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RESEARCH ARTICLE

Novel Hybrid Molecules of Isoxazole Chalcone Derivatives: Synthesis and Study of *In Vitro* Cytotoxic Activities

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ARTICLE HISTORY

Received: May 15, 2017
Revised: August 17, 2017
Accepted: August 28, 2017

DOI:
10.2174/1570180814666170914121740

Abstract: ***Background:*** Now-a-days, the model of "hybrid drugs" has acquired recognition in medicine due to their significant role in the treatment of different health problems.

Methods: We have synthesized new series of isoxazole-chalcone conjugates (**14a-m**) by the Claisen-Schmidt condensation of suitable substituted acetophenones with isoxazole aldehydes (**12a-d**). *In vitro* cytotoxic activity of the synthesized compounds was studied against four different selected human cancer cell lines by using sulforhodamine B (SRB) method.

Results: The adopted scheme resulted in good yields of new series of isoxazole-chalcone conjugates (**14a-m**). Potent cytotoxic activity was observed for compounds **-14a, 14b, 14e, 14i, 14j** and **14k** against prostate DU-145 cancer cell line.

Conclusion: The observed potent cytotoxic activities were due to the presence of 3,4,5-trimethoxyphenyl group.

Keywords: l-(3,4,5-trimethoxyphenyl)ethanone, isoxazole-chalcone, hybrid molecules, antiproliferative activity, claisen-schmidt condensation, cytotoxic activities.

1. INTRODUCTION

Hybrid molecules are designed by chemical hybridization wherein two drug pharmacophores are incorporated in a single molecule with an objective to put forth twin drug action [1]. For example, one of the pharmacophores possibly will target explicitly tumor vessels, whereas another may be the active agent [2]. Hence, hybrid molecules are also known as multifunctional or conjugated drugs. Hybrid molecules may also exhibit synergetic effect compared to the individual pharmacophores [3].

Natural products and their derivatives have been widely used in cancer chemotherapy as Microtubule-Binding Agents (MBA) [4-6]. There are two classes of MBAs. Either they stabilize microtubules to promote polymerization or destabilize the microtubules to promote depolymerization [7-10]. Both types interfere with the mitotic spindle assembly during cell division, resulting in cell death. Studies have shown that most MBAs have anti vascular effects from anti-angiogenetic or vascular disrupting activities, or both [11-14]. The combretastatins, such as combretastatin A-4 (CA-4, **1**) and combretastatin A-l (CA-1, **2**) (Fig. **1**), have attracted much interest as anti-cancer agents [15-19].

It was reported that substituted triazoles, oxadiazoles including 3,4-disubstituted-1,2,5-oxadiazole (combretafurazan) [20] and 2,5-disubstituted-l,3,4-oxadiazole [21] (**3** and **4**) are more potent than combretastatin itself. A key structural factor for tubulin affinity is the presence of the double bond or a suitable linker, forcing the two aromatic rings to stay within an appropriate distance. To overcome the problem of the isomerization of the active *cis* double bond into an inactive *trans* form, heterocyclic rings were used in place of the ethene bridge. The synthesis and biological activities of a series of CA-4 analogues with a five-membered heterocycles include isoxazole as linker of the two aromatic rings of CA-4 (**5** and **6**) have been reported [22].

Combretastatin A-4 (CA-4) analogs exhibiting comparable activity have been developed to avoid problem of isomerization of cis double bond [23]. There is always an equal effort to make a more active drug than combretastatin A-4, and to achieve this heterocyclic ring was placed in between the two aromatic rings with retaining activity. Survey of literature reveals that, a variety of chalcones exhibited potent cytotoxicity activities against human cancer cell lines [24, 25]. So by keeping in mind about the advantages of linking of CA-4 with heterocyclic rings and

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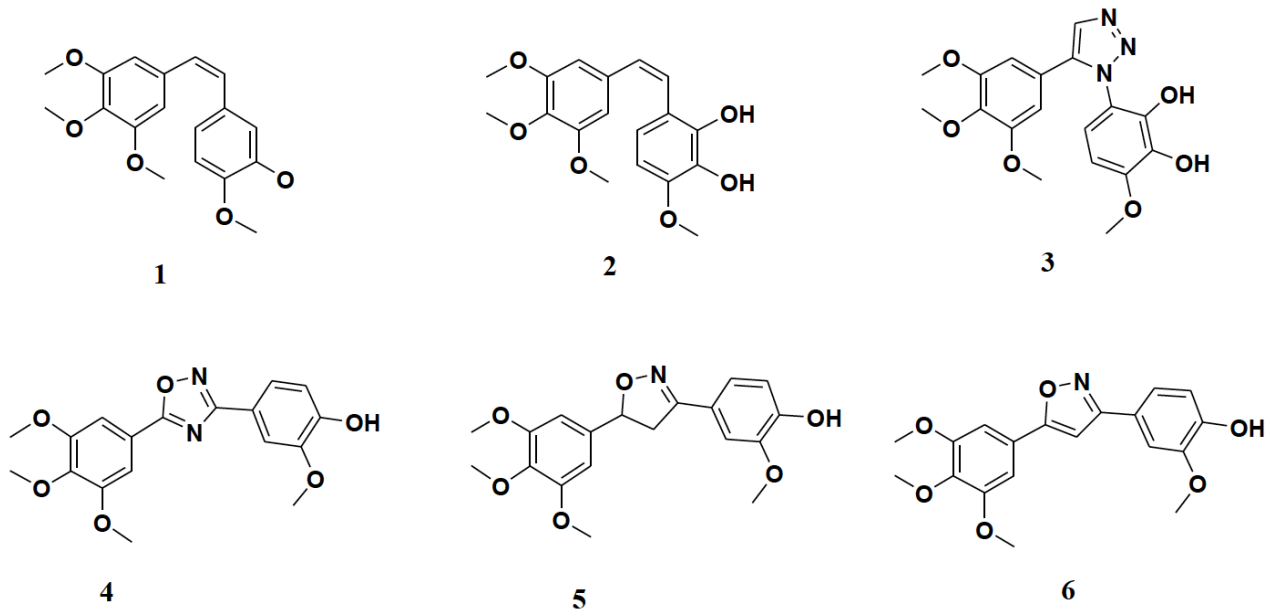


Fig. (1). Chemical structures of microtubule-binding agents, potent oxadiazole and isoxazole derivatives.

the chalcones for usage as anticancer agents, we have designed and synthesized a series of isoxazole-chalcones which contain structural features of combretastatin A-4 and chalcones. These conjugates have not been reported so far to the best of our knowledge, where we can expect these molecules as potent inhibitors of tubulin polymerization [26].

2. EXPERIMENTAL SECTION

All the reactants, reagents and solvents were obtained from commercial sources and were of analytical grade. Melting points were determined in open glass capillaries on a Fisher–Johns melting point apparatus and are uncorrected. ELEMENTAR/Vario EL Cube was used to carry out elemental analysis. NMR (^{1}H 400 MHz; ^{13}C 100 MHz) were recorded at room temperature in $CDCl_3$ as solvent and TMS as an internal standard (δ = 0 ppm), and the values were reported in the following order: Chemical shift (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, qq = quartet of quartet, brs= broad singlet) coupling constants (J in Hz), and integration. Mass spectra were recorded on a VG micromass70-70H instrument. All the reactions were monitored by Thin Layer Chromatography (TLC) on precoated silica gel GF-254 (100-200 mesh); spots were visualized under UV light at 254 nm.

2.1. Typical Experimental Procedure for the Synthesis of (E)-l-(3,4,5-Trimethoxyphenyl)-3-(5-(3,4,5-trimethoxyphenyl) isoxazol-3-yl)prop-2-en-l-one (14a)

A mixture of l-(3,4,5-trimethoxyphenyl)ethanones **(13a)** (210 mg, 1 mmol) and 5-(3,4,5-trimethoxyphenyl)isoxazole-3-carbaldehyde **(12a)** (263 mg, 1 mmol) was dissolved in 10 mL ethanol. To this mixture, sodium hydroxide (40%, 1 mL) was added at 0-5^{o}C. The reaction mixture was stirred at room temperature for 2 h. Then this reaction mixture was poured over crushed ice and acidified with dilute hydrochloric acid. The obtained solid was filtered, washed with water and dried. The residue was purified on column chromatography (silica gel with 50% ethyl acetate in hexane) to afford compound (**14a**) as a yellow solid, Yield: 77%; MR: 220-222°C; DIPMS: m/z=455.97. Elemental analysis: Calculated (%) for $C_{24}H_{25}NO_8$: C-63.29, H-5.53, and N-3.08; found (%) C-63.18, H-5.49, and N-3.01;^{1}H NMR (400 MHz, $CDCl_3$): δ3.9 (brs, 18H, OCH_3), 6.85 (s, 1H, ArH), 6.90 (s, 2H, ArH), 7.23-7.29 (m, 2H, ArH), 7.55 (d, 1H, *J*=15.6 Hz, C=C), 7.76 (d, 1H, *J*=15.6 Hz, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ186.5, 165.8, 159.3, 150.2, 150.6, 142.3, 136.3, 131.8, 124.3, 122.8, 121.9, 98.7, 98.2, 55.3, 53.4 ppm.

Following the same procedure as depicted for **14a**, the other isoxazole-chalcone derivatives **14b–m** were prepared.

(E)-1-(3,4-Dimethoxyphenyl)-3-(5-(3,4,5-trimethoxyphenyl) isoxazol-3-yl)prop-2-en-l-one (14b)

Yellow solid, Yield: 72%;MR: 196-198°C; DIPMS :m/z=425.14; $C_{23}H_{23}O_7NNa$ $[M+Na]^+$ 448.13; found: 448.13; Elemental analysis: Calculated (%) for $C_{23}H_{23}NO_7$: C-64.93, H-5.45, and N-3.29; found (%) C-64.87, H-5.38, and N-3.23^{1}H NMR (400 MHz, $CDCl_3$): δ 3.91 (s, 3H, OCH_3), 3.97 (s, 6H, OCH_3), 4.13 (s, 6H, OCH_3), 6.85 (s, 1H, ArH), 6.93 (d, 1H, *J*=8.3 Hz, ArH), 7.30-7.40 (m, 3H, ArH), 7.45 (d, 1H,*J*=8.3 Hz,ArH), 7.64 (d, 1H, *J*=15.1 Hz, C=C), 7.78 (d, 1H, *J*=15.1 Hz, C=C), ^{13}C NMR (100 MHz, $CDCl_3$): δ185.6, 165.3, 158.2, 151.6, 149.3, 135.3, 131.8, 123.2, 120.7, 119.2, 118.6, 108.3, 103.1, 97.2, 96.4, 56.8, 52.3 ppm.

(E)-l-(4-Methoxyphenyl)-3-(5-(3,4,5-trimethoxyphenyl) isoxazol-3-yl)prop-2-en-l-one (14c)

Yellow solid, Yield: 76%; MR: 173-175°C; DIPMS:m/z=395.13;$C_{22}H_{21}O_6NNa$ $[M+Na]^+$ 418.12; Elemental analysis: Calculated (%) for $C_{22}H_{21}NO_6$: C-66.83, H-5.35, and N-3.54; found (%) C-66.76, H-5.30, and N-3.47^{1}H NMR (400 MHz,

$CDCl_3$): δ3.84 (s, 6H, OCH_3), 3.92 (s, 6H, OCH_3), 6.88 (s, 1H, ArH), 6.94-7.01 (m, 4H, ArH), 7.60 (d, 1H, *J*=15.8 Hz, C=C), 7.79 (d, 1H, *J*=15.8 Hz, C=C), 8.04 (d, 2H, *J*=3.8 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 184.6, 164.3, 162.3, 158.6, 149.2, 135.2, 131.8, 125.8, 125.2, 123.8, 120.5, 120.4, 110.6, 97.2, 96.6, 56.8, 52.2 ppm.

(E)-l-(4-Fluorophenyl)-3-(5-(3,4,5-trimethoxyphenyl) isoxazol-3-yl)prop-2-en-l-one (14d)

Yellow solid, Yield: 81%; MR: 179-181°C;DIPMS :m/z=383.11; Elemental analysis: Calculated (%) for $C_{21}H_{18}O_5FN$: C-65.79, H-4.73, and N-3.65; found (%) C-65.71, H-4.69, and N-3.57; ^{1}H NMR (400 MHz, $CDCl_3$): δ3.88 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3), 6.51 (s, 1H, ArH), 6.95 (s, 2H, ArH), 7.10-7.18 (m, 2H, ArH), 7.96-8.07 (m, 4H, ArH, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ185.8,164.8, 164.2, 158.7, 149.3, 135.3, 131.8, 129.7, 127.7, 127.1, 123.2,120.7, 112.2, 111.6, 97.6, 95.1, 56.7, 52.4 ppm.

(E)-3-(5-(3,4-Dirnethoxyphenyl)isoxazol-3-yl)-l-(3,4,5-trimethoxyphenyl)prop-2-en-l-one (14e)

Yellow solid, Yield: 80%; MR: 180-182°C; DIPMS:m/z=425.32;$C_{23}H_{23}O_7NNa$ $[M+Na]^+$ 448.13; Elemental analysis: Analysis calculated for $C_{23}H_{23}O_7N$: C-64.93, H-5.45, and N-3.29; found C-64.84, H-5.38, and N-3.21; ^{1}H NMR (400 MHz, $CDC1_3$):δ3.91 (s, 12H, OCH_3), 3.98 (s, 3H, OCH_3), 6.70 (s, 1H, ArH), 7.23 (s, 2H, ArH), 7.34 (s, 1H, ArH), 7.41 (dd, 2H, *J*=6.0 Hz, *J*=2.3 Hz, ArH), 7.61 (d, 1H, *J*=15.8 Hz, C=C), 7.75 (d, 1H, *J*=15.8 Hz, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.7,165.3, 158.6, 149.6, 144.7, 144.1, 141.2, 131.8, 124.6, 123.8, 119.7, 115.2, 107.2, 104.8, 96.4, 95.6, 94.7, 55.7, 52.2 ppm.

(E)-1-(3,4-Dimethoxyphenyl)-3-(5-(3,4-dimethoxyphenyl) isoxazol-3-yl)prop-2-en-1-one (14f)

Yellow solid, Yield: 79%; MR: 203-205°C; DIPMS: m/z=395.136; Elemental analysis: Calculated (%)for $C_{22}H_{21}O_6N$: C-66.83, H-5.35, and N-3.54; found (%) C-66.76, H-5.30, and N-3.46^{1}H NMR (400 MHz, $CDCl_3$): δ3.95 (s, 6H, OCH_3), 3.98 (s, 6H, OCH_3), 6.70 (s, 1H, ArH), 6.88-6.94 (m, 2H, ArH), 7.24-7.30 (m, 2H, ArH), 7.41 (d, 2H, *J*=6.0 Hz, *J*=2.3 Hz, ArH), 7.66 (d, IH, *J*=15.8 Hz, C=C), 7.75 (d, 1H, *J*=15.8 Hz, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ185.3, 164.6, 158.6, 151.6, 146.2, 145.9, 131.7, 123.1, 119.8, 119.1, 118.2, 117.6,115.1, 107.2, 104.8, 103.8, 95.9, 52.7, ppm.

(E)-3-(5-(3,4-Dimethoxyphenyl)isoxazol-3-yl)-l-(4-methoxyphenyl)prop-2-en-l-one (14g)

Yellow solid, Yield: 84%; MR: 187-189°C; DIPMS: m/z=365.12; Elemental analysis: Calculated (%) for $C_{21}H_{19}O_5N$: C-69.03, H-5.24, and N-3.83; found (%) C-68.96, H-5.19, and N-3.76; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.83 (s, 3H, OCH_3), 3.91 (s, 6H, OCH_3), 6.65 (s, 1H, ArH), 6.78 (d, 1H, *J*=8.2 Hz, ArH), 7.17 (d, 2H, *J*=8.6 Hz, ArH), 7.35-741 (m, 2H, ArH), 7.49 (d, 2H, *J*=8.6 Hz, ArH), 7.61 (d, 1H, *J*=15.6 Hz, C=C), 7.73 (d, 1H, *J*=15.6 Hz, C=C) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ183.9, 164.3, 162.2, 158.6, 145.6,144.9, 131.8,126.7, 125.7, 124.8, 122.8, 119.6, 114.6, 110.2, 109.4, 107.1, 106.4, 103.9, 95.6, 52.1, 50.8 ppm.

(E)-3-(5-(3,4-Dimethoxyphenyl)isoxazol-3-yl)-l-(4-fluorophenyl)prop-2-en-l-one (14h)

Yellow solid, Yield: 72%;MR: 194-196°C; DIPMS: m/z=353.10; Elemental analysis: Calculated (%) for $C_{20}H_{16}O_4NF$: C-67.98, H-4.56, and N-3.96; found (%) C-67.91, H-4.49, and N-3.87; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.92 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.65 (s, 1H, ArH), 6.91 (d, IH, *J*=8.0 Hz, ArH), 7.10-7.18 (m, 2H, ArH), 7.23 (s, 1H, ArH), 7.29-7.35 (dd, 1H, *J*=6.8 Hz, J=1.5 Hz, ArH), 7.98-8.07 (m, 4H, ArH, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ184.9, 164.6, 163.1, 158.6, 145.9, 145.3, 131.8, 129.8, 127.9, 126.7, 123.1, 119.8, 115.8, 112.3, 106.9, 104.8, 95.9, 51.8 ppm.

(E)-3-(5-(4-Methoxyphenyl) isoxazol-3-yl)-l-(3,4,5-trimethoxyphenyl)prop-2-en-l-one (14i)

Yellow solid, Yield: 75%; MR: 192-194°C;DIPMS: m/z=395.13; $C_{22}H_{21}O_6NNa$ $[M+Na]^+$ 418.12; Elemental analysis: Calculated (%) for $C_{22}H_{21}O_6N$: C-66.83, H-5.35, and N-3.54; found (%) C-66.76, H-5.29, and N-3.49; ^{1}H NMR (400 MHz, $CDC1_3$): δ 3.88 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.96 (s, 6H, OCH_3), 6.67 (s, 1H, ArH), 7.01 (d, 2H, *J*=8.9 Hz, ArH), 7.28 (s, 2H, ArH), 7.60 (d, 1H, *J*=15.8 Hz, C=C), 7.70 (d, 1H, *J*=15.8 Hz, C=C), 7.77 (d, 2H, *J*=8.9 Hz, ArH); ^{13}C NMR (100 MHz, $CDC1_3$): δ185.4, 164.8, 158.7, 155.9, 149.8, 141.3, 131.2,124.1, 123.2,122.6, 121.8, 114.5, 110.7, 109.6, 96.6, 94.6, 56.1, 51.8, 50.6 ppm.

(E)-1-(3,4-Dimethoxyphenyl)-3-(5-(4-methoxyphenyl) isoxazol-3-yl)prop-2-en-l-one (14j)

Yellow solid, Yield: 78%;MR: 163-165°C; DIPMS: m/z=365.12; Elemental analysis: Calculated (%) for $C_{21}H_{19}O_5N$: C-69.03, H-5.24, and N-3.83; found (%) C-68.97, H-5.18, and N-3.80; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.98 (s, 6H, OCH_3), 6.67 (s, 1H, ArH), 6.93-6.96 (m, 1H, ArH), 7.01 (d, 2H, *J*=8.9 Hz, ArH), 7.60-7.65 (m, 1H, ArH), 7.66-7.71 (m, 2H, ArH, C=C), 7.74 (d, 1H, *J*=15.8 Hz, C=C), 7.76 (d, 2H, *J*=8.9 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ185.1, 164.4, 158.3, 155.6, 151.7, 146.1, 131.8, 123.7, 122.8, 119.8, 118.7, 114.1, 108.6, 103.1, 100.4, 95.8, 51.1, 50.6 ppm.

(E)-l-(4-Methoxyphenyl)-3-(5-(4-methoxyphenyl) isoxazol-3-yl) prop-2-en-1-one (14k)

Yellow solid, Yield: 78%;MR: 190-192°C; DIPMS: m/z=335.15;$C_{20}H_{17}O_4NNa$ $[M+Na]^+$ 358.10; Elemental analysis: Calculated (%) for $C_{20}H_{17}O_4N$: C-71.63, H-5.10, and N-4.18; found (%) C-71.57, H-5.03, and N-4.11; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.67 (s, 1H, ArH), 6.99 (d, 2H, *J*=2.4 Hz, ArH), 7.02 (d, 2H, *J*=2.4 Hz, ArH), 7.64 (d, 1H, *J*=15.9 Hz, C=C), 7.73 (d, 1H, *J*=15.9 Hz, C=C), 7.76 (d, 2H, *J*=8.8 Hz, ArH), 8.05 (d, 2H, *J*=8.8 Hz, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ185.2, 165.2, 161.9, 158.4, 155.8, 131.8, 126.1, 125.5, 124.7, 123.6, 122.3, 121.4, 114.6, 110.2, 95.8, 51.3 ppm.

(E)-1-(4-Fluorophenyl)-3-(5-(4-methoxyphenyl) isoxazol-3-yl) prop-2-en-l-one (141)

Yellow solid, Yield: 70%; MR: 178-180°C; DIPMS: m/z=323.09;Elemental analysis: Calculated (%) for

$C_{19}H_{14}O_3NF$: C-70.58, H-4.36, and N-4.33; found (%) C-70.51, H-4.28, and N-4.27; ^{1}H NMR (400 MHz, $CDCl_3$): δ3.84 (s, 3H, OCH_3), 6.41 (s, 1H, ArH), 6.94 (d, 2H, 7=9.0 Hz, ArH), 7.10-7.16 (m, 2H, ArH), 7.66 (d, 2H, *J*=9.0 Hz, ArH), 7.99-8.04 (m, 4H, ArH, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ184.6, 165.6, 164.2, 158.6, 156.1, 132.2, 129.6, 127.2, 123.2, 122.5,114.3, 112.3, 110.9, 96.6, 51.8 ppm.

(E)-3-(5-(4-Fluorophenyl) isoxazol-3-yl)-l-(3,4,5-trimethoxyphenyl)prop-2-en-l-one (14m)

Yellow solid, Yield: 75%; MR: 169-171^{o}C; DIPMS: m/z=383.11;Elemental analysis: calculated (%) for $C_{21}H_{18}O_5NF$: C-65.79, H-4.73, and N-3.65; found (%) C-65.71, H-4.68, and N-3.59; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.81 (s, 3H, OCH_3), 3.87 (s, 6H, OCH_3), 6.81 (s, 1H, ArH), 6.86 (d, 2H, *J*=8.4 Hz, ArH), 7.25 (s, 2H, ArH), 7.54-7.68 (m, 4H, ArH, C=C); ^{13}C NMR (100 MHz, $CDCl_3$): δ184.9, 165.1, 158.6, 157.4, 149.8, 141.2, 131.8, 124.6,123.2,118.6, 111.8, 110.5, 95.7, 94.8, 56.1, 51.8 ppm.

2.2. Experimental Procedure for Biological Studies

2.2.1. Fixation Protocol

200 µl of culture medium was taken in each well and added 50% TCA (50 µl, 4^{o}C) solution on it. 50% TCA (50 µl, 4^{o}C) solution was gently added without any fluid shearing forces to each well to avoid any loss / detachment of cells. Microplates were incubated at 4^{o}C for 30 min and then washed with deionized for five times. Later microplates were allowed to dry at room temperature for about 24 h [27, 28].

2.2.2. SRB Assay

In the present study, SRB assay was performed as per protocol of Skehan *et al*. [29, 30]. Sulforhodamine B (0.4% w/v) solution was prepared in 1% acetic acid solution. 70 µl Sulforhodamine B solution was transferred to each well and allowed for twenty minutes at room temperature. Dilute acetic acid (1%) was used to wash the plates for five times after the removal of SRB and air dried. Unbuffered tris-base solution (200 ml of 10 mM) was used to solubilise the bound SRB while leaving the plates for about 10 min on a plate shaker. Absorbance in a 96-well plate reader (Anthos-2001, Anthos Labteck Instruments, A-5022, Salzburg) was measured at 492 nm by subtraction of background readings measured at 620 nm. Statistical analysis (coefficient of variation and mean values of six replicate wells) was carried out using Excel 7.0 software.

2.2.3. In Vitro Cytotoxicity Assay

Exponential growth of cells was initiated at 37^{o}C for 24 hours by inoculating 100 µl per cell *i.e.*, 10,000 cells /well. Two microplates were used for each cell line and twice repeated the experiments. Complete culture medium was used to dilute after 24 h. For each concentration of synthesized compound, six replicate wells were used. SRB assay was used to evaluate the cytotoxicity after 24h. Regression analysis was used on dose–response curves to estimate IC_{50} values (50% inhibitory concentration) [29].

3. RESULTS AND DISCUSSIONS

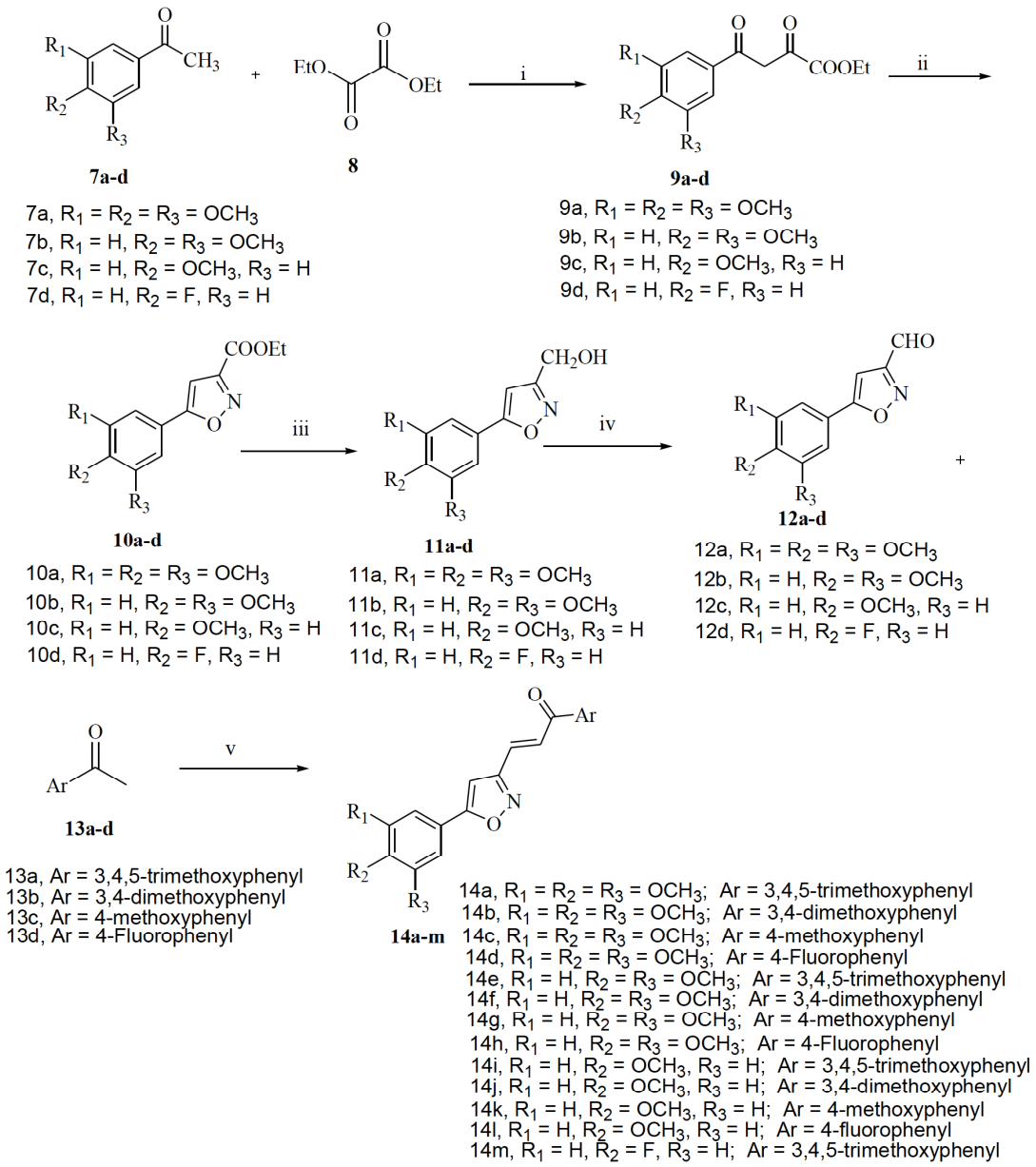
3.1. Chemistry

The synthesis of isoxazole-chalcone (**14a-m**) derivatives is sketched in Scheme **1**, in which the derivatives were obtained by the Claisen-Schmidt condensation of suitable substituted acetophenones with isoxazole aldehydes. The key intermediates 5-substituted phenyl-isoxazol-3-carbaldehydes were prepared in four sequential steps. Initially substituted acetophenones (**7a-d**) reacted with diethyl oxalate **(8)** in the presence of sodium ethanoate in ethanol to obtain ethyl 2,4-dioxo-4-(substituted phenyl)butanoates **(9a-d)**. This was further cyclized with hydroxylamine hydrochloride in ethanol to produce ethyl 5-substituted phenyl-isoxazol-3-carboxylates in good yields. The obtained carboxylates were reduced to (5-substitutedphenyl-isoxazol-3-yl) methanol with $LiAlH_4$ in THF. These were selectively oxidized to 5-substituted phenyl-isoxazol-3-carbaldehydes by IBX (2-iodoxy benzoic acid) in DMSO. The obtained carbaldehydes were condensed with 1-(3, 4, 5-trimethoxyphenyl) ethanone (**13a-d**) in presence of 10% aqueous sodium hydroxide solution to form a compound 14a-m.

3.2. *In Vitro* Cytotoxic Studies

These synthesized isoxazole-chalcone compounds (**14a-m**) were evaluated for their anticancer activity in selected human cancer cell lines like DU145 (prostate carcinoma), MDA MB-231, MCF-7 (breast cancer) and A549 (non-small cell lung cancer) cell lines (Table **1**) by using sulforhodamine B (SRB) method [27, 28] and shown in IC_{50} values. IC_{50} means that the concentration at which minimum 50% of the cancer cell lines shall be destroyed The trimethoxy chalcone (TMC) was used as positive control for screening of anticancer activities [30-32] and TMC derivatives are also exhibited potent anticancer activities against different human cancer cell lines [33-35]. The synthesized compounds exhibited anticancer potency with IC_{50} values ranging from 0.96 to 26.29 µM. Interestingly, almost all these new compounds showed significant activity towards DU-145 cancer cell line compared to other cell lines. According to structure-activity relationship studies, the presence of electron donating groups like methoxy, dimethoxy or trimethoxy substituents on either of phenyl rings showed enhanced anti-cancer activities especially on DU-145 and MDA-MB-231 cancer cell lines. The present observations are in similar lines to previous reports by other researchers in which the presence of 3,4,5-trimethoxyphenyl group in chalcones was ascribed to their exhibited anticancer activity [31].

Among the 13 series of the compounds, the compounds **14i** and **14j** exhibited potent cytotoxic activities against DU-145 prostate cancer cell line with IC_{50} values of 0.96 µM and 1.06 µM as compared to the positive control (IC_{50} value 4.10 µM). So these two compounds may be identified as promising drug lead compounds. The compounds **14a, 14b, 14c, 14d, 14e** and **14k** showed promising cytotoxic activities against DU145 cell line with IC_{50} values of 1.80 µM, 1.72 µM, 2.32 µM, 2.32 µM, 1.23 µM and 1.93 µM respectively. While the compounds, **14c**, **14e** and **14i** showed good cytotoxic activities against MDA-MB-231 cell line with IC_{50}



Reagents & Conditions: **(i)** Na metal, EtOH, rt; 4-5 h **(ii)** $NH_2OH.HCl$, EtOH, reflux, 2-3 h, **(iii)** LAH, THF, rt, 2-3 h, **(iv)** IBX, DMSO, 0^oC, 2-3 h, **(v)** 10% aq. NaOH, 1-2 h, rt.

Scheme 1. Synthesis of novel hybrid isoxazole chalcone derivatives (14a-m).

Table 1. Anticancer activity (IC_{50} values in µM)[a] for compounds 14a-m in selected human cancer cell lines.

Compound	IC_{50} values (µM)[a]			
	DU-145[b]	MDA MB-231[c]	MCF-7[c]	A-549[d]
14a	1.80	3.67	5.28	5.99
14b	1.72	3.15	4.25	6.35
14c	2.32	2.49	3.92	5.97
14d	2.32	4.99	6.84	7.48
14e	1.23	2.42	3.11	5.36

(Table 1) contd….

Compound	IC_{50} values (μM)[a]			
	DU-145[b]	MDA MB-231[c]	MCF-7[c]	A-549[d]
14f	15.62	19.43	26.29	17.52
14g	8.06	11.83	21.48	19.64
14h	11.36	18.42	20.54	21.89
14i	0.96	2.18	3.12	3.29
14j	1.06	5.60	4.36	3.98
14k	1.93	4.91	5.92	6.28
14l	16.66	20.41	19.31	15.36
14m	4.54	8.91	10.87	11.89
TMC	4.10	5.25	4.61	2.95

[a]Concentration required for 50% inhibition and the values are mean of three determinations, [b]prostate cancer, [c]breast cancer, [d]lung cancer.

values of 2.49 μM, 2.42 μM and 2.18 μM respectively. The compounds 14b, 14c, 14e, 14i, 14j and 14k showed better activities against breast cancer cell line MCF-7 with IC_{50} values of 4.25 μM, 3.92 μM, 3.11 μM, 3.12 μM, 4.36 μM and 5.92 μM respectively. All the fourteen compounds showed less cytotoxic activities against lung cancer cell line A-549.

CONCLUSION

In the present study, we have synthesized new series of isoxazole-chalcone conjugates (**14a-m**) and evaluated them for their *in vitro* cytotoxic activity. It was observed that compounds -**14a, 14b, 14e, 14i, 14j** and **14k** exhibited potent cytotoxic activity against prostate DU-145 cancer cell line. The observed cytotoxic studies were related to the presence of electron donating groups like methoxy, dimethoxy or trimethoxy substituents (especially 3,4,5-trimethoxyphenyl group) on either of phenyl rings.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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