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SYNTHESIS OF NOVEL COUMARIN–CHALCONE HYBRIDS AND SCREENED IN VITRO ANTIMICROBIAL AND ANTIMYCOBACTERIAL ACTIVITIES AS BIOLOGICALLY ACTIVE PHARMACOPHORES

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Abstract: Based on the pragmatic biological activities of coumarin and chalcone, we have synthesized coumarin–chalcone hybrids with the aim of evaluating their antimicrobial properties. By applying standard Vilsmeier-Haack reaction condition, reaction of 4-hydroxy coumarin **(1)** with Vilsmeier-Haack reagents gives 4-chloro-2-oxo-2*H*-chromene-3-carbaldehyde **(2)** which on further react with morpholine **(3)** gives 4-morpholino-2-oxo-2*H*-chromene-3-carbaldehyde **(4)** which on further react with 4-aminoacetophenone **(5)** gives chalcones **(6).** Structural variations were selected by introducing various amine at chloro acetyl group as well as various isothiocyanate and isocyanate at amine group. The structures of all the newly synthesised compounds were confirmed by their FTIR, ^{1}H NMR, mass spectral as well as elemental analysis data. All the newly synthesized compounds were screened for non automated in vitro antimicrobial and antimycobacterial activity against selected pathogens. Some of the newly synthesized compounds exhibited excellent antimicrobial activity and said to be the most proficient members of the series compared to standard drugs and for future scope.

Keywords: 4-Hydroxycoumarin,Chalcone, Vilsmeier-Haack reaction, Antimicrobial activity.

Introduction

Fighting disease with drugs is the timeless struggle. Its beginnings are echoed out of the primeval jungle. Today the conflict continues unabated in the laboratory and clinic. The scientific approach to this struggle is pharmacology. The great expansion in medicinal research in past has contributed much to the unparalleled progress of medicine during that period. Improved and basically more meaningful biological test procedures and methods of diagnosis have provided better guidance in drug discovery by pointing out suggestive observations which could be used in the design of new prophylactic and therapeutic agents.

The design and synthesis of hybrid molecules surrounding two pharmacophores in one molecular scaffold is well established approach to the synthesis of more potent drugs with

dual activity. In our continuing search for and efficacious antimicrobial agents, structurally interesting coumarin-chalcone derivatives were synthesized and evaluated in vitro antimicrobial and antimycobacterial activity [I-V]. In the search for novel drug leads, the hybrid approach is a promising one since it can effectively target multi factorial diseases. Due to the high potential of natural products to exhibit pronounced biological activities, natural products have been one of the major sources of components in hybrid molecules [VI]. coumarins and chalcones are a family of natural and synthetic compounds that drawn much attention due to its broad pharmacological activities. Coumarins constitute an important class of oxygen hetrocycles [VII]. Many compounds containing the coumarin nucleus, both naturally occurring and synthetic, are known to exhibit pharmacological activity [VIII-XIII]. Substituted coumarin-chalcon derivatives are an important class of compounds having anti-tumoragent [XIV], antiviral [XV], anti-coagulant [XVI], antidepressant [XVII], antimicrobial [XVIII], anti-oxidant [XIX], and anti-inflammatory [XX]. Furthermore substituted coumarin-chalcon derivatives have been used as NLO (Non-Linear Optical) materials. Compounds with two or more heterocycles play a very important role in natural and synthetic bioactive compounds [XXI]. Within this framework, we describe the synthesis of various compounds featuring different heterocyclic rings fused onto the coumarin ring with morpholine the aim of obtaining more potent pharmacologically active compounds.

In view of these conceptions, the aim of the present work is to design and synthesize novel Coumarin derivatives incorporated with various chalcone as bioactive scaffold. The structural variations were selected by introducing various amine at chloro acetyl group as well as various isothiocyanate and isocyanate at amine group.

Experimental

Melting points were determined in open capillaries and were uncorrected. Reactions were monitored by thin-layer chromatography (TLC) carried out on silica gel plates (GF 254) using UV light as visualizing agent. Column chromatography was performed with silica gel mesh size 60-120. ^{1}H NMR spectra were recorded on Jeol-400 (1H, 500 MHz) spectrometer at ambient temperature, using DMSO-d^6 as solvents. Chemical shifts are reported in parts per million (ppm) with TMS as an internal reference. Mass spectrometric data were recorded at Waters Micromass Q-Tof Micro. Elemental analysis was done with Thermo Scientific (Flash 2000) analyzer. Ethylacetate : Hexane and chloroform: methanol were the adopted solvent systems for mobile phase used in TLC.

General procedure for the synthesis of N - (substituted phenyl) - 2 - ((4 - (3 - (4 – morpholino – 2 – oxo - 2H - chromen-3 - yl) acryloyl) phenyl) amino) acetamide (5a-5e)

Compound **(4)** (3.39 mmole, 1.0 equivalent) and KOH (4.72 mmole, 1.2 equivalent) in acetonitrile (10 ml) was stirred for 30 min until the anion was formed (appearance of yellow colour). This mixture was added to a solution of substituted arylacetamide (4.33 mmole, 1.1 equivalent) in acetonitrile (5ml) and refluxed for 6-8 hours. The progress of reaction was monitored by TLC using hexane : ethylacetate (6:4) as eluent. After the completion of reaction, yellow crystals were separated out by filtration. The crude product was purified by crystallization form absolute alcohol to get the title compound **(5a-e).**

***(E)*-2-((4-(3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino) -N-phenylacetamide(5a):**

Light-yellow solid, M.P.: 150-155^{o}C; Yield: 71%; Anal. Calcd. for $C_{30}H_{27}N_3O_5$: C, 70.71; H, 5.34; N, 8.25%. Found C, 70.74; H, 5.30; N, 8.22%; IR (KBr, Vmax/cm^{-1}): 3425 (-NH str., 2^{0}amine), 1600 (-C=O str., pyrane),1674(-C=O str., amide), 1118 (-C-O-C str., morpholine), 1713 (-C=O str., coumarin), 1621 (-C=C- str., alkenyl), 3101 (-CH str., in Aromatic), 1415

(-C=C str., aromatic), 820 (-C-N str., morpholine); ^{1}H NMR (500 MHz, DMSO, δ ppm): 2.36 (s, 1H, CH), 2.34 (s, 1H, CH), 2.64 (t, 4H, CH_2), 2.77 (t, 4H, CH_2), 2.26 (dd, 2H, CH_2), 4.44 (s, 1H, -CH in D_2O exchanger), 7.12 (dd, 1H, Ar-H),7.15 (dd, 1H, Ar-H), 7.24 (ddd, 1H, Ar-H), 7.25 (dd, 1H, Ar-H), 7.38 (dd, 1H, Ar-H), 7.61 (d, 2H, Ar-H), 7.74 (d, 2H, Ar-H), 7.98 (d, 2H, Ar-H), 8.06 (d, 2H, Ar-H), 8.97 (s, 1H, Ar-H); MS m/z 508.20 (M^+ +1).

***(E)*-N-(2-chlorophenyl)-2-((4-(3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino) acetamide (5b):**

Light-yellow solid, M.P.: 102-105 ^{o}C; Yield: 68%; Anal. Calcd. For $C_{30}H_{26}N_3O_5$: C, 66.24; H, 4.82; N, 7.72%. Found C, 66.21; H, 4.85; N, 7.77%; IR (KBr, Vmax/cm^{-1}): 3072 (-NH str., 2^{0}amine), 1652 (-C=O str., amide), 1097 (-C-O-C str., morpholine), 1711 (-C=O str., coumarin), 1622 (-C=C- str., alkenyl), 1468 (-C=C- str., aromatic), 1335 (-C-N str., morpholine), 755 (C-Cl str.); ^{1}H NMR (500 MHz, DMSO, δppm): 2.32 (s, 1H, CH) , 2.37 (s, 1H, CH), 2.63 (t, 4H, CH2), 2.76 (t, 4H, CH2), 2.25 (dd, 2H, CH2), 4.45 (s, 1H, CH D_2O exchanger), 7.12 (dd, 1H, Ar-H), 7.23 (ddd, 1H, Ar-H), 7.28 (dd, 1H, Ar-H), 7.37 (dd, 1H, Ar-H), 7.68 (d, 2H, Ar-H), 7.77 (d, 2H, Ar-H), 7.96 (d, 2H, Ar-H), 8.08 (d, 2H, Ar-H), 8.97 (s, 1H, Ar-H); MS m/z 542.16 (M^+ +1).

***(E)* -N- (4-chlorophenyl) - 2- ((4- (3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino) acetamide (5c):**

Light-yellow solid, M.P.: 131-134^{o}C; Yield: 64%; Anal. Calcd. For $C_{30}H_{26}N_3O_5$: C, 66.24; H, 4.82; N, 7.72%. Found C, 66.28; H, 4.88; N, 7.76%; IR (KBr, Vmax/cm^{-1}) : 3070 (-NH str., 2^{0}amine), 1650 (-C=O str., amide), 1097 (-C-O-C str., morpholine), 1712 (-C=O str., coumarin), 1620 (-C=C- str., alkenyl), 1469 (-C=C- str., aromatic), 1335 (-C-N str., morpholine), 759 (C-Cl str.); ^{1}H NMR (500MHz, DMSO, δppm): 2.32 (s, 1H, CH), 2.35 (s, 1H, CH), 2.67 (t, 4H, CH2), 2.74 (t, 4H, CH2), 2.27 (dd, 2H, CH2) ,4.47 (s, 1H, CH D_2Oexchanger), 7.13 (dd, 1H, Ar-H), 7.25 (ddd, 1H, Ar-H), 7.27 (dd, 1H, Ar-H) ,7.39 (dd, 1H, Ar-H), 7.65 (d, 2H, Ar-H), 7.78 (d, 2H, Ar-H), 7.97 (d, 2H, Ar-H), 8.07 (d, 2H, Ar-H), 8.95 (s, 1H, Ar-H); MS m/z 542.16 (M^+ +1).

***(E)*-2- ((4- (3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino) -N- (o-tolyl) acetamide (5d):**

Light-yellow solid, M.P.: 138-140 ^{o}C; Yield: 56%; Anal. Calcd. For $C_{31}H_{29}N_3O_5$: C, 71.11; H, 5.58; N, 8.03%. Found C, 71.16; H, 5.52; N, 8.10%; IR (KBr, Vmax/cm^{-1}): 3446 (-NH str., 2^{0}amine), 1610 (-C=O str., pyrane), 1672 (-C=O str., amide), 1116 (-C-O-C str., morpholine), 1711 (-C=O str., coumarin), 1621 (-C=C- str., alkenyl), 3104 (-CH str., aromatic), 1418 (-C=C str., in aromatic), 821 (-C-N str., morpholine); ^{1}H NMR (500 MHz, DMSO, δppm): 2.32 (s, 1H, CH), 2.37 (s, 1H, CH), 2.63 (t, 4H, CH2), 2.76 (t, 4H, CH2), 2.19 (d, 3H, CH3), 2.25 (dd, 2H, CH2), 4.45 (s, 1H, CH D_2O exchanger), 7.12 (dd, 1H, Ar-H), 7.23 (ddd, 1H, Ar-H), 7.28 (dd, 1H, Ar-H), 7.37 (dd, 1H, Ar-H), 7.66 (d, 2H, Ar-H), 7.77 (d, 2H, Ar-H), 7.98 (d, 2H, Ar-H), 8.05 (d, 2H, Ar-H), 8.98 (s, 1H, Ar-H); MS m/z 522.21 (M^+ +1).

***(E)* -N- (4-methoxyphenyl) -2- ((4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino) acetamide (5e):**

Light-yellow solid, M.P.: 130-133 ^{o}C; Yield: 66%; Anal. Calcd. For $C_{31}H_{29}N_3O_6$: C, 69.00; H, 5.42; N, 7.79%. Found C, 69.06; H, 5.48; N, 7.85%; IR (KBr, Vmax/cm^{-1}): 3074 (-NH str., 2^0 amine), 6055 (-C=O str., amide), 1098 (-C-O-C str., morpholine), 1711 (-C=O str., coumarin), 1622 (-C=C- str., alkenyl), 1468 (-C=C- str., aromatic), 1335 (-C-N str., morpholine), 1132 (-OCH3 Str.) ; ^{1}H NMR (500 MHz, DMSO, δ ppm): 2.34 (s, 1H, CH), 2.39 (s, 1H, CH), 2.65 (t, 4H, CH2), 2.78 (t, 4H, CH2), 3.93 (s, 3H, OCH3), 2.23 (dd, 2H, CH2), 4.44 (s, 1H, CH D_2O exchanger), 7.14 (dd, 1H, Ar-H), 7.21 (ddd, 1H, Ar-H), 7.26 (dd,

1H, Ar-H), 7.34 (dd, 1H, Ar-H), 7.66 (d, 2H, Ar-H) 8.98 (s, 1H, Ar-H); MS m/z 538.50 (M^+ +1).

General procedure for the synthesis of N-(substituted phenyl) -2- ((4- (3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino) amino) thiourea (5f-j)

To a mixture of 3-(3-(4-aminophenyl)-3-oxoprop-1-en-1-yl)-4-morpholino-2H-chromen-2-one (1.61 g, 10 mmole) and phenylisothiocyanate (10 mmol) in absolute ethanol (25 ml) was added K_2CO_3 (1g) was added and heated under reflux for 8 hours. The progress of reaction was monitored by TLC using hexane : ethylacetete (6:4) as eluent. After the completion of reaction yellow crystal separate out. The crude product was purified by crystallization form absolute alcohol to get the title compound. Similarly other compounds were prepared from intermediate **(4)** with various substituted isothiocyanates.

***(E)*-1-(4-(3-(4-morpholino-2-oxo-2H-chromen-3-yl)acryloyl)phenyl)-3-phenylthiourea (5f):**

Light-yellow solid, M.P.: 96-98 ^oC; Yield: 66%; Anal. Calcd. For $C_{29}H_{25}N_3O_4S$: C, 68.95; H, 5.18; N, 7.99%. Found C, 68.59; H, 5.13; N, 7.95%; IR (KBr, Vmax/cm^{-1}): 3426 (-NH str., 2^0 amine), 1620 (-C=O str., pyrane), 1032 (-C-O-C str., morpholine), 1732 (-C=O str., coumarin), 1682 (-C=C- str., alkenyl), 3062 (-CH str. aromatic), 1415 (-C=C- str., aromatic), 1312 (-C-N str., morpholine), 955 (-C=S str. thiourea), 3000 (-CH str. alkane); ^1H NMR (500 MHz, DMSO, δppm): 2.35 (s, 1H , CH), 2.37 (s, 1H, CH), 2.67 (t, 4H, CH), 2.75 (t, 4H, CH), 6.99 (d, 2H, Ar-H), 7.32 (d, 2H, Ar-H), 7.47 (ddd, 1H, Ar-H), 7.54 (dd, 1H, Ar-H), 7.76 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 7.97 (d, 2H, Ar-H), 8.29 (dd, 1H, Ar-H), 8.45 (dd, 1H, Ar-H), 8.47 (ddd, 1H, Ar-H), 8.8 (d, 1H, Ar-H), 9.3 (s, 1H, Ar-H), 9.7 (s, 1H, Ar-H); MS m/z 215.0 (M^+ +1).

***(E)*-1-(4-(3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) -3- (p-tolyl) thiourea (5g):**

Light yellow solid, M.P.: 112-115^0C; Yield: 62%; Anal. Calcd. For $C_{30}H_{27}N_3O_4S$: C, 69.01; H, 5.42; N, 7.79%. Found C, 69.07; H, 5.39; N, 7.75%; IR (KBr, Vmax/cm^{-1}): 3420 (-NH str., 2^0 amine), 1644 (-C=O str., pyrane), 1035 (-C-O-C str., morpholine), 1733(-C=O str., coumarin), 1685 (-C=C- str., alkenyl), 3064 (-CH str. aromatic), 1419 (-C=C- str., aromatic), 1311 (-C-N str., morpholine), 955 (-C=S str. thiourea); ^1H NMR (500 MHz, DMSO, δ ppm): 2.37 (s, 1H, CH), 2.39 (s, 1H, CH), 2.17 (d, 3H, CH3), 2.66 (t, 4H, CH), 2.74 (t, 4H, CH), 6.97 (d, 2H, Ar-H), 7.46 (ddd, 1H, Ar-H), 7.55 (dd, 1H, Ar-H), 7.75 (d, 2H, Ar-H), 7.84 (d, 2H, Ar-H), 7.98 (d, 2H, Ar-H), 8.28 (dd, 1H, Ar-H), 8.43 (dd, 1H, Ar-H), 8.45 (ddd, 1H, Ar-H), 8.6 (d, 1H, Ar-H), 9.2 (s, 1H, Ar-H), 9.8 (s, 1H, Ar-H); MS m/z 510.55 (M^+ +1).

***(E)* -1- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) -3- (o-tolyl) thiourea (5h):**

Light-yellowsolid, M.P.: 100-104^0C; Yield: 58%; Anal. Calcd. For $C_{30}H_{27}N_3O_4$: C, 69.01; H, 5.42; N, 7.79%. Found C, 69.08; H, 5.46; N, 7.75%; IR (KBr, Vmax/cm^{-1}): 3424 (-NH str., 2^0amine), 1646 (-C=O str., pyrane), 1033 (-C-O-C str., morpholine), 1735 (-C=O str., coumarin), 1682 (-C=C- str., alkenyl), 3061 (-CH str., aromatic), 1418 (-C=C- str., aromatic), 1311 (-C-N str., morpholine), 953 (-C=S str., thiourea); ^1H NMR (500MHz, DMSO, δppm): 2.35 (s, 1H, CH), 2.37 (s, 1H, CH), 2.18 (d, 3H, CH3), 2.67 (t, 4H, CH), 2.75 (t, 4H, CH), 6.99 (d, 2H, Ar-H), 7.47 (ddd, 1H, Ar-H), 7.54 (dd, 1H, Ar-H), 7.76 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H), 7.97 (d, 2H, Ar-H), 8.29 (dd, 1H, Ar-H), 8.45 (dd, 1H, Ar-H), 8.47 (ddd, 1H, Ar-H), 8.8 (d, 1H, Ar-H), 9.3 (s, 1H, Ar-H), 9.7 (s, 1H, Ar-H); MS m/z 510.55 (M^+ +1).

***(E)*-1- (2-chlorophenyl) -3- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) thiourea (5i):**

Light-yellow solid, M.P.: 125-130 ^{0}C; Yield: 60%; Anal. Calcd. For $C_{29}H_{24}ClN_3O_4S$: C, 64.34; H, 4.68; N, 7.51%. Found C, 64.37; H, 4.62; N, 7.47% ; IR (KBr, Vmax/cm^{-1}): 3422 (-NH str., 2^0 amine), 1 645 (-C=O str., pyrane), 1033 (-C-O-C str., morpholine), 1732 (-C=O str., coumarin), 1683 (-C=C- str., alkenyl), 3062 (-CH str. aromatic), 1417 (-C=C- str., aromatic), 1311 (-C-N str., morpholine), 954(-C=S str., thiourea); ^{1}H NMR (500 MHz, DMSO, δppm): 2.36 (s, 1H, CH), 2.37 (s, 1H, CH), 2.63 (t, 4H, CH), 2.76 (t, 4H, CH), 6.98 (d, 2H, Ar-H), 7.44 (ddd, 1H, Ar-H), 7.51 (dd, 1H, Ar-H), 7.73 (d, 2H, Ar-H), 7.83 (d, 2H, Ar-H), 7.96 (d, 2H, Ar-H), 8.27 (dd, 1H, Ar-H), 8.42 (dd, 1H, Ar-H), 8.44 (ddd, 1H, Ar-H), 8.7 (d, 1H, Ar-H), 9.1 (s, 1H, Ar-H), 9.5 (s, 1H, Ar-H); MS m/z 545.04 (M^+ +1).

***(E)* -1- (4-fluorophenyl) -3- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) thiourea (5j):**

Light-yellow solid, M.P.: 120-124 ^{0}C; Yield: 59%; Anal. Calcd. For $C_{29}H_{24}FN_3O_4S$: C, 66.28; H, 4.82; N, 7.73%. Found C, 66.24; H, 4.87; N, 7.77%; IR (KBr, Vmax/cm^{-1}): 3424 (-NH str., 2^{0}amine), 1646 (-C=O str., pyrane), 1031 (-C-O-C str., morpholine), 1734 (-C=O str., coumarin), 1685 (-C=C- str., alkenyl), 3063 (-CH str. aromatic), 1418 (-C=C- str., aromatic), 1311 (-C-N str., morpholine), 956(-C=S str. thiourea); ^{1}H NMR (500 MHz, DMSO, δppm): 2.38 (s, 1H, CH), 2.39 (s, 1H, CH), 2.62 (t, 4H, CH), 2.75 (t, 4H, CH), 6.97 (d, 2H, Ar-H), 7.43 (ddd, 1H, Ar-H), 7.50 (dd, 1H, Ar-H), 7.72 (d, 2H, Ar-H), 7.84 (d, 2H, Ar-H), 7.98 (d, 2H, Ar-H), 8.26 (dd, 1H, Ar-H), 8.44 (dd, 1H, Ar-H), 8.46 (ddd, 1H, Ar-H), 8.8 (d, 1H, Ar-H), 9.2 (s, 1H, Ar-H), 9.6 (s, 1H, Ar-H); MS m/z 528.53 (M^+ +1).

General procedure for the syntheses of N-(Aryl)-2- ((4-(3-(4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) amino)urea (5k-o)

To a mixture of 3- (3- (4-aminophenyl) -3-oxoprop-1-en-1-yl) -4-morpholino-2H-chromen-2-one (1.61 g, 10 mmole) and phenylisocyanate (10 mmol) in absolute ethanol (25ml), added K_2CO_3 (0.1g),was heated under reflux for 8 hours. The progress of reaction was monitored by TLC using hexan:ethylacetete (6:4) as eluent. After the completion of reaction, yellow crystals were separated out. The crude product was purified by crystallization form absolute alcohol to get the title compound. Similarly other compounds were prepared from intermediate **(4)** with various substituted isocyanates.

***(E)* -1- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) -3-phenylurea (5k):**

Light-yellow solid, M.P.: 117-114 ^{0}C; Yield: 74%; Anal. Calcd. For $C_{30}H_{27}N_3O_5$: C, 70.71; H, 5.34; N, 8.25%. Found C, 70.75; H, 5.38; N, 8.20%; IR (KBr, Vmax/cm^{-1}): 3292 (-NH str.,2^{0}Amine), 1635 (-C=O str., pyrane), 1682 (-C=O str. amide) 1227 (-C-O-C str., morpholine), 1721 (-C=O str., coumarin), 1636 (-C=C- str., alkene), 3060 (CH str. Aromatic), 1587 (-C=C- str., aromatic), 1117 (-C-N str., morpholine); ^{1}H NMR (500 MHz, DMSO, δppm): 2.37 (s, 1H, CH), 2.36 (s, 1H, CH), 2.63 (t, 4H, CH2), 2.77 (t, 4H, CH2), 6.95 (d, 1H, Ar-H), 7.06 (dd, 1H, Ar-H), 7.13 (dd, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 7.33 (d, 2H, Ar-H) 7.36 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.83 (d, 2H, Ar-H), 7.98 (d, 2H, Ar-H), 9.1 (s, 1H, Ar-H), 9.4 (s, 1H, Ar-H); MS m/z 494.17 (M^+ +1).

***(E)* -1- (2-chlorophenyl) -3- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) urea (5l):**

Light-yellow solid, M.P.: 119-125 ^{0}C; Yield: 70%; Anal. Calcd. For $C_{30}H_{26}ClN_3O_5$: C, 66.24; H, 4.82; N, 7.72%. Found C, 66.20; H, 4.87; N, 7.77%; IR (KBr, Vmax/cm^{-1}): 3290 (-NH str.,2^{0}amine), 1684 (-C=O str., pyrane), 1645 (-C=O str. amide) 1034 (-C-O-C str., morpholine), 1732 (-C=O str., coumarin), 1682 (-C=C- str., alkene), 3065 (CH str. aromatic),

1417 (-C=C- str., aromatic), 1117 (-C-N str., morpholine), 757 (C-Cl str.); ^{1}H NMR (500 MHz, DMSO, δppm): 2.37 (s, 1H, CH), 2.36 (s, 1H, CH), 2.63 (t, 4H, CH2), 2.77 (t, 4H, CH2), 6.95 (d, 1H, Ar-H), 7.06 (dd, 1H, Ar-H), 7.13 (dd, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 7.33 (d, 2H, Ar-H), 7.42 (d, 2H, Ar-H), 7.83 (d, 2H, Ar-H), 7.98 (d, 2H, Ar-H), 9.1 (s, 1H, Ar-H), 9.4 (s, 1H, Ar-H); MS m/z 528.12 (M^+ +1).

***(E)* -1- (3-chlorophenyl) -3- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) urea (5m):**

Light-yellow solid, M.P.: 110-114 ^{0}C; Yield: 75%; Anal. Calcd. For $C_{30}H_{26}ClN_3O_5$: C, 66.24; H, 4.82; N, 7.72%. Found C, 66.28; H, 4.87; N, 7.78%; IR (KBr, Vmax/cm^{-1}): 3292 (-NH str.,2^{0}amine), 1683 (-C=O str., pyrane), 1644 (-C=O str. amide) 1034 (-C-O-C str., morpholine), 1732 (-C=O str., coumarin), 1682 (-C=C- str., alkene), 3065 (CH str. aromatic), 1417 (-C=C- str., aromatic), 1117 (-C-N str., morpholine), 755 (C-Cl str.); ^{1}H NMR (500 MHz, DMSO, δppm): 2.35 (s, 1H, CH), 2.36 (s, 1H, CH), 2.65 (t, 4H, CH2), 2.79 (t, 4H, CH2), 6.92 (d, 1H, Ar-H), 7.05 (dd, 1H, Ar-H), 7.14 (dd, 1H, Ar-H), 7.15 (d, 1H, Ar-H), 7.35 (d, 2H, Ar-H), 7.44 (d, 2H, Ar-H), 7.82 (d, 2H, Ar-H), 7.99 (d, 2H, Ar-H), 9.3 (s, 1H, Ar-H), 9.6 (s, 1H, Ar-H); MS m/z 528.12 (M^+ +1).

***(E)* -1- (2-fluorophenyl) -3- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) urea (5n):**

Light-yellow solid, M.P.: 105-107 ^{0}C; Yield: 65%; Anal. Calcd. For C30H26FN3O5: C, 68.31; H,4.49; N,7.97%. Found C, 68.35; H, 4.42; N, 7.94%; IR (KBr, Vmax/cm^{-1}): 3294 (-NH str., 2^{0}amine), 1632 (-C=O str., pyrane), 1682 (-C=O str. amide) 1227 (-C-O-C str., morpholine), 1722 (-C=O str., coumarin), 1637 (-C=C- str., alkene), 3064 (CH str. aromatic), 1584 (-C=C- str., aromatic), 1118 (-C-N str., morpholine), 1288 (C-F str.); ^{1}H NMR (500 MHz, DMSO, δppm): 2.38 (s, 1H, CH), 2.36 (s, 1H, CH), 2.65 (t, 4H, CH2), 2.77 (t, 4H, CH2), 6.96 (d, 1H, Ar-H), 7.07 (dd, 1H, Ar-H), 7.10 (dd, 1H, Ar-H), 7.14 (d, 1H, Ar-H), 7.32 (d, 2H, Ar-H), 7.44 (d, 2H, Ar-H), 7.83 (d, 2H, Ar-H), 7.99 (d, 2H, Ar-H), 9.2 (s, 1H, Ar-H), 9.5 (s, 1H, Ar-H); MS m/z 512.18 (M^+ +1).

***(E)* -1- (4-fluorophenyl) -3- (4- (3- (4-morpholino-2-oxo-2H-chromen-3-yl) acryloyl) phenyl) urea (5o):**

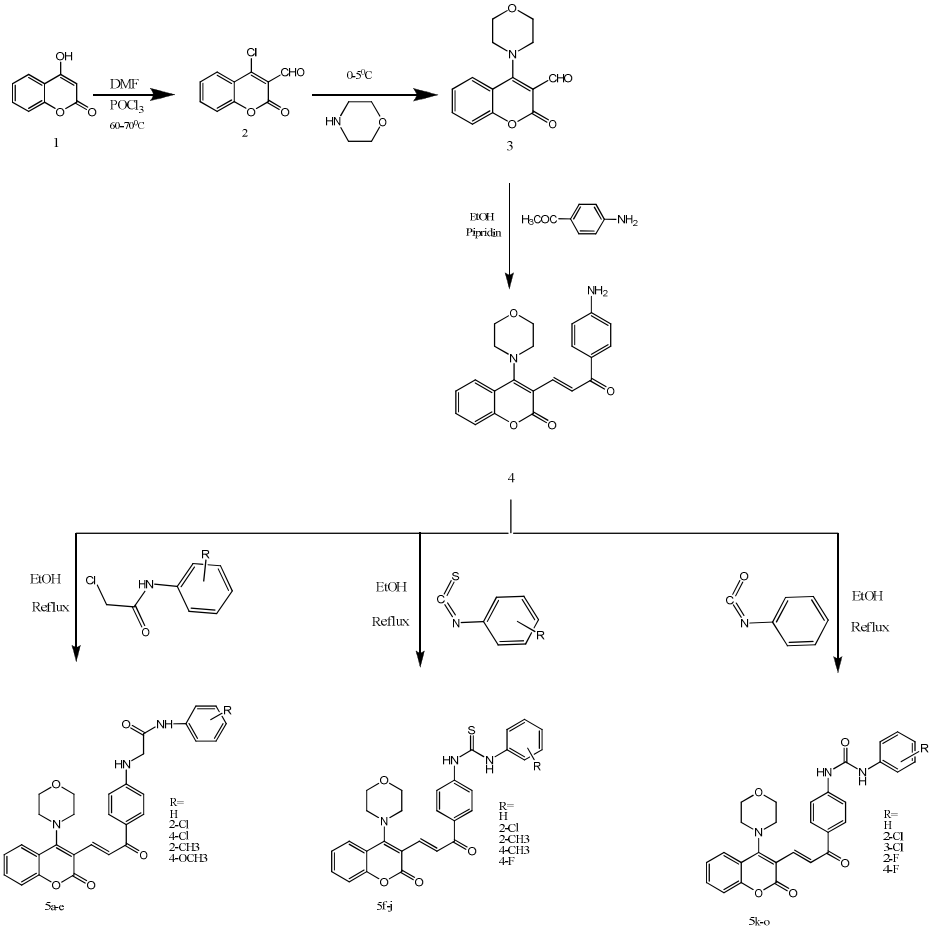
Light-yellow solid, M.P.: 112-116 ^{0}C; Yield: 63% ; Anal. Calcd. For $C_{30}H_{26}FN_3O_5$: C, 68.31; H, 4.49; N, 7.97%. Found C, 68.37; H, 4.52; N, 7.90%; IR (KBr, Vmax/cm^{-1}): 3292 (-NH str.,2^{0}amine), 1635 (-C=O str., pyrane), 1683 (-C=O str. amide) 1225 (-C-O-C str., morpholine), 1722 (-C=O str., coumarin), 1635 (-C=C- str., alkene), 3062 (CH str. aromatic), 1585 (-C=C- str., aromatic), 1117 (-C-N str., morpholine), 1286 (C-F str.); ^{1}H NMR (500 MHz, DMSO, δppm): 2.36 (s, 1H, CH), 2.37 (s, 1H, CH), 2.64 (t, 4H, CH2), 2.76 (t, 4H, CH2), 6.94 (d, 1H, Ar-H), 7.09 (dd, 1H, Ar-H), 7.11 (dd, 1H, Ar-H), 7.13 (d, 1H, Ar-H), 7.34 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H), 7.81 (d, 2H, Ar-H), 7.99 (d, 2H, Ar-H), 9.1 (s, 1H, Ar-H), 9.5 (s, 1H, Ar-H); MS m/z 512.18 (M^+ +1).

Results and discussion

Chemistry

The synthesis of the target **(5a-e), (5f-j)** and **(5k-o)** compounds were carried out as outlined in **Scheme 1**. The structures of all synthesised compounds were inferred from their analytical and spectral data. The formation of titled compound was confirmed by their FT-IR, ^{1}H NMR, mass spectra as well as elemental analysis. As an example, in the IR spectrum of compound **5c**, characteristic is the -C=C- stretching in alkenyl stretching vibration, which appear as an intense band at 1620 cm^{-1}. The stretching vibration band appeared at 1335 cm^{-1} due to the stretching of C-N in morpholine moreover 1097 cm^{-1}due to the stretching of -C-O-C in morpholine. The stretching vibration band appeared at 3070 cm^{-1} due to –NH stretching in

secondary amine, C=O stretching in coumarin ring at 1712 cm^{-1} and C=O stretching vibration in amide at 1650 cm^{-1}. The ^1H NMR spectrum of compound **5a** showed two singlet peak at 2.32 δ ppm and 2.38 δ ppm due to presence of –CH=CH, several peak appered two triplet between 2.64-2.80 δ ppm due to the presence of morpholine. Moreover mass spectra of all the compound showed molecular ion peak M^+ corresponding to their mass. The obtained elemental analysis values are in good agreement with theoretical data.



Scheme 1. Systematic path for the synthesis of **(5a-e)**, **(5f-j)** and **(5k-o)**

In vitro antimicrobial activity

In vitro antibacterial and antifungal activity of newly synthesized compounds **(5a-o)** was carried out by micro broth dilution method according to National Committee for Clinical Laboratory Standards (NCCLS, 2002) [XXII]. A panel of selected pathogens Gram positive (Staphylococcus aureus MTCC 96 and Bacilluscereus MTCC 430) and Gram negative (Escherichia coli MTCC 443 and Klebsiella pneumonia MTCC 109) bacterial species used for antibacterial activity whereas for antifungal activity, a panel of selected fungal pathogens (Candida albicans MTCC 227, Aspergillus niger MTCC 282 and Aspergillus calavatus MTCC 1323) species were used. 2% DMSO solution was used as diluent to get desired concentration of drugs to test upon standard bacterial and fungal strains. The zone of inhibition produced by each compound was measured in µg/ml. The minimum inhibitory concentration (MIC) was determined and recorded at the lowest concentration inhibiting growth of the organism. Ciprofloxacin and Chloramphenicol were used as standard antibiotic drugs for antibacterial activity while Greseofulvin and Nystatin were used as standard drug

for antifungal activity. All the test compounds containing Coumarin as the core unit structure substituted with morpholine, 4-amino acetophenone, various acetamides, ureas as well as thioureas were tested for their in vitro antibacterial efficacy.

In vitro antitubercular activity

In vitro antitubercular activity of all the newly synthesized compounds was determined by using Lowenstein-Jensen medium (conventional method) against Mycobacterial tuberculosis H37Rv strain as described by Rattan [XXIII]. The observed results are presented in Table 1 in the form of inhibition (%), relative to that of standard antitubercular drugs Isoniazid and Rifampicin. Compounds demonstrating more than 80% inhibition in the primary screening were retested at lower concentration (MIC) in a Lowenstein-Jensen medium and evaluated for their MIC values. Four compounds exhibiting more than 80% inhibition were again screened to get their MIC values (Table 2).

Table 1. In vitro antimicrobial and antitubercular activity data of the compounds **(5a-e)**, **(5f-j)**, and **(5k-o)**

Compd.	**Antimicrobial activity (MIC) µg/ml**							**Antitubercular activity**
	Antibacterial activity				**Antifungal activity**			
	Gram Positive Bacteria		**Gram Negative Bacteria**		**Fungus**			**% Inhibition at 250 µg/ml**
	S. aureus.	***B. cereus.***	***E. coli***	***K. pneumonia.***	***C. albican***	***A. niger***	***A. clavatus***	***M. tuberculosis H37Rv***
5a	250	125	100	62.5	>1000	>1000	>1000	46
5b	100	62.5	250	100	250	500	500	45
5c	125	100	62.5	125	1000	1000	1000	47
5d	62.5	125	100	62.5	>1000	>1000	>1000	85
5e	62.5	250	100	125	>1000	>1000	>1000	45
5f	100	250	62.5	125	1000	500	500	60
5g	62.5	100	100	250	500	>1000	>1000	34
5h	100	125	100	250	>1000	>1000	>1000	35
5i	250	62.5	125	100	>1000	250	250	84
5j	125	250	62.5	100	1000	1000	1000	37
5k	62.5	100	250	125	500	>1000	>1000	45
5l	250	125	62.5	100	1000	>1000	>1000	80
5m	100	62.5	250	125	>1000	>100	>1000	48

						0		
5n	100	250	125	62.5	250	500	500	32
5o	250	125	250	100	>1000	500	500	84
A	50	50	25	25	-	-	-	-
B	50	50	50	50	-	-	-	-
C	-	-	-	-	500	100	100	-
D	-	-	-	-	100	100	100	-
E	-	-	-	-	-	-	-	99
F	-	-	-	-	-	-	-	98

A=Ciprofloxacin, B= Chloramphenicol, (Standard Drugs for antibacterial activity) C = Greseofulvin, D = Nystatin (Standard Drugs for antifungal activity) F=Isoniazid and G= Rifampicin. (Standard Drugs for antitubercular activity)

Table 2: In vitro antitubercular activity data of the synthesised compounds exhibiting greater inhibition against M. tuberculosis H37Rv (MICs, µg/ml)

Compd.	**% Inhibition**	**MIC (µg/ml)**
5d	84	50
5i	81	100
5l	85	62.5
5o	84	62.5
A	99	0.20
B	98	40

A: Isoniazid and **B:** Rifampicin

Conclusion

The present work is focused on the synthesis of novel heterocyclic compounds.with the aim of discovering innovative structure leads serving as potent antimicrobial and antitubercular agents. The screening results revealed that all the compounds exhibited moderate to excellent activities against all the pathogenic strains. Among the synthesized scaffolds of mostly the compounds containing either halogen atom i.e., chloro group or electron donating group i.e., methyl and methoxy group showed higher potential against the specific bacterium.Rest of the other compounds exhibited less or moderate activity among the synthesized compounds. None of the compound has reached up to the level of standard drugs Ciprofloxacin and Chloramphenicol. Based on the above results, it can be concluded that electron donating substitutent i.e., o-methyl and p-methoxy group may be helpful to inhibit the growth of Gram negative microorganisms, while halo substituent as well as electron donating group may be beneficial for the inhibition of Gram positive microorganisms. Compound **5d**, **5i, 5l** and **5o** displayed excellent anti tubercular activity.

From the above discussion it may be concluded that it is worthwhile to pursue further investigation on coumarin derivatives.

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