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Synthesis and Antibacterial Study of Novel Piperazine Linked Methylene-*bis*-Coumarins

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ABSTRACT

A series of new 6-(2-oxo-3-[(4-arylpiperazino)carbonyl]-2*H*-6-chromenyl-methyl)-3-[(4-arylpiperazino)carbonyl]-2*H*-2-chromenone **9(a-j)** have been synthesized and tested for their antibacterial activity against human pathogenic strains. The antibacterial evaluation data revealed that the compounds containing 4-methoxyphenyl, 4-fluorophenyl, 4-nitrophenyl and 4-hydroxyphenyl moieties at 4-position of the piperazine ring exhibited potent inhibitory activity towards all the tested bacterial strains. Further, the compounds containing phenyl and 4-methylphenyl moieties showed good activity towards *P. aeruginosa* and *C. violaceum*. The 4-nitrophenyl moiety also showed potent activity towards *B. subtilis* and *B. sphaericus*.

KEYWORDS

Methylene-*bis*-coumarin, Piperazine, Antibacterial activity.

INTRODUCTION

The heterocyclic compounds participate in important biochemical processes and are the constituents of main substances in living cells. The heterocyclic ring comprises the core of the active moiety or pharmacophore. More attention is given to nitrogen containing heterocyclic compounds, as they possess a broad spectrum of biological activities and are used in various fields of pharmacy [1-3]. Various biologically active synthetic compounds have six member, two nitrogen containing heterocyclic ring in their structures [4], such important class of compound is piperazine, which is of great significance to the rational drug design. This moiety can be found in various well known drugs with the desired therapeutic uses [5] (Fig. 1) and shows considerable physiological effect such as antituberculosis [6], anthelmenitics [7], antianginal [8], anticancer [9], analgesic [10], antidepressant [11], antipsychotic [12], antidiabetic [13], antihistamines [14], hypolipidemic [15]. The modification of substitution pattern on the piperazine moiety facilitates a significance difference in the biological and pharmacological effect of the resultant molecules and this moiety has encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents [16].

Similarly, coumarins are the class of compounds and are naturally occurring [17] and involved in the actions of plant growth hormones [18] and growth regulators [19], the control

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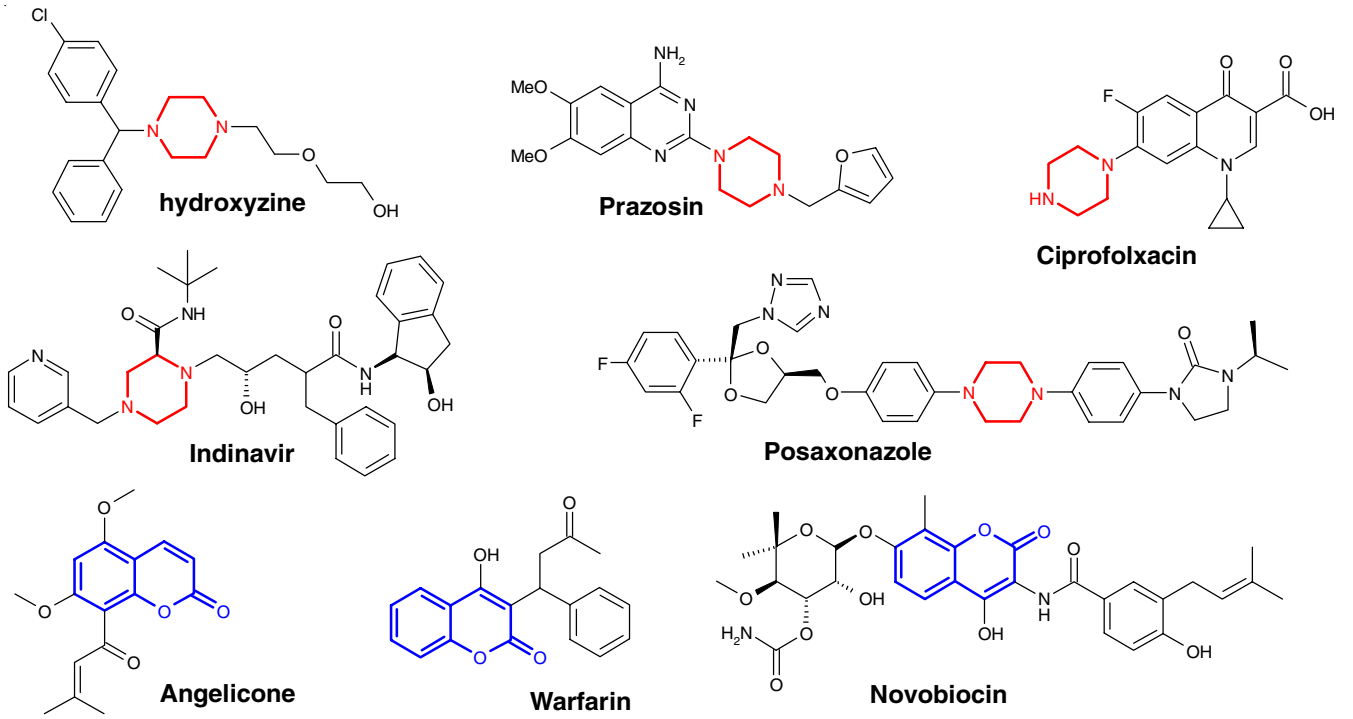


Fig. 1. Clinically used drugs with piperazine/coumarin rings

of respiration [20], photosynthesis [21], as well as defence against infection [22]. Also, they have important effects in plant biochemistry [23], physiology [24] and acting as antioxidants [25], enzyme inhibitors [26] and precursors of toxic substances [27]. The coumarin and its derivatives are medicinal candidates for drugs with strong pharmacological activity, low toxicity and side effects, fewer drug resistance, high bioavailability, broad spectrum, better curative effects and are used as analgesics [28], anticoagulant [29], specific inhibitors of α-chymotripsin [30], human leukocyte elastase [31], diuretics [32], platelet aggregation [33], anticancer [34], inhibitor of HIV-1 protease [35] and antibacterial [36]. They are also present or used in perfumes and cosmetics [37], alcoholic beverages [38] and laser dyes [39]. The large conjugated system in the coumarin ring, with electron rich and charge transport properties, is important in the interaction of this scaffold with molecules or ions. Moreover, the unique and versatile oxygen containing heterocyclic structure makes coumarin compounds occupy an important place in medicinal chemistry. Further, there are important drugs containing the coumarin ring, which are in clinical use are depicted in Fig. 1.

Further, in spite of a large number of antibiotics and chemotherapeutic agents available for medical use, but the treatment of infectious diseases still challenging problem [40] because of emerging infectious diseases [41] and the microbial pathogens have turning out to be more resistant towards antibiotics/chemotherapeutics [42], resulted a significant universal health failure [43]. Therefore, the broad spectrum potency is preferred for newly found antimicrobial agents, the recent efforts have been made towards the investigation of more potential antimicrobial agents [44]. Further, the synthesis of a variety of *bis*-heterocyclic compounds has received great attention [45] not only as main chain polymers but also because many biologically active natural [46] and synthetic products have molecular symmetry [47]. Thus, *bis*-heterocyclic compounds may eliminate the microbial resistance. In the synthesis of *bis*-heterocyclic, researchers tried to extend the existing method by using a broad range of protocols to improve the various scope and limitations regarding their yield, purity and mostly on the various biological applications [48].

Owing to the immense importance and varied biological activities exhibited by coumarins and piperazines and in continuation of our ongoing research on biologically active *bis*-heterocyclic compounds [49-53], it was considered to synthesize the *bis*-heterocyclic compounds containing coumarin and piperazine pharmacophores in one molecule for enhancing biological activity (Fig. 2). In the present study, the synthesis and antibacterial screening of new series of novel 6-(2-oxo-3-[(4-arylpiperazino)carbonyl]-2*H*-6-chromenylmethyl)-3-[(4-arylpiperazino)carbonyl]-2*H*-2-chrome- none **9(a-j)** with a view to explore their potential biological activity. The antibacterial activities of the compounds have also been evaluated.

EXPERIMENTAL

All the chemical and solvent were of commercial grade and were used as supplied or were prepared according to procedures described in literature. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F_{254} plates from Merck and compounds visualized either by exposure to UV light. Melting points were determined in open capillary tube on a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded using KBr disk on a Perkin–Elmer FTIR spectrometer. The NMR spectra were recorded on a Varian Gemini spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Mass spectra were recorded on a VG micro mass 7070H spectrometer. The structure of all the synthesized compounds was assigned on basis of their analytical data and spectral data.

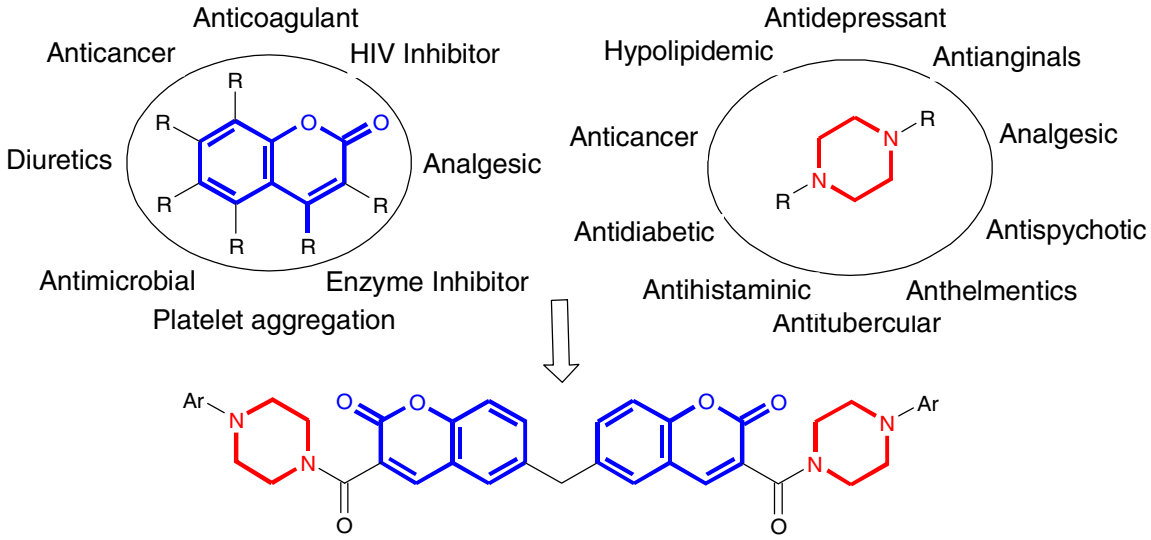


Fig. 2. Title compounds containing coumarin and piperazine moieties

Preparation of 5-(3-formyl-4-hydroxybenzyl)-2-hydroxybenzaldehyde (2): Trioxane (0.2 mol) was mixed to the solution of salicylaldehyde (**1**) (0.65 mol in 50 mL of glacial acetic acid). To this a mixture of 0.5 mL of conc. sulfuric acid and 2.5 mL of glacial acetic acid was added slowly with stirring in a nitrogen atmosphere at a temperature of ≈ 90 °C. This temperature was maintained for 24 h, with constant stirring solution. The reaction mixture was then poured into ice cold water and allowed to stand overnight. The deposited solid was filtered and extracted twice with ether and the solid obtained was purified by crystallization in acetone to give pure **2** as white solid in 62 % yield, m.p.132-134. IR (KBr, ν_{max}, cm^{-1}): 3435, 2736, 1665, 843, 813; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.99 (s, 2H, CH_2), 7.32 (m, 4H, ArH), 7.40 (d, *J* = 8.1 Hz, 2H, ArH), 9.93 (s, 2H, CHO), 10.9 (s, 2H, OH); MS: *m/z* 256 (M^+), 227, 181, 152, 135, 77.

Preparation of ethyl 6-[3-(ethoxycarbonyl)-2-oxo-2*H*-6-chromenyl]methyl-2-oxo-2*H*-3-chromene carboxylate (3): A mixture of compound **2** (0.005 mol), diethylmalonate (0.01 mol), piperidine (0.05 mol) and glacial acetic acid (0.005 mL) were added to 30 mL anhydrous methanol. The mixture was refluxed for 4 h and then the resultant was poured into 50 mL hot water. The water was cooled down to room temperature and the residue was filtered and recrytallized with 95 % alcohol to give compound **3** in 48 % yields.

Preparation of 6-[(3-carboxy-2-oxo-2*H*-6-chromenyl)-methyl]-2-oxo-2*H*-3-chromene carboxylic acid (4): A solution of compound **3** (0.02 mol) and 0.5 % NaOH (250 mL) solution were added to ethylalcohol (50 mL). The mixture was refluxed for 3 h and then it was acidified with HCl to pH 2. The mixture was cooled down to 0 °C and crystallized with ethanol to give **4** in 66 % yield. IR (KBr, ν_{max}, cm^{-1}): 3341, 3037, 2841, 1716, 1682, 1444, 1229, 1028, 755; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.08 (s, 2H, CH_2), 7.15-7.20 (m, 6H, Ar), 7.68 (s, 2H, CH=C), 10.90 (s, 2H, COOH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.1, 118.4, 119.9, 120.1, 122.8, 125.3, 132.2, 138.0, 151.5, 157.8, 166.1; MS: *m/z* 392 (M^+).

Preparation of 6-[(3-chlorocarbonyl-2-oxo-2*H*-6-chromenyl)methyl]-2-oxo-2*H*-3-chromene-carbonyl chloride (5): To a dried compound **4** (0.002 mol), thionyl chloride (5 mL) was added. The mixture was refluxed for 4 h and then the resultant was removed with simple distillation to give compound **5** in 59 % yields. The compound can be used directly without purification. IR (KBr, ν_{max}, cm^{-1}): 3043, 2832, 1721, 1687, 1682, 1458, 1237, 1032, 755, 686; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.11 (s, 2H, CH_2), 7.01 (d, *J* = 6.9 Hz, 2H, ArH), 7.25-7.30 (m, 4H, ArH), 8.66 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 45.8, 118,7, 121.9, 122.6, 124.2, 132.6, 136.0, 154.5, 158.6, 159.2; MS: *m/z* 429 (M^+).

General procedure for the synthesis of 1-arylpiperazine 8(a-j): The mixture of substituted aniline (10 g, 78.4 mmol), compound **7** (14.7 g, 82.4 mmol) and *p*-TsOH (0.5, 3 %) in xylene (44 mL) was heated to reflux at 140-145 ºC for 12-24 h. When the reaction was completed, the mixture was cooled to room temperature to crystallize. The crystal was filtered and recrystallized in the distilled water.

1-Phenylpiperazine (8a): IR (KBr, ν_{max}, cm^{-1}): 3398, 3032, 2982; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.39-2.43 (m, 4H, CH_2-N of piperazine), 3.10-3.16 (m, 4H, CH_2-N of piperazine), 4.37 (bs, 1H, NH of piperazine), 6.80-6.90 (m, 3H, ArH), 7.19 (d, 2H, *J* = 8.1 Hz, ArH); MS: *m/z* 162 (M^+).

General procedure for the synthesis of 6-(2-oxo-3-[(4-arylpiperazino)carbonyl]-2*H*-6-chromenylmethyl)-3-[(4-arylpiperazino)carbonyl]-2*H*-2-chromenone (9a-j): To a mixture of compound **5** (0.001 mol), corresponding arylpiperazine derivatives (**8**) (0.0022 mol) and potassium carbonate (0.003 mol), a mixture of solvent containing acetone (25 mL) and ethyl alcohol (25 mL) was added. The mixture was refluxed for 8 h, after completion of the reaction the solvent was evaporated. The resultant compounds **9(a-j)** were purified by chromatography using EtOAc/hexane.

6-(2-Oxo-3-[(4-phenylpiperazino)carbonyl]-2*H*-6-chromenylmethyl)-3-[(4-phenylpiperazino)carbonyl]-2*H*-2-chromenone (9a): IR (KBr, ν_{max}, cm^{-1}): 3042, 2831, 1717, 1692, 1680, 1469, 1234, 1030, 754; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.37-3.42 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 4.17 (s, 2H, CH_2), 6.90-7.10 (m, 6H, ArH), 7.25-7.30 (m, 4H, ArH), 7.40-7.50 (m, 6H, ArH), 8.07

(s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.1, 42.4, 55.7, 113.5, 114.3, 114.8, 121.0, 121.5, 123.5, 124.6, 132.3, 136.8, 152.7, 154.2, 161.3, 164.1; MS: *m/z* 680 (M^+).

3-[4-(4-Methylphenyl)piperazino]carbonyl-6-[(3-[4-(4-methylphenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)-methyl]-2*H*-2-chromenone (9b): IR (KBr, ν_{max}, cm^{-1}): 3046, 2835, 1712, 1694, 1686, 1471, 1231, 1039, 757; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.31 (s, 3H, CH_3), 3.38-3.45 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 4.14 (s, 2H, CH_2), 6.90-7.10 (m, 8H, ArH), 7.40-7.50 (m, 6H, ArH), 8.10 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 23.7, 36.4, 42.6, 54.6, 114.1, 114.2, 114.9, 121.1, 121.8, 122.9, 123.5, 130.1, 136.8, 144.7, 154.3, 162.3, 164.4; MS: *m/z* 708 (M^+).

3-[4-(4-Methoxyphenyl)piperazino]carbonyl-6-[(3-[4-(4-methoxyphenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)methyl]-2*H*-2-chromenone (9c): IR (KBr, ν_{max}, cm^{-1}): 3032, 2817, 1721, 1691, 1682, 1465, 1239, 1069, 754; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.40-3.45 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 3.85 (s, 3H, OCH_3), 4.11 (s, 2H, CH_2), 6.70 (d, *J* = 6.9 Hz, 4H, ArH), 6.85 (d, *J* = 6.9 Hz, 4H, ArH), 7.40-7.45 (m, 6H, ArH), 8.12 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.4, 42.5, 56.1, 57.2, 113.3, 113.9, 114.4, 121.2, 122.1, 125.3, 123.7, 135.7, 147.6, 152.5, 153.4, 163.0, 164.3; MS: *m/z* 730 (M^+).

3-[4-(4-Fluorophenyl)piperazino]carbonyl-6-[(3-[4-(4-fluorophenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)-methyl]-2*H*-2-chromenone (9d): IR (KBr, ν_{max}, cm^{-1}): 3032, 2817, 1721, 1691, 1682, 1465, 1239, 1069, 752; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.35-3.40 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 4.16 (s, 2H, CH_2), 6.75 (d, *J* = 7.1 Hz, 4H, ArH), 6.95 (d, *J* = 7.1 Hz, 4H, ArH), 7.40-7.50 (m, 6H, ArH), 8.09 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 35.1, 42.2, 55.3, 113.8, 114.1, 115.0, 121.2, 121.1, 123.7, 125.1, 136.4, 144.8, 154.4, 160.1, 161.2, 164.5; MS: *m/z* 716 (M^+).

3-[4-(4-Bromophenyl)piperazino]carbonyl-6-[(3-[4-(4-bromophenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)-methyl]-2*H*-2-chromenone (9e): IR (KBr, ν_{max}, cm^{-1}): 3028, 2820, 1716, 1689, 1683, 1462, 1241, 1069, 755; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.35-3.40 (m, 8H, CH_2 of piperazine), 3.65-3.70 (m, 8H, CH_2 of piperazine), 4.10 (s, 2H, CH_2), 6.75 (d, *J* = 7.2 Hz, 4H, ArH), 7.22 (d, *J* = 7.2 Hz, 4H, ArH), 7.40-7.50 (m, 6H, ArH), 8.10 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.2, 42.7, 55.0, 113.7, 114.2, 117.2, 121.2, 121.4, 122.8, 126.7, 132.7, 135.7, 149. 7, 153.1, 161.7, 164.6; MS: *m/z* 838 (M^+).

3-[4-(3-Nitrophenyl)piperazino]carbonyl-6-[(3-[4-(3-nitrophenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)-methyl]-2*H*-2-chromenone (9f): IR (KBr, ν_{max}, cm^{-1}): 3044, 2835, 1722, 1691, 1686, 1459, 1372, 1234, 1072, 752; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.40-3.45 (m, 8H, CH_2 of piperazine), 3.65-3.70 (m, 8H, CH_2 of piperazine), 4.12 (s, 2H, CH_2), 6.90-7.10 (m, 6H, ArH), 7.40-7.50 (m, 8H, ArH), 8.12 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 34.9, 42.1, 55.4, 109.9, 112.2, 113.6, 114.3, 117.4, 121.5, 121.9, 123.5, 127.5, 136.8, 146.8, 149.1, 154.3, 161.4, 164.5; MS: *m/z* 770 (M^+).

3-[4-(4-Nitrophenyl)piperazino]carbonyl-6-[(3-[4-(4-nitrophenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)-methyl]-2*H*-2-chromenone (9g): IR (KBr, ν_{max}, cm^{-1}): 3048, 2832, 1720, 1692, 1684, 1458, 1377, 1234, 1072, 752; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.35-3.40 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 4.09 (s, 2H, CH_2), 7.21 (d, *J* = 7.7 Hz, 4H, ArH), 7.35-7.45 (m, 6H, ArH), 7.87 (d, *J* = 7.7 Hz, 4H, ArH), 8.10 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.1, 42.4, 55.7, 111.6, 113.5, 114.8, 121.0, 121.5, 123.5, 124.8, 136.8, 137.9, 154.1, 155.0, 161.1, 164.5; MS: *m/z* 770 (M^+).

3-[4-(2,5-Difluorophenyl)piperazino]carbonyl-6-[(3-[4-(2,5-difluorophenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)methyl]-2*H*-2-chromenone (9h): IR (KBr, ν_{max}, cm^{-1}): 3051, 2812, 1714, 1690, 1681, 1464, 1237, 1062, 751; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.40-3.45 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 4.13 (s, 2H, CH_2), 6.70-6.75 (m, 4H, ArH), 6.90-7.00 (m, 2H, ArH), 7.40-7.45 (m, 6H, ArH), 8.16 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.4, 42.6, 55.5, 107.4, 108.0, 116.8, 113.5, 114.8, 121.0, 121.5, 123.5, 129.8, 136.8, 154.2, 158.1, 161.3, 162.2, 164.3; MS: *m/z* 752 (M^+).

3-[4-(3-Hydroxyphenyl)piperazino]carbonyl-6-[(3-[4-(3-hydroxyphenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)methyl]-2*H*-2-chromenone (9i): IR (KBr, ν_{max}, cm^{-1}): 3428, 3041, 2870, 1707, 1694, 1682, 1459, 1236, 1062, 757; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.40-3.45 (m, 8H, CH_2 of piperazine), 3.60-3.65 (m, 8H, CH_2 of piperazine), 4.15 (s, 2H, CH_2), 6.50-6.60 (m, 8H, ArH, OH), 7.40-7.50 (m, 6H, ArH), 8.14 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.7, 42.2, 55.6, 103.2, 106.6, 110.5, 113.5, 114.8, 121.0, 121.5, 123.5, 128.1, 136.8, 141.3, 154.2, 158.2, 161.3, 164.1; MS: *m/z* 712 (M^+).

3-[4-(4-Hydroxyphenyl)piperazino]carbonyl-6-[(3-[4-(4-hydroxyphenyl)piperazino]carbonyl-2-oxo-2*H*-6-chromenyl)methyl]-2*H*-2-chromenone (9j): IR (KBr, ν_{max}, cm^{-1}): 3431, 3037, 2877, 1705, 1691, 1680, 1455, 1232, 1061, 756; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.35-3.40 (m, 8H, CH_2 of piperazine), 3.60-3.70 (m, 8H, CH_2 of piperazine), 4.14 (s, 2H, CH_2), 5.07 (s, 2H, OH), 6.70-6.75 (m, 8H, ArH), 7.40-7.50 (m, 6H, ArH), 8.16 (s, 2H, CH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3, 42.2, 55.5, 113.8, 114.2, 116.1, 121.1, 121.4, 122.3, 123.6, 136.9, 147.1, 153.3, 154.7, 161.2, 164.4; MS: *m/z* 712 (M^+).

RESULTS AND DISCUSSION

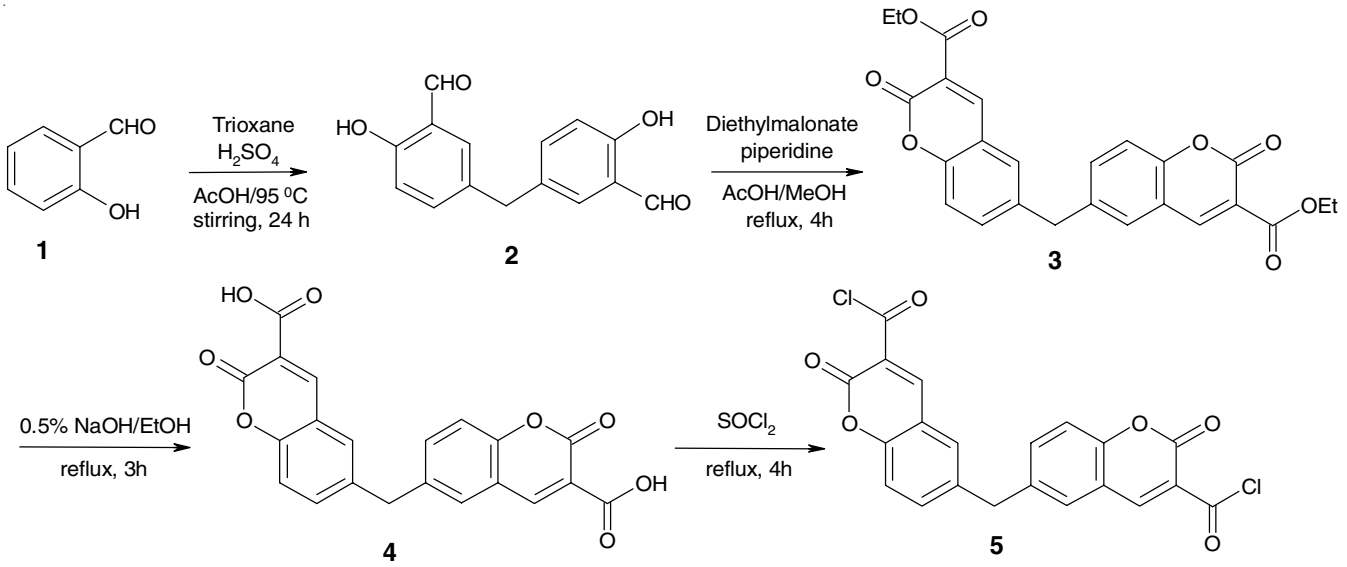
Condensation of salicylaldehyde (**1**) with trioxane in the presence of a mixture of conc. sulphuric acid and acetic acid gave 5-(3-formyl-4-hydroxybenzyl)-2-hydroxybenzaldehyde (**2**) in 62 % yield [54]. The cyclo-condensation of compound **2** with diethylmalonate in the presence of piperidine in a mixture of acetic acid and ethyl alcohol at reflux for 5 h resulted the ethyl 6-[3-(ethoxycarbonyl)-2-oxo-2*H*-6-chromenyl]methyl-2-oxo-2*H*-3-chromenecarboxylate (**3**) in 56 % of yield [55]. Further, hydrolysis of compound **3** in the presence of 0.5 % NaOH in ethyl alcohol at reflux for 3 h led to the formation of 6-[(3-carboxy-2-oxo-2*H*-6-chromenyl)methyl]-2-oxo-2*H*-3-chromenecarboxylic acid (**4**) in 66 % of yield, which was reacted with thionylchloride at reflux for 4 h led to the formation of 6-[(3-chlorocarbonyl-2-oxo-2*H*-6-chromenyl)methyl]-

2-oxo-2*H*-3-chromenecarbonyl chloride (**5**) in 59 % of yields (**Scheme-I**). The structures of compounds were confirmed by its EI mass, IR, ^{1}H NMR and ^{13}C NMR spectral data.

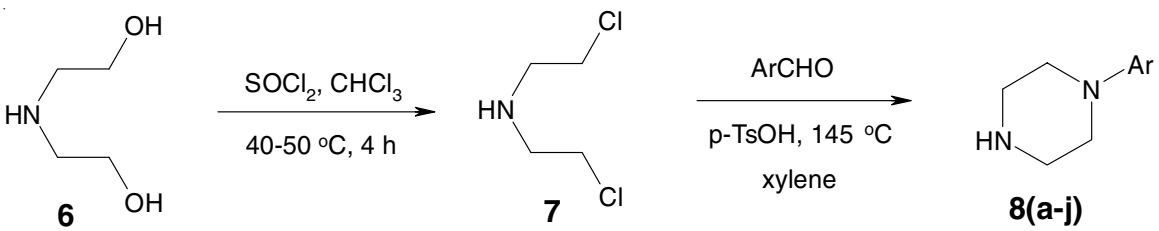
The IR spectrum of **2** showed the absorption band at 2736, 1665, 813 and a broad band in the 3435 cm^{-1} region. Its protons NMR spectrum showed resonances at δ 10.9 as singlet for two protons corresponding to hydroxyl protons, at δ 9.93 as singlet for two protons of carboxyl group, at δ 7.40 as doublet, 7.32 as multiplet corresponding to aromatic protons and at δ 3.99 as singlet for two protons corresponding to methylene protons. Its mass spectrum showed a signal at *m/z* 256 corresponding to molecular ion, at *m/z* 227 corresponding to the ion, which resulting by the loss of formyl radical, at *m/z* 181 corresponding the ion resulted subsequent loss of water and carbon monoxide from the molecular ion and the other signals at *m/z* 152, 135 and 77 confirming its structure. The IR spectrum of **4** showed absorption bands appeared at 3341 (OH), 1710 (C=O, lactone) and 1682 (C=O) cm^{-1}. Its proton NMR spectrum showed a prominent signals corresponding to protons of methylene bridge appeared at δ 4.08 as a singlet for two protons, the carboxylic proton appeared at δ 10.9 ppm as singlet. Its ^{13}C NMR spectra showed the signals corresponding to the carbons of coumarin ring appeared at δ 118.4, 119.9, 122.8, 151.5, 157.8, the signal of carboxyl carbon in acid group appeared at δ 166.1 ppm. The IR spectrum of compound **5** showed absorption bands appeared at 1721 (C=O, lactone) and 1682 (C=O) cm^{-1}. Its proton NMR spectrum showed a prominent signals corresponding to protons of methylene bridge appeared at δ 4.11 as a singlet for two protons, the proton of alkene of coumarin ring appeared at δ 8.66 ppm as singlet. Its ^{13}C NMR spectra showed the signals corresponding to the carbons of coumarin ring appeared at δ 118.7, 121.9, 122.6, 154.5, 158.6, the signal of carboxyl carbon in acid group appeared at δ 158.2 ppm.

Further, the chlorination of diethanolamine (**6**) by thionyl chloride at 40-50 ^{0}C in chloroform for 4 h, led to the formation of *N*,*N*-di(2-chloroethyl)amine (**7**), which on cyclo-condensation with various arylamines in the presence of *p*-TsOH in xylene at reflux temperature for 12-24 h, to afford 1-arylpiperazine (**8a-j**) (**Scheme-II**). The IR spectrum of compound **7a** showed absorption bands at 3398 and 3032 cm^{-1} due to O-H and Ar-H. Its proton NMR spectra showed the signals corresponding to the aromatic protons at δ 6.80-6.90 and 7.19 ppm as multiplet for three protons and doublet for two protons respectively. The proton corresponding to the -NH group of piperazine ring appeared at δ 4.37as a broad singlet, the protons of piperazine ring appeared at δ 2.39-2.43 and δ 3.10-3.16 ppm as multiplets.

The condensation of compound **5** with the corresponding arylpiperazine derivatives (**8a-j**) [26] in the presence of potasium carbonate, in a mixture of solvent containing acetone and ethyl alcohol (1:1) at reflux for 8 h, resulted the corresponding compounds 6-(2-oxo-3-[(4-arylpiperazino)carbon-yl]-2*H*-6-chromenyl-methyl)-3-[(4-arylpiperazino)carbonyl]-2*H*-2-chromenone (**9a-j**) (**Scheme-III**). The structure of compounds

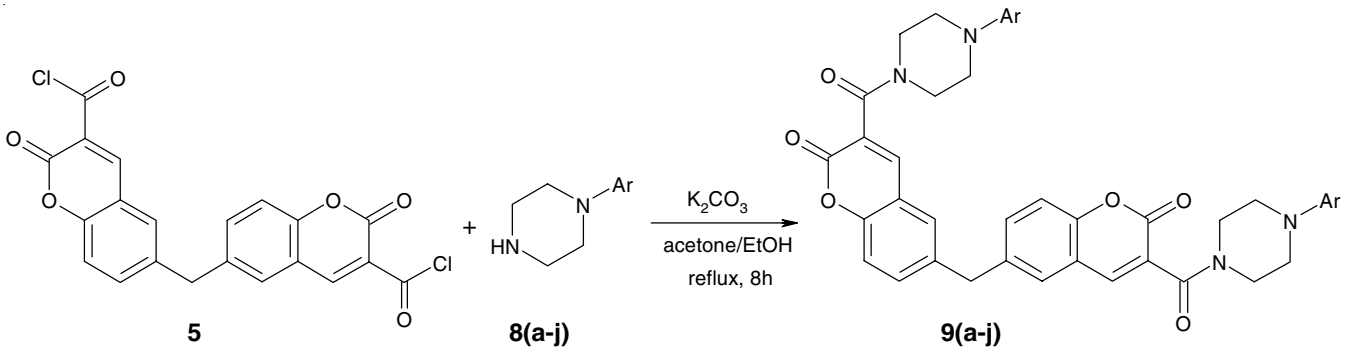


Scheme-I



8: Ar = a) C_6H_5; b) 4-CH_3-C_6H_4; c) 4-OCH_3-C_6H_4; d) 4-F-C_6H_4; e) 4-Br-C_6H_4; f) 3-NO_2-C_6H_4; g) 4-NO_2-C_6H_4; h) 2,5-$(F)_2$- C_6H_3; i) 3-OH-C_6H_5; j) 4-OH-C_6H_4

Scheme-II



8: Ar = a) C_6H_5; b) 4-CH_3-C_6H_4; c) 4-OCH_3-C_6H_4; d) 4-F-C_6H_4; e) 4-Br-C_6H_4; f) 3-NO_2-C_6H_4; g) 4-NO_2-C_6H_4; h) 2,5-$(F)_2$- C_6H_3; i) 3-OH-C_6H_5; j) 4-OH-C_6H_4

Scheme-III

TABLE-1
ANTIBACTERIAL ACTIVITY OF COMPOUNDS **9(a-j)**

Compound	Minimum inhibitory concentration (MIC, µg/mL)					
	B. subtilis	*B. sphaericus*	*S. aureus*	*P. aeruginosa*	*K. aerogenes*	*C. violaceum*
9a	25	24	25	25	30	25
9b	29	26	30	25	27	20
9c	13	17	18	19	21	16
9d	12	18	19	20	18	19
9e	17	19	30	25	24	25
9f	24	30	29	30	30	30
9g	14	17	15	19	18	15
9h	26	27	27	30	26	28
9i	30	20	21	22	27	24
9j	12	16	15	21	23	14
Penicillin	26	25	26	20	30	20

were confirmed by its EI mass, IR, ^{1}H and ^{13}C NMR spectral data. The IR spectra of **9a**, appearance of bands at 1717 (C=O, lactone) cm^{-1} and the absence of -OH (acid) and -NH (piperazine) stretching vibrations provided the evidence for condensation, involving piperazine-NH and triazole-COOH groups. Similarly, the absence of signals for the -OH and -NH protons in the ^{1}H NMR spectra followed by the presence of piperazine protons in the region of δ 3.37-3.42 and 3.60-3.70, the aromatic protons in the region of δ 6.90-7.10, 7.25-7.30 and 7.40-7.50 ppm well support the structures. The proton of alkene at coumarin ring appear at δ 8.07. In the ^{13}C NMR spectra, the signals of coumarin carbons observed nearly at δ 113.5, 121.0, 121.5, 152.7 and 161.3 ppm, similarly the signals corresponding to the C=O were observed at δ 164.1. In summary all the newly synthesized compounds exhibited satisfactory spectral data consistent with their molecular structures.

Antibacterial activity: The antibacterial activity of compounds **9(a-j)** was evaluated using the tube-dilution method [56] by measuring the minimum inhibitory concentration (MIC) in mg/mL, against six representative organisms *viz. B. subtilis, B. sphaericus, S. aureus, P. aeruginosa, K. aerogenes* and *C. violaceum*. Standard antibacterial agent penicillin was also screened under identical conditions for comparison. The minimum inhibitory concentrations are given in Table-1. It has been observed that the test compounds exhibited an interesting biological activity however, with a degree of variation.

Antibacterial evaluation revealed that the compounds **9(a-j)** were showed good antibacterial activity towards all the tested strains. Further, compounds containing 4-methoxyphenyl (**9c**), 4-fluorophenyl (**9d**), 4-nitrophenyl (**9g**) and 4-hydroxyphenyl (**9j**) moieties at 4-position of the piperazine ring exhibited potent inhibitory activity towards all the tested microorganisms. Further, the compounds containing phenyl (**9a**) and 4-methylphenyl (**9b**) moieties showed good activity towards *P. aeruginosa* and *C. violaceum*. Compound **9e** containing 4-nitrophenyl moiety, also showed potent activity towards *B. subtilis* and *B. sphaericus.*

Conclusion

In conclusion, a new series of novel 6-(2-oxo-3-[(4-phenylpiperazino)carbonyl]-2*H*-6-chromenylmethyl)-3-[(4-phenylpiperazino)carbonyl]-2*H*-2-chromenone (**9a-j**) has been synthesized and evaluated their antibacterial activity. Among the screened, the compounds **9c**, **9d**, **9g** and **9j** showed more antibacterial activity compared to the standard drugs.

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