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Research article

Design, synthesis and validation of anti-microbial coumarin derivatives: An efficient green approach

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ABSTRACT

An ecofriendly itinerary for the synthesis of newly substituted chromene-3-carboxamide derivatives was undertaken to avoid impurities, usage of toxic solvents, toxic catalyst, and having improved quantitative yields. The green synthesis involves the condensation of substituted salicylaldehyde with N-(substituted)phenyl malonic acid in the presence of a base catalyst, piperidine. All reported compounds were assessed for their antimicrobial activities which clearly suggested their therapeutic implications to address antimicrobial pathogenesis. The synthesized coumarin compounds were examined for their antimicrobial activity against 7 fungal strains and 2 bacterial strains at concentration 125–1000 μg/mL. In particular, the compounds 4 and 5 showed lower minimum inhibitory concentration value (125 μg/mL) against maximum microbial strains. Further, docking of all the synthesized compounds was performed with the enzymes lanosterol 14α-demethylase and glucosamine-6-phosphate synthase and a significant binding affinity was observed which supports *in vivo* antimicrobial study. In addition, the thermal analysis revealed good thermal stability of compounds up to 250 °C. The compounds showed abroad absorption spectrum between 280-550 nm establishing them to be good UV absorbers.

1. Introduction

Due to the increase in the number of immune-compromised hosts, the more cases of microbial infections have been reported drastically. Most of the microorganisms develop resistance over a period of time against available drugs. Hence, the available medicines are either less effective or ineffective. Therefore, there is an urgent need to develop alternative antimicrobial agents. Coumarin compounds are the important class in the domain of natural products and organic synthesis. They possess an oxygen heterocyclic benzopyran system belonging to the lactone family [1]. The benzopyran-2-one, or chromen-2-one ring system is present in the natural products, and exhibit interesting pharmacological properties. Thus, researchers are fascinated from decades to investigate important biological properties of such natural coumarins or to synthesize their analogs for therapeutic purposes [2].

The coumarins analogs (synthetic or natural) are an essential component in the cosmetics and perfumes industry [3]. Biologically active natural products possess coumarin skeleton and used as an intermediate for the synthesis of bioactive heterocyclic compounds which revealed it's antimicrobial [4], antifungal [5], anti-inflammatory [6, 7], anti-cancer [3, 7], anti-tubercular [8], antioxidant [9], and anticoagulant [10] properties. Few synthetic compounds with a coumarin ring have been reported to be active against multi-drug resistant (MDR) bacteria [11, 12]. The literature has also highlighted the potential role of FXII (factor XII) in pathological thrombogenesis. Robert et.al. synthesized a new series of 3-carboxamide-coumarins that is the first potent and selective nonpeptidic inhibitors of FXIIa [13].

Several synthetic strategies for coumarin compounds have already been proposed. Coumarin derivatives are prepared by Perkin reaction, Pechmann reaction or by Knoevenagel condensation of salicylaldehydes with malonic acid or Meldrum's acid [14, 15]. The coumarins can be of versatile use including as an intermediate in the synthesis of furocoumarins, chromenes, coumarones and 2-acylresorcinol [16]. Fluorescent chemodosimeter based on coumarin and salicylaldehyde

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functionality is used for selective detection of cyanide anions in the water at biological pH [17]. 3-(2-benzothiazolyl) based coumarins were studied by Zhou and coworkers [18] and they explained the fluorescent properties of the compound in which the coumarin acts as a donor while the benzothiazole moiety acts an acceptor. Moeckli [19] has synthesized a few new red fluorescent coumarin molecules by keeping the electrons donating group on 7th position of the coumarin ring and withdrawing group on 3rd position.

Chemchem et al. designed and reported the novel coumarin-thiophene-derived Schiff base for selective fluorescent detection of cyanide anions at a concentration as low as 0.32 μM, which is lower than the WHO guideline (2.7 μM) for cyanide in aqueous solution. Additionally, they proposed the mechanism in this study as an ICT involving di-deprotonation of the acidic OH protons in chemosensor. They also revealed in their study that the chemosensor can intercalate into dublex DNA and it can be evaluated using a DNA detection agent [20]. The chemosensors with a chromophoric group act as a powerful tool for detecting cyanide and fluoride ions in aqueous solution. The coumarin based derivatives with acidic NH proton binds with fluoride and cyanide ions both via H-bonding and produces fluorescence change in a solution containing the probe through a change in their photophysical properties [21].

The hybrid framework of coumarin nucleus with other moieties and the improved pharmacological property have been a topic of interest. In view of the above observations, the synthesis of some newly halogen-substituted coumarin derivatives by facile greener method has been reported herein. The series of the synthesized 10 coumarin derivatives are not yet reported and have not been assessed for its biological activity. The molecular docking study of these 10 coumarin compounds with proposed targets glucosamine-6-phosphate synthase (GlcN-6-P synthase) and lanosterol 14α-demethylase have also been performed. The binding energy of coumarin compounds with the above enzymes is estimated and reported.

2. Experimental

2.1. General information

Analytical grade dimethylsulfoxide (DMSO), dimethylformamide (DMF), tetrahydrofuran (THF), chloroform, methanol, carbon tetrachloride, acetonitrile, benzyl alcohol, dioxane, acetone, carbon disulfide, salicylaldehyde, sodium chloride, agar, tryptone, Yeast extract were purchased from LobaChemie (India). All the substituted salicylaldehyde required as a precursor for the synthesis of compounds (1–10) were procured from Sigma Aldrich (USA).

The reactions were monitored by thin-layer chromatography (TLC) using aluminium sheets of Merck silica gel 60 F254. The melting points (mp) were recorded on Electrothermal 9002 apparatus. UV-visible spectroscopy was performed on UV double beam spectrophotometer Systronics T2201, with the band width of 2nm in DMSO solvent (0.25 mg/mL) from 200-800 nm, using 1cm quartz cells. Fluorescence spectra of compounds 1–10 were recorded on Cary Eclipse MY13340005 at scan rate 600 nm/min and λ_{ex} varying from 315 to 325 nm. The fluorescence spectra were recorded in DMSO at a concentration of 0.25 mg/mL and were recorded using excitation into the maximum of the longest wavelength absorption band program. The fluorescence of solution was measured in a 1 cm^3 cuvette in the right-angle arrangement. FTIR spectra (KBr) were recorded on a Perkin Elmer System 2000 spectrophotometer in KBr pellet in the 4000-400 cm^{-1} range at room temperature. ^{1}H and ^{13}C-NMR spectra were recorded at 400 MHz on Bruker Spectrometer using TMS as an internal standard. Chemical shifts are expressed as δ unit (ppm). Elemental analysis was performed using Thermo Scientific (FLASH 2000) CHN Elemental Analyser. Elemental analytical data (in accordance with the calculated values) were obtained from microanalytical unit. TGA/DTA (thermogravimetric analysis) and DSC (differential scanning calorimetry) were performed using Perkin Elmer, Diamond TG/DTA at 20 °C/min in a nitrogen atmosphere. Mass spectrometry was recorded on a Velos Pro Ion Trap mass Spectrophotometer from Thermo Scientific 21 (San Jose, CA, USA) using ESI (electrospray ionization).

2.2. Chemistry

2.2.1. General procedure for the synthesis of 3-(4-bromo-2-fluorophenylamino)-3-oxopropanoic acid

The precursor 3-(4-bromo-2-fluorophenylamino)-3-oxopropanoic acid was synthesized in the laboratory [22]. A mixture of 4-bromo-2--fluoro aniline (1.89 gm, 1 mol) and freshly distilled diethyl malonate (3.2 gm, 2 mol) was refluxed in the round bottom flask using an upright air condenser for 90 min, allowing the alcohol formed to escape and the ester to flow back. After cooling, ethanol (20 mL) was added and the contents were kept overnight. A crystalline substance was separated out and filtered. The filtrate was mixed with sodium carbonate (5 gm) dissolved in water (20 mL) and the steam was blown through it for 60 min. On cooling, dianilide as a product was separated. This was filtered off and the filtrate was acidified with hydrochloric acid. A white precipitate was obtained which was filtered and purified from sodium bicarbonate and hydrochloric acid. It was identified as 3-(4-bromo-2--fluorophenylamino)-3-oxopropanoic acid.

Colour and physical state of precursor was white amorphous powder; Yield 81.45%; mp 153 °C; IR (KBr) υ_{max} cm^{-1}: 3293-3121 (N–H, stret. H-bonded), 3064 Aromatic (C–H, stret.), 2986-2925 Aliphatic (C–H, stret.), 1715 (-C=O, stret.), 1605 (HN–C=O, stret., amide-II), 1335 (C–O, stret.), 1184 (ArC-F, stret.,), 1071-1021 (ArC-Br, sret.); ^{1}H-NMR (300 MHz, $CDCl_3$) in δppm: 7.26 ($CDCl_3$), 1.30–135 (t, 3H, CH_3), 3.49–3.50 (s, 2H, CH_2), 4.24–4.31 (q, 2H, OCH_2), 7.24–8.25 (m, 3H, Ar–H), 9.61 (s, 1H, CONH); Anal. calcd. for $C_9H_7O_3NBrF$ (276), Calc: C 39.13, H 2.53, N 5.07. Found: C 39.20, H 2.55, N 5.06.

2.2.2. General procedure for the synthesis of substituted coumarin derivatives (1–10)

A mixture of substituted salicylaldehyde (1 mM) and N-(4-bromo-2-fluoro)phenyl malonic acid (1mM) were mixed and placed in a round bottom flask. Piperidine (2–3 drops) was used as a catalyst and the mixture was heated in an oil bath at 105–110 °C for 4h. The reaction mixture was allowed to cool at room temperature and filtered off. The residue was then boiled with absolute ethanol (20 mL) and filtered hot. It was recrystallized with acetone and absolute alcohol to afford the target coumarin derivatives.

2.2.3. 6-Bromo-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide (1)

A yellow powder, Yield 64.73%, mp 245 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (chromone ring) 790, C–Br (phenyl ring) 740, C–F (phenyl ring) 1409, N–H 3103, –CO–O– 1737, C–H 3045, C–H (def.) 831, C=C(Arom) 1614, –CONH– 1670; 1H-NMR (DMSO-d6 400MHz, in δppm): 8.99 (s, 1H, NH), 8.34 (s, 1H, coum. H_4), 7.47 (d, J = 9.2 Hz, 1H, Ar–H), 7.55 (d, J = 8.8 Hz, 2H, Ar–H), 7.79 (d, J = 8 Hz, 1H, Hcoum.), 7.88–7.96 (m, 2H, Hcoum.);^{13}C-NMR (DMSO-d_6 400MHz, δppm): 160, 159(C_{11} = O), 153(C_2 = Ocoum), 151, 147, 136(C_4coum.), 132, 127, 125, 123, 120, 119, 118, 115.; MS (ESI) (m/z): 441; Anal. calcd. for $C_{16}H_8O_3NBr_2F$, C 44.53, H 1.81, N 3.17. Found: C 44.60, H 1.78, N 3.18. (see supplementary Fig S1, Fig S2 and Fig S3).

2.2.4. 6-Chloro-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide (2)

A yellow powder; Yield 72.22%; mp 230 °C; IR (KBr) υmax cm-1:C–Br (phenyl ring) 758, C–F (phenyl ring) 1411, C–Cl 815, N–H 3197, –CO–O-1716, C–H 3047, C–H (def.)829, C=C(Arom) 1616,–CONH– 1664; 1H-NMR (DMSO-d6 400MHz): 9.01 (s, 1H, NH), 8.21 (s, 1H, coum. H_4), 7.49 (d, J = 8.8 Hz, 1H, Ar–H), 7.73–7.85 (m, 2H, Ar–H), 7.72 (d, J = 5.6 Hz, 1H, Hcoum.) 8.31–8.38 (m, 2H, Hcoum.);^{13}C-NMR (DMSO-d_6 400MHz, δppm): 162, 159.5(C_{11} = O), 158(C_2 = Ocoum.), 148, 143,

138.6(C_4coum.), 128.5, 128, 127.5, 126, 124, 123, 121.9, 120, 119; MS (ESI) (m/z): 397 [M+1]; Anal. calcd. for $C_{16}H_8O_3NBrClF$, C 48.42, H 2.02, N 3.53. Found C 48.50, H 2.10, N 3.55. (see supplementary Fig S4 and Fig S5).

2.2.5. 6-Nitro-N-{4-bromo-2-fluoropheny}-2-oxo-2H-chromene-3-carboxamide (3)

A yellow powder; Yield 70.81%; mp 182 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (phenyl ring) 754,C–F (phenyl ring) 1479, N–H 3084, –CO–O- 1714, C–O 1298, C–NO_2 1573 & 1346 C–H (def.) 860, C=C(Arom) 1618, –CONH– 1573; ^{1}H-NMR (DMSO-d_6 400MHz): 9.05 (s, 1H, NH), 8.69 (s, 1H, coum. H_4), 7.04–7.56 (m, 3H, Ar–H), 7.80–8.43 (m, 3H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 165, 162(C_{11} = O), 160.1(C_2 = Ocoum.), 156, 147, 139(C_4coum.), 127, 126, 124, 123, 122, 120, 118; MS (ESI) (m/z): 406.9 [M]+; Anal. calcd. for $C_{16}H_8O_5N_2BrF$, C 47.17, H 1.96, N 6.87. Found: C 47.22, H 1.90, N 6.86. (see supplementary Fig S6, Fig S7 and Fig S8).

2.2.6. 6,8-Dibromo-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(4)

A light orange powder; Yield 54.68%; mp 164 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (chromone ring) 864, C–Br (phenyl ring) 740, C–F (phenyl ring) 1408, N–H 3471,–CO–O- 1718, Ar–H 3064, C–H (def) 864, C=C(Arom) 1614, –CONH– 1654; ^{1}H-NMR (DMSO-d_6 400MHz): 9.10 (s, 1H, NH), 8.34 (s, 1H, coum. H_4), 7.45 (d, J = 5.2 Hz, 1H, Ar–H), 7.75–7.64 (m, 2H, Ar–H), 7.90 (d, J = 2.4 Hz 1H, Hcoum.), 8.00 (d, J = 2.4 Hz, 1H, Hcoum.);^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 164, 157.7(C_2 = O coum.), 154(C_{11} = O), 138(C_4coum.), 147, 134,128.9, 125.8, 122, 120, 120.9, 120.5, 111.9,; MS (ESI) (m/z): 397 [M]+; Anal. calcd. for $C_{16}H_7O_3NBr_3F$, C 36.92, H 1.35, N 2.69. Found: C 36.95, H 1.39, N 2.64. (see supplementary Fig S9, Fig S10 and Fig S11).

2.2.7. 6,8-Dichloro-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(5)

A light orange powder; Yield 66.16%; mp 170 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (phenyl ring) 742,C–Cl 825, C–F (phenyl ring) 1452, N–H 3383,–CO–O- 1722,Ar–H 3070,C–H (def.)964, C=C(Arom) 1616,–CONH– 1543; ^{1}H-NMR (DMSO-d_6 400MHz): 9.12 (s, 1H, NH), 8.98 (s, 1H, coum. H_4), 7.73–7.78 (m, 3H, Ar–H), 7.50–7.59 (m, 2H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 164, 159.6(C_2 = O coum.), 155(C_{11} = O), 154, 133.8(C_4coum.),133, 132.7, 130.7, 127.5, 125.5, 122, 121, 120.9, 120, 119.7; MS (ESI) (m/z): 430 [M]+; Anal. calcd. for $C_{16}H_7O_3NBrCl_2F$, C 44.65, H 1.63, N 3.25. Found: C 44.72, H 1.69, N 3.27. (see supplementary Fig S12, Fig S13 and Fig S14).

2.2.8. 6-Chloro-8-nitro-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(6)

A intense orange powder; Yield 64.57%; mp 216 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (phenyl ring) 771, C–F (phenyl ring) 1413,C–Cl 839,C–NO_2 1523,N–H 3182,–CO–O- 1718,Ar–H 3086, C–H (def.)891, C=C(Arom) 1595, –CONH– 1718; ^{1}H-NMR (DMSO-d_6 400MHz): 10.74 (s, 1H, NH), 9.01 (s, 1H, coum. H_4), 7.48 (d, J = 8.8 Hz, 1H, Ar–H), 7.60–7.74 (m, 2H, Ar–H), 8.55 (d, J = 9.2 Hz, 1H, Hcoum.), 8.25 (d, J = 8.4 Hz, 1H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 158.9, 154(C_2 = O coum.), 146(C_{11} = O), 144, 136.9, 134(C_4coum.),134.5,130, 127.9,125, 123,121.7, 119.8, 118.9; MS (ESI) (m/z): 441 [M]+; Anal. calcd. for $C_{16}H_7O_5N_2BrClF$, C 43.59, H 1.59, N 6.34. Found: C 43.60, H 1.59, N 6.36. (see supplementary Fig S15, Fig S16, Fig S17 and Fig S18).

2.2.9. 6,8-dinitro- N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(7)

A yellow powder; Yield 62.99%; mp 262 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (phenyl ring) 740,C–F (phenyl ring)1411, C–NO_2 1541,N–H 3253,–CO–O- 1745,Ar–H 3084,C–H (def.)912, C=C(Arom) 1618, –CONH– 168; ^{1}H-NMR (DMSO-d_6 400MHz): 9.10 (s, 1H, NH), 8.83 (s, 1H, coum. H_4), 7.25–7.50 (m, 3H, Ar–H), 8.95 (d, J = 3.2 Hz, 1H, Hcoum.), 8.57 (d, J = 2.4 Hz, 1H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400MHz, δppm): 162, 155.5 (C_2 = Ocoum.), 154, 148(C_{11} = O), 140.5, 139.6(C_4coum.), 133,127.5, 126,124.8, 122, 120.5, 120, 119, 115; MS (ESI) (m/z): 452 [M]+; Anal. calcd. for $C_{16}H_7O_7N_3BrF$, C 42.48, H 1.55, N 9.29. Found: C 42.52, H 1.60, N 9.42. (see supplementary Fig S19 and Fig S20).

2.2.10. 6-Bromo-8-nitro-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(8)

A yellowish orange powder; Yield 58.80%; mp 280 °C; IR (KBr) υ_{max} cm^{-1}: C–Br (phenyl ring) 740, C–F (phenyl ring) 1411, C–NO_2 1529, N–H 3338, –CO–O- 1739, Ar–H 3074, C–H (def.)877, C=C(Arom.) 1614, –CONH– 1681; ^{1}H-NMR (DMSO-d_6 400MHz): 9.08 (s, 1H, NH), 8.81 (s, 1H, coum. H_4), 7.52–7.33 (m, 3H, Ar–H), 8.17 (d, J = 2.4 Hz, 1H, Hcoum.), 7.89 (d, J = 2.6 Hz, 1H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 168, 165(C_{11} = O), 156.6 (C_2 = O coum.), 148, 145.4, 139.6(C_4coum.), 136.4, 128.7,128.6, 126.9,125.8, 121.6, 120.8, 120, 119.7, 114.8; MS (ESI) (m/z): 486 [M]+; Anal. calcd. for $C_{16}H_7O_5N_2Br_2F$, C 39.51, H 1.44, N 5.76. Found: C 39.60 H 1.48 N 5.80. (see supplementary Fig S21 and Fig S22).

2.2.11. 6,8-Diiodo-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(9)

A yellow powder; Yield 46.01%; mp 256 °C; IR (KBr) υ_{max} cm^{-1}: C–Br(Phenyl ring) 740,C–F(Phenyl ring) 1400, C–I680, N–H 3280,–CO–O- 1728, Ar–H 3045, C–H (def.)875, C=C(Arom) 1602, –CONH– 1658; ^{1}H-NMR (DMSO-d_6 400MHz): 9.11 (s, 1H, NH), 8.78 (s, 1H, coum. H_4), 7.85–7.39 (m, 3H, Ar–H), 7.70 (d, J = 2.5 Hz, 1H, Hcoum.), 7.21 (d, J = 2.5 Hz, 1H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 166, 164(C_{11} = O), 161(C_2 = Ocoum.),145.9, 145.7, 139.4(C_4coum.), 134.4, 127.6, 125.4, 126.4, 121, 120.9, 119.5, 114.6, 96.4, 94.4; MS (ESI) (m/z): 614 [M]+; Anal. calcd. for $C_{16}H_7O_3NBrFI_2$, C 31.27, H 1.14, N 2.28. Found: C 31.40, H 1.20, N 2.28. (see supplementary Fig S23 and Fig S24).

2.2.12. 8-Nitro-N-{4-bromo-2-fluorophenyl}-2-oxo-2H-chromene-3-carboxamide(10)

A yellow powder; Yield 70.45%; mp 203 °C; IR (KBr) υ_{max} cm^{-1}: C–Br(Phenyl ring) 754,C–F(Phenyl ring)1413,C–NO_2 1523,N–H 3223,–CO–O- 1718,Ar–H 3086,C–H (def.)914, C=C(Arom.) 1618, –CONH– 1672; ^{1}H-NMR (DMSO-d_6 400MHz): 9.12 (s, 1H, NH), 8.58 (s, 1H, coum. H_4), 7.35–7.52 (m, 3H, Ar–H), 7.63–8.56 (m, 3H, Hcoum.); ^{13}C-NMR (DMSO-d_6 400 MHz, δppm): 166.4, 163.4(C_{11} = O), 159(C_2 = Ocoum.),144.9, 143, 138.4(C_4coum.),133.7, 127, 125, 124.5, 126.8, 121.7,121, 120.6, 118.7, 114.5; MS (ESI) (m/z): 407 [M]+; Anal. calcd. for $C_{16}H_8O_5N_2BrF$, C 47.17, H 1.96, N 6.88. Found: C 47.30, H 2.01, N 6.90. (see supplementary Fig S25 and Fig S26).

2.3. Antimicrobial activity

Bacterial species *Escherichia coli* (Gram –ve) and *Bacillus cereus* (Gram +ve) were grown on the nutrient agar medium in the laboratory. Fungal species *Aspergillus niger, Aspergillus flavus, Aspergillus fumigates, Penniciillium, Candida albicans, Rhizopus, Mucor* were grown in the laboratory on sabouraud dextrose agar (SDA) media.

Antibacterial activity was investigated using agar well diffusion method. The activity of synthesized coumarin compounds (1–10) was studied against *E.coli* and *Bacillus cereus*. The solution of 1 mg/mL concentration of each coumarin compound (1–10) and the reference drug was prepared in DMSO. Five different concentrations of compounds viz., 1000 μg/mL, 750 μg/mL, 500 μg/mL, 250 μg/mL and 125 μg/mL were prepared. Bacteria from a 24 h culture containing approximately 104–106 CFU/mL were spread on the surface of nutrient agar (tryptone 1%, yeast extract 0.5%, sodium chloride 0.5%, agar 1%, 1000 mL of distilled water, pH 7.0) which was autoclaved under 121 °C for at least 20 min. Wells was created in agar medium with the help of sterile

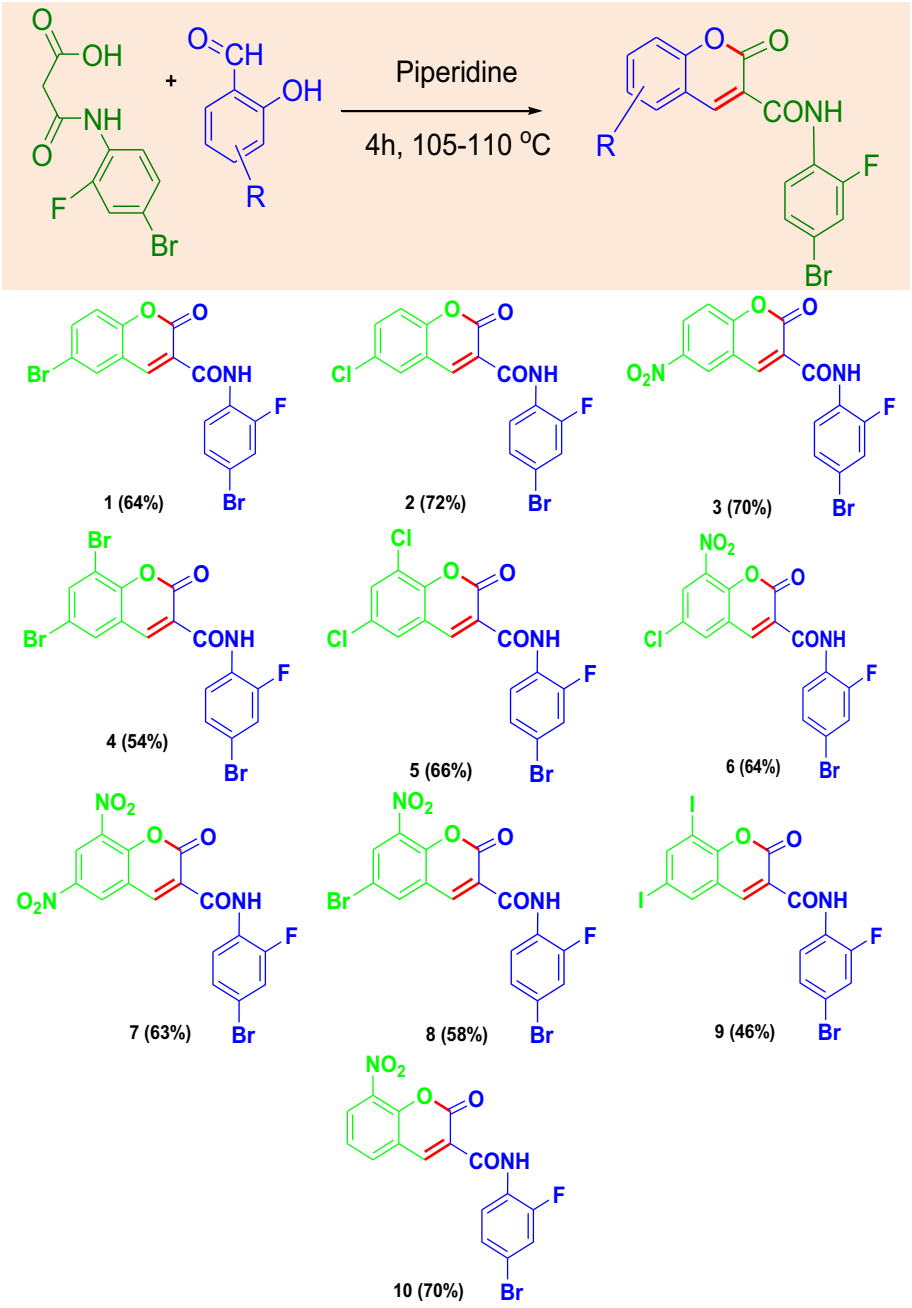

Fig. 1. Reaction scheme for the synthesis of coumarin compounds and their structure and yield % (where, R=(1); 6-Br, (2); 6-Cl, (3); 6-NO_2, (4); 6,8-dibromo, (5); 6,8-dichloro, (6); 6-Cl, 8-NO_2, (7); 6,8-NO_2, (8); 6-Br, 8-NO_2, (9); 6,8-diiodo, (10); 8-NO_2).

metallic bores and then cooled down to 45 °C. The activity was determined on the basis of the inhibition zone (in mm). The wells were loaded with each concentration of a compound of the plates. All compounds were dissolved in DMSO. DMSO was used as a negative control. Ciprofloxacin (1000 μg/mL) was used as a reference. The plates were kept in an incubator at 37 °C overnight and then examined for inhibition zone at these different concentrations of compounds. The experiment was performed in triplicate and the inhibition zone was measured by caliper to get an average value. Minimum Inhibitory Concentration (MIC) was determined for each bacterial strain.

Synthesized coumarin compounds (1–10) were screened separately for their antifungal activity against various fungal strains. The antifungal activity was performed by agar well diffusion method as per following procedure, a homogeneous mixture of glucose-peptone-agar (40:10:15) was sterilized by autoclaving at 121 °C and 15 lb/cm^2 for 20 min. The sterilized solution (25 mL) was poured in each sterilized Petri dish in laminar flow and left for 20 min for solidification of SDA plate. These plates were kept inverted at 30 °C in an incubator.

Fungal strains were grown in 5 mL SD broth (glucose: peptone, 40:10) for 3–4 days to achieve 105 CFU/mL cells. The fungal culture was spread out uniformly on SDA plates by a sterilized triangular folded glass rod. Plates were left for 5–10 min so that culture is properly adsorbed on the surface of SDA plates. Small wells of size (4 mm) were cut into the plates with the help of sterile metallic bores. Five different concentrations of compounds viz., 1000 μg/mL, 750 μg/mL, 500 μg/mL, 250 μg/mL and 125 μg/mL were prepared. Wells were loaded with each concentration of synthesized coumarin compounds on the plate. All the compounds were prepared in DMSO and DMSO was loaded as a negative control.

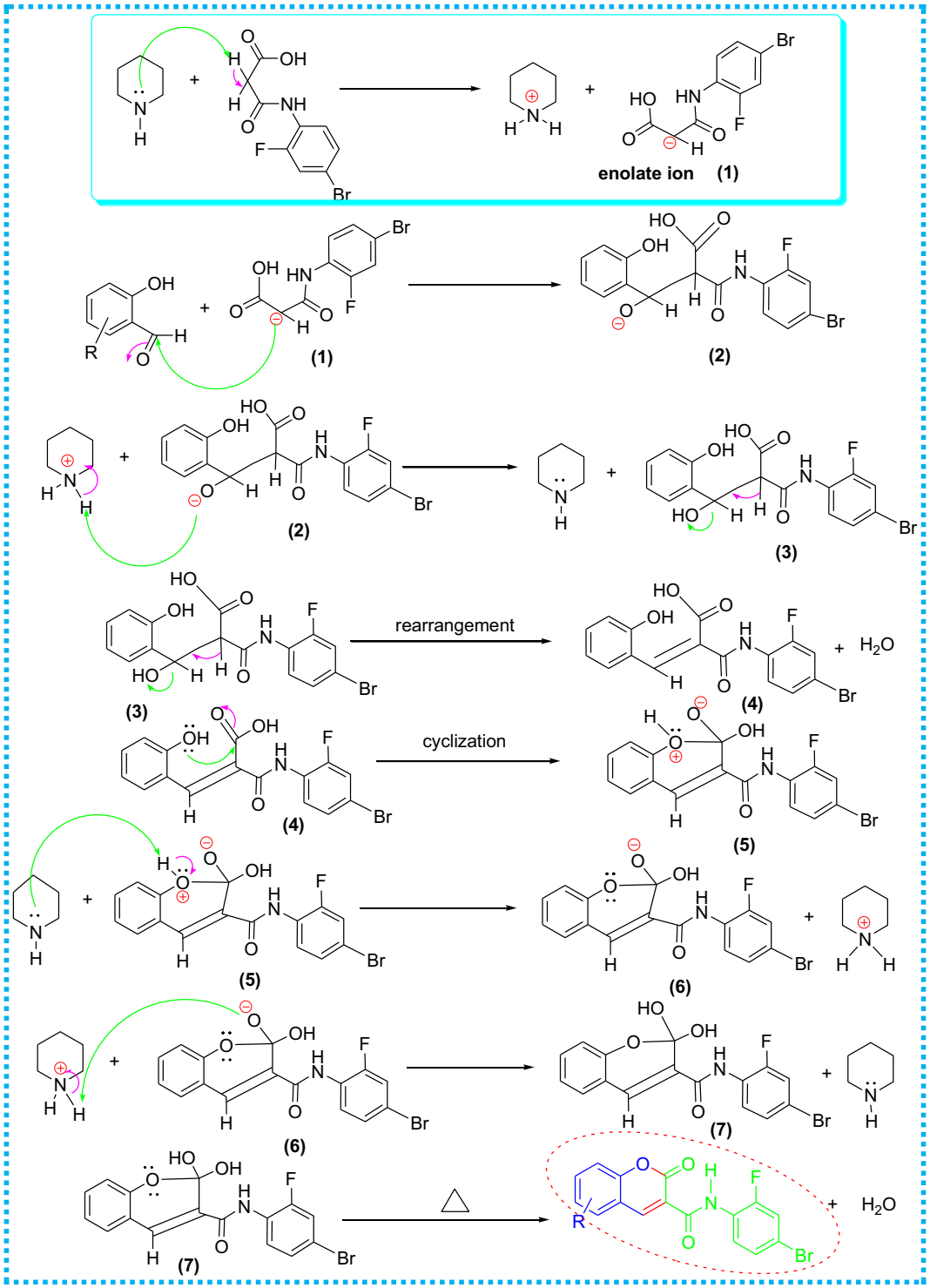

Fig. 2. Mechanistic approach for synthesized compounds (1–10).

Fluconazole (1000 μg/mL) was used as a reference. The plates were kept for incubation at 30 °C overnight and after incubation inhibition zone was measured. All measurements were made in triplicate. MIC values were determined for each fungal strain.

2.4. Molecular docking

Here, the ligands were synthesized coumarin compounds (1–10) whereas enzymes used were Glc N-6-P synthase, protein database ID (PDB ID: 2vf5) and lanosterol 14α-demethylase (PDB ID: 3jus and 2juv). The atomic coordinates of both enzymes were taken from the Protein Data Bank (PDB) (www.rcsb.org). Graphical user interface program AutoDock Tool (ADT) was used to prepare pdbqt files for CAII for ligands and grid box creation. Auto Dock 4.2 was used to dock the compounds with CAII using the Lamarckian genetic algorithm (LGA) to deal with protein-ligand interactions. Both protein and ligands were considered as rigid during docking. The most favourable free energy of binding and docking orientation lying within the range less than 1.0 Å in root-mean-square deviation (RMSD) were clustered together and ranked accordingly. The binding affinity was extracted and aligned with the receptor

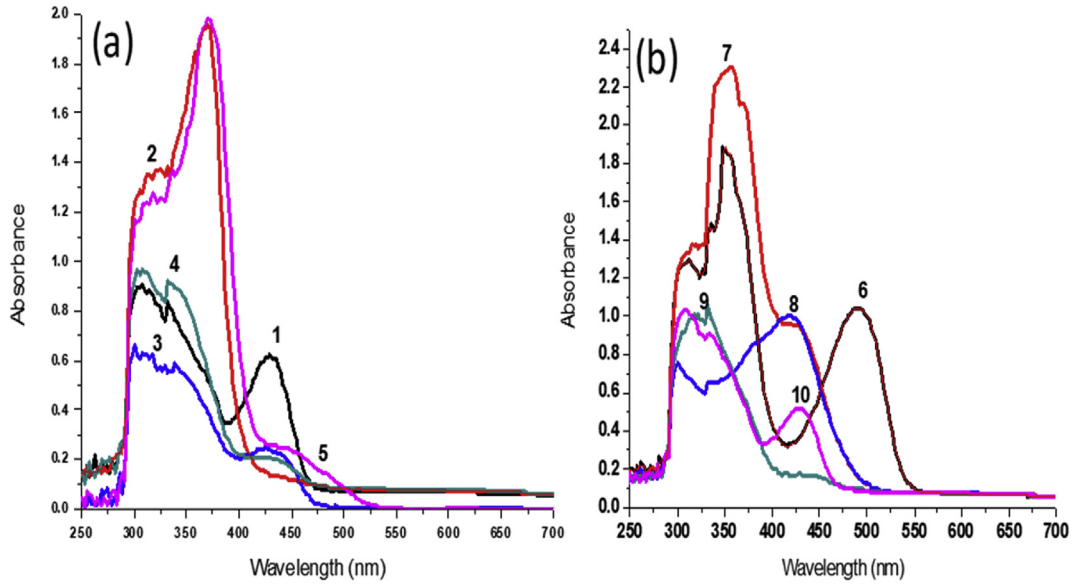

Fig. 3. UV-visible spectrum of (a) compounds (1–5) and (b) compounds (6–10).

Table 1
Electronic absorption (UV λ_{max}), emission wavelength (Emλ_{max}), molar absorptivity coefficient (ε) and Stoke's shift of coumarin compounds **(1–10)**.

Compound	λ_{max} (nm)	Molar Absorptivity Coefficient (ε) $Lmol^{-1}cm^{-1}$	Emission Wavelength (nm)	Stoke's Shift (nm)
1	438	107.14	475	160
2	375	317.46	425	110
3	425	48.18	525	210
4	430	41.66	575	260
5	380	344.82	560	245
6	495	196.42	570	255
7	355	418.18	525	210
8	425	196.07	525	210
9	420	48.78	415, 500	100,185
10	432	81.96	525	210

structure for further analysis. The molecular interaction between protein atoms and compounds were visualized by PyMOL.

3. Results and discussion

3.1. Chemistry

Here, the target compounds were synthesized by condensation of substituted salicylaldehyde with N-(substituted)phenyl malonic acid via Knoevenagel condensation reaction (KCR) [23], with a catalytic amount of piperidine. KCR method explained the condensation between aldehyde and malonic compound containing an active methylene group. The reaction involved the formation of stable enolate ion which is cleanly and catalytically formed by the reaction of β-dicarbonyl C–H acid compound with a weak base. In this reaction, carbanion attack to the slightly positive carbon on the salicylaldehyde, and the carbonyl oxygen recaptures the original proton which was extracted in the formation of enolate. The

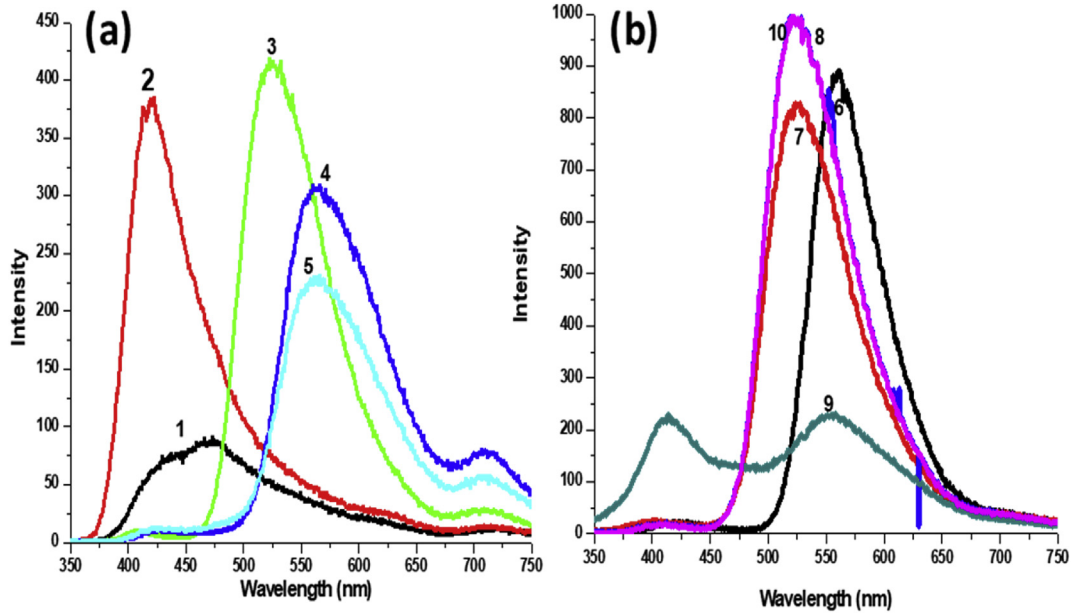

Fig. 4. Fluorescence spectrum of (a) compounds (1–5) and (b) compounds (6–10).

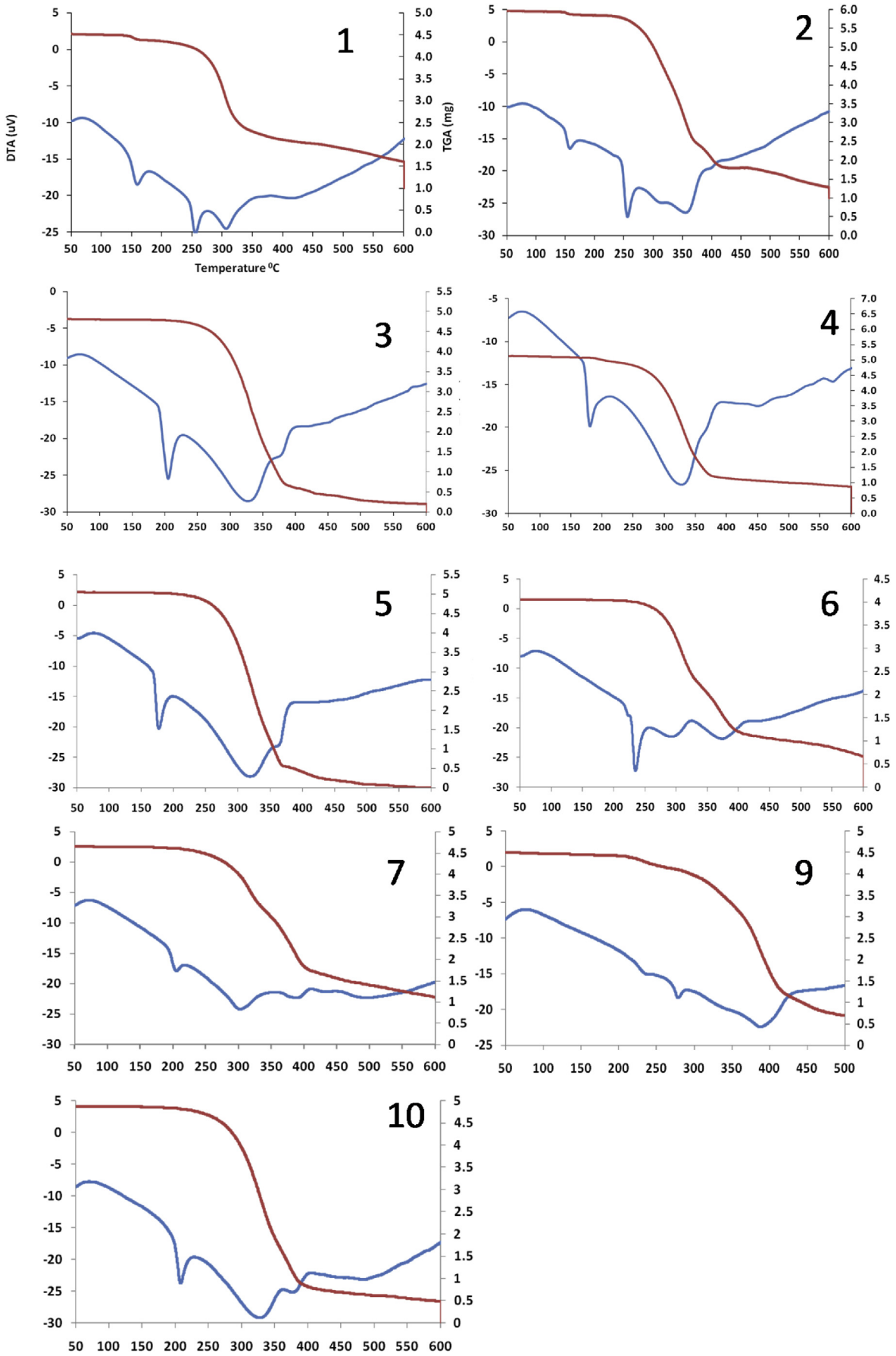

Fig. 5. Thermogravimetric analysis of synthesized Coumarin compounds1-7 and 9-10.

Table 2
In vitro antimicrobial activity of target coumarin compounds **(1–10)**.

Test strains	(Coumarin compounds) Minimum Inhibitory Concentration (MIC) (μg/mL)									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
A. niger	500	500	500	1000	500	1000	750	750	1000	1000
A. Fumigatus	>1000	>1000	750	125	125	250	750	>1000	>1000	1000
A. flavus	500	1000	250	<125	125	500	250	750	750	250
Rhizopus	1000	1000	500	125	<125	1000	750	1000	750	500
Mucor	1000	1000	250	250	1000	250	500	1000	1000	250
Pennicillium	500	750	500	750	750	500	750	>1000	500	500
Candida albicans	>1000	250	1000	1000	750	1000	1000	750	750	750
Bacillus cereus	>1000	>1000	500	250	250	250	500	750	750	250
E. coli	750	1000	250	250	250	250	1000	750	750	250

lone pair on the o-hydroxyl group of salicylaldehyde removes the hydrogen from the hydroxyl group of acid and forms an intermediate anion cyclizing ring followed by elimination of water molecule from intermediate during heating and coumarin ring was formed. All reactions were monitored by TLC and the melting point was reported as observed. The % yields of all compounds were above 60% except compounds 4, 6 and 9. The reaction scheme, structures and mechanism of the coumarin compounds (1–10) are shown in Figs. 1 and 2.

3.2. Characterization

UV-visible spectra of the compounds (1–10) are shown in Fig. 3. Compounds showed strong absorption in the range 280–550 nm. λ_{max} value and molar absorptivity coefficient(ε) for all the compounds has been discussed in Table 1. Absorption at wavelength 315 nm is almost common in all the compounds and absorption at another wavelength differs from compound to compound depending upon the substituent moieties. Molar absorptivity coefficient(ε) values at variable λ_{max} showed that compounds 2, 5 and 7 have a maximum intensity of absorption.

The compounds (1–10) showed characteristic absorption due to coumarin chrome tautomerism which significantly influences the spectra. All 10 compounds have coumarin moiety with bromo-fluoro-substituted phenyl ring at C3 position joined by –CONH– group. Hence, absorption by the coumarin ring as well as absorption maximum by the phenyl substituent at a longer wavelength was observed [24]. New derivatives of coumarin showed absorption in the broad region of 280–550 nm depending upon the properties of substituents and the common chromophores. These coumarin derivatives with a wide range of UV spectrum act as a potential candidate for UV absorbers.

The fluorescence excitation wavelengths of all the synthesized compounds (1–10) are listed in Table 1. The introduction of a strong electron-donating group (dibromo, dichloro and chloro nitro) at C6/C8 positions in compounds 4, 5 and 6, respectively with characteristic orange color have provided them a significant increase in fluorescence intensity (λ_{em} = 575, 560 and 570 nm). Compound 9 with di-substitution of iodine groups at C6/C8 positions shows two emission wavelengths at 415 and 500 nm. The compound 3, 7, 8 and 10 show maximum emission wavelength at 525 nm as shown in Fig. 4.

FTIR spectra of all compounds (1–10) show a strong absorption band around 3084-3471 cm^{-1} assigned to the N–H stretching, while a strong absorption at 1543-1718 cm^{-1} supported the presence of –CONH– amide stretching shows FTIR of coumarin derivatives (1–10). In addition, the stretching vibration was observed at 1714–1745 cm^{-1}due to a carbonyl group (C=O) of coumarin. Stretching vibrations due to C=C (aromatic) and C–NO_2 were observed at 1618-1595 cm^{-1} and 1573-1523 cm^{-1}, respectively. A band at 1479-1400 cm^{-1} appeared due to C–F stretch. Stretching vibrations due to C–Br (chromone ring) and C–Br (phenyl ring) were observed at 864-790 cm^{-1} and 758-740 cm^{-1}, respectively. Absorption bands at 839-815 $cm^{-1}$and 680 cm^{-1}indicated the presence of C–Cl stretch and C–I stretch, respectively.

^{1}H-NMR spectra were recorded by using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed as δ unit (ppm). ^{1}H-NMR of these derivatives revealed the presence of singlet at 9.19–8.99 ppm for –NH–. The derivatives of coumarin ring proton (Ccoum.H4) were observed as singlet 8.98–8.20 ppm. All the other aromatics protons were observed within the expected regions. ^{13}C-NMR of the compounds (1–10) was in concordance with the proposed structure of substituted coumarin derivatives. A chemical shift at 153 ppm was attributed to the carbonyl carbon (C=O coum.) of coumarin ring in addition to the new signal at 159 ppm relative to the amide carbon (–CONH–) which was introduced at the third position of coumarin ring. The two new signals at 115 and 136 ppm show carbon of coumarin (C3 coum. and C4 coum.), respectively.

3.3. Thermal analysis

Thermal analysis of coumarin compounds (1–7) and (9–10) was performed to study the change in their physical properties with respect to temperature. Thermal analysis of Sample 8 was not done since there was shortage of sample. TGA thermograms of compounds showedthe loss in weight of compounds between 150–500 °C. A maximum weight loss is observed in compounds between 260–410 °C. It is to be noted that the compounds showed a slight change in the % weight upto 250 °C. The % weight loss for compound 1 and 2 are 66% and 70%,respectively at 600 °C whereas the maximum % weight loss is observed in compound 3 (96%) and 5 (100%) at 600 °C. In rest of compounds, the weight loss is between 70–80% at 600 °C. DTA thermogram show similar pattern of three-step degradation of compounds, the degradation at 150–210 °C is due to the loss of residual solvent. In second step degradation of compounds (1–7) and (9–10), an endothermic dip is observed between 250-330 °C which may be due to loss of phenyl ring attached to coumarin ring. In third step degradation of compounds (1–7) and (9–10), endothermic dip is observed at 400–410 °C due to decomposition of coumarin ring. The figure for thermal analysis of compounds is given in (Fig. 5).

3.4. Antimicrobial evaluation

3.4.1. Antibacterial evaluation

All the synthesized coumarin compounds (1–10) were screened for their *in vitro* antibacterial activity against the two bacterial strains, *E. coli* (Gram –ve) and *B. cereus* (Gram + ve) by agar well diffusion method using DMSO as negative control and ciprofloxacin was used as reference against both bacterial strains [25, 26]. Table 2 represents the values of MIC in μg/mL for synthesized coumarin compounds. The result revealed that compounds (3–10) showed good MIC values against *E. coli* and *B. cereus*. The synthesized compounds (1–10) possess lower antibacterial activity against *E. coli* (Gram–ve) bacteria, while compounds 1 and 2 showed no inhibition at 1000 μg/mL concentration against *B. cereus*

Table 3
In vitro antimicrobial activity of tested compounds in term of the zone of inhibition diameter (mm), Fluconazole and Ciprofloxacin are used as reference drugs.

Compound number	Test strains								
	Fungal strains							Bacterial strain	
	A. niger	*A. fumigatus*	*A. flavus*	*Rhizopus*	*Mucor*	*Pennicillium*	*Candida albicans*	*E. coli*	*Bacillus cereus*
1	11	0	12	4	6	12	4	8	0
2	11	0	4	6	6	8	14	7	0
3	10	6	14	10	14	13	6	12	10
4	8	16	27	18	14	8	6	12	12
5	11	18	20	27	6	10	8	15	16
6	6	14	12	6	14	10	6	10	12
7	8	7	14	10	12	8	6	6	10
8	8	0	8	6	4	0	8	8	8
9	4	0	7	8	7	10	8	8	8
10	6	2	16	10	16	8	8	11	12
DMSO	0	0	0	0	0	0	0	0	0
Fluconazole	0	0	0	6	0	0	0	- - - - -	- - - -
Ciprofloxacin	- - - - -	- - - -	- - - - -	- - - -	- - - - -	- - - -	- - - - -	32	32

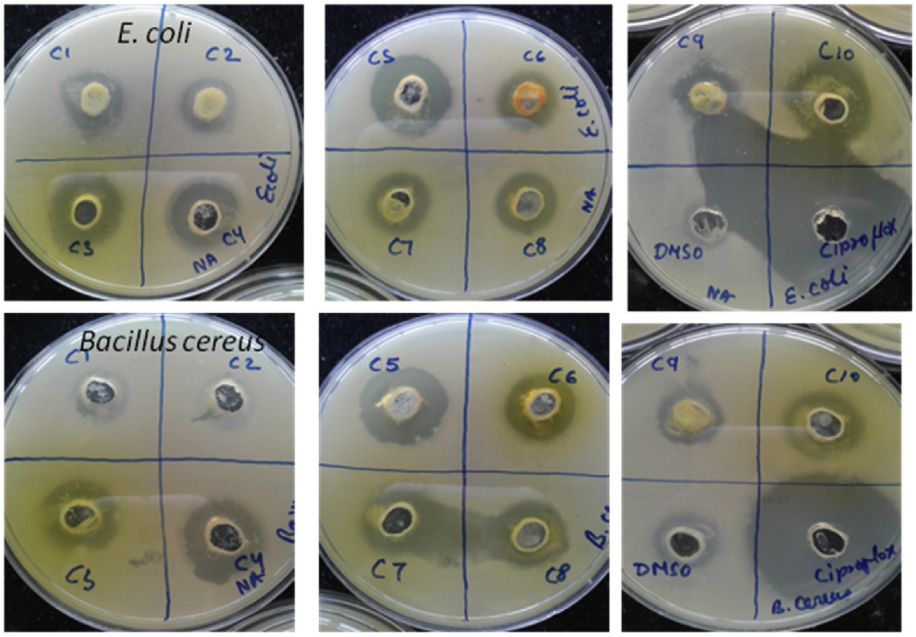

Fig. 6. Antibacterial activity of synthesized coumarin compounds (1–10).

(Gram + ve) bacteria. The five compounds (3–6 and 10) showed appreciable *in vitro* antibacterial activity against both bacterial strains at 1000 μg/mL concentration with a zone of inhibition of 10–16 mm (as shown in Table 3). It is to be noted that compounds 1 and 2 with mono substitution of bromo and chloro group at C6 position of chromene ring did not show any growth inhibition of Gram + ve bacteria but with di-substitution of bromo and chloro groups as in compounds 4 and 5 showed good growth inhibition of *B. cereus*. Furthermore, the response of compound 5 with dichloro substitution showed maximum growth inhibition in comparison to the rest of the compounds against both bacterial strains. The synthesized coumarin compounds (3–6 and 10) showed broad-spectrum antibacterial potency because of the presence of bromo, chloro or nitro group substituent at C6 and C8 position of chromene ring. The figure showing the antibacterial activity of compounds is given in (Fig. 6).

3.4.2. Antifungal activity

The promising broad-spectrum antibacterial activity of synthesized coumarin compounds has encouraged authors to test further for antifungal activity. Synthesized compounds (1–10) were screened for their antifungal activity against 7 fungal strains viz., *A. niger, A. fumigatus, A. flavus, Rhizopus, Mucor, Penicillium,* and *Candida albicans* by agar well diffusion method. Fluconazole was used as the reference against all fungal strains and DMSO was used as a negative control [27, 28]. The result of the antifungal activity of tested compounds (1–10) in term of MIC is shown in Table 2. Most of the coumarin compounds possess a moderate to good activity against the fungal strains (as shown in Table 3). Compounds 1, 2, 8 and 9 showed no antifungal activity upto 1000 μg/mL concentrations against *A. fumigatus*. Compound 1 revealed moderate antifungal activity (MIC 500 μg/mL) against *A. niger, A. flavus,* and *Penicillium*. Compound 2 also showed moderate activity against *A. niger* and *Candida albicans,* MIC 500 and 250 μg/mL, respectively. Compound 3 showed moderate antifungal activity against *A. niger, A. flavus, Rhizopus, Mucor,* and *Penicillium.* Compound 6 showed lower MIC value against *A. fumigatus, A. flavus, Mucor* and *Penicillium*. Similarly, compound 7 revealed a moderate antifungal activity against *A. flavus, Rhizopus* and *Mucor.* Results indicated that compounds 3–7 showed good activity against all the fungal strains studied. However, compounds 4 and 5 showed excellent MIC values i.e. 125 μg/mLor<125 μg/mL against *A. fumigates, A. flavus,* and *Rhizopus*. These two compounds contain chromene ring with disubstituted bromo and chloro moiety at C6 and C8, respectively. The inhibition of fungal strains viz., *A. flavus, Rhizopus,*

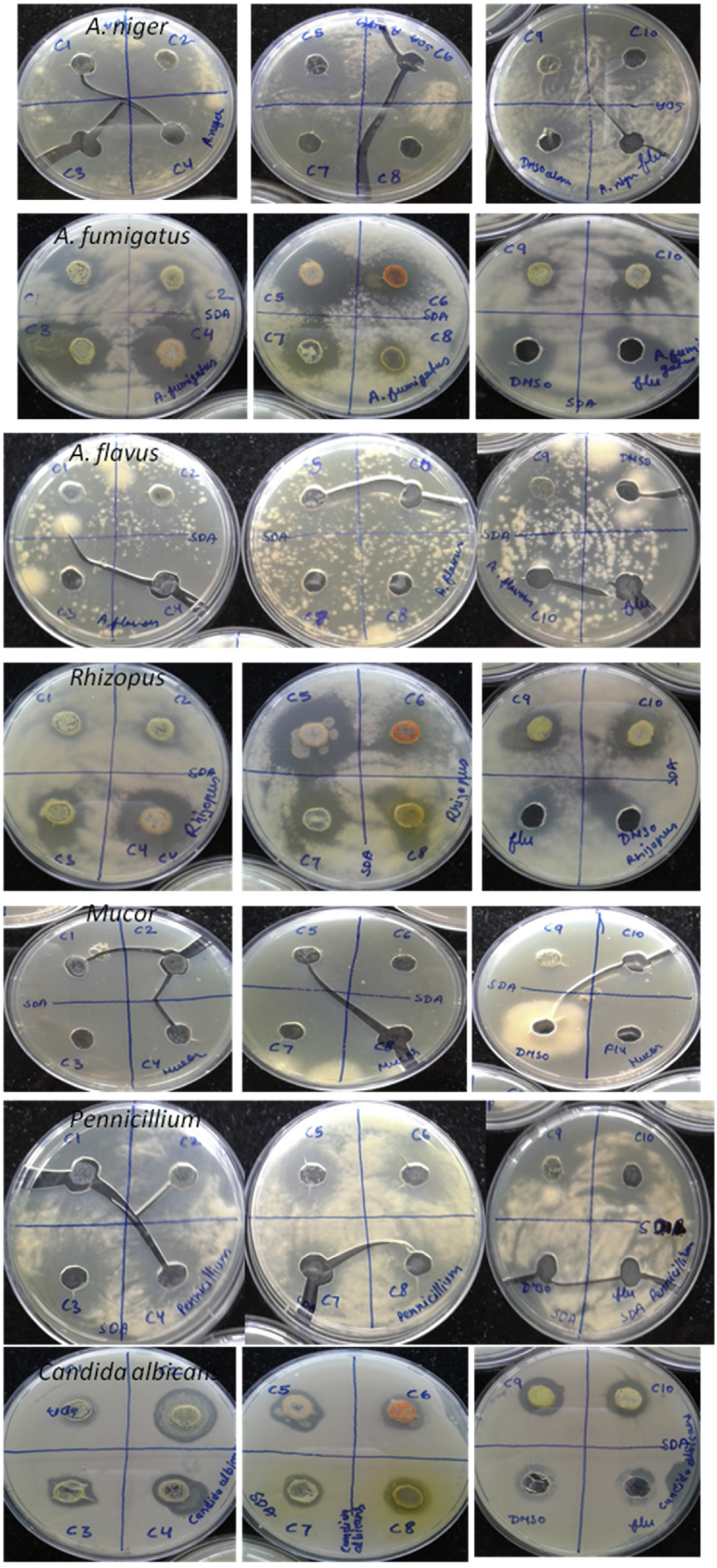

Fig. 7. Antifungal activity of synthesized coumarin compounds (1–10).

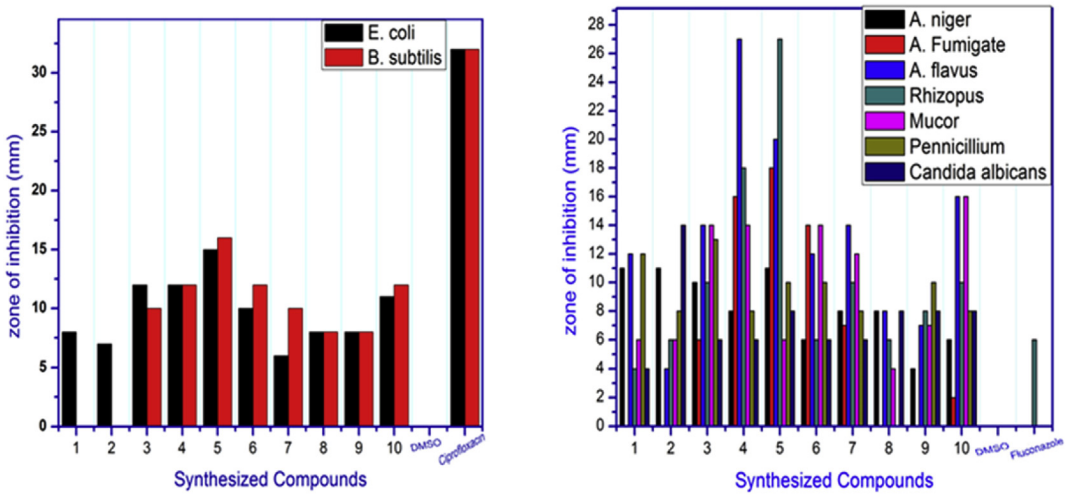

Fig. 8. *In vitro* antimicrobial activity of target coumarin compounds (1–10).

Table 4
Docking study data of compounds **(1–10)** showing the binding energy between ligands and enzymes.

Compound name	2vf5 (GlcN-6-P synthase) Δ*G* (kcal/mol)	3jus (lanosterol 14α-demethylase (CYP51) Δ*G* (kcal/mol)	3juv (lanosterol 14α-demethylase (CYP51) Δ*G* (kcal/mol)
1	-42.26	-15.67	-23.42
2	-26.13	-14.38	-14.53
3	-25.53	-18.22	-13.35
4	-39.86	-20.05	-16.26
5	-27.63	-12.53	-12.82
6	-26.79	-13.13	-13.33
7	-24.50	-12.79	-13.88
8	-40.08	-16.25	-14.62
9	-11.09	-11.04	-10.69
10	-26.40	-15.05	-11.46

Mucor, A. niger and Candida albicans by all synthesized coumarin derivatives (1–10) have proposed them as potential candidate for antifungal activity (Fig. 7). Antimicrobial activity of target coumarin compounds as shown in (Fig. 8).

3.5. Molecular docking

For molecular docking study, the enzyme GlcN-6-P synthase was selected as a potential target for *in silico* analysis since it is essentially required for bacterial growth [25]. GlcN-6-P synthase enzyme is responsible for the biosynthesis of N-acetylglucosamine (NAG),glycoproteins and mucopolysaccharides in bacteria, which are necessary for the bacterial growth and viability [28,29]. On the other hand, the enzyme lanosterol 14α-demethylase was selected as a target molecule for determination of antifungal property. Lanosterol 14α-demethylase (CYP51) belongs to cytochrome P450 superfamily and essential for fungal viability [30]. This enzyme is a specific target to design effective antifungal agent as it catalyzes a key step in ergosterol biosynthesis in fungi in which methyl group is removed from the C14 position in the sterol molecule [31, 32]. Ergosterol is the key component of the fungal cell wall. Docking analysis showed that the synthesized coumarin compounds have good binding affinities with both target molecules. Docking results showed the compounds have a better affinity towards GlcN-6-P synthase in comparison to lanosterol 14α-demethylase. The binding affinities of all these compounds (1–10) with the proposed targets are listed in Table 4.

4. Conclusions

The ten new coumarin derivatives were synthesized by a facile and efficient method with a fairly good yield. The hybrid framework of the compounds bears a coumarin ring with amide moiety at C3 position and substitution at C6/C8 positions. The compounds showed good thermal stability upto 250 °C. The compounds also showed a broad absorption spectrum between 280-550 nm establishing them to be good UV absorbers. The newly designed and synthesized compounds were showing moderate to good antibacterial and antifungal activities. Five compounds 3, 5, 6, 9 and 10 have shown broad-spectrum antimicrobial activity. The *in silico* study has given an insight of binding efficiency of compound 1–10 with target enzymes (GlcN-6-P synthase and lanosterol 14α-demethylase) which support the *in-vitro* antimicrobial study. The study is significant for establishing new coumarin derivatives structurally and proposing them a potential broad-spectrum antimicrobial agent in the field of pharmaceutical sciences.

Declarations

Author contribution statement

Mohd Shahnawaz Khan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Ranu Agrawal: Performed the experiments; Wrote the paper.

Mohammad Ubaidullah: Performed the experiments; Analyzed and interpreted the data.

Md. Imtaiyaz Hassan: Performed the experiments.

Nazia Tarannum: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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