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Synthesis, characterization, molecular docking and evaluation of antimicrobial activity of some 3-heteroaryl substituted chromen-2-one derivatives

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Abstract: Chromen-2-ones commonly called coumarins are important synthetic and phytogenic bioactive molecules. Owing to their diverse biological activities, synthesis of substituted coumarin derivatives has been tried as antimicrobial agent in current investigation. Series of 1-(substituted phenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea derivatives (CTU1-12) and 3-(5-substituted phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one derivatives (COD1-9) were synthesized. Docking studies were carried out with Autodock vina, using MGL Tools 1.5.6. Docking studies in Autodock vina suggested hydrogen bond interaction between carbonyl group of coumarin ring or carbonyl group of urea substituent with key residue Asn46 for compounds from CTU series and hydrogen bond interaction between nitrogen of oxadiazole ring and Asn46. Compounds CTU1, CTU4, CTU7 and CTU10 showed moderate antibacterial activity against gram positive organisms.
Keywords: Coumarin, 1,3-thiazole, 1,3,4-oxadiazole, Autodock vina, Antimicrobial.

Introduction

Chromen-2-ones commonly called as coumarins are widely found phyto constituents. Its presence in some plants confer them defence against pathogens, insects, pests and herbivores[1]. Coumarins possess derivatives diverse pharmacological activities including antitumor [2, 3], antivascular [4], antimicrobial[5], antioxidant [6], TNF-α inhibitor[7], antifungal[8], anticoagulant[9], estrogenic[10], antiviral[11], anthelmintic[12], anti-HIV[13], antitubercular[14], anti-inflammatory[15], herbicidal[16], analgesic[17] and anticonvulsant[18, 19] activity. Anticoagulant coumarin derivatives like dicoumarol, warfarin, phenoprocoumon and acenocoumarol, photosensitizing agents used in psoriasis and vitiligo like methoxsalen, trioxsalen, antibacterial agents like novobiocin and clorobiocin and antioxidant and antiproliferative agent ellagic acid have been approved as a drugs [20]. Substituted 1, 3-thiazole derivatives[21, 22] and 1, 3, 4- oxadiazole[23, 24] derivatives have been reported as antimicrobial agents. Microbial resistance has posed a series threat to mankind as many new strains of bacteria have been reported which are resistant to currently available antibacterials and antibiotics [25]. In view of this, 1, 3- thiazole substituted and 1, 3, 4- oxadiazole substituted coumarin derivatives were synthesised and characterized by FT-IR, ^{1}H-NMR and mass spectrometry. The docking studies, evaluation of antimicrobial activity against two gram-positive organisms *viz*. *Bacillus subtilis* and *Staphylococcus aureus* and two gram-negative organisms *viz*. *Escherichia coli* and *Pseudomonas aeruginosa* of the synthesised compounds are reported in this paper.

Materials and Methods

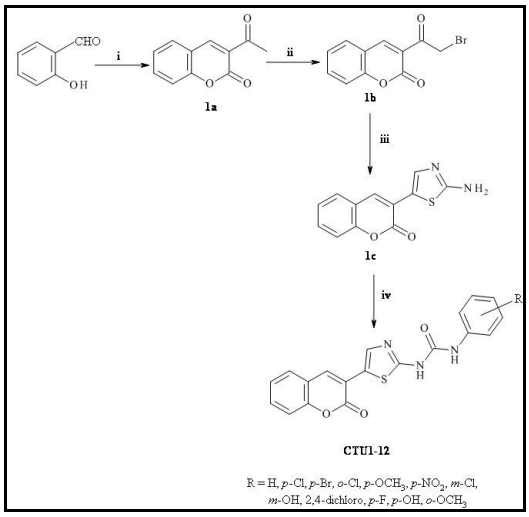
Docking studies

In the present investigation, the X-ray crystal structure of the antimicrobial agent Clorobiocin bound to topoisomerase II DNA gyrase was obtained from the RCSB Protein Data Bank (PDB ID: 1KZN). The protein with resolution 2.30 A^0 with 205 amino acid residues was processed by removing water and clorobiocin.

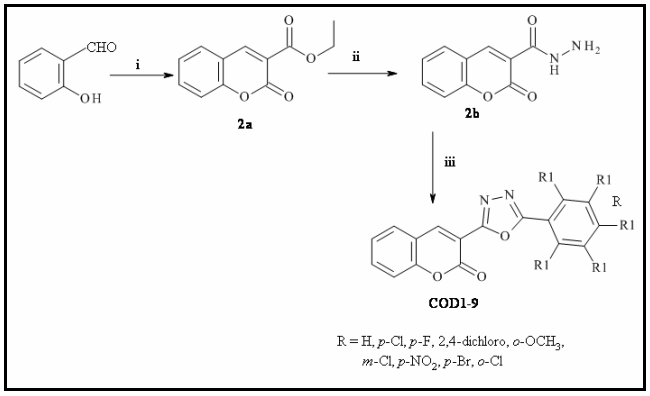
Energy minimization of this clean protein was carried out UCSF Chimera [26] with Amber ff12SB force field and combination of 10,000 steepest descent and conjugate gradient steps with 0.02 A^0 step size. 2D and 3D structures of all the synthesized compounds were drawn in Marvin Sketch (a structure drawing program). Geometry optimization of these 3D structures was carried out in ArgusLab 4.0.1 (from Thomson and Planaria Software LLC) on semi empirical quantum mechanical basis with parameterized model number 3 (PM3) hamiltonian, until restricted closed shell hartree-fock self consistent field formalism converses to 10^{-10} kcal/mol and steepest descent geometry search criteria until gradient converses to 10^{-6} kcal/mol. Gasteiger partial atomic charges for optimized molecules were computed in UCSF chimera. The energy minimized protein and geometry optimized structures of compounds were pre processed in MGLtools1.5.4 [27]. Docking simulation was carried out in Autodock Vina [28] using the grid box of size 18 x 18 x 18 with 1 A^0 spacing was defined along x, y and z axis. The analysis of binding free energy and interactions of ligands with residues at active site was carried out by using Pymol and Discovery studio 3.5.

Chemistry

The reagents and solvents used during synthesis were of laboratory grade obtained from Thomas Baker and Loba Chemie. The melting point of the compound was determined by open capillary method, expressed in°C. The reactions were monitored preparative TLC from Merck with the solvent system Chloroform: methanol in the ratio of 9:1. IR spectra were recorded on Shimadzu FT-IRAffinity-1 spectrophotometer by KBr pellet technique and are expressed in cm^{-1}. ^{1}H-NMR spectra was recorded on Bruker Avance 400 MHz FT-NMR spectrometer using $CDCl_3$ or DMSO as solvent and TMS as internal standard (δ ppm). The chemical shifts are expressed in δ ppm and splitting patterns are designated as s: singlet; d: doublet; q: quartet; m: multiplet. Mass spectra were recorded using Waters Quatropole-TOF Micro Mass (Electro spray ionization) Spectrometer. The strategy adopted in the synthesis of compounds is shown in **Scheme 1** and **Scheme 2**.



Scheme 1: Synthesis of 1-(substituted phenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea derivatives; (i) Ethylacetoacetate, piperidine, RT; (ii) Bromine, dioxane; (iii) Thiourea, reflux; (iv) Ar-COOH, Phenyl chloroformate, Dimethoxyethane, Sodium azide, 70 ^{0}C



Scheme 2: Synthesis of 3-(5-substituted phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one;
(i) Diethylmalonate, piperidine, glacial acetic acid; (ii) Hydrazine hydrate (99%), reflux; (iii) Ar-COOH, $POCl_3$

Synthesis of 3-acetylcoumarin (1a)

Piperidine (0.1 ml) was added dropwise with stirring to the mixture of salicylaldehyde (18 mmol) and ethylacetoacetate (24 mmol) in 1 ml ethanol. The solid obtained was recrystallized from ethanol to yield 72 % pale yellow solid of 1a; m.p. 118-119 ^{0}C.

Synthesis of 3-(2-bromoacetyl)-2H-chromen-2-one (1b)

In the solution of 15 mmol of **1a** in 15 ml dioxane, bromine (0.7 ml, 15 mmol) was added with continuous stirring. The solid separated was filtered and air dried. The crude **1b** was recrystallized from benzene-petroleum ether mixture in 60 % yield, m.p. 122-125 ^{0}C.

Synthesis of 3-(2-amino-1,3-thiazol-5-yl)-2H-chromen-2-one (1c)

Compound **1b** (10 mmol) and thiourea (10 mmol) was dissolved 30 ml of absolute ethanol and refluxed for 4 hrs. Solvent was evaporated on rotary evaporator and the residue was poured over crushed ice. Ammonia was added to make the solution distinctly basic, the solid obtained was filtered and washed with cold water. The crude **1c** was recrystallized from ethanol to obtain pale yellow solid in 55 % yield; m.p. 138-140 ^{0}C. ^{1}H NMR ($CDCl_3$): (ppm) 4.97 (2H, s, -NH_2), 7.28-7.78 (5H, m, Ar-H), 8.51 (1H, pyran-H); MS: m/z = 245.0520 (M^++1).

General procedure for the synthesis of 1-(substituted phenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea derivatives (CTU1-12)

To the solution of sodium azide (0.110 gm, 1.7 mmol), sodium acetate (12.30 mg, 0.15 mmol) and appropriate aromatic acids (1 mmol) in dimethoxyethane (DME) (10 ml); phenylchloroformate (140 µl, 1.10 mmol) was added at room temperature and the mixture was stirred for 8 hrs. Compound **1c** (366 mg, 1.50 mmol) was added at 75 ^{0}C and the solution was stirred at this temperature for 16 hrs in Radlys Carousel 6 place reaction station. The reaction mixture was allowed to cool to room temperature. Hexane 40 ml and water 10 ml was added to the cooled reaction mixture and stirred for 20 min. The solid obtained was filtered and air dried.

Synthesis of ethyl 2-oxo-2H-chromene-3-carboxylate (2a)

The mixture of salicyladehyde 3.6 mmol and diethylmalonate 7.2 mmol was stirred at room temperature. Piperidine 0.25 ml was slowly added to the mixture under stirring and stirring was continued for 30 min. The resulting solution was acidified with glacial acetic acid. The solid obtained was filtered and recrystallized from ethylacetate to obtain **2a** in 60 % yield.

Synthesis of ethyl 2-oxo-2H-chromene-3-carbohydrazide (2b)

The mixture of compound **2a** (1.9 gm), hydrazine hydrate (2 ml) in 15 ml absolute ethanol was refluxed for 6hr. The solid obtained on cooling the reaction mixture was filtered and recrystallized from ethanol to obtain **2b** in 40 % yield.

General procedure for the synthesis of 3-(5-substituted phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (COD1-9)

Compound **2b (**2.4 mmol) was dissolved in 10 ml phosphorous oxychloride. Appropriate aromatic acids (2.4 mmol) were added and the mixture was refluxed for 6 hrs. The reaction mixture was cooled and poured over crushed ice and the solution was neutralized with 20% sodium bicarbonate solution. The solid separated was filtered, air dried and recrystallised from methanol to afford 37-55% of **COD1-9.**

3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]-1-phenylurea (CTU1)

Pale green solid, Yield: 45 %, m.p. 201-203 ^{0}C, Mol. Wt. 363.39, 1102 (C-O-C, str.), 1506 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3100 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.28 (2H, s, NH_2), 7.00-7.92 (9H, m, Ar-H), 8.14 (1H, s, thiazole-H), 8.51 (1H, s, pyran-H). MS: m/z = 363 (M^+)

1-(4-chlorophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU2)

Pale yellow solid, Yield: 57 %, m.p. 168-170 ^{0}C, Mol. Wt. 397.02, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.27 (2H, s, NH_2), 7.04-8.18 (8H, m, Ar-H), 8.50 (1H, s, thiazole-H), 8.59 (1H, s, pyran-H).

1-(4-bromophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU3)

Pale green solid, Yield: 43 %, m.p. 175-177 ^{0}C, Mol. Wt. 442.28, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.29 (2H, s, NH_2), 6.99-7.72 (8H, m, Ar-H), 8.13 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(2-chlorophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU4)

Pale green solid, Yield: 45 %, m.p. 158-160 ^{0}C, Mol. Wt. 397.83, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.29 (2H, s, NH_2), 7.00-8.12 (8H, m, Ar-H), 8.49 (1H, s, thiazole-H), 8.57 (1H, s, pyran-H).

1-(4-methoxyphenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU5)

Pale green solid, Yield: 50 %, m.p. 210-212 ^{0}C, Mol. Wt. 393.41, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 2.54 (3H, s, CH_3), 3.28 (2H, s, NH_2), 7.01-7.71 (8H, m, Ar-H), 7.73 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(4-nitrophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU6)

Pale green solid, Yield: 57 %, m.p. 196-198 ^{0}C, Mol. Wt. 408.38, 1102 (C-O-C, str.), 1506 (Ar C=C), 1665 (N-H, bend), 1604 (-C=N), 1696 (C=O), 3100 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.28 (2H, s, NH_2), 7.03-7.73 (8H, m, Ar-H), 8.16 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(3-chlorophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU7)

Pale green solid, Yield: 40 %, m.p. 181-183 ^{0}C, Mol. Wt. 397.83, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.28 (2H, s, NH_2), 7.00-7.72 (8H, m, Ar-H), 8.14 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(3-hydroxyphenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU8)

Pale green solid, Yield: 43 %, m.p. 163-165 ^{0}C, Mol. Wt. 379.38, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3200 (O-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 2.55 (1H, s, OH), 3.30 (2H, s, NH_2), 6.92-7.68 (8H, m, Ar-H), 8.05 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(2, 4-dichlorophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU9)

Pale green solid, Yield: 51 %, m.p. 178-180 ^{0}C, Mol. Wt. 432.28, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.30 (2H, s, NH_2), 6.93-7.69 (7H, m, Ar-H), 8.07 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(4-fluorophenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU10)

Pale green solid, Yield: 40 %, m.p. 116-118 ^{0}C, Mol. Wt. 381.38, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.29 (2H, s, NH_2), 6.97-7.68 (8H, m, Ar-H), 7.70 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(4-hydroxyphenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU11)

Pale green solid, Yield: 48 %, m.p. 149-151 ^{0}C, Mol. Wt. 379.38, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3200 (O-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 2.55 (1H, s, OH), 3.31 (2H, s, NH_2), 6.86-7.66 (8H, m, Ar-H), 8.00 (1H, s, thiazole-H), 8.50 (1H, s, pyran-H).

1-(2-methoxyphenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea (CTU12)

Pale green solid, Yield: 42 %, m.p. 198-200 ^{0}C, Mol. Wt. 393.41, 1102 (C-O-C, str.), 1539 (Ar C=C), 1665 (N-H, bend), 1696 (C=O), 3154 (Ar-C-H), 3384 (N-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.28 (2H, s, NH_2), 3.86 (3H, s, CH_3), 6.96-7.68 (8H, m, Ar-H), 7.68 (1H, s, thiazole-H), 8.49 (1H, s, pyran-H).

3-(5-phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one (COD1)

Pale green solid, Yield: 52 %, m.p. 172-175 ^{0}C, Mol. Wt. 290.27, 1152 (C-O-C, str.), 1570 (Ar C=C), 1623 (N-H, bend), 1740 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.92-8.14 (9H, m, Ar-H), 8.92 (1H, s, pyran-H). MS: m/z = 291 (M+H)

3-[5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD2)

Pale yellow solid, Yield: 45 %, m.p. 198-200 ^{0}C, Mol. Wt. 324.71, 1195 (C-O-C, str.), 1573 (Ar C=C), 1696 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.93-8.17 (8H, m, Ar-H), 8.96 (1H, s, pyran-H).

3-[5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD3)

Pale yellow solid, Yield: 40 %, m.p. 186-188 ^{0}C, Mol. Wt. 308.26, 1195 (C-O-C, str.), 1573 (Ar C=C), 1696 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.96-8.83 (9H, m, Ar-H), 8.95 (1H, s, pyran-H).

3-[5-(2,4-dichlorophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD4)

Pale yellow solid, Yield: 55 %, m.p. 201-203 ^{0}C, Mol. Wt. 359.16, 1255 (C-O-C, str.), 1581 (Ar C=C), 1696 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.93-8.15 (7H, m, Ar-H), 8.98 (1H, s, pyran-H).

3-[5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD5)

Pale yellow solid, Yield: 48 %, m.p. 138-140 ^{0}C, Mol. Wt. 320.29, 1174 (C-O-C, str.), 1490 (Ar C=C), 1734 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 3.31 (3H, s, -OCH_3), 6.96-8.13 (8H, m, Ar-H), 8.90 (1H, s, pyran-H).

3-[5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD6)

Pale yellow solid, Yield: 37 %, m.p. 145-147 ^{0}C, Mol. Wt. 324.71, 1152 (C-O-C, str.), 1573 (Ar C=C), 1700 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.93-8.17 (8H, m, Ar-H), 8.96 (1H, s, pyran-H).

3-[5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD7)

Pale yellow solid, Yield: 55 %, m.p. 193-195 ^{0}C, Mol. Wt. 335.27, 1111 (C-O-C, str.), 1540 (Ar C=C), 1683 (C=O), 3100 (Ar-C-H).; 1H NMR (CDCl3): (ppm) 6.96-8.44 (8H, m, Ar-H), 8.70 (1H, s, pyran-H).

3-[5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD8)

Pale yellow solid, Yield: 51 %, m.p. 171-173 ^{0}C, Mol. Wt. 369.16, 1123 (C-O-C, str.), 1570 (Ar C=C), 1681 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.97-8.31 (8H, m, Ar-H), 8.96 (1H, s, pyran-H).

3-[5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl]-2H-chromen-2-one (COD9)

Pale yellow solid, Yield: 46 %, m.p. 127-129 ^{0}C, Mol. Wt. 324.71, 1152 (C-O-C, str.), 1573 (Ar C=C), 1700 (C=O), 3100 (Ar-C-H).; ^{1}H NMR ($CDCl_3$): (ppm) 6.96-8.21 (8H, m, Ar-H), 8.97 (1H, s, pyran-H).

Evaluation of antimicrobial activity

The antimicrobial activity of all the synthesized compounds was examined against different Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) by measuring zone of inhibition. The antimicrobial activity was carried out by agar cup plate method at the concentration level 25 µg/ml. Ofloxacin was used as standard at concentration 25 µg/ml. Nutrient agar was used as culture media for antibacterial activity. Twenty four hrs old culture of bacterial pathogen was placed in nutrient agar and spread throughout the plate by spread plate technique. Wells were bored using sterile borer at equidistance. The plates were kept at room temperature for 30 minutes. The test compounds, standard and control were placed in respective wells and plates were incubated at 37^{0}C for 36 hrs. Zone of inhibition was measured by zone reader.

Results and Discussion

Docking

The docking protocol adopted in this investigation was validated by docking of clorobiocin to the energy minimized gyrase protein. The residues Asp73, Asn46 and Arg136 are important in making hydrogen bond and Arg76, Pro79, Ile78, Ile90, Thr165, Val43, Val71, Ala47, Val120 and Gly77 are important in hydrophobic interactions. The best conformer generated in docking showed same interactions as shown in Figure 1.

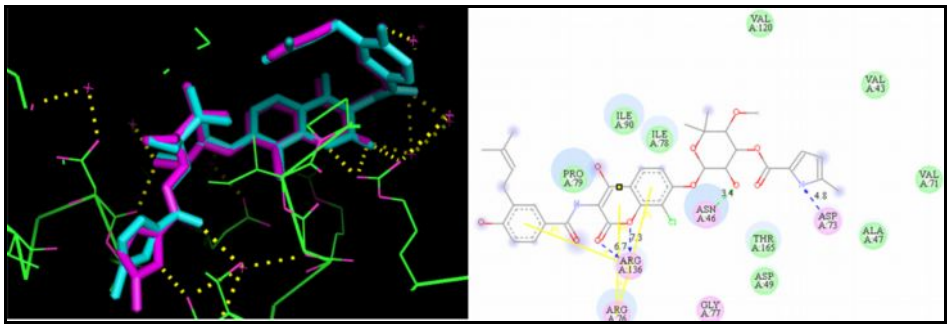


Figure 1: Docked conformer of Clorobiocin (docked conformer shown in cyan and original pose of clorobiocin shown in megenta color)

After docking designed molecules (CTU1-12 & COD1-9) most of the compounds show interaction with Asn46 along with other hydrophobic interactions. The extra hydrogen bonding interactions were not observed for designed compounds as these compounds may not access deep hydrophobic pocket of gyrase protein. The binding free energy in Kcal/mole and interactions are presented in (Table 1). Compounds CTU 1, CTU4, CTU7, CTU10 showed two hydrogen bond interactions. Compounds CTU8, CTU9, COD5, COD8 showed no hydrogen bond interactions. The 2D digram of important interactions between active site residues and compound atoms is shown in figure 2 and 3.

Table 1: Docking score (Binding free energy in kcal/mol) and important interaction with residues

Sr. No.	Compound	Docking score (Binding free energy) kcal/mol	Interactions
1	CTU1	-8.3	**Asn46 (H), Arg76**
2	CTU2	-8.3	**Asn46 (H)**
3	CTU3	-8.0	**Asn46 (H)**
4	CTU4	-8.2	**Asn46 (H), Arg76**
5	CTU5	-7.9	**Asn46 (H)**
6	CTU6	-7.9	**Asn46 (H)**
7	CTU7	-8.5	**Asn46 (H), Arg76**
8	CTU8	-7.5	Asn46
9	CTU9	-8.6	-
10	CTU10	-8.2	**Asn46 (H), Arg76**
11	CTU11	-9.0	**Asn46 (H)**
12	CTU12	-7.9	**Asn46 (H)**
13	COD1	-8.7	**Asn46 (H)**
14	COD2	-9.0	**Asn46 (H)**
15	COD3	-8.9	**Asn46 (H)**
16	COD4	-9.3	**Asn46 (H)**
17	COD5	-8.0	Arg76
18	COD6	-8.9	**Thr165 (H),** Arg76
19	COD7	-8.7	**Asn46 (H)**
20	COD8	-8.7	-
21	COD9	-9.0	**Asn46 (H)**
22	Clorobiocin	-6.4	**Asn46 (H), Asp73 (H), Arg136 (H),** Arg76

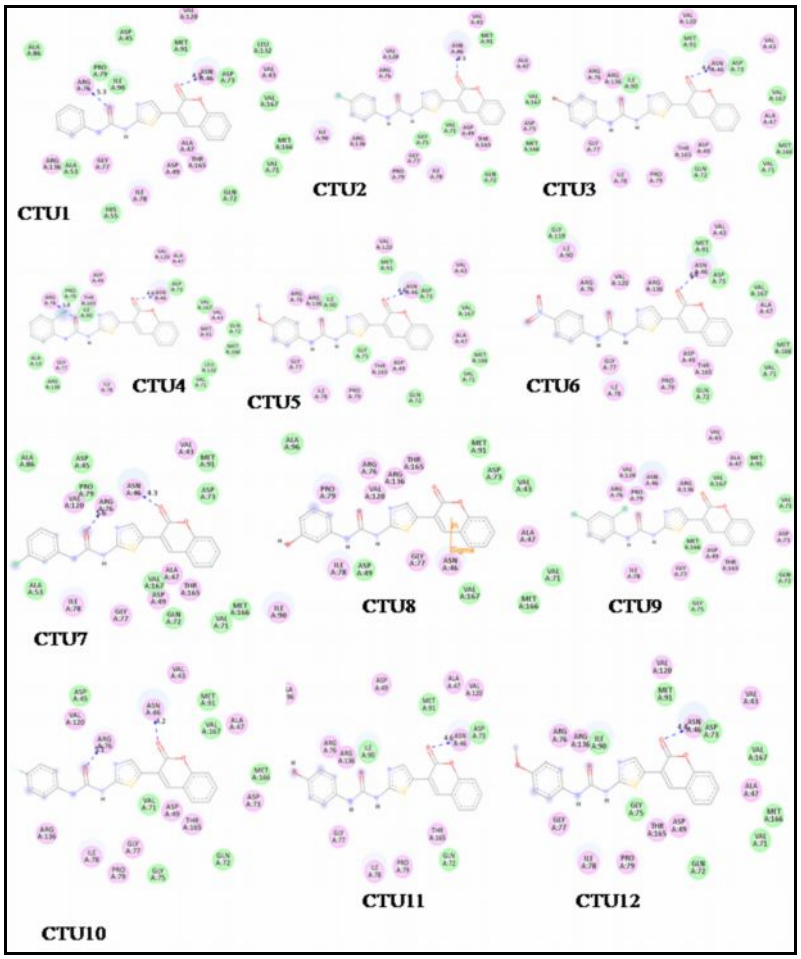


Figure 2: 2D interaction diagram for CTU series compounds

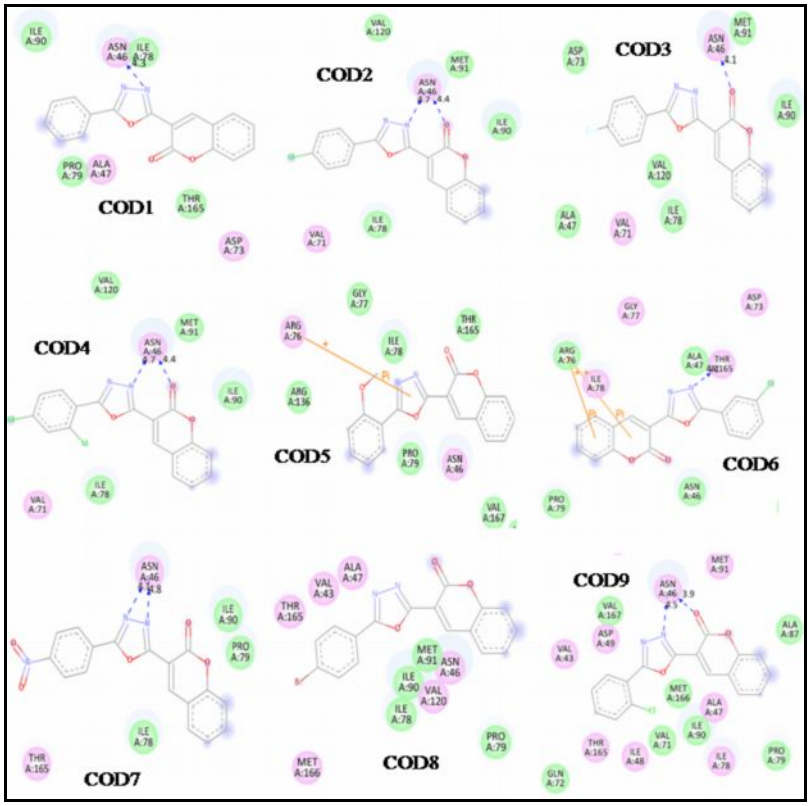


Figure 3: 2D interaction diagram for COD series compounds

Antimicrobial activity

The synthesised compounds were evaluated for antibacterial activity using agar cup plate method. Ofloxacin, a well known topoisomerase II DNA gyrase inhibitor, was used as a standard. The results are presented (Table 2). The results show that compounds form CTU series are more active than COD series. The presence of thiazole ring with hydrophobic urea substituent may be the possible reason for better antimicrobial activity of these compounds. CTU 7 was found most active against *S. Aureus.* Docking scores and hydrogen bonds formation with important residues are in good agreement with the antimicrobial activity. All the compounds were found poorly active against gram negative *E. coli and P. aeruginosa*. Compound CTU 1 possess good activity against all the test organisms used in the study, suggesting the importance of unsubstituted phenyl ring on urea nucleus. When compared with the standard ofloxacin, all the synthesised compounds exhibited poor to moderate activity against gram positive and gram negative organisms.

Table 2: Antimicrobial activity of synthesized compounds

Zone of Inhibition in mm (millimetre)				
Compounds	**B. subtilis**	**S. aureus**	**E. coli**	**P. aeruginosa**
CTU1	17.3 ± 0.3	16 ± 0.4	16.4 ± 0.6	15.5 ± 0.4
CTU2	13.2 ± 0.2	14.1 ±0.8	11.0 ± 1.0	11.9 ± 0.3
CTU3	13.6 ± 0.3	13.3 ± 0.1	9.2 ± 0.9	11.6 ± 0.6
CTU4	17.0 ± 1.7	17.8 ± 0.3	11.4 ± 1.0	14.5 ± 0.4
CTU5	13.8 ± 0.2	14.7 ± 0.2	8.4 ± 0.1	11.5 ± 0.2
CTU6	14.7 ± 0.2	15.6 ± 0.5	7.1 ± 0.3	11.5 ± 0.4
CTU7	18.3 ± 0.2	16.9 ± 0.5	13.2 ± 0.7	14.7 ± 0.2
CTU8	13.8 ± 0.9	14.4 ± 0.5	7.5 ± 0.9	11.3 ± 0.2
CTU9	12.6 ± 0.1	13.1 ± 0.6	6.6 ± 0.5	9.7 ± 0.1
CTU10	17.8 ± 0.7	17.1 ± 0.2	13.5 ± 0.9	15.1 ± 0.5
CTU11	14.4 ± 0.1	13.3 ± 0.5	9.3 ± 1.0	12.0 ± 0.1
CTU12	15.0 ± 0.2	14.5 ± 0.3	11.3 ± 0.6	12.9 ± 0.1
COD1	12.2 ± 0.3	12.9 ± 0.6	10.7 ± 0.2	11.4 ± 0.3
COD2	13.8 ± 0.6	13.2 ± 0.9	9.8 ± 0.3	11.6 ± 0.2
COD3	13.9 ± 0.7	14.5 ± 0.3	8.9 ± 0.4	11.6 ± 0.1
COD4	12.2 ± 0.3	13.4 ± 0.4	9.2 ± 0.05	10.9 ± 0.1
COD5	10.3 ± 0.4	10.9 ± 0.8	9.7 ± 1.0	9.6 ± 0.4
COD6	9.4 ± 0.3	10.6 ± 0.4	11.6 ± 1.0	9.7 ± 0.4
COD7	14.1 ± 0.1	13.2 ± 0.4	12.1 ± 0.2	12.1 ± 1.2
COD8	9.7 ± 0.4	10.6 ± 0.2	9.6 ± 0.7	9.5 ± 0.5
COD9	13.2 ± 0.3	12.4 ± 0.3	10.3 ± 0.1	11.3 ± 0.3
Ofloxacin	31.0 ± 0.711	28.8 ± 0.849	28.2 ± 0.205	27.9 ± 0.216

Data presented in Mean ± SD (N=3)

Conclusion

Series of 1-(substituted phenyl)-3-[5-(2-oxo-2H-chromen-3-yl)-1,3-thiazol-2-yl]urea derivatives (CTU1-12) and 3-(5-substituted phenyl-1,3,4-oxadiazol-2-yl)-2H-chromen-2-one derivatives were synthesized. Docking studies in Autodock vina suggested hydrogen bond interaction between carbonyl group of coumarin ring or carbonyl group of urea substituent with key residue Asn46 for compounds from CTU series and hydrogen bond interaction between nitrogen of oxadiazole ring and Asn46. Compounds CTU1, CTU4, CTU7 and CTU10 showed moderate antibacterial activity against gram positive organisms.

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