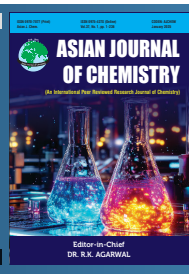
Asian Journal of Chemistry; Vol. 37, No. 4 (2025), 858-864

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.33225



Design and Synthesis of Novel Coumarin-Piperazine Derivatives as Potential Antimicrobial Agents

Mo. Shahanawaz[1], Kuldeep Singh[1,*], Suvaiv[1], Syed Misbahul Hasan[1], Praveen Kumar[2], Arun Kumar[1] and Iffat Zareen Ahmad[3]

[1]Faculty of Pharmacy, Integral University, Lucknow-226026, India
[2]Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai-206130, India
[3]Department of Bioengineering, Integral University, Lucknow-226026, India

*Corresponding author: E-mail: kuldeep@iul.ac.in

Received: 18 December 2024; *Accepted*: 8 March 2025; *Published online*: 29 March 2025; AJC-21947

Coumarin piperazine acetamide analogues have shown great potential as drug candidates due to their wide range of biological activities. A series of novel coumarin-piperazine derivatives were synthesized and performed the *in vitro* antibacterial activity and ADME profiling. Compounds **MS1**, **MS2**, **MS5** and **MS6** exhibited docking scores higher than the standard ranging from -8.6 to -7.6 Kcal mol^{-1}. Compound **MS6** showed the best zone of inhibition, measuring 17 mm at 150 µg/mL against *Staphylococcus epidermidis*. Molecular docking and *in vitro* study of compound suggest that the presence of halogen on the aromatic ring, attached to piperazine might responsible for a good antibacterial potential. ADME profiling of the synthesized compounds showed the log S ESOL value between -2.39 to -4.52, number of H bond acceptors between 4-5, No. of H bond donors 1, high GIT absorption and adhered to the Lipinski, Ghose and Veber drug-likeness rules.

Keywords: Coumarin, Piperazine, Microbial resistance, ADME, *In vitro* antibacterial activity.

INTRODUCTION

Microbial resistance has emerged as a significant health issue, posing a formidable challenge for researchers worldwide [1]. World Health Organization (WHO) reports an alarming statistic, noting that approximately 50,000 individuals succumb to microbial infections on a daily basis [2]. In 1990, 16 million people lost their lives to such infections, a number that slightly decreased to 15 million in 2010 and is anticipated to further decline to 13 million by 2050 [3]. Centre for Disease Control and Prevention (CDC) highlights the prevalence of approximate 2.8 million bacterial infections annually, causing about 35,000 deaths in the US alone [4]. Recent efforts in antimicrobial agent development must confront significant challenges such as heightened toxicity risks, inadequate antimicrobial efficacy and the ongoing emergence of microbial resistance. Heterocyclic compound chemistry has long been a focal point in the exploration of antimicrobial agents [5,6].

Gram-negative and Gram-positive bacteria are prevalent culprits in a variety of diseases, including lower respiratory tract infections, urinary tract infections, intra-abdominal infections and skin-soft tissue infections [7,8]. Antibiotics are the crucial in addressing bacterial infections, yet their widespread and inappropriate use contributes to the rapid proliferation of antibiotic resistance, diminishing the efficacy of empiric antimicrobial treatments [9].

Coumarin has become an important part of medicinal chemistry due to its wide range of pharmacological applications and low toxicity to normal cells [10]. Numerous heterocyclic members, including warfarin, phenprocoumon and acenocoumarol, are renowned for their potent anticoagulant properties [11]. Moreover, compounds like novobiocin and clorobiocin exhibited antibiotic used to treat microbial infections [12]. Coumarins analogues have been discovered before as an antibacterial agent. Some important class of coumarin derivatives including some hydroxylated variations were tested for antibacterial and antibiofilm activity against *R. solanacearum* [13]. Previous studies on coumarins reveals that coumarin (2-oxo-2*H*-chromene) and its analogues show a wide spectrum of pharmacological such as antitumor, antibacterial, antifungal, anticoagulant, antioxidant and anti-inflammatory [14], *etc*.

Piperazine comes in class of heterocyclic organic compound, having ring of six-membered containing two nitrogen atoms at opposite positions of each other. Piperazine is a weak base in nature having two pK_a values of 5.35 and 9.73 at 25 ºC [15]. Piperazine is used as an anthelmintic agent to treat roundworm and pinworm infections in humans and domestic animals. It is also used as a solvent for uric acid [16]. Nitrogen containing polycyclic structures have been associated with a wide-ranging of physiological effects, comprising antimicrobial, anthelmintic, anti-inflammatory, anti-tumour, AChE inhibitors, antidepressant, antipsychotic, antidiabetic, antihistaminic and flavouring properties [17]. This wide array of pharmacological effects has spurred medicinal chemists to synthesize numerous novel chemotherapeutic agents based on these frameworks [18].

EXPERIMENTAL

Waters Alliance e2695/HPLC-TQD Mass spectrometer was used for ESI/MS, LC-ESI/MS, LC-MS/MS and Agilent QTOF spectrometer was used for HR-ESI/MS analysis. The mass spectral data confirmed the expected molecular structures of the targeted compounds analyzed, as indicated by correlation between the observed mass spectral data and the anticipated structures.

Synthetic route for the synthesis of targeted compound MS1-MS6: Compounds **MS1-MS6** was synthesized by reacting different piperazines with intermediate compound **d** (1.0 mmol) in ethanol (10 mL) and anhydrous potassium carbonate (0.3 g) under reflux at 80 ºC for 4 h. After the reaction was completed, the mixture was filtered, dried and recrystall-ized using ethanol. The final product was then dried using a rotary evaporator to obtain the targeted compounds **MS1-MS6**.

6-Aminocoumarin (c): Brownish-yellow crystals, m.p.: 160 ºC (Lit. 158 ºC) [19]. Yield: 76%. TLC solvent-chloroform: methanol 6:4. ESI/MS: *m/z* [M^+] 162, *m/z* [M^+] +1 163, *m/z* [M^+] +2 164.

2-Chloro-*N*-(2-oxo-2*H*-chromen-6-yl)acetamide (d): Fine brown crystals, yield: 75%, m.p.: 220 ºC, [M^+] +1: 237, Solubility: methanol, acetone. TLC solvent:chloroform:ethyl acetate 5:5. IR (KBr, ν_{max}, cm^{-1}): 3165.43 (>NH *str.*), 3102.36 (Ar.-CH *str.*), 2995.86 and 2594.51 (>CH_2 *str.*), 1765.84 and 1701.91 (C=O peaks), 825.83 (>CH-Cl *str.*). ESI/MS: *m/z* [M^-] 236.2, *m/z* [M^+] +1 238.2, *m/z* [M^+] +2 239.2.

2-(4-(2-Chlorophenyl)piperazin-1-yl)-*N*-(2-oxo-2*H*-chromen-6-yl)acetamide (MS1): Light yellow, m.p.: 110-112 ºC, yield: 65%, R_f: 0.6; IR (KBr, ν_{max}, cm^{-1}): 3521 (>NH *str.*), 3089 (Ar.-CH *str.*), 2835 (>CH_2 *str.*), 1719 (C=O peaks), 550 (>C-Cl *str.*). HRMS (ESI+) *m/z* calcd. for $C_{21}H_{20}ClN_3O_5$+H 398.1193, found 398.1727 [M+H$^+$]; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.200 (s, 1H, -NH), 8.146 (s, 1H, Ar-H, 2H-chromen-2-one), 7.803 (d, Ar-H, 2H-chromen-2-one), 7.389 (d, 1H, Ar-H, 2H-chromen-2-one), 7.360 (d, Ar-H, 2H-chromen-2-one), 6.499 (d, 2H, Ar-H, phenyl), 6.467 (d, 1H, Ar-H, 2H-chromen-2-one), 3.766 (2H, piperazine), 2.679 (s, 1H, >CH_2), 2.500 (d, 2H, piperazine).

***N*-(2-Oxo-2*H*-chromen-6-yl)-2-(4-(pyridin-2-yl)piperazin-1-yl)acetamide (MS2):** Dull white, m.p.: 150-152 ºC, yield: 70%, R_f: 0.5; IR (KBr, ν_{max}, cm^{-1}): 3236 (>NH *str.*), 3036 (Ar.-CH *str.*), 2833 (>CH_2 *str.*), 1732 (C=O peaks), 1245 (>C-N *str.*). HRMS (ESI+) *m/z* calcd. for $C_{20}H_{20}N_4O_3$+H 365.1535, found 365.2397 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.025 (s, 1H, -NH), 8.004 (d, 2H, Ar-H, pyridine), 8.004 (d, 2H, Ar-H, pyridine), 7.559 (d, 2H, Ar-H, pyridine), 7.205 (s, 1H, Ar-H, 2H-chromen-2-one), 7.155 (d Ar-H, 2H-chromen-2-one), 7.048 (d, 1H, Ar-H, 2H-chromen-2-one), 6.455 (d, 1H, Ar-H, 2H-chromen-2-one), 3.425 (2H, piperazine), 3.055 (s, 1H, >CH_2), 2.025 (d, 2H, piperazine).

2-(4-Methylpiperazin-1-yl)-N-(2-oxo-2*H*-chromen-6-yl)acetamide (MS3): White, m.p.: 185-187 ºC, yield: 60%, R_f: 0.4; IR (KBr, ν_{max}, cm^{-1}): 3356 (>NH *str.*), 3150 (Ar.-CH *str.*), 2920 (>CH_2 *str.*), 1632 (C=O peaks), 1450 (>C=C *str.*), 1150 (>C-N *str.*). HRMS (ESI+) *m/z* calcd. for $C_{16}H_{19}N_3O_3$+H 302.1426, found 302.1811 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.015 (s, 1H, -NH), 7.869 (d, 1H, Ar-H, 2H-chromen-2-one),7.665 (s, 1H, Ar-H, 2H-chromen-2-one), 7.543 (d, Ar-H, 2H-chromen-2-one), 7.015 (d, 1H, Ar-H, 2H-chromen-2-one), 6.755 (d, 1H, Ar-H, 2H-chromen-2-one), 3.425 (2H, piperazine), 3.245 (s, 1H, >CH_2), 2.525 (d, 2H, piperazine), 1.985 (s, 1H, >CH_3).

2-(4-Ethylpiperazin-1-yl)-N-(2-oxo-2*H*-chromen-6-yl)-acetamide (MS4):While, m.p.: 210-212 ºC, yield: 60%, R_f: 0.7; IR (KBr, ν_{max}, cm^{-1}): 3420 (>NH *str.*), 3055 (Ar.-CH *str.*), 2820 (>CH_2 *str.*), 1620 (C=O peaks), 1560 (>C=C *str.*), 1050 (>C-N *str.*). HRMS (ESI+) *m/z* calcd. for $C_{17}H_{21}N_3O_3$+H316.1583, found 316.1978 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.998 (s, 1H, -NH), 7.759 (d, 1H, Ar-H, 2H-chromen-2-one), 7.435 (s, 1H, Ar-H, 2H-chromen-2-one), 7.324 (d, Ar-H, 2H-chromen-2-one), 7.215 (d, 1H, Ar-H, 2H-chromen-2-one), 6.456 (d, 1H, Ar-H, 2H-chromen-2-one), 3.515 (2H, piperazine), 3.025 (s, 1H, >CH_2), 2.019 (d, 2H, piperazine), 1.886 (s, 1H, >CH_2), 1.055 (s, 1H, >CH_3).

2-(4-(2-Cyanophenyl)piperazin-1-yl)-N-(2-oxo-2*H*-chromen-6-yl)acetamide (MS5): Grey, m.p.: 166-167 ºC, yield: 70%, R_f: 0.4; IR (KBr, ν_{max}, cm^{-1}): 3310 (>NH *str.*), 3031 (Ar.-CH *str.*), 2821 (>CH_2 *str.*), 1724 (C=O peaks), 1233 (>C-N *str.*). HRMS (ESI+) *m/z* calcd. for $C_{22}H_{20}N_4O_3$+H 389.1535, found 389.1884 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 9.775 (s, 1H, -NH), 7.505 (s, 1H, Ar-H, 2H-chromen-2-one), 7.400 (d Ar-H, 2H-chromen-2-one), 7.149 (d, 1H, Ar-H, 2H-chromen-2-one), 7.045 (d, Ar-H, 2H-chromen-2-one), 6.635 (d, 2H, Ar-H, Phenyl), 6.330 (d, 2H, Ar-H, phenyl), 6.250 (d, 1H, Ar-H, 2H-chromen-2-one), 3.345 (2H, piperazine), 3.045 (s, 1H, >CH_2), 2.645 (d, 2H, piperazine).

***N*-(2-Oxo-2*H*-chromen-6-yl)-2-(4-phenylpiperazin-1-yl)acetamide (MS6):** Brown, m.p.: 175-177 ºC, yield: 65%, R_f: 0.6; IR (KBr, ν_{max}, cm^{-1}): 3410 (>NH *str.*), 3021 (Ar.-CH *str.*), 2812 (>CH_2 *str.*), 1710 (C=O peaks), 1212 (>C-N *str.*). HRMS (ESI+) *m/z* calcd. for $C_{21}H_{21}N_3O_3$+H 364.1616, found 364.2397 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 10.245 (s, 1H, -NH), 7.745 (s, 1H, Ar-H, 2H-chromen-2-one), 7.635 (d Ar-H, 2H-chromen-2-one), 7.539 (d, 1H, Ar-H, 2H-chromen-2-one), 7.245 (d, Ar-H, 2H-chromen-2-one), 6.075 (d, 2H, Ar-H, Phenyl), 6.024 (d, 2H, Ar-H, Phenyl), 6.015 (d, 1H, Ar-H, 2H-chromen-2-one), 3.435 (2H, piperazine), 3.235 (s, 1H, >CH_2), 2.535 (d, 2H, piperazine).

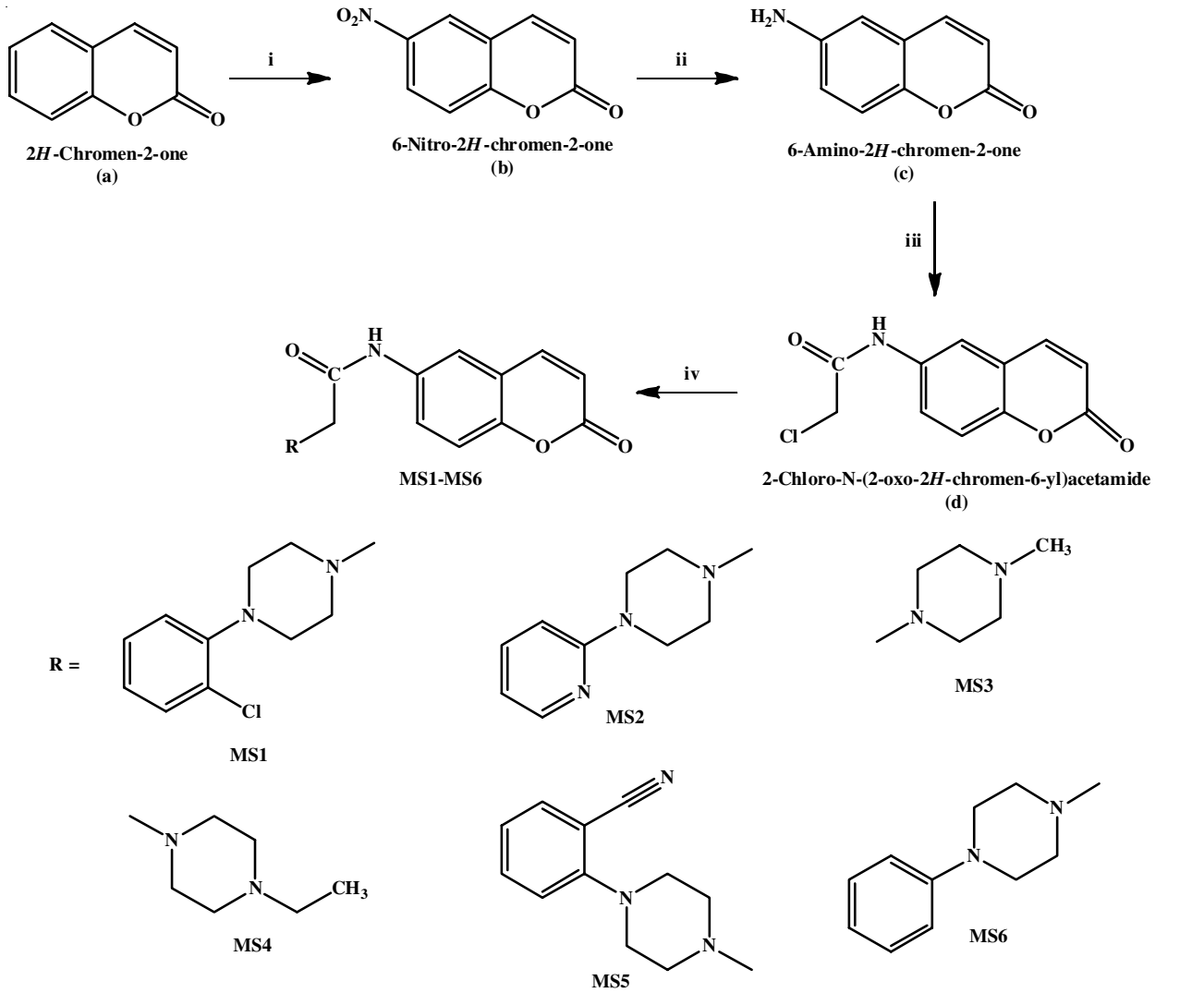
***In vitro* antibacterial screening:** All the synthesized coumarin-piperazine acetamide derivatives (**MS1-MS6**) were evaluated for their *in vitro* antimicrobial activities using the agar well diffusion method [20]. The zone of inhibition was measured at the concentrations of 50 µg/mL, 100 µg/mL and 150 µg/mL against Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*).

Docking studies: Docking studies: Docking of designed compounds (**MS1-MS6**) was performed by PyRx 0.8 software for anti-bacterial screening on DNA gyrase subunit b receptor (PDB: 1KZN) [21]. The three-dimensional structure of the DNA gyrase subunit B receptor (PDB: 1KZN) was downloaded from the Protein Data Bank (https://www.rcsb.org/). The structure of the target protein was visualized using the BIOVIA Discovery Studio 2016 software. The docking process was performed using the Vina Wizard feature integrated into PyRx 0.8, which predicts the binding affinity of the designed compounds [22]. Clorobiocin contains coumarin in its structure and norfloxacin contains piperazine, both of which show structural similarity with the synthesized compounds, therefore were chosen as standards for the docking studies.

ADME profiling: ADME profiling of synthesized compounds was performed by the Swiss-ADME open-access database [23]. Canonical SMILES of synthesized compounds was generated by the Chem-Draw software. For ADME profiling, the physico-chemical properties, lipophilicity, water solubility, pharmacokinetics and drug likeness were measured.

RESULTS AND DISCUSSION

Targeted derivatives of coumarin-piperazine acetamide derivatives (**MS-MS6**) were synthesized in four steps, as outlined in **Scheme-I**. Nitration of coumarin (**a**) was achieved by nitrating agent HNO_3 in conc. H_2SO_4 (50 mL) at –5 ºC and then 24 mL nitrating mixture (HNO_3 and H_2SO_4 1:3 volume ratio) was added. After the completion of stirring crushed ice was added in to the mixture to obtain white precipitate of 6-nitrocoumarin (**b**) [19,24]. In second step, 6-nitrocoumarin (**b**)



Scheme-I: Synthesis of coumarin-piperazine acetamide derivatives (**MS1-MS6**), (i) HNO_3, H_2SO_4, -5 ºC (ii) HCl, methanol, stannous chloride, 150 ºC, 7 h, (iii) acetone, chloroacetyl chloride, K_2CO_3, 45 ºC, (iv) Solvent and temperature varied as per derivatives (see experimental section) R = substituted piperazine derivatives

(40.00 g, 0.20 mol) and stannous chloride dihydrate (80.00 g, 0.36 mol) was mixed in conc. HCl (172 mL, 202.96 g) for time period of 15 min at 80 ºC. After 15 min of stirring methanol (250 mL) was added at 150 ºC to get clear solution and then stirred at 150 ºC for 17 h till completion of the reaction, finally refrigerated it overnight. After cooling overnight, a yellow precipitate formed, which was filtered and dissolved in distilled water. The solution was then filtered again to obtain a clear yellow liquid. This solution was made alkaline by adding anhydrous $NaHCO_3$ powder, leading to the precipitation of free amine. The precipitated amine was filtered and extracted with acetone in five separate portions of 200 mL each. Extract was concentrated under reduced pressure using rotatory evaporator to get yellow crystals of 6-amino-2*H*-chrome-2-one (**c**) (6.5 g, 39%) [19]. 6-Amino-2*H*-chrome-2-one (**c**) (0.44 g, 0.002 mol), chloroacetyl chloride (0.17 mL, 0.002 mol) and anhydrous K_2CO_3 (0.5 g) placed in 150 mL of RBF, acetone (10 mL) was added and the mixture was refluxed for 3 h. The precipitate was formed, filtered off the precipitate and recrystallized with ethanol after evaporation of ethanol by rotatory evaporator to obtain titled compound 2-chloro-N-(2-oxo-2*H*-chromen-6-yl) acetamide (**d**) [25]. The resulting intermediate was then reacted with various substituted piperazines to yield the desired compounds **MS1-MS6**. The characterization of structure of synthesized compound **MS1-MS6** was done by FT-IR, Nuclear magnetic resonance (NMR) spectroscopy, Mass spectral studies, TLC and physico-chemical properties.

The IR spectra of 6-aminocoumarin (**c**) showed a characteristic peak of C=O group at peak at 1637.40 cm^{-1}, whereas the peak at 1550.25 cm^{-1} signify the presence of C–H and C=C group in the aromatic ring. The presence of primary amine in 6-aminocoumarin (**c**) was directed by the peak at 3225.20 cm^{-1} and 3410.30 cm^{-1} respectively. The IR spectra of 2-chloro-N-(2-oxo-2*H*-chromen-6-yl) acetamide (**d**) showed the peak at 1620.34 cm^{-1}, which is attributed due to the presence of C=O group, whereas the peak at 1520.20 cm^{-1} confirmed the presence of C–H and C=C group in aromatic ring. The presence of secondary amine in intermediated **d** was confirmed by the peak at 3360.30 cm^{-1}.

The existence of –NH functional group in compounds **MS1-MS6** was showed by the peak at the range between 9.5-10.25 ppm in ^{1}H NMR spectra. The existence of Ar-H of coumarin in compounds **MS1-MS6** was showed by the peak at the range between 7.4-8.0 ppm in ^{1}H NMR spectra. The existence of Ar-H of aromatic piperazine in compounds **MS1-MS6** was showed by the peak at 6.5-7.4 ppm. The existence of H-atom of piperazine ring in compounds **MS1-MS6** was showed by the peak at 2.7-7.4 ppm. The existence of –C=O functional group in compounds **MS1-MS6** was showed by the bands between 1700-1600 cm^{-1} in IR spectra. Similarly, the existence of 2º-amine in compounds **MS1-MS6** was showed by the bands between 3400-3300 cm^{-1}. IR bands at 3150-3000 cm^{-1} directed the presence of C–H in the aromatic structure and 1550.25 cm^{-1} confirmed the presence of C–H and C=C group in aromatic ring of compounds **MS1-MS6**.

Antibacterial activity: *in vitro* antibacterial activity revealed that compounds **MS1**, **MS2**, **MS4** and **MS6** demonstrated good antibacterial activity, establishing themselves as strong candidates for further development as an antimicrobial agent. The ZOI values of the synthesized compounds are shown in Table-1. Ofloxacin, streptomycin, doxycycline and ciprofloxacin were employed as the standards. Ofloxacin and ciprofloxacin were chosen as standard drugs due to the presence of a piperazine ring in their structure, which shows the structural

TABLE-1
ANTIBACTERIAL DATA OF SYNTHESIZED COMPOUNDS (**MS1-MS6**)

Compounds	Concentrations (µg/mL)	Zone of inhibition (mm)			
		Staphylococcus aureus	*Bacillus subtilis*	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*
MS1	50	12	10	11	10
	100	14	12	13	14
	150	16	15	14	16
MS2	50	–	10	10	10
	100	10	10	11	12
	150	12	13	12	15
MS3	50	–	10	10	–
	100	10	11	10	10
	150	10	13	11	11
MS4	50	–	10	–	–
	100	10	10	10	12
	150	12	12	10	13
MS5	50	10	10	12	10
	100	13	11	14	12
	150	14	14	15	13
MS6	50	12	10	13	10
	100	14	11	15	13
	150	16	13	17	14
Ofloxacin	5 µg/disc	24	22	22	30
Streptomycin	300 µg/disc	22	25	27	33
Doxycycline	30 µg/disc	16	27	27	29
Ciprofloxacin	10 µg/disc	30	26	28	34

similarity with the synthesized compounds. Ofloxacin (5 µg/disc) showed the best zone of inhibition (ZOI) of 34 mm against *P. aeruginosa*, followed by ciprofloxacin (10 µg/disc) with a ZOI of 34 mm, streptomycin (300 µg/disc) with a ZOI of 33 mm and doxycycline (30 µg/disc) with a ZOI of 33 mm against *P. aeruginosa*. Notably, all compounds demonstrated antibacterial activity *in vitro* at varying concentrations 50 µg/mL, 100 µg/mL and 150 µg/mL. Among them, compound **MS6** emerged as the most potent, with ZOI of 17 mm, against *Staphylococcus epidermidis*. The remaining compounds **MS1**, **MS2**, **MS4** and **MS6** exhibited zones of inhibition ranging from 14-17 mm against all bacterial strains at 150 µg/mL. Consequently, they could serve as lead compounds for the development of other coumarin-piperazine acetamide derivatives as antibacterial agents.

Docking studies: The docking results of the synthesized compounds showed that compound **MS6** displayed the best binding score of -8.6 kcal/mol, while compounds **MS1** and **MS2** also displayed good binding scores of -8.5 and -8.3 kcal/mol, respectively (Table-2). Clorobiocin and norfloxacin displayed binding scores of -7.7 and -7.6 kcal/mol, respectively, which were found lower than the binding scores of some compounds **MS1**, **MS2**, **MS5** and **MS6**. On the basis of the chemical structure of the synthesized compounds, it is found that the presence of aromatic substitution on the piperazine ring led to an increase in binding affinity. Compounds **MS1-MS5** showed the conventional hydrogen bonding with the oxygen of acetamide and the asparagine amino acid (ASN A: 46), while compound **MS6** showed conventional hydrogen bonding with the arginine amino

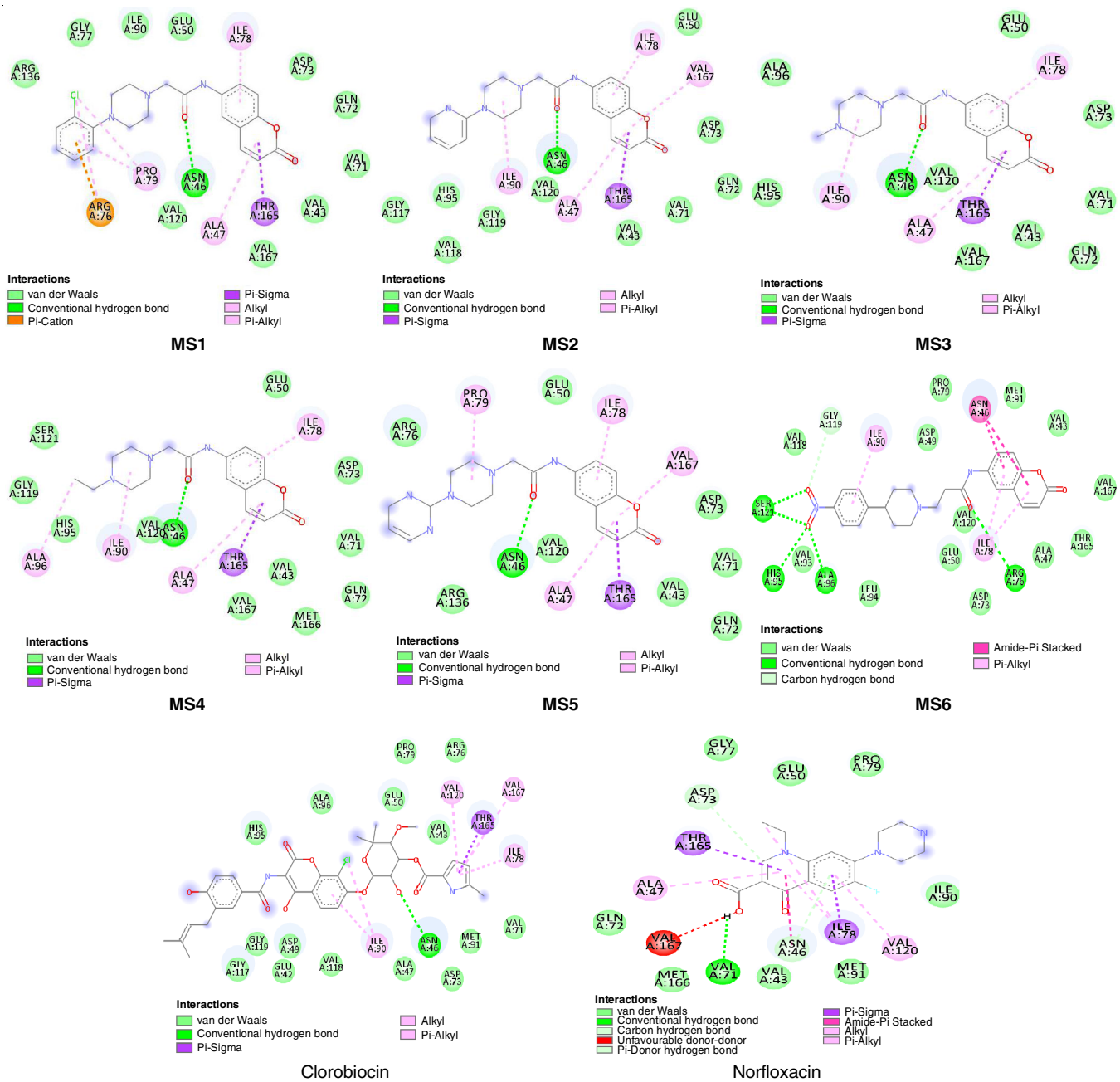


Fig. 1. 2D interaction of compounds **MS1-MS6**, clorobiocin and norfloxacin with 1KZN

TABLE-3
PHYSICO-CHEMICAL PROPERTIES, LIPOPHILICITY, WATER SOLUBILITY, PHARMACOKINETICS AND DRUG LIKENESS OF SYNTHESIZED **MS1-MS6**

Compounds code	log S ESOL	H bond donors	H bond acceptors	GIT absorption	Lipinski rule	Veber rule
MS1	-4.52	1	4	High	Yes	Yes
MS2	-3.47	1	5	High	Yes	Yes
MS3	-2.39	1	5	High	Yes	Yes
MS4	-2.62	1	5	High	Yes	Yes
MS5	-3.88	1	5	High	Yes	Yes
MS6	-4.04	1	5	High	Yes	Yes

acid (ARG A: 76). Due to the presence of an oxygen-containing group on the phenyl piperazine, compound **MS6** showed the conventional hydrogen bonding with other amino acids such as SER A: 121, HIS A: 95, and ALA A: 96, which contributed to its increased potency in terms of binding affinity. (Fig. 1).

TABLE-2
DOCKING SCORES OF DESIGNED COMPOUNDS (**MS1-MS6**) FOR THE TARGET 1KZN COMPLEX WITH CLOROBIOCIN AND STANDARD DRUG (NORFLOXACIN)

Compd.	Docking score affinity (Kcal/mol)	Compd.	Docking score affinity (Kcal/mol)
MS1	-8.5	**MS5**	-8.3
MS2	-8.3	**MS6**	-8.6
MS3	-7.6	Clorobiocin	-7.7
MS4	-7.7	Norfloxacin	-7.6

ADME studies: Due to the presence of aromatic substitution on the piperazine ring, Compounds MS1 and MS6 showed log S ESOL values of -4.52 and -4.04, respectively, indicating poor water solubility. In contrast, compounds **MS2-MS5** exhibited log S ESOL values ranging from -3.88 to -2.39, indicating higher water solubility compared to compounds **MS1** and **MS6**. Compounds **MS1-MS6** followed both Lipinski's rule and Veber's rule of drug-likeness. Similarly, Compounds **MS1-MS6** exhibited one H-bond donor and high gastrointestinal (GIT) absorption. Compound **MS1** had four H-bond acceptors, while compounds MS2-MS6 had five H-bond acceptors, which influenced their water solubility (Table-3). These properties predict the compounds' absorption and bioavailability and found satisfactory (Table-3).

Conclusion

A new series of coumarin piperazine acetamide derivatives (**MS1-MS6**) was synthesized and characterized using spectral analysis, high-resolution mass spectrometry (HRMS),NMR and FTIR spectroscopic techniques. These compounds were evaluated for their *in vitro* antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis* and *Staphylococcus epidermidis*) as well as Gram-negative bacteria (*Pseudomonas aeruginosa*). Compound **MS6** showed the best zone of inhibition, measuring 17 mm at 150 µg/mL against *Staphylococcus epidermidis*. Active compounds **MS1**, **MS2**, **MS4** and **MS6** exhibited zones of inhibition ranging from 14-17 mm against all bacterial strains at 150 µg/mL. Compound **MS6** showed the highest binding score -8.6 against the target DNA gyrase (PDB-1KZN).

ACKNOWLEDGEMENTS

The authors extend their sincere gratitude to the Integral University, Lucknow, India, for generously providing the research facilities and assigning the manuscript communication number to this article IU/R&D/2024-MCN0002975. Moreover, the authors also express their appreciation to the Council of Science and Technology, Uttar Pradesh Government for their invaluable support in facilitating this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. F. Prestinaci, P. Pezzotti and A. Pantosti, *Pathog. Glob. Health*, **109**, 309 (2015); https://doi.org/10.1179/2047773215Y.0000000030
2. R.S. Cheke, H.M. Patel, V.M. Patil, I.A. Ansari, J.P. Ambhore, S.D. Shinde, A. Kadri, M. Snoussi, M. Adnan, P.S. Kharkar, V.R. Pasupuleti and P.K. Deshmukh, *Antibiotics*, **11**, 566 (2022); https://doi.org/10.3390/antibiotics11050566
3. GBD 2021 Antimicrobial Resistance Collaborators, *Lancet*, **404**, 1199 (2024); https://doi.org/10.1016/S0140-6736(24)01867-1
4. I. Górniak, R. Bartoszewski and J. Króliczewski, *Phytochem. Rev.*, **18**, 241 (2019); https://doi.org/10.1007/s11101-018-9591-z
5. M. Fesatidou, A. Petrou and G. Athina, *Curr. Pharm. Des.*, **26**, 867 (2020); https://doi.org/10.2174/1381612826666200206093815
6. S. Sandhu, Y. Bansal, O. Silakari and G. Bansal, *Bioorg. Med. Chem.*, **22**, 3806 (2014); https://doi.org/10.1016/j.bmc.2014.05.032
7. M. Garland, S. Loscher and M. Bogyo, *Chem. Rev.*, **117**, 4422 (2017); https://doi.org/10.1021/acs.chemrev.6b00676
8. K.S. Suvaiv, S.M. Hasan, S.P. Kushwaha, A. Kumar, I.Z. Ahmad and P. Kumar, *Indian J. Heterocycl. Chem.*, **33**, 249 (2023); https://doi.org/10.59467/IJHC.2023.33.249
9. D.C. Nwobodo, M.C. Ugwu, C.O. Anie, M.T.S. Al-Ouqaili, J.C. Ikem, U.V. Chigozie and M. Saki, *J. Clin. Lab. Anal.*, **36**, e24655 (2022); https://doi.org/10.1002/jcla.24655
10. K. Singh, S. Mujeeb, B. Yogi, V. Ansari and S. Sinha, *Mini Rev. Med. Chem.*, **22**, 1064 (2022); https://doi.org/10.2174/1389557521666210927124511
11. M.V. Kulkarni, G.M. Kulkarni, C.H. Lin and C.M. Sun, *Curr. Med. Chem.*, **13**, 2795 (2006); https://doi.org/10.2174/092986706778521968
12. F.J. Reen, J.A. Gutiérrez-Barranquero, M.L. Parages and F.O. Gara, *Appl. Microbiol. Biotechnol.*, **102**, 2063 (2018); https://doi.org/10.1007/s00253-018-8787-x
13. J. Chen, Y. Yu, S. Li and W. Ding, *Molecules*, **21**, 1501 (2016); https://doi.org/10.3390/molecules21111501

14. L. Balewski, S. Szulta, A. Jaliñska and A. Kornicka, *Front. Chem.*, **9**, 781779 (2021); https://doi.org/10.3389/fchem.2021.781779
15. M. Rizwan, S. Noreen, S. Asim, Z. Liaqat, M. Shaheen and H. Ibrahim, *Chem. Inorg. Mater.*, **2**, 100041 (2024); https://doi.org/10.1016/j.cinorg.2024.100041
16. K.R. Pawar, P.G. Kale, M.S. Nalawade, T. Patil, T. Patil and S. Gaikwad, *Res. J. Pharm. Technol.*, **17**, 1196 (2024); https://doi.org/10.52711/0974-360X.2024.00186
17. J. Ansari, S.P. Kushwaha, V.A. Ansari, K. Singh and S.M. Hasan, *Int. J. Bot. Stud.*, **6**, 836 (2021); https://doi.org/10.52711/0974-360X.2024.00186
18. M. Asif, *Int. J. Adv. Sci. Res.*, **1**, 05 (2015); https://doi.org/10.7439/ijasr.v1i1.1766
19. K.M. Amin, A.M. Taha, R.F. George, N.M. Mohamed and F.F. Elsenduny, *Arch. Pharm.*, **351**, 1700199 (2018); https://doi.org/10.1002/ardp.201700199
20. D. Patel, R. Patel, P. Kumari and N. Patel, *Med. Chem. Res.*, **21**, 1611 (2012); https://doi.org/10.1007/s00044-011-9676-3
21. R. Wardhani, C. Darsih, A. C. Iwansyah, A. Indriati, H.A. Hamid and D.R. Husain, *J. Comput. Biophys. Chem.*, **23**, 161 (2024); https://doi.org/10.1142/S2737416523500564
22. D.R. Husain and R. Wardhani, *Iran. J. Microbiol.*, **13**, 537 (2021); https://doi.org/10.18502/ijm.v13i4.6981
23. A. Daina, O. Michielin and V. Zoete, *Scient. Rep.*, **7**, 42717 (2017); https://doi.org/10.1038/srep42717
24. A.Y. Mohammed and L.S. Ahamed, *Chem. Methodol.*, **6**, 813 (2022); https://doi.org/10.22034/CHEMM.2022.349124.1569
25. C.R. Sahoo, J. Sahoo, M. Mahapatra, D. Lenka, P.K. Sahu, B. Dehury and S.K. Paidesetty, *Arab. J. Chem.*, **14**, 102922 (2021); https://doi.org/10.1016/j.arabjc.2020.102922