoline ring H-4'' appeared at δ 4.62 (d, *J* = 8.8 Hz) and H-5'' at δ 6.08 (d, *J* = 8.8 Hz). Ethyl ester protons appeared at δ 0.95 (t, -COOCH$_2$CH$_3$) and δ 3.95 (qq, -COOCH$_2$CH$_3$), phenyl protons attached to isoxazoline at δ 7.40-7.41 (m, H-2''' & H-6'''). H-5 and H-6 protons appeared at 7.6 (d, *J* = 8.8 Hz) and 6.96 (d, *J* = 9.2 Hz). 4-CH_3 and rest of the aromatic protons appeared at 2.54 (s), 7.31-7.4 (m). Mass spectrum was consistent with assigned structure showing 518 (M+1), 520 (M+1+2). IR spectra of compound **11a** reveals absorption band in the region 1721 cm^{-1} corresponding to -C=O stretching and 1682 due to -$COOCH_3$. The proof for the regioselectivity of the 3-chlorinated product, ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazolecarboxylate (**11a**) rather than other isomer ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl]-3-chloro-4-phenyl-4,5-dihydro-5-isoxazole carboxylate **(12a)** is from the analysis of NOESY spectrum. The isoxazoline proton H-5'' at δ 6.08 (d, *J* = 8.8 Hz) showed a strong nOe with the H-4'' of the isoxazoline ring at δ 4.62 (d, *J* = 8.8 Hz) & medium nOe with the protons of the phenyl ring protons 7.4-7.41 (m, H-2''' & H-6'''). The H-4'' at δ 4.62 (d, *J* = 8.8 Hz) showed a strong nOe with the H-5'' at δ 6.08 (d, *J* = 8.8 Hz). These nOe values are favouring the formation of the regioisomer ethyl-3-[7-(benzyloxy)-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (**11a**).

Conclusion

Herewith, we report the simple and efficient method for regioselective synthesis of novel mixed heterocyclic compounds, ethyl-3-(7-benzyloxy-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**9a-e**) and ethyl-3-(7-benzyloxy-3-chloro-4-methyl-2-oxo-2*H*-8-chromenyl)-5-aryl-4,5-dihydro-4-isoxazole carboxylates (**11a-e**). Some of these compounds have shown good antimicrobial activity. The present 1,3-dipolar cycloaddition reaction strategy, which is the first example in coumarin chemistry can be further explored for the synthesis of various heterocyclic-coumarin derivatives.

ACKNOWLEDGEMENTS

The authors thank Head, Department of Chemistry and Director-CFRD, Osmania University, Hyderabad, India for providing the necessary research facilities.

REFERENCES

1. K.N. Venugopala, V. Rashmi and B. Odhav, *BioMed Res. Int.*, **Article ID 963248** (2013); https://doi.org/10.1155/2013/963248.

2. S. Sandhu, Y. Bansal, O. Silakari and G. Bansal, *Bioorg. Med. Chem.*, **22**, 3806 (2014); https://doi.org/10.1016/j.bmc.2014.05.032.
3. R. Choure and K.S. Pitre, *Can. J. Chem. Eng. Technol.*, **1**, 7 (2010).
4. H.B. Lad, R.R. Giri and D.I. Brahmbhatt, *Chin. Chem. Lett.*, **24**, 227 (2013); https://doi.org/10.1016/j.cclet.2013.01.041.
5. A. Lacy and O.R. Kennedy, *Curr. Pharm. Des.*, **10**, 3797 (2004); https://doi.org/10.2174/1381612043382693.
6. A. Witaicenis, L.N. Seito, A. da Silveira Chagas, L.D. de Almeida, A.C. Luchini, P. Rodrigues-Orsi, S.H. Cestari and L.C. Di Stasi, *Phytomedicine*, **21**, 240 (2014); https://doi.org/10.1016/j.phymed.2013.09.001.
7. T.O. Olomola, R. Klein, N. Mautsa, Y. Sayed and P.T. Kaye, *Bioorg. Med. Chem.*, **21**, 1964 (2013); https://doi.org/10.1016/j.bmc.2013.01.025.
8. M.O. Karatas, B. Alici, U. Cakir, E. Cetinkaya, D. Demir, A. Ergün, N. Gencer and O. Arslan, *J. Enzyme Inhib. Med. Chem.*, **28**, 299 (2013); https://doi.org/10.3109/14756366.2012.677838.
9. K.V. Sairam, B.M. Gurupadayya, R.S. Chandan, D.K. Nagesha and B. Vishwanathan, *Curr. Drug Deliv.*, **13**, 186 (2016); https://doi.org/10.2174/1567201812666150702102800.
10. M. Khoobi, A. Foroumadi, S. Emami, M. Safavi, G. Dehghan, B.H. Alizadeh, A. Ramazani, S.K. Ardestani and A. Shafiee, *Chem. Biol. Drug Des.*, **78**, 580 (2011); https://doi.org/10.1111/j.1747-0285.2011.01175.x.
11. J. Grover and S.M. Jachak, *RSC Adv.*, **5**, 38892 (2015); https://doi.org/10.1039/C5RA05643H.
12. K. Zheng, W. Lin, L. Tan, H. Chen and H. Cui, *Chem. Sci.*, **5**, 3439 (2014); https://doi.org/10.1039/C4SC00283K.
13. T.D. Penning, J.J. Talley, S.R. Bertenshaw, J.S. Carter, P.W. Collins, S. Docter, M.J. Graneto, L.F. Lee, J.W. Malecha, J.M. Miyashiro, R.S. Rogers, D.J. Rogier, S.S. Yu, G.D. Anderson, E.G. Burton, J.N. Cogburn, S.A. Gregory, C.M. Koboldt, W.E. Perkins, K. Seibert, A.W. Veenhuizen, Y.Y. Zhang and P.C. Isakson, *J. Med. Chem.*, **40**, 1347 (1997); https://doi.org/10.1021/jm960803q.
14. J. Elguero, eds.: A.R. Katrizky and C.W. Rees, Comprehensive Heterocyclic Chemistry II; Pergamon: Oxford, vol. 3, p. 1 (1996).
15. L. Meng, B.A. Lorsbach, T.C. Sparks, J.C. Fettinger and M.J. Kurth, *J. Comb. Chem.*, **12**, 129 (2010); https://doi.org/10.1021/cc900133k.
16. A. Nefzi, J.M. Ostresh and R.A. Houghten, *Chem. Rev.*, **97**, 449 (1997); https://doi.org/10.1021/cr960010b.
17. D.A. Horton, G.T. Bourne and M.L. Smythe, *Chem. Rev.*, **103**, 893 (2003); https://doi.org/10.1021/cr020033s.
18. A. Nefzi, J.M. Ostresh, J. Yu and R.A. Houghten, *J. Org. Chem.*, **69**, 3603 (2004); https://doi.org/10.1021/jo040114j.
19. M.A. Gallop, R.W. Barrett, W.J. Dower, S.P.A. Fodor and E.M. Gordon, *J. Med. Chem.*, **37**, 1233 (1994); https://doi.org/10.1021/jm00035a001.
20. Y.-S. Lee and B. Hyean Kim, *Bioorg. Med. Chem. Lett.*, **12**, 1395 (2002); https://doi.org/10.1016/S0960-894X(02)00182-8.
21. S. Srivastava, L.K. Bajpai, S. Batra, A.P. Bhaduri, J.P. Maikhuri, G. Gupta and J.D. Dhar, *Bioorg. Med. Chem.*, **7**, 2607 (1999); https://doi.org/10.1016/S0968-0896(99)00188-1.
22. D. Simoni, G. Grisolia, G. Giannini, M. Roberti, R. Rondanin, L. Piccagli, R. Baruchello, M. Rossi, R. Romagnoli, F.P. Invidiata, S. Grimaudo, M.K. Jung, E. Hamel, N. Gebbia, L. Crosta, V. Abbadessa, A. Di Cristina, L. Dusonchet, M. Meli and M. Tolomeo, *J. Med. Chem.*, **48**, 723 (2005); https://doi.org/10.1021/jm049622b.
23. J.J. Talley, D.L. Brown, J.S. Carter, M.J. Graneto, C.M. Koboldt, J.L. Masferrer, W.E. Perkins, R.S. Rogers, A.F. Shaffer, Y.Y. Zhang, B.S. Zweifel and K. Seibert, *J. Med. Chem.*, **43**, 775 (2000); https://doi.org/10.1021/jm990577v.
24. S. Dadiboyena, J. Xu and A.T. Hamme II, *Tetrahedron Lett.*, **48**, 1295 (2007); https://doi.org/10.1016/j.tetlet.2006.12.005.
25. S. Dadiboyena and A. Nefzi, *Eur. J. Med. Chem.*, **45**, 4697 (2010); https://doi.org/10.1016/j.ejmech.2010.07.045.
26. A.P. Kozikowski, *Acc. Chem. Res.*, **17**, 410 (1984); https://doi.org/10.1021/ar00108a001.
27. B.B. Shankar, D.Y. Yang, S. Girton and A.K. Ganguly, *Tetrahedron Lett.*, **39**, 2447 (1998); https://doi.org/10.1016/S0040-4039(98)00237-8.
28. R.E. Sammelson, T. Ma, L.J.V. Galietta, A.S. Verkman and M.J. Kurth, *Bioorg. Med. Chem. Lett.*, **13**, 2509 (2003); https://doi.org/10.1016/S0960-894X(03)00482-7.
29. G. Bal, P. Van der Veken, D. Antonov, A.-M. Lambeir, P. Grellier, S.L. Croft, K. Augustyns and A. Haemers, *Bioorg. Med. Chem. Lett.*, **13**, 2875 (2003); https://doi.org/10.1016/S0960-894X(03)00579-1.
30. A. Gopalsamy, M. Shi, J. Golas, E. Vogan, J. Jacob, M. Johnson, F. Lee, R. Nilakantan, R. Petersen, K. Svenson, R. Chopra, M.S. Tam, Y. Wen, J. Ellingboe, K. Arndt and F. Boschelli, *J. Med. Chem.*, **51**, 373 (2008); https://doi.org/10.1021/jm701385c.
31. R. Huisgen, ed.: A. Padwa, 1,3-Dipolar Cycloaddition Chemistry, Wiley: NewYork, vols. 1 and 2 (1984).
32. K.B.G. Torssell, Nitrile Oxides, Nitrones and Nitronates in Organic Synthesis; VCH: New York (1988).
33. V. Jaeger and P.A. Colinas, ed.: A. Padwa, Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Towards Heterocycles and Natural Products, Wiley: Hoboken, vol. 59, p. 361 (2002).
34. K.S. Atwal, G.J. Grover, S.Z. Ahmed, F.N. Ferrara, T.W. Harper, K.S. Kim, P.G. Sleph, S. Dzwonczyk and A.D. Russell, *J. Med. Chem.*, **36**, 3971 (1993); https://doi.org/10.1021/jm00076a027.
35. W.A. Ayer and K. Nozawa, *Can. J. Microbiol.*, **36**, 83 (1990); https://doi.org/10.1139/m90-016.
36. W. Pfefferle, H. Anke, M. Bross, B. Steffan, R. Vianden and W. Steglich, *J. Antibiot.*, **43**, 648 (1990); https://doi.org/10.7164/antibiotics.43.648.
37. D.R. Bender, D. Kanne, J.D. Frazier and H. Rapoport, *J. Org. Chem.*, **48**, 2709 (1983); https://doi.org/10.1021/jo00164a015.