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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design and synthesis of new coumarin–chalcone/NO hybrids of potential biological activity

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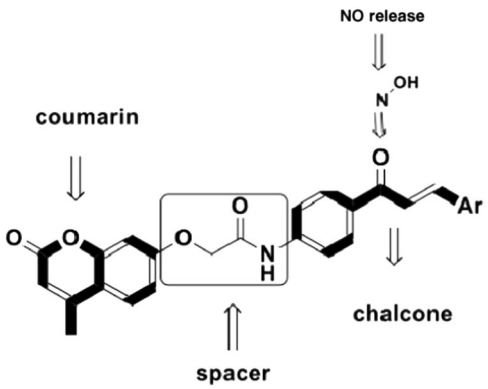
Abstract This study aims at investigating a synthesis approach based on molecular hybridization strategy through grafting an nitric oxide-releasing moiety, oxime, to coumarin–chalcone hybrids. In vitro anti-proliferative activity of some of the prepared compounds showed moderate activity (growth inhibition values = 45.85, 40.86, 39.25 for compound **8a** against leukemia, Central Nervous system and breast cancer cells, respectively). Also, IC_{50} = 9.62 and 14.40 for compounds **8h** and **8f**, respectively against breast Michigan Cancer Foundation-7 cell lines. The antibacterial screening results suggest a possible role for nitric oxide in enhancement of the antibacterial activity where nitric oxide is not the only factor but other factors like physicochemical properties should be investigated for their potential role on the activity.

Graphical abstract



Keywords Coumarins · Chalcones · Anti-proliferative · Antibacterial · Nitric oxide donors · Oximes

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Introduction

Flavonoids compose large group of plant secondary metabolites that can be found in many plants such as ferns, conifers and flowering plants (Di Carlo et al. 1999, Rackova et al. 2005; Middleton et al. 2000). They usually contribute to plant color, and they also display several pharmacological benefits (e.g., anticancer, anti-inflammatory, anti-allergic, etc.) and are known as effective antioxidants, metal chelators, and free radical scavengers (Pick et al. 2011; Amaral et al. 2009; Gong et al. 2009). Natural and synthetic flavonoids therefore drawn much interest in the development of novel therapeutic agents for various diseases and

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are generally believed to be non-toxic compounds (Rackova et al. 2005; Amaral et al. 2009).

The chromone ring system, 1-benzopyran-4-one, is the core fragment in several flavonoids, such as flavones, flavonols and isoflavones (Joule and Mills 2010). The importance of such compounds arisen from the pharmacological activities that they display (Borges et al. 2005; Rao and Tangeti 2012), such as antimicrobial (Matos et al. 2012a), monoamine oxidase inhibition (Matos et al. 2011, 2012b; Secci et al. 2011), antitumor (Serra et al. 2012; Singh et al. 2014), adenosine receptor antagonist (Vazquez-Rodriguez et al. 2013), and antioxidant (Kostova et al. 2011) among others. The prominent anti-tumor activity of coumarin is believed to be due to its metabolites (e.g., 7-hydroxycoumarin). 7-hydroxy coumarin inhibits the release of Cyclin D1, which is overexpressed in many types of cancer. This knowledge may lead to its use in cancer therapy (Lacy and O'Kennedy 2004). Esculetin (coumarin derivative) inhibits growth and cell cycle progression by inducing arrest of the G1 phase in HL-60 leukemia cells, resulting from the inhibition of retinoblastoma protein phosphorylation (Lacy and O'Kennedy 2004). Several drugs such as Khellin, sodium cromoglycate, diosmin, and flavoxate (Fig. 1) belonging to this chemical class are available in the market for treatment of various diseases. Coumarins are known to possess antibacterial activity. Novobiocin is an important naturally occurring antibiotic in which the coumarin nucleus is present in its skeleton, which is mainly active against Gram-positive bacteria. It can antagonize the B subunit of the essential *E. coli* DNA gyrase super twisting activity in vitro and the bacterial multiplication (Riveiro et al. 2010; Sahu et al. 2012).

Moreover, chalcones (1,3-diaryl-2-propen-1-onesthe precursors of flavonoids and isoflavonoids are widely present in edible plants. Chemically, they consist of open-chain

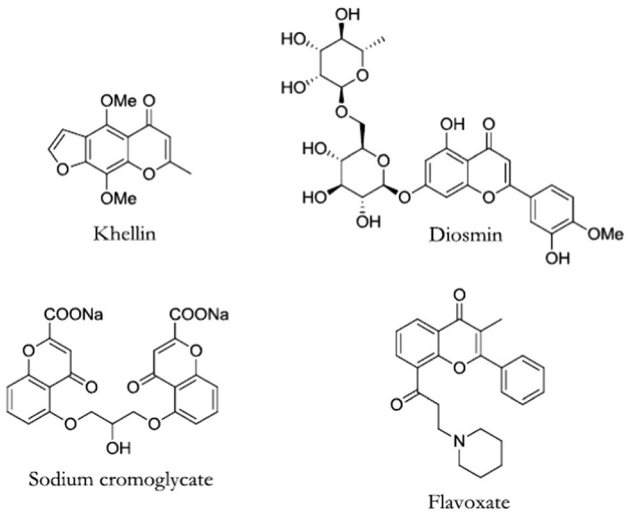


Fig. 1 Examples of some important chromone-based drugs

flavonoids in which the two aromatic rings are joined by a three-carbon α, β-unsaturated carbonyl system (Sahu et al. 2012; Go et al. 2005; Bandgar et al. 2009). Among the flavonoids, chalcones are extensively investigated due to their broad spectrum of biological activities, including anti-inflammatory (Kachadourian et al. 2012), anti-tumor (Kumar et al. 2011; Valdameri et al. 2012), and anti-bacterial (Vicini et al. 2006) activity. They are regarded as promising anticancer agents against most human cancers. Previous literature suggests that chalcones are capable of inducing apoptosis and also have the ability to uncouple mitochondrial respiration and thus collapse mitochondrial membrane potential. Other proposed mechanism for this anti-cancer activity is tubulin polymerization prevention via binding to the colchicine-binding site (Go et al. 2005; Ducki 2009; Dyrager et al. 2011).

On the other hand, nitric oxide (NO) is an important gas mediator that was found to be biosynthesized using enzymatic and non-enzymatic reactions (Andreadou et al. 2015; Moncada and Higgs 1993). It has a range of biological activities including regulation of vascular tone, inhibition of platelet aggregation, and neurotransmission (Moncada and Higgs 1993; Torres-Rasgado et al. 2007; Knowles and Moncada 1994). NO has long been associated with cancer. Interestingly, various studies have shown that NO can both promote and inhibit tumor progression and metastasis (Vasudevan and Thomas 2014). The effects of NO in tumors seem to depend on the activity and localization of nitric oxide synthesases isoforms, concentration and duration of NO exposure, and cellular sensitivity to NO (Vasudevan and Thomas 2014; Fukumura et al. 2006). NO releasing drugs have been evaluated in clinical and pre-clinical settings to arrest tumor growth [Maciag et al. 2013; Hickok and Thomas 2013). Mechanisms of NO-mediated apoptosis include inhibition of mitochondrial respiration, activation of caspase signaling and accumulation of the tumor suppressor protein (p53) (Forrester et al. 1996). NO is also shown to have a role in fighting bacterial infections and inflammation. Production of NO by phagocytic cells is augmented in response to inflammation. The reaction of NO with metal centers and thiols inhibits bacterial respiration, DNA replication and specific metabolic pathways including the tricarboxylic acid cycle (Fang 2012).

Based on the aforementioned data, and on the fact that the design and development of new bioactive agents based on molecular hybridization strategy involving the integration of two or more pharmacophoric units having different mechanisms of action in the same molecule became a rationally attractive approach (Decker 2011), hybrids of coumarin and chalcones are recently investigated for potential synergism of biological activities (Pérez-Cruz et al. 2013; Pingaew et al. 2014; Singh et al. 2014). Herein, we report the design and synthesis of new coumarin–chalcone

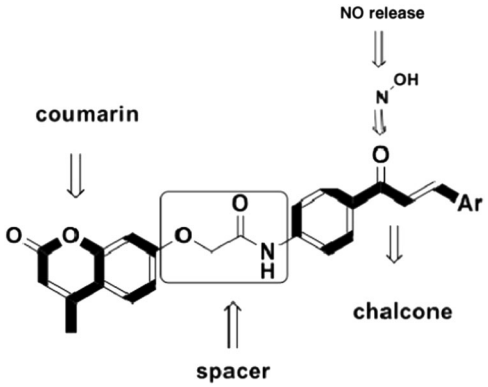


Fig. 2 General structure of the designed coumarin−chalcone/NO hybrids

hybrids linked together through a simple oxyacetamide linkage (Fig. 2). Hybridization with the NO releasing group, oxime, was then done for evaluating the potential beneficial impact of NO on the designed coumarin–chalcone hybrids (Fig. 2) as both potential anti-cancer and antibacterial agents.

Materials and methods

Chemistry

Reactions were monitored by thin layer chromatography analysis using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) with F_{254} indicator thin layer plates. Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. Infrared (IR) spectra were recorded as KBr disks on a Shimadzu S8400 IR spectrophotometer. ^{1}H nuclear magnetic resonance (NMR) spectra were carried out on 400 MHz Brucker spectrometer, using tetramethylsilane as an internal reference. Chemical shift (δ) values are given in parts per million (ppm) relative to $CDCl_3$ (7.29) or dimethyl sulfoxide (DMSO)-d_6 (2.5) and coupling constants (J) in Hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. Accurate masses were obtained on mMicromass LCT mass spectrometer. Elemental analyzes were performed on Perkin Elmer 2400 CHN Elemental Analyzer. Chalcones **3a–h** were prepared as reported (Chun Wai et al. 2014; Rtishchev et al. 2001).

General procedure for synthesis of 2-bromo-N-*{4-[3-arylacryloyl]-phenyl}acetamides (**4a–h**)*

To a stirred mixture of chalcone derivatives **3a–h** (4.20 mmol) in dichloromethane (20 mL), potassium carbonate (6.30 mmol, 0.868 g) in water (100 mL) was added. The mixture was cooled in an ice bath; bromoacetyl bromide (0.928 g, 4.60 mmol) in dichloromethane (30 mL) was added in a drop-wise manner with stirring over 30 min. Stirring was continued for 2 h at 0–5 °C, and after that at rt overnight. The reaction mixture was extracted with dichloromethane (2 × 60 mL). The organic layer was washed with distilled water (2 × 40 mL), dried over anhydrous sodium sulfate, filtered, evaporated under vacuum and the residue was filtered off, washed with ethanol, and recrystallized from ethanol (Abdel-Aziz et al. 2013).

2-Bromo-*N*-(4-((*E*)-3-phenylacryloyl) phenyl)acetamide (**4a**) Pale yellow powder (1.8 g, 83.72% yield); mp 155–157 (lit mp 157–158 °C, Abdel-Aziz et al. 2013), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.34 (s, 2H, CH_2), 7.46–7.48 (m, 3H, Ar–H), 7.75 (d, 1H, $J = 15.2$ Hz, CO–CH=CH), 7.8 (d, 2H, $J = 8$ Hz, Ar–H), 7.89–7.91 (m, 2H, Ar–H), 7.98 (d, 1H, $J = 15.2$ Hz, CH=CH–Ar), 8.18 (d, 2H, $J = 8$ Hz, Ar–H), 10.77 (bs, 1H, NH): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$ (CH_2), 119 (**CH** = CH–Ar), 122 ($C_{2,6}$), 129 ($C_{2,6}'$), 130 (C_4'), 131 ($C_{3,5}'$), 133 ($C_{3,5}$), 135 (C_4, C_1'), 143 (C_1), 144 (CH = CH–Ar), 166 (CONH), 188 (CO–Ar).

2-Bromo-*N*-(4-((*E*)-3-(4-bromophenyl)acryloyl)phenyl) acetamide (**4b**) Pale yellow powder (2.23 g, 83.72% yield), mp 175–177 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.3 (s, 2H, CH_2), 7.65– 7.71 (m, 3H, Ar–H and CO–CH = CH), 7.78 (dd, 2H, $J = 8.4$ Hz, $J = 20.4$ Hz, Ar–H) 7.85 (dd, 2H, $J = 8.4$ Hz, $J = 20.4$ Hz, Ar–H), 7.98 (d, 1H, $J = 15.2$ Hz, CH = CH–Ar), 8.18 (d, 2H, $J = 8$ Hz, Ar–H), 10.73 (bs, 1H, NH)): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta =$ 44 (CH_2), 119 (**CH**=CH–Ar), 123 ($C_{2,6}$), 124 (C_4'), 130 ($C_{2,6}'$), 131 ($C_{3,5}$), 132 ($C_{3,5}'$), 134 (C_4), 141 (C_1'), 142 (C_1), 143 (CH=**CH**–Ar), 166 (CONH), 187 (CO).

2-Bromo-N-(4-((E)-3-(4-methoxyphenyl)acryloyl)phenyl) acetamide (**4c**) Paleorange crystal (1.85 g, 79% yield), mp 161–162 °C (lit. mp 160–162 °C)$^{(Reference)}$, ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.83 (s, 3H, OCH_3), 4.33 (s, 2H, CH_2), 7.04 (d, 2H, $J = 8$ Hz, Ar–H), 7.69 (d, 1H, $J = 16$ Hz, CO–CH=CH), 7.77–7.87 (m, 5H, CH=CH–Ar and Ar–H) 8.18 (d, 2H, $J = 8$ Hz, Ar–H), 10.77 (bs, 1H, NH)): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$ (CH_2), 55 (OCH_3), 114 ($C_{3,5}'$), 119 (**CH**=CH–Ar), 120 ($C_{2,6}$), 127 ($C_{2,6}'$), 130 (C_1'), 131 ($C_{3,5}$), 133 (C_4), 143 (C_1), 144 (CH=**CH**–Ar), 161 (C_4'), 165 (CONH), 187 (CO–Ar) (Abdel-Aziz et al. 2013).

2-Bromo-*N*-(4-((*E*)-3-(2-methoxyphenyl)acryloyl)phenyl) acetamide (**4d**) Pale orange crystal (1.9 g 81.13% yield), mp 168–170 °C, ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.89 (s, 3H, OCH_3), 4.32 (s, 2H, CH_2), 7.01–7.11 (m, 2H, Ar–H), 7.42–7.46 (m, 1H, Ar–H),7.79 (d, 2H, $J = 8.4$ Hz,

Ar–H), 7.87 (d, 1H, $J = 16$ Hz, CH=CH–Ar), 7.95 (d, 1H, $J = 7.6$ Hz, Ar–H), 8.04 (d, 1H, $J = 16$ Hz, CH=CH–Ar), 8.15 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.75 (bs, 1H, NH): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$ (CH_2), 56 (OCH_3), 112 (C_3'), 119 (C_1'), 121 (C_5'), 122 (**CH** = CH–Ar), 123 ($C_{2,6}$), 128 (C_6'), 130 (C_4'), 132 ($C_{3,5}$), 133 (C_4), 138 (C_1), 143 (CH=**CH**–Ar), 158 (C_2'), 165 (CONH), 188 (CO–Ar).

2-Bromo-*N*-(4-((*E*)-3-(2,4-dichlorophenyl)acryloyl)phenyl) acetamide (**4e**) Pale orange crystal (1.9 g, 76% yield), mp 182–184 °C, ^{1}H NMR (400 MHz, DMSO- *d_6*) δ (ppm): 4.32 (s, 2H, CH_2), 7.55– 7.77 (m, 2H, Ar–H), 7.79 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.94 (d, 1H, $J = 15.6$ Hz, CH = CH–Ar), 8.03 (d, 1H, $J = 15.6$ Hz, CH = CH–Ar), 8.19 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.25 (d,1H, Ar–H), 10.72 (bs, 1H, NH): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$ (CH_2), 119 (**CH** = CH–Ar), 125 ($C_{2,6}$), 128 (C_5'), 129 (C_6'), 130 ($C_{3,5}$), 131 (C_3'), 132 (C_1'), 133 (C_2'), 135 (C_4), 136 (C_4'), 137 (C_1), 143 (CH = **CH**–Ar), 165 (CONH), 187 (CO–Ar).

2-Bromo-N-(4-((E)-3-(3,4,5 trimethoxyphenyl)acryloyl) phenyl)acetamide (**4f**) Yellow powder (2.3 g 84.56% yield), mp 165–167 °C (lit. mp 166–167 °C), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.71 (s, 3H, OCH_3), 3.87 (s, 6H, $2OCH_3$), 4.33 (s, 2H, CH_2), 7.24 (s, 2H, Ar–H), 7.69 (d, 1H, $J = 16$ Hz, CO–CH=CH), 7.8 (d, 2H, $J = 8$ Hz, Ar–H), 7.91 (d, 1H, $J = 16$ Hz, CH=CH–Ar), 8.2 (d, 2H, $J = 8$ Hz, Ar–H), 10.72 (bs, 1H, NH): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$(CH_2), 56, 60 (OCH_3), 106 ($C_{2,6}'$), 119 (**CH**=CH–Ar), 121 ($C_{2,6}$), 130 (C_1'), 131 ($C_{3,5}$), 133 (C_4), 140 (C_4'), 143 (C_1), 144 (CH=**CH**–Ar), 153 ($C_{3,5}'$), 165 (CONH), 187 (CO–Ar) (Abdel-Aziz et al. 2013).

2-Bromo-N-(4-((E)-3-(4-chlorophenyl)acryloyl)phenyl) acetamide (**4g**) Pale yellow powder (1.81 g, 76% yield), mp 190–191 (lit mp 190–192 °C), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.30 (s, 2H, CH_2), 7.65– 7.71 (m, 3H, Ar–H and CO–CH = CH), 7.72 (dd, 2H, $J = 8.4$ Hz, $J =$ 20.4 Hz, Ar–H) 7.80 (dd, 2H, $J = 8.4$ Hz, $J = 20.4$ Hz, Ar–H), 7.98 (d, 1H, $J = 15.2$ Hz, CH = CH–Ar), 8.18 (d, 2H, $J = 8$ Hz, Ar–H), 10.73 (bs, 1H, NH)): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$ (CH_2), 119 (**CH**=CH–Ar), 124 ($C_{2,6}$), 125 ($C_{2,6}'$), 130 ($C_{3,5}'$), 131 ($C_{3,5}$), 132 (C_1'), 134 (C_4), 141 (C_4'), 142 (C_1), 143 (CH=**CH**–Ar), 166 (CONH), 187 (CO–Ar) (Abdel-Aziz et al. 2013).

2-Bromo-N-(4-((E)-3-(2-furyl)acryloyl)phenyl)acetamide (**4h**) Pale yellow powder (1.57 g 75% yield), mp 137–139 °C (lit mp 138–139 °C), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 4.33 (s, 2H, CH_2), 6.7 (d, 2H, $J = 15.8$ Hz, CO–CH=CH–Ar), 7.11 (d, 2H, $J = 15.8$ Hz, CO–CH=CH–Ar), 7.56 (s, 2H, Ar–H), 7.78 (d, 2H, $J = 8$ Hz, Ar–H), 7.93 (s, 1H, Ar–H), 8.11 (d, 2H, $J = 8$ Hz, Ar–H), 10.71 (bs, 1H, NH): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 44$ (CH_2), 113 (C_3'), 117 (C_4'), 119 ($C_{2,6}$), 120 (**CH**=CH–Ar), 130 ($C_{3,5}$), 131 (CH=**CH**–Ar), 133 (C_4), 143 (C_1), 146 (C_5'), 151 (C_2'), 165 (CONH), 187 (CO–Ar) (Abdel-Aziz et al. 2013).

Synthesis of 7-hydroxy-4-methylcoumarine (**7**) Compound **7** was synthesized according to the reported procedure (Thimons et al. 1998). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.33 (s, 3H, CH_3), 4.1 (bs, 1H, OH), 6.09 (s, 1H, H-3 pyran), 6.68–7.55 (m, 3H, Ar–H): ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18.54$ (CH_3), 102 (C_9), 110 (C_3), 112 (C_7), 113 (C_5), 127 (C_6), 153 (C_{10}), 155 (C_4), 160 (C_8), 161 (CO).

*General procedure for synthesis of 2-(4-methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-phenylacryloyl)phenyl) acetamide (**8a–h**)*

A mixture of compounds **4a–h** (0.015 mol), compound **7** (0.01 mol, 1.76 g), and K_2CO_3 (0.04 mol, 5.5 g) in dimethyl formamide (DMF) (5 mL), was stirred at room temperature for 6–48 h, then water was added and the formed precipitate was filtered, washed with water, and recrystallized from methanol–chloroform mixture (9:1) (El-koussi and Abdel-rahman 2006).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-phenylacryloyl) phenyl)acetamide (**8a**) Pale yellow powder (4.15 g, 94.5% yield), mp 230–231 °C; IR (KBr) υ_{max} (cm^{-1}) 3350 (NH), 1712 (C=O-coumarin), 1693 (C=O-ketone), 1660 (C=O-amide), ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 4.93 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.84 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.05–7.08 (m, 2H, Ar–H), 7.46–7.47 (m, 3H, Ar–H), 7.71–7.75 (m, 2H, Ar–H), 7.88–7.97 (m, 3H, Ar–H and –CH=CH–), 8.19 (d, 2H, $J = 8.8$ Hz, Ar–H), 10.61 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 114 (C_5), 119 (**CH**=CH–Ar), 122 ($C_{2,6}''$), 127-($C_{2,6}'''$), 129 (C_6'), 130 -(C_4'''), 131-($C_{3,5}'''$), 133 ($C_{3,5}''$), 134 (C_4''),135-(C_1'''),143 -(C_1''), 144 (CH=**CH**–Ar), 153 (C_{10}'), 155 (C_4), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 188 (CO–Ar).

2–(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(4-bromophenyl)acryloyl) phenyl)acetamide (**8b**) White powder (3.9 g, 75.28% yield), mp 290–291 °C; IR (KBr) υ_{max} (cm^{-1}) 3365 (NH), 1712 (C=O-coumarin), 1695 (C=O-ketone), 1651 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.05–7.08 (m, 2H, Ar–H), 7.66 (d, 2H, Ar–H), 7.19–7.87 (m, 6H, Ar–H and CH=CH–Ar), 7.99 (d, 1H, $J = 15.6$ Hz, CH=CH–Ar), 8.17 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.62 (bs, 1H, NH); ^{13}C NMR (100

MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 114 (C_5), 119 (**CH**=CH–Ar), 123 ($C_{2,6}''$), 124-(C_4'''), 127 (C_6'), 130-($C_{2,6}'''$), 131 ($C_{3,5}''$), 132-($C_{3,5}'''$), 133 (C_4''), 134-(C_1'''), 142 (C_1''), 143 (CH=**CH**–Ar), 153 (C_{10}'), 155 (C_4), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 188 (CO–Ar).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(4-methoxyphenyl) acryloyl)phenyl)acetamide (**8c**) Pale yellow powder (3.46 g, 73.77% yield), mp 253–255 °C; IR (KBr) υ_{max} (cm^{-1}) 3332 (NH), 1716 (C=O-coumarin), 1683 (C=O-ketone), 1651 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.05–7.08 (m, 2H, Ar–H), 7.65–7.74 (m, 3H, Ar–H), 7.83–7.87 (m, 5H, Ar–H and CH=CH–Ar), 7.99 (d, 1H, $J = 15.6$ Hz, CH=CH–Ar), 8.17 (d, 2H,$J = 8.4$ Hz, Ar–H), 10.56 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 19$ (CH_3), 61 (OCH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 113 (C_5), 114 -($C_{3,5}'''$), 119 (**CH**=CH–Ar), 126 ($C_{2,6}''$), 124-($C_{2,6}'''$), 127-(C_1'''), 130 (C_6'), 132 ($C_{3,5}''$), 134 (C_4''), 142 (C_1''), 143 (CH=**CH**–Ar), 153 (C_{10}'), 155 (C_4), 160-(C_4'''), 161 (C_8'), 167 ($CO_{coumarin}$), 172 (CONH), 187 (CO–Ar).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(2-methoxyphenyl)acryloyl)phenyl)acetamide (**8d**) Pale yellow powder (3.33 g, 71% yield), mp 233–234 °C; IR (KBr) υ_{max} (cm^{-1}) 3354 (NH), 1714 (C=O-coumarin), 1695 (C=O-ketone), 1651 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 3.90 (s, 3H, OCH_3), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.04–7.31 (m, 4H, Ar–H), 7.45–7.75 (m, 2H, Ar–H), 7.83 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.89 (d, 1H, $J = 16$ Hz, CH=CH–Ar), 7.97 (d, 1H, $J = 7.6$ Hz, Ar–H), 8.04 (d, 1H, $J = 16$ Hz, CH=CH–Ar), 8.15 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.55 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_d) $\delta = 19$ (CH_3), 61 (OCH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 113 (C_5), 114-(C_3'''), 119-(C_1'''), 121-(C_5'''), 122 (**CH**=CH–Ar), 123 ($C_{2,6}''$), 126-(C_6'''), 128 (C_6'), 130-(C_4'''), 132 ($C_{3,5}''$), 133 (C_4''), 138 (C_1''), 143 (CH=**CH**–Ar), 153 (C_{10}'), 155 (C_4), 158-(C_2'''), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 188 (CO–Ar).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(2,4-dichlorophenyl)acryloyl)phenyl)acetamide (**8e**) Pale yellow powder (3.74 g, 73.62 % yield), mp 280–281 °C; IR (KBr) υ_{max} (cm^{-1}) 3396 (NH), 1724 (C=O-coumarin), 1705 (C=O-ketone), 1662 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.05–7.08 (m, 2H, Ar–H), 7.55–7.76 (m, 3H, Ar–H), 7.84 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.95 (d, 1H, $J = 15.6$ Hz, –CH=CH–Ar), 8.05 (d, 1H, $J = 16.4$ Hz, –CH=CH–Ar), 8.20 (d, 2H, $J = 8.8$ Hz, Ar–H), 8.26 (d, 1H, Ar–H), 10.58 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 19$ (CH_3), 67 (CH_2), 102 (C_9'), 112 (C_7'), 113 (C_3), 114 (C_5), 119 (**CH**=CH–Ar), 126 ($C_{2,6}''$), 127-(C_5'''), 128 (C_6'), 129-(C_6'''), 130 ($C_{3,5}''$), 131-(C_1'''), 132-(C_3'''), 135-(C_2'''), 136(C_4''), 137-(C_4'''), 142 (C_1''), 143 (CH=**CH**–Ar), 153 (C_{10}'), 155 (C_4), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 188 (CO–Ar).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(3,4,5- trimethoxyphenyl)acryloyl)phenyl)acetamide (**8f**) Yellow powder (5 g, 94.3% yield), mp 267–268 °C; IR (KBr) υ_{max} (cm^{-1}) 3338 (NH), 1710 (C=O-coumarin), 1672 (C=O-ketone), 1654 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 3.86 (s, 6H, $2OCH_3$), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.05–7.23 (m, 4H, Ar–H), 7.66–7.75 (m, 2H, Ar–H and CH=CH–Ar), 7.85 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.90 (d, 1H, $J = 15.6$ Hz, CH=CH–Ar), 8.19 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.58 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 56, 60 (OCH_3), 67 (CH_2), 102(C_9'), 105-($C_{2,6}'''$), 111 (C_7'), 112 (C_3), 116 (C_5), 119 (**CH**=CH–Ar), 124 ($C_{2,6}''$), 125 (C_6'), 126-(C_1'''), 131 ($C_{3,5}''$), 132 (C_4''), 134-(C_4'''), 135 (C_1''), 143 (CH=**CH**–Ar), 147 –($C_{3,5}'''$), 154 (C_{10}'), 158 (C_4), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 189 (CO–Ar).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(4-chlorophenyl)acryloyl)phenyl)acetamide (**8g**) Pale yellow powder (3.5 g, 73.99% yield), mp 240–242 °C; IR (KBr) υ_{max} (cm^{-1}) 3346 (NH), 1703 (C=O-coumarin), 1685 (C=O-ketone), 1656 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.08–7.09 (m, 2H, Ar–H), 7.53 (d, 2H, Ar–H), 7.70–7.75 (m, 2H, Ar–H and CH=CH–Ar), 7.84 (d, 2H, $J = 8.8$ Hz, Ar–H), 7.93 (d, 2H, $J = 8.4$, Ar–H), 7.98 (d, 1H, $J = 16$ Hz, CH=CH–Ar) 8.18 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.56 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 114 (C_5), 119 (**CH**=CH–Ar), 123 ($C_{2,6}''$), 127 (C_6'), 129-($C_{2,6}'''$), 130-($C_{3,5}'''$), 131 ($C_{3,5}''$), 133-(C_1'''), 134 (C_4''),135-(C_4'''), 142 (C_1''), 143 (CH=**CH**–Ar), 153 (C_{10}'), 155 (C_4'), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 188 (CO–Ar).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-((*E*)-3-(furan-2-yl)acryloyl)phenyl)acetamide (**8h**) Pale brown powder (3.46 g, 80.65% yield), mp 228–230 °C; IR (KBr) υ_{max} (cm^{-1}) 3346 (NH), 1734 (C=O-coumarin), 1683 (C=O-ketone), 1654 (C=O-amide); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.92 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 6.69 (s, 1H, Ar–H), 7.04–7.10 (m, 3H, Ar–H), 7.56 (s, 2H, Ar–H), 7.73 (d, 1H, $J = 8.4$ Hz, CH=CH–Ar) 7.81–7.91 (m, 3H, Ar–H and CH=CH–Ar),

8.09 (d, 2H, $J = 8.4$ Hz, Ar–H), 10.55 (bs, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3'''), 113 (C_3), 114 (C_4'''), 117 (C_5), 120 ($C_{2,6}''$), 127 (**CH**=CH–Ar), 130 (C_6'), 133 ($C_{3,5}''$), 143 (CH=**CH**–Ar), 146 (C_4''), 151 ($C_{1\text{-}}''$, C_2'''), 153 (C_{10}', C_5'''), 155 (C_4), 160 (C_8'), 161 ($CO_{coumarin}$), 167 (CONH), 187 (CO–Ar).

*General procedure for synthesis of 2-(4-methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-(1-(hydroxyimino)-3-(phenyl)-allyl) phenyl)acetamides (**9a–h**)*

A mixture of compounds **8a–h** (0.01 mol), hydroxylamine hydrochloride (0.03 mol, 2.07 g), was heated at reflux in pyridine for 2–3 h, pyridine was then neutralized using HCl (1N). The formed precipitate was filtered, washed with distilled water crystallized from methanol (Luo et al. 2012).

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-(1-(hydroxyimino)-3-(phenyl)-allyl)phenyl)acetamide (**9a**) Pale yellow powder (2.74 g, 60.42% yield), mp 187–188 °C; IR (KBr) υ_{max} (cm^{-1}) 3350 (OH), 3200–3280 (NH), 1712 (C=O-coumarin), 1705 (C=O-amide), 1676 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.90 (s, 2H, CH_2), 6.23 (s, 1H, H-3 pyran), 7.04–7.08 (m, 2H, Ar–H), 7.33–7.38 (m, 4H, Ar–H and CH=CH–Ar), 7.45 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.55–7.59 (m, 2H, Ar–H), 7.70–7.74 (m, 3H, Ar–H and CH=CH–Ar), 10.38 (bs, 1H, NH), 11.58 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 114 (C_5), 117 (**CH**=CH–Ar), 120 ($C_{2,6}''$), 127 ($C_{2,6}'''$), 128 (C_6'), 129 (C_4'''), 130 ($C_{3,5}'''$), 131 (C_4''), 136 ($C_{3,5}''$), 137 (C_1'''), 139 (C_1''), 141 (CH=**CH**–Ar), 149 (C_{10}'), 154 (C_4), 155(C_8), 160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS(EI) *m/z* (%) 454 (16) [M^+], 131 (35), 105 (100), 91 (24), 77 (70). Anal. calcd for $C_{27}H_{22}N_2O_6$: C,71.35;H, 4.88; N, 6.16. Found: C, 71.52; H, 4.92; N, 6.24.

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-(3-(4-bromophenyl)-1-(hydroxyimino)-allyl)phenyl)acetamide (**9b**) Pale yellow powder (2.42 g, 44.56% yield), mp 173–175 °C; IR (KBr) υ_{max} (cm^{-1}) 3355 (OH), 3290–3323 (NH), 1730 (C=O-coumarin), 1690 (C=O-amide), 1615 (C=C-chalcone); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 6.24 (s, 1H, H-3pyran), 6.71–6.75 (m, 1H, Ar–H), 7.03–7.38 (m, 3H, Ar–H), 7.45 (d, 1H, $J = 8.4$ Hz, Ar–H), 7.47–7.71 (m, 6H, Ar–H, CH=CH–Ar and CH=CH–Ar–H), 8.60–8.61 (m, 2H, Ar–H), 10.35 (bs, 1H, NH), 11.65 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 113 (C_3), 114 (C_5), 120 (**CH**=CH–Ar), 122 ($C_{2,6}''$), 124 (C_4'''), 127 (C_6'), 129 ($C_{2,6}'''$), 130 (C_4''), 132 ($C_{3,5}''$), 135 ($C_{3,5}'''$), 136 (C_1'''), 137 (C_1''), 139, (CH=**CH**–Ar), 150 (C_{10}'), 153 (C_4), 155 (C_8'), 160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS (EI) *m/z* (%) 534 (8) [M + 2], 532 (25)[M^+], 515(5), 543(9), 334(36), 180 (42), 148(41), 139(100), 131(59), 111(54), 103(72), 91(47), 77(63), 64(41), 51(38). Anal. calcd for $C_{27}H_{21}$ BrN_2O_6: C,60.80; H, 3.97; N, 5.25. Found: C, 60.96; H, 4.12; N, 5.41.

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-(1-(hydroxyimino)-3-(4-methoxyphenyl)-allyl)phenyl)acetamide (**9c**) Pale yellow powder (1.70 g, 35.12 % yield), mp 150–152 °C; IR (KBr) υ_{max} (cm^{-1}) 3444–3421 (OH), 3354 (NH), 1722 (C=O-coumarin), 1668 (C=O-amide), 1625 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.92 (s, 2H, CH_2), 6.23 (s, 1H, H-3pyran), 6.92–6.94 (m, 2H, Ar–H), 7.41–7.55 (m, 5H, Ar–H and CH=CH–Ar), 7.73 (d, 1H, $J = 8.4$ Hz, Ar–H), 8.04–8.08 (m, 2H, Ar–H and CH=CH–Ar), 8.57–8.61 (m, 1H, Ar–H), 8.93 (d, 2H, $J = 5.2$, Ar–H), 10.55 (bs,1H, NH), 11.5 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 19$ (CH_3), 61 (OCH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 113 (C_5), 114 ($C_{3,5}'''$), 118 (**CH**=CH–Ar), 119 ($C_{2,6}''$), 126 ($C_{2,6}'''$), 128 (C_1'''), 129 (C_6'), 130 (C_4''), 133 ($C_{3,5}''$),135 (C_1''), 139 (CH=**CH**–Ar), 150 (C_{10}'), 153 (C_4), 155 (C_4'''), 158 (C_8'), 160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS (EI) *m/z* (%) 484(3.51) [M^+], 334(39), 267(41), 176(54), 148(47), 135(96), 131 (52), 103(51), 91(97.5), 77(100), 65(51), 64(75), 51(50). Anal. calcd for $C_{28}H_{24}N_2O_6$: C,69.41; H,4.99; N, 5.78. Found: C, 69.60; H, 5.04; N, 5.89.

2-(4-Methyl-2-oxo-*2H*-chromen-7-yloxy)-*N*-(4-(1-(hydroxyimino)-3-(2-methoxyphenyl)-allyl)phenyl)acetamide (**9d**) Pale yellow powder (1.56 g, 32.23 % yield), mp 146–148 °C; IR (KBr) υ_{max} (cm^{-1}) 3440–3421 (OH), 3350 (NH), 1722 (C=O-coumarin), 1668 (C=O-amide), 1625 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 3.75 (s, 3H, OCH_3), 4.89 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 6.98–7.09 (m, 5H, Ar–H), 7.44 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.55 (d, 1H, $J = 16.8$ Hz, CH=CH–Ar), 7.70–7.75 (m, 5H, Ar–H and CH=CH–Ar), 10.36 (bs, 1H, NH), 11.5 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 19$(CH_3), 61 (OCH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 113 (C_5), 114 (C_3'''), 118 (C_1'''), 119 (**CH**=CH–Ar), 120 (C_5'''), 122 ($C_{2,6}''$), 126 (C_6'''), 128 (C_6'), 129 (C_4''), 132 (C_4''') 133 ($C_{3,5}''$),135 (C_1''), 139 (CH=**CH**–Ar), 150 (C_{10}'), 153 (C_4), 155 (C_2'''), 156 (C_8'),160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS (EI) *m/z* (%) 484 (3.51) [M^+], 334 (39), 267 (41), 176 (54), 148 (47), 135 (96), 131 (52), 103 (51), 91 (97.5), 77 (100), 65 (51), 64 (75), 51 (50). Anal. calcd for $C_{28}H_{24}N_2O_6$: C,69.41; H, 4.99; N, 5.78. Found: C, 69.63; H, 5.02; N, 5.91.

2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*-(4-(3-(2,4-dichlorophenyl)-1-(hydroxyimino)-allyl)phenyl)acetamide (**9e**) Pale yellow powder (4.06 g, 77.62% yield), mp 195–196 °C; IR (KBr) υ_{max} (cm^{-1}) 3448 (OH), 3244–3232 (NH), 1716 (C=O-coumarin), 1681 (C=O-amide), 1614 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.90 (s, 2H, CH_2), 6.23 (s, 1H, H-3 pyran), 7.01–7.082 (m, 3H, Ar–H), 7.47 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.55 (d, 1H, $J = 17.2$ Hz, CH=CH–Ar), 7.65 (d, 1H, $J = 16.4$ Hz, CH=CH–Ar), 7.73 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.93 (d, 2H, $J = 8.4$, Ar–H), 8.93 (s, 1H, Ar–H), 10.42 (bs, 1H, NH), 11.7 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3), 114 (C_5), 119 (**C**H=CH–Ar), 121 ($C_{2,6}''$), 127 (C_5'''), 128 (C_6'), 129 (C_4''), 130 (C_6'''), 131 ($C_{3,5}''$), 132 (C_3'''), 133(C_1''), 134 (C_2'''), 139 (C_4), 144 (C_1''), 145 (CH=**C**H–Ar), 153 (C_{10}'), 154 (C_4), 155 (C_8'), 160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS (EI) *m/z* (%) 524 (7) [M + 2], 522 (15) [M^+], 334(67), 176(92), 175(68), 173(100), 159(49), 148(76), 147(64), 131(97), 103(99), 91(68), 75(61), 74(58), 51(49). Anal. calcd for $C_{27}H_{20}Cl_2N_2O_5$: C, 61.96; H, 3.85; N, 5.35. Found: C, 62.08; H, 3.83; N, 5.43.

2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*-(4-(1-(hydroxyimino)-3-(3,4,5-trimethoxyphenyl)-allyl)phenyl)acetamide (**9f**) Pale yellow powder (3.08 g, 56.72% yield), mp 180–181 °C; IR (KBr) υ_{max} (cm^{-1}) 3402 (OH), 3200–3269 (NH), 1728 (C=O-coumarin), 1716 (C=O-amide), 1693 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 3.79 (s, 6H, $2OCH_3$), 4.89 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 6.85 (s, 2H, Ar–H), 7.03–7.08 (m, 2H, Ar), 7.43 (d, 2H, $J = 8$ Hz, Ar–H), 7.51 (d, 1H, $J = 16.8$ Hz, CH=CH–Ar), 7.72–7.76 (m, 4H, Ar–H and CH=CH–Ar), 10.35 (bs, 1H, NH), 11.5 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 57, 61 (OCH_3), 67 (CH_2), 102(C_9'), 111($C_{2,6}'''$), 112(C_7'), 113(C_3), 114 (C_5), 119 (**C**H=CH–Ar), 122($C_{2,6}''$), 127(C_6'), 129 (C_4''), 130($C_{3,5}''$), 132(C_1'''), 133(C_4'''), 134(C_1''), 139 (CH=**C**H–Ar), 144($C_{3,5}'''$), 153(C_{10}'), 154(C_4), 155(C_8'), 160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS (EI) *m/z* (%) 544(22) [M^+], 335(47) 334(64), 196(51), 195(76), 176(81), 148(73), 131(97), 103(100), 91 (70), 77(99), 65 (56), 51(51). Anal. calcd for $C_{30}H_{28}N_2O_8$: C, 66.17; H, 5.18; N, 5.14. Found: C, 60.96; H, 5.37; N, 5.43.

2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*-(4-(3-(4-chlorophenyl)-1-(hydroxyimino)-allyl)phenyl)acetamide (**9g**) Pale yellow powder (2.36 g, 48.36% yield), mp 190–191 °C; IR (KBr) υ_{max} (cm^{-1}) 3350 (OH), 3288–3323 (NH), 1722 (C=O-coumarin), 1689 (C=O-amide), 1612 (C=C); ^{1}H NMR (400 MHz, DMSO- d_6) δ (ppm): 2.40 (s, 3H, CH_3), 4.89 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 7.03–7.08 (m, 3H, Ar–H), 7.41–7.48 (m, 4H, Ar–H), 7.60–7.66 (m, 3H, Ar–H and CH=CH–Ar), 7.72–7.78 (m, 3H, Ar–H and CH=CH–Ar), 10.36 (bs, 1H, NH), 11.63 (bs, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 18$ (CH_3), 67 (CH_2), 102 (C_9'), 111(C_7'), 113(C_3), 114(C_5), 120 (**C**H=CH–Ar), 122 ($C_{2,6}''$), 126(C_6'), 127($C_{2,6}'''$), 129($C_{3,5}'''$, C_4''), 133($C_{3,5}''$), 135(C_1'''), 136(C_4'''), 139(C_1''), 149(CH=**C**H–Ar), 150 (C_{10}'), 153(C_4), 155 (C_8'), 160 ($CO_{coumarin}$), 161 (C=NOH), 166 (CONH); MS (EI) *m/z* (%) 490 (10) [M + 2], 488 (31) [M^+], 487(43), 176(42), 148(41), 139 (100), 131(59), 111 (54), 103(72), 91(47), 77(63), 64(41), 51(38). Anal. calcd for $C_{27}H_{21}ClN_2O_5$: C, 66.33; H, 4.33; N, 5.73. Found: C, 66.45; H, 4.37; N, 5.91.

2-(4-Methyl-2-oxo-2*H*-chromen-7-yloxy)-*N*-(4-(3-(furyl)-1-(hydroxyimino)-allyl)phenyl)acetamide (**9h**) Light brown powder (2.86 g, 64.41% yield), mp 204–205 °C; IR (KBr) υ_{max} (cm^{-1}) 3412 (OH), 3344 (NH), 1722 (C=O-coumarin), 1668 (C=O-amide), 1625 (C=C); ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.41 (s, 3H, CH_3), 4.90 (s, 2H, CH_2), 6.24 (s, 1H, H-3 pyran), 6.54–6.69 (m, 2H, Ar–H), 7.03–7.08 (m, 2H, Ar–H), 7.35 (d, 1H, $J = 16$ Hz, CH=CH–Ar), 7.41 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.70–7.77 (m, 3H, Ar–H and CH=CH–Ar), 7.99–7.02 (m, 1H, Ar–H), 8.89–8.90 (m, 1H, Ar–H), 10.55 (s, 1H, NH). 11.58 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) $\delta = 19$ (CH_3), 68 (CH_2), 102 (C_9'), 111 (C_7'), 112 (C_3'''), 113 (C_3), 114 (C_4'''), 115 (C_5), 120 ($C_{2,6}''$), 124 (**C**H=CH–Ar), 127 (C_6'), 129 (CH=**C**H–Ar, C_4''), 130 ($C_{3,5}''$), 139 (C_1''), 143 (C_5'''), 144 (C_2'''), 152(C_{10}'), 153(C_4), 155(C_8'), 160 $CO_{coumarin}$), 161 (C=NOH), 167 (CONH); MS (EI) *m/z* (%) 444 (29)[M^+], 176(40), 148(40), 147(35), 131(59), 121(100), 103(74), 95 (51), 91(53), 77(60), 65(59), 64(50), 57(37), 51(37). Anal. calcd for $C_{25}H_{20}N_2O_6$: C,67.56; H, 4.54; N, 6.30. Found: C, 67.70; H, 4.61; N, 6.39. (Note: a bold or underline means that the marked proton is the one described in that sentence).

NO release assay

A solution of the appropriate compound (20 mL) in DMSO was added to 2 mL of 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) with MeOH, containing 5×10^{-4}M of L-cysteine. The final concentration of drug was 10^{-4} M. After 1 h at 37 °C, 1 mL of the reaction mixture was treated with 250 mL of Griess reagent [sulfanilamide (2 g), *N*-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid Tsikas (2007) (10 mL) in distilled water (final volume: 100 mL)]. After 10 min at room temperature, the absorbance was measured at $\lambda = 546$ nm. Sodium nitrite standard solutions (10–80 nmol/mL) were used to construct the calibration curve. The same procedure was repeated using different solutions of the test compounds under the

same conditions using 0.1 N HCl of pH 1 instead of phosphate buffer of pH 7.4.

The results were expressed as amount of NO released relative to a theoretical maximum release of 1 mol NO/mol of test compound.

Biological evaluation

cytotoxic activity

The NCI-60 anticancer drug screening The methodology of the NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov). Briefly, the primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases, in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, SRB. Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically vs. controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compound were tested in vitro against the full panel of about 60 human tumor cell lines at ten-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. Using the seven-absorbance measurements [time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: $[(Ti - Tz)/(C - Tz)] \times 100$ for concentrations for which $Ti > Tz$, and $[(Ti - Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$.

Three-dose response parameters were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from $[(Ti - Tz)/(C - Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in TGI was calculated from $Ti = Tz$. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from $[(Ti - Tz)/Tz] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The log GI_{50}, log TGI, and log LC_{50} were then determined, defined as the mean of the logs of the individual GI_{50}, TGI, and LC_{50} values. The lowest values are obtained with the most sensitive cell lines. Compound having log GI_{50} values -4 and <-4 was declared to be active.

Anti-cancer drug screening using viability assay Human colon carcinoma (HCT-116) and human breast carcinoma (Michigan Cancer Foundation-7 (MCF-7)) cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week (Gangadevi and Muthumary 2007).

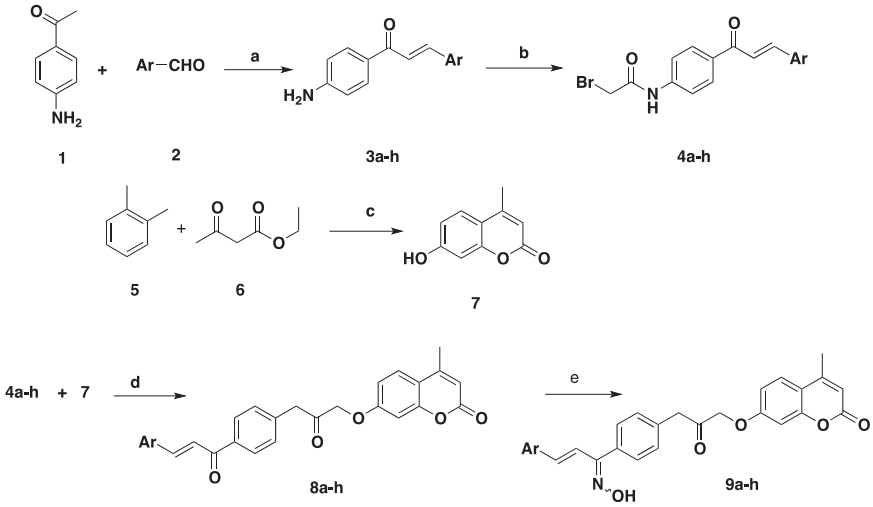
The antitumor activity was evaluated on tumor cells. The cells were grown as monolayers in growth RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The monolayers of 10,000 cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µL from different dilutions of tested sample in fresh maintenance medium and incubated at 37 °C. A control of untreated cells was made in the absence of tested sample. A positive control containing Doxroubcin drug was also tested as reference drug for comparison. Six wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590 nm using ELISA reader (SunRise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation.

The number of viable cells was determined using ELISA reader as previously mentioned before and the percentage of viability was calculated as [1−(ODt/ODc)] x 100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots.

Antibacterial activity

The synthesized compounds **8a–h** and **9a–h** and levofloxacin were screened for their in vitro antibacterial activity against a group of Gram positive bacteria as *Staphylococcus aureus* and a group of Gram negative bacteria such as *Escherichia*

Scheme 1 Synthesis of the designed coumarin–chalcone/NO hybrids



coli, Pseudomonas aeruginosa, and *Klebsiella pneumonia*, using agar well diffusion method (Gemeda et al. 2008).

Solutions of the tested compounds, **8a–h** and **9a–h** and levofloxacin (reference drug) in DMSO were prepared at an initial concentration of 5 µg/mL, then serial diluted using two-fold dilution technique to give (5, 2.5, 1.25, and 0.625 µg/mL) concentrations. Recultured bacterial strains were supplied from department of Microbiology, Faculty of Pharmacy, Minia University.

Microorganisms (0.5 mL) of 1×10^6 colonu foming unit/mL (0.5 Mcfarland turbidity) were plated in sterile petri dishes then 20 mL of sterile, molten and cooled (45 °C) Muller Hinton agar media was added to all petri dishes. The plates then were rotated slowly to ensure uniform distribution of the microorganisms and then allowed to solidify on a flat surface. After solidification, four equidistant and circular wells of 10 mm diameter were carefully punched using a sterile cork borer. Each sample (5 mL) was applied as triplicate. The plates were allowed to stand for 1 h for pre diffusion of the extract to occur then incubated overnight at 37 °C. All plates were examined and zones of inhibition were recorded. This method was adopted for all microbiological experiments in this study

The average of the zones of inhibition was calculated. The minimum inhibitory concentration (MIC) was calculated by plotting the natural logarithm of the concentration of compounds against the square of zones of inhibition. A regression line was drawn through the points. The antilogarithm of the intercept on the logarithm of concentration axis gave the MIC value.

Results

Chemistry

The target coumarin–chalcone hybrids **8a–h** were efficiently synthesized in three steps (Scheme 1). Initially, chalcones **3a–h** were synthesized via base catalyzed Clasien–Schmidt condensation of various aromatic aldehydes **2a–h** with *p*-aminoacetophenon, followed by acetylation of the free amino group with bromoacetyl bromide. Acylation with bromomacetyl bromide to give the intermediates **3a–h** presented a very simple synthetic way for spacer attachment to the chalcone moiety.

Subsequently, resorcinol readily undergoes Von Pechmann condensation with β-keto ester (ethyl acetoacetate) in presence of sulphuric acid to afford 7-hydroxy-4-methyl coumarin **7**. Finally, coupling the two intermediates **7** and **3a–h** together in DMF afforded the designed coumarin-chalcone hybrids **8a–h** (Scheme 1).

The structural formula of hybrids **8a–h** is characterized by ^{1}H NMR spectra, which showed the existence of singlet signal for one proton at 10.55–10.62 ppm interpreted for the NH group in the oxyacetamide spacer. Two protons for CH_2 of the acetamide spacer were detected via a singlet signal at 4.90–4.92 ppm. The existence of chalcone was confirmed by the doublet signals at 7.89–8.19 ppm with a coupling constant of 15.6–16 Hz indicating a *trans* configuration for the chalcone double bond. On the other hand, the presence of coumarin was identified through CH_3 singlet signal at 2.40–2.41 ppm and singlet at 6.24 ppm for C-3 proton of coumarin ring.

The NO hybrids **9a–h** were designed by changing the chalcone carbonyl oxygen into an oxime group, which was synthesized via refluxing coumarin–chalcones **8a–h** with hydroxylamine hydrochloride in pyridine (Scheme 1). Trials using ethanol/sodium acetate reflux procedure to form the oxime were not successful.

An additional singlet peak at 11.50–11.70 ppm in ^{1}H NMR spectra of compounds **9a–h** confirmed the existence of the oxime group and indicated the presence of a mixture of both E and Z isomers. Mass spectrum of those compounds showed moderate abundance of the molecular ion peak relative to coumarin and chalcone fragments.

Table 1 The amount of NO released from tested compounds **9a–h** in phosphate buffer pH = 7.4 (%mol/mol)

Comp#	Amount of NO released (% mol/mol)					
	1 h	2 h	3 h	4 h	5 h	6 h
9a	0.178 ± 0.001	0.192 ± 0.005	0.234 ± 0.003	0.257 ± 0.007	0.443 ± 0.004	0.354 ± 0.021
9b	0.060 ± 0.001	0.071 ± 0.002	0.094 ± 0.007	0.164 ± 0.007	0.285 ± 0.044	0.241 ± 0.004
9c	0.071 ± 0.003	0.109 ± 0.041	0.112 ± 0.016	0.189 ± 0.014	0.263 ± 0.087	0.189 ± 0.006
9d	0.094 ± 0.001	0.098 ± 0.002	0.123 ± 0.005	0.166 ± 0.002	0.354 ± 0.010	0.269 ± 0.031
9e	0.125 ± 0.008	0.135 ± 0.001	0.192 ± 0.004	0.196 ± 0.010	0.204 ± 0.088	0.176 ± 0.010
9f	0.078 ± 0.001	0.111 ± 0.004	0.128 ± 0.001	0.166 ± 0.001	0.296 ± 0.068	0.231 ± 0.002
9g	0.107 ± 0.001	0.140 ± 0.001	0.205 ± 0.002	0.330 ± 0.006	0.400 ± 0.019	0.356 ± 0.013
9h	0.133 ± 0.005	0.153 ± 0.006	0.176 ± 0.001	0.230 ± 0.002	0.375 ± 0.026	0.259 ± 0.019

Nitric oxide release

The NO-releasing properties of the target the starting ketones **8a–h** NO donating oximes **9a–h** were assessed in both phosphate buffer of pH 7.4 and pH 1 using 0.1 N HCl with Griess reagent (reference). The reaction was carried out in the presence of *N*-acetyl cysteine as a source of the SH group. The amount of NO released from the tested compounds was measured relative to NO released from standard sodium nitrite solution, calculated as the amount of NO (mol/mol) released and are listed in Table 1.

The results in Table 1 indicates that; the starting ketone derivatives **8a–h** did not release any amount of NO neither at phosphate buffer of pH 7.4 nor at pH 1 and this is a proof that oximes are the main source of NO release.

The NO-donating oximes **9a–h** were found to release moderate amounts of NO compared to the sodium nitrite standard solution reaching their maximum release after 5 h. On the other hand there is no release of NO at pH 1 and this may support the fact that NO-donating moieties (oximes) are weakly hydrolyzed in the gastric lumen and this confirms that the suggested actions of NO is mediated systemically (Velázquez et al. 2005).

Biological activity

Cytotoxic activity

The National Cancer Institute (NCI), USA, selected compound **8a** for evaluation for preliminary anti-proliferative activity evaluation at single concentration of 10^{-5} M towards panel of sixty cancer cell lines. The compound was added at a single concentration and the cell culture was incubated for 48 h. End point determinations were made with a protein binding dye, sulforhodamine B (SRB). The results are reported as the percent growth of treated cells compared to untreated control cells. Compound **8a** showed only a moderate cytotoxic activity against leukemia; RPMI-8226 cell line, Central Nervous system cancer; U251 cell line and breast cancer MCF7 cell line with GI value 45.85, 40.86, and 39.25 %, respectively (Fig. 3). (What about other tested compounds?)

Further independent cytotoxic evaluation were done for compounds **8c–f**, **8h**, **9c–f**, and **9h** against breast cancer cell line MCF-7 was carried out.

The tested compounds were added at six concentrations compared to doxrubcin as reference drug. End point determinations were made with a protein binding dye, crystal violet. The results for each compound are reported as the concentration required to cause toxic effects to 50% of viable cells (IC_{50}). Results are listed in Table 2 and Fig. 4.

The results showed that some of the tested compounds showed moderate anti-cancer activity compared to the standard drug; doxorubicin. The best substitution among the coumarin–chalcone hybrids was the furyl substitution **8h** (IC_{50} 9.62 μg) followed by the trimethoxy derivative **8f** (IC_{50} 14.4 μg) then the 4-methoxy derivative **8c** (IC_{50} 24 mg). Compound **8e** with the dichloro substitution was the least active.

These results go with a previous report that associated anticancer activity to methoxy coumarin chalcone hybrids (Jamier et al. 2014). The cytotoxic activity of compounds **9c–f** and **h** are listed in Table 2. It had a similar pattern of activity with the derivatives carrying methoxy groups being the most active (IC_{50} 20.9 and 22.82 μg for compounds **9c** and **f**, respectively).

The presence of the NO donating group enhanced the activity of compounds **9c**, **d**, and **e** compared to their corresponding ketones **8c**, **d**, and **e** by 12.92, 23.61, and 23.40% of of their corresponding ketones. Meanwhile it decreased the activity of compounds **9f** and **h** by 36.84 and 77.73 % of the activity of their corresponding ketones **8f** and **h**. These results suggest a possible role of NO in the activity of compounds **9c–e**. This difference in activity could also be structure based, i.e., the oxime derivatives could form additional interactions with certain biological targets. The increased activity of some ketones also indicates a contribution of the carbonyl group in the anti-cancer activity of such compounds or other factors like physico-chemical parameters.

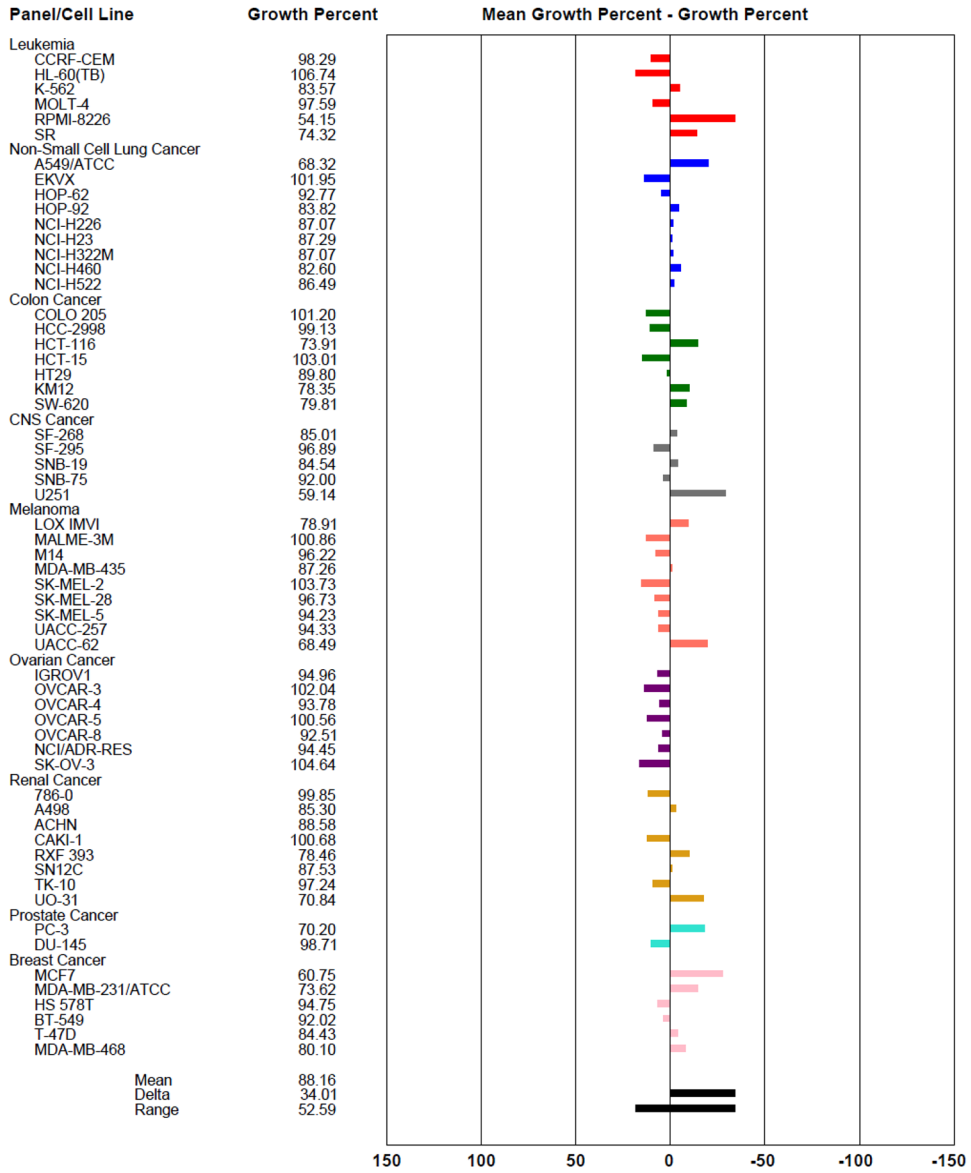


Fig. 3 One dose mean graph of nine different cancer cell types of compound **8a**

Antibacterial evaluation

The newly synthesized compounds **8a–h**, **9a–h** and levofloxacin were screened for their in vitro antibacterial activity against a group of Gram positive bacteria as *S. aureus* (ATTC 6538) and a group of Gram negative bacteria such as *E. coli (*ATTC 7839*), P. aeruginosa* (ATTC 10145) and *Klebsiella pneumonia* (ATTC 10031), using agar well diffusion method. The results were recorded as the MIC of the tested compounds that caused an inhibition of the growth of the examined microorganisms (MIC, μg/mL) and listed in Table 3.

In the series of chalcone–coumarin amide hybrids **8a–f**, compound **8f** was the most active against *Staph. aureus* with MIC of 35.8 μg/mL comparable to that of levofloxacin (MIC of 45 μg/mL). Compounds **8a–e**, **g**, and **h** showed very weak activity with MICs of 111 224, NA, 400, 108, 108, and 260 μg/mL, respectively. Incorporation of NO releasing oxime group into that series (compounds **9a–f**) generally resulted in enhancement of antibacterial activity except for compound **8g**.

Detailed comparison between the NO hybrids and their respective ketones gave the following results, compound **9a** has MIC of 38 vs. 111 μg/mL for **8a**, **9b** have MIC of 32 vs. 224 μg/mL for **8b**, **9c**, **9d** have MIC of 1.1 vs. 400 μg/mL for **8d**, **9e** have MIC of 97 vs. 108 μg/mL for **8e**, **9f** have MIC of 9.8 vs. 35.8 μg/mL for **8f** and **9h** have MIC of 8.2 vs. 260 μg/mL for **8b**.

These results suggest a possible role for NO in the antibacterial activity of this series against *S. aureus*. The fact that nearly all compounds release almost similar amounts of NO (Table 3) but the antibacterial is not affected

Table 2 Cell viability and IC_{50} of breast cancer cell line MCF-7 treated with compounds **8c–f**, **h** and **9c–f**, **h**

Sample conc. (μg)	Cell viability %										
	8c	**8d**	**8e**	**8f**	**8h**	**9c**	**9d**	**9e**	**9f**	**9h**	Doxorubicin
50	19.84	32.98	45.87	21.52	16.38	20.85	34.29	35.12	30.48	43.69	3.24
25	48.72	67.52	80.48	39.16	28.49	36.29	52.83	61.69	45.91	66.52	6.55
12.5	65.39	81.93	89.76	51.98	37.87	78.42	71.54	76.14	68.83	79.63	11.74
6.25	81.85	90.46	95.19	72.83	64.15	89.53	86.28	85.28	79.19	91.43	17.22
3.125	90.41	98.84	99.07	85.14	78.93	94.18	94.72	94.42	90.64	98.12	21.18
1.56	97.19	100	100	93.22	87.54	97.61	98.04	98.67	96.07	100	30.86
IC_{50} μg	24	37.7	47	14.4	9.62	20.9	28.8	36	22.8	43.1	0.426

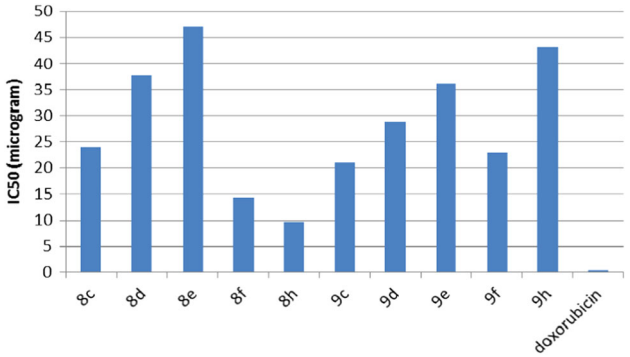


Fig. 4 IC_{50} of different coumarin–chalcones **8c–f**, **h**, their corresponding oximes **9c–f**, **h** and doxorubicin against MCF-7 breast cancer cell line

in the same way, suggests that NO is not the only factor enhancing the activity, but the individual structural properties of the hybrids also have their own contributions.

Activity of the same series against Gram-ve bacteria was also evaluated. Compounds **8c**, **f**, and **g** were highly active against *E. coli* with MICs of 7, 9.6, and 1.3 μg/mL, respectively compared to 29.2 μg/mL for levofloxacin. **8a** and **b** have MICs similar to that of levofloxacin (24, 21 μg/mL, respectively) (Table 3).

Compounds **8e**, **f**, and **h** show moderate activity against *K. Pneumonia* with MIC of 9.6 μg/mL for all compared to 1.57 μg/mL for levofloxacin (Table 3). Compounds **8e** and **d** only showed also a moderate activity with MIC of 9.6 μg/mL for both compared to 2.6 μg/mL for levofloxacin (Table 3).

Forming NO releasing hybrids (compounds **9a–h**) generally abolished activity against *E. coli* and *K. pneumonia* suggesting the importance of the chalcone carbonyl group in the activity against those two microorganisms.

Meanwhile, the addition of the oxime group caused a variation in the antibacterial activity against the Gram –ve *P. aueriginosa*. It enhanced the activity of compounds **9c**, **f**, and **g** compared to their starting **8c**, **f**, and **g**, while decreased the activity for the rest of compounds (Table 3).

Table 3 MICs (μg/mL) of compounds **8a–h** and **9a–h** against *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumonia*, and *Pseudomonus aeruginosa*

	Minimum inhibitory concentration (MIC) in μg/mL			
Comp#	*S. aureus*	*E. coli*	*K. pneumonia*	*P. aeruginosa*
Levofloxacin	45	29.2	1.57	2.6
8a	111.4	24.16	108.22	238.66
8b	224.66	21.15	430.9	108.22
8c	NA	7.17	279.7	108.22
8d	400.26	95.07	50.85	9.66
8e	108.22	193.8	9.66	9.66
8f	35.8	9.66	9.66	102.7
8g	108.22	1.26	108.22	108.22
8h	260.5	NA	9.66	108.22
9a	38.33	NA	NA	NA
9b	32.79	NA	NA	NA
9c	3.6	NA	NA	78.11
9d	1.15	NA	NA	98.9
9e	97.4	NA	NA	45.85
9f	19.82	NA	NA	37.49
9g	243.4	NA	8.89	37.49
9h	8.17	NA	NA	2.07

NA no measurable activity

Generally the coumarin–chalcone hybrids looks like an interesting group of compounds having a good spectrum of antibacterial activity that needs to undergo further investigations for proper SAR conclusions and determination of the exact mechanism of action of such derivatives.

Conclusions

Hybrids of coumarin–chalcones with the nitric oxide donor oxime are prepared and investigated for their potential synergism of biological activities. All the tested oximes released moderate amount of NO. Most of the tested compounds showed moderate anti-proliferative activity against

the used cancer cell lines. The presence of the NO donating group caused enhancement in the activity of compounds **9c**, **d**, and **e** compared to their corresponding ketones by 12.92, 23.61, and 23.40% of their original activity. Meanwhile, it decreased the activity of compounds **9f** and **h** by 36.84 and 77.73% of the activity of their corresponding ketones. Moreover, incorporation of NO releasing oxime group generally resulted in enhancement of antibacterial activity against *S. aureus* except for compound **8g**. The addition of the oxime group caused a variation in the antibacterial activity against the Gram–ve *P. aueriginosa*. It enhanced the activity of compounds **9c**, **f**, and **g** compared to their starting **8c**, **f**, and **g**, while decreased the activity for the rest of compounds. These results suggest a possible role of NO in the biological activity of the hybrids **9c–e** where it is not the only contributing factor where other factors like physicochemical parameters should be investigated for their potential role in activity.

Generally, the coumarin–chalcone-NO hybrids looks promising candidates with anti-proliferative and antibacterial activity that needs to undergo further investigations for proper SAR conclusions and determination of the exact mechanism of action of such derivatives.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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