**N-[coumarin-6-yl] spiro-indoloazetidin-2-ones/thiazolidin-4-ones의 합성과 항균검사**

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**Synthesis and antibacterial screening of N-[coumarin-6-yl] spiro-indoloazetidin-2-ones/thiazolidin-4-ones**

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요 약. **N-[Coumarin-6-yl]carbamic acid hydrazide 2a-c와 이사틴을 축합하여 indole-2-oxo-3-(2’-oxo-2'H-benzopyran-6-yl)-semicarbazone 3a-c를 얻었다. 무수 ZnCl2의 촉매하에서 두소 1,4-dioxane용매에서 Thiglycollic acid치 리를 한 화합물 3a-c는 3-(2’-oxo-2'H-benzopyran-6-yl)-spiro-3H-[indole-(1H,2H)-3,2-(4H)-thiazolidin-1-yl]-2,4-dioxo-urea 4a-c를 제공했고, 화합물 3a-c는 3-(2’-oxo-2'H-benzopyran-6-yl)-spiro-3H-[indole-(1H,2H)-3-chloro-2,4-dioxoazetidin-1-yl]-urea를 얻기 위해 1-chloroacetyl chloride를 처리했다. 제조된 화합물은 스피로-미셀의 방출시료를 기초로 확인되었다. 합성된 화합물 3a-c, 4a-c와 5a-c는 gram-양성과 gram-음성 박테리아에 대한 항균 활성을 검사 했고, 상당한 항균 활성을 보였다.**

주제어: 6-아미노-코마린, 스피로 화합물, 티아졸리딘, 아제티딘, 생물학적 활성도

**ABSTRACT.** **N-[Coumarin-6-yl]carbamic acid hydrazide 2a-c on condensation with isatin yields indole-2-oxo-3-(2’-oxo-2'H-benzopyran-6-yl)-semicarbazone 3a-c. Compound 3a-c on treatment with thiglycollic acid in dry 1,4-dioxane in presence of catalytic amount of anhydrous ZnCl2 affords 3-(2’-oxo-2'H-benzopyran-6-yl)-spiro-3H-[indole-(1H,2H)-3,2-(4H)-thiazolidin-1-yl]-2,4-dioxo-urea 4a-c, compound 3a-c were also treated with chloracetyl chloride to afford 3-(2’-oxo-2'H-benzopyran-6-yl)-spiro-3H-[indole-(1H,2H)-3-chloro-2,4-dioxoazetidin-1-yl]-urea. All the newly synthesized compounds have been confirmed on the basis of their spectral and analytical data. The synthesized compounds 3a-c, 4a-c and 5a-c were screened for the antibacterial activities against Gram positive and Gram negative bacteria and have been found to exhibit significant antibacterial activities.**

**Keywords:** 6-Amino-coumarin, Spiro Compound, Thiazolidin, Azetidine, Biological Activity

**INTRODUCTION**

Coumarins are nowadays an important group of organic compounds that are used as bactericides, fungicides, anti-inflammatory, and antimutagen agents. These pharmacological properties of coumarins aroused our interest in synthesizing several new compounds featuring different heterocyclic rings fused onto the coumarin moiety with the aim of obtaining more potent pharmacologically active compounds. Various indole derivatives show a wide range of biochemical properties. It has been reported that if the indole ring is joined to other heterocyclic compounds through a spiro-carbon atom, the resulting compounds show increased spectrum of biological activities. Spiro nuclei
have also drawn considerable attention of the chemist because of their antiseptic, analgesic, and broad-spectrum antimicrobial activities. Hence, introduction of spiro nucleus to such vital molecules will enhance their biological activity.

By observing the importance of the above heterocycles, we planned to synthesize spiro-indoloazetidin-4-one and spiro-indoloazetidin-2-ones ring system from 6-aminocoumarin as starting materials which may possess some of the above biological activity.

RESULTS AND DISCUSSION

Ethyl-N-(coumarin-6-yl)carbamate was obtained by refluxing 6-aminocoumarin with chloroethylformate in presence of triethyl amine to afford compound 1a-e which was subsequently converted into its acid hydrazide 2a-e. The IR spectrum of 1e in KBr showed band at 1730 cm\(^{-1}\) for coumarin stretching and at 1720 cm\(^{-1}\) for carbonyl stretching of -NH-C=O. In its \(^1H\) NMR it shows signals as triplet for CH\(_3\) protons at 1.40 and as a quartet at 4.35 for methylene protons and a peak at 8.30 for -NH group which is D\(_2\)O exchangeable. IR spectrum in KBr of compound 2e showed band at 3100-3300 cm\(^{-1}\) for -NH stretching. The \(^1H\) NMR showed absence of signals for methyl protons and methylene protons which were observed in compound 1a-e as a triplet and quartet indicating the formation of its acid hydrazide.

Compound 2a-e was treated with isatin in ethanol to give indole-2-oxo-3-(2-oxo-2H-benzopyran-6-yl)-semicarbazone 3a-e. The IR spectrum of compound 3e in KBr showed bands at 3456 cm\(^{-1}\) and 3430 cm\(^{-1}\) for -NH stretching, at 1605 cm\(^{-1}\) for -CH aromatics stretching, at 1723 cm\(^{-1}\) for carbonyl carbon. \(^1H\) NMR spectrum in CDCl\(_3\) shows peak at 12.5 ppm for -NH of indole nucleus which is D\(_2\)O exchangeable, along with other peaks. Compound 3a-e separately on refluxing with thiaglycollic acid in dry 1,4-dioxane and with chloroacetyl chloride gave 3-(2-oxo-2H-benzopyran-6-yl)-spiro-3H-[indole-(1H,2H)]-3,2-(4H)-thiazolidin-1-yl]-2,4-dioxo-urea 4a-e, and 3-(2-oxo-2H-benzopyran-6-yl)-spiro-3H-[indole-(1H,2H)]-3-chloro-2,4-dioxo-azetidin-1-yl]-urea 5a-e respectively. The IR spectrum of compound 4e in KBr showed bands at 3430 cm\(^{-1}\) for -NH groups, at 1721 cm\(^{-1}\) for carbonyl group. \(^1H\) NMR in CDCl\(_3\) showed a singlet at 4.10 for two protons of -S-CH\(_2\), a singlet at 12.5 for one proton of -NH which is D\(_2\)O exchangeable. In its \(^13C\) NMR showed signals at 45.6 for spiro-carbon atom, and at 159.6 for carbonyl of coumarin, at 175.5 for carbonyl of indole nucleus and at 182.5 for carbonyl of thiaglycollic ring, etc. Similarly compound 5e showed a band at 3450 cm\(^{-1}\) for -NH group in its IR spectrum. \(^1H\) NMR in CDCl\(_3\), showed a singlet at 6.23 ppm for one proton of C-\(\delta\)-H and peak at 5.09 ppm for -CH-Cl. In its \(^13C\) NMR, it showed signals at 38.6 for spiro-carbon atom, a signal at 60.1 for CH-Cl, at 160.0 for carbonyl of Coumarin and at 174.6 for carbonyl of indole nucleus. The mass spectrum of compound 5e shows M+ at 452 and M+2 at 454 indicating the presence of chlorine atom. It also gives positive Beilstein and Lasagnes sodium fusion tests, indicating the presence of halogen. The structures of all the compounds were in agreement with spectral and analytic data and all the synthesized compounds were screened in vitro for antibacterial activity (Scheme 1).

Biological Evaluation

In vitro anti-bacterial evaluation of newly synthesized compounds was done against four bacterial strains viz. *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* by cup plate method. The results indicated that compound 3a-c, 4a-e and 5a-e showed total inhibition of bacterial growth at 100 \(\mu\)g/ml concentration. (Table 1). The zones of inhibition of norfloxacin was taken as 100% and the observed zones of inhibition of the newly synthesized compounds have been expressed as related to the standard. From the antibacterial screening of the compounds 3a-c, 4a-e and 5a-e it has been observed that presence of methyl group in coumarin ring increases the activity.

CONCLUSION

In conclusion we here report the synthesis of...
some novel spiro-azetidin and spiro thiazolidin derivatives of amino coumarin under milder operating conditions. Among the tested compounds compound 5c showed the maximum activity. Rest of the compounds shows moderate to good biological activity.

EXPERIMENTAL

Melting points were taken in open capillaries and were uncorrected. IR spectra (ν in cm⁻¹) were recorded on Perkin Elmer FTIR and NMR (¹H and ¹³C) was recorded on Jeol 300 MHz using TMS as
Table 1. In vitro anti-bacterial spectrum of coumarin derivative.

<table>
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<th>Compound</th>
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Std. Used: Norfloxacin, 100% inhibition at each concentration.

standard. Mass spectra (GC-MS) on a Shimadzu GC-MS QP-2010. All products are purified by recrystallisation. The reaction are followed up and purity of the products is carried out on pre-coated TLC plates (Silica gel 60 F254, Merck), visualizing the spots in ultraviolet light. Column chromatography is performed on Merck silica gel (60-120 mesh). All the compounds gave satisfactory elemental analysis.

**General Method for the synthesis of Ethyl-N-[Coumarin-6-yl] carbamate 1a-e**

To a solution solution 6-amino-coumarin (0.01 mole) in DMF (60 mL), was added chloroethylformate (0.015 moles) and methyl amine (TEA) (0.015 mole) under cold condition. After the addition reaction mixture was refluxed on oil bath for 10-12 hrs. and monitored by TLC, after the reaction is complete, reaction mixture was poured with stirring into ice/cold water containing HCl. The solid obtained was filtered washed with water and dried, then purified by column chromatography using Ethyl acetate-Hexane (2:8) as eluent to give desired product 1a-e.

(1a): yield (62%); m.p 176-178 ºC; IR (KBr, cm⁻¹): 3405(NH), 1730(COOEt), 1720(CO); ¹H NMR (CDCl₃): δ 1.30 (t, 3H, J = 4.20Hz, CH₃-CH₂), 4.26 (q, 2H, J = 6.60Hz, CH₂-CH₂), 6.37(d, 1H, J = 9Hz, C₆-H₇), 7.21(d, 1H, J = 9Hz, C₆-H₃), 7.23(d, 1H, J = 9Hz, C₆-H₁), 7.29(s, 1H, C₆-H₂), 7.75(d, 1H, J = 9Hz, C₆-H₁), 8.28(s, 1H, NH). Elemental analysis [Cal. (Obs.)]: C; 61.80% (61.71%), H; 4.75% (4.71%), N; 6.01% (6.09%).

(1b): yield (58%); m.p 192-194 ºC; IR (KBr, cm⁻¹): 3435 (NH), 1735 (COOEt), 1725 (CO); ¹H NMR (CDCl₃): δ 1.35 (t, 3H, J = 4.20Hz, CH₃-CH₂), 2.25 (s, 3H, CH₃), 4.30 (q, 2H, J = 6.60Hz, CH₂-CH₂), 6.26 (d, 1H, J = 9Hz, C₆-H₇), 7.15 (s, 1H, C₆-H₃), 7.3(s, 1H, C₆-H₁), 7.72(d, 1H, J = 9Hz, C₆-H₂), 8.32 (s, 1H, NH, D₂-O-exchangeable); Elemental analysis [Cal. (Obs.)]: C; 63.15% (63.01%), H; 5.30% (5.27%), N; 5.66% (5.59%).

(1c): yield (55%); m.p 185-187 ºC; IR (KBr, cm⁻¹): 3450 (NH), 2950 (CH-arom.), 1723 (-CO), 1730 (COOEt); ¹H NMR (CDCl₃): δ 1.40 (t, 3H, J = 4.20Hz, CH₂-CH₂), 2.36 (s, 3H, CH₃), 2.40 (s, 3H, CH₃).
CH₃), 4.35 (q, 2H, J = 6.60Hz, CH₃-CH₃), 6.23 (s, 1H, C=H), 7.10 (s, 1H, C=H), 7.25 (s, 1H, C=H), 8.50 (s, 1H, NH, D,O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 64.36% (64.15%), H; 3.79% (5.76%), N; 5.36% (5.29%); MS, m/z (%): M⁺ 261(100), 233(20), 216(30), 188(25), 160(65), 77(20).

**General procedure for N-[coumarin-6-yl]-carbamic acid hydrazide 3a-c**

Compound 1a-c (0.01 mole) and hydrazine hydrate (99.9%) 0.04 mole were refluxed in ethanol for 10 hrs. after the completion of the reaction, excess of ethanol was distilled off and the solid compound was well washed with water and recrystallized from ethanol.

**(2a):** yield; (68%); m.p 196°C; IR (KBr, cm⁻¹): 3384 (NH), 1719 (CO), 1645 (NHCO); ¹H NMR (CDCl₃): δ 3.45 (s, 2H, NH, D,O-exchangable), 4.15 (s, 1H, NH, D,O-exchangable), 6.26 (d, 1H, J = 9Hz, C=H), 7.21(d, 1H, J = 9Hz, C=H), 7.30(d, 1H, J = 9Hz, C=H), 7.35(s, 1H, C=H), 7.78(d, 1H, J = 9Hz, C=H), 8.30(s, 1H, NH); Elemental analysis [Cal. (Obs.)]: C; 54.80% (54.63%), H; 4.14% (4.23%), N; 19.17% (19.27%).

**(2b):** yield; (62%); m.p 205°C; IR (KBr, cm⁻¹): 3432 (NH), 1725 (CO), 1635 (NHCO); ¹H NMR (CDCl₃): δ 2.42 (s, 3H, CH₃), 3.55 (s, 2H, NH, D,O-exchangable), 4.12 (s, 1H, NH, D,O-exchangable), 6.25(d, 1H, J = 9Hz, C=H), 7.12(s, 1H, C=H), 7.28(s, 1H, C=H), 7.76(d, 1H, J = 9Hz, C=H), 8.28(s, 1H, NH, D,O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 56.65% (56.47%), H; 4.73% (4.71%), N; 18.02% (17.97%).

**(2e):** Yield; (65%); m.p 200°C; IR (KBr, cm⁻¹): 3500 (NH), 3100-3300 (NH), 1720 (-CO), 1688 (NHCO); ¹H NMR (CDCl₃): δ 2.20 (s, 3H, CH₃), 2.40 (s, 3H, CH₃), 3.60 (s, 2H, NH, D,O-exchangable), 4.15 (s, 1H, NH, D,O-exchangable), 6.20 (s, 1H, C=H), 6.85 (s, 1H, C=H), 7.20 (s, 1H, C=H), 8.20 (s, 1H, NH, D,O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 58.29% (58.17%), H; 5.30% (5.24%), N; 16.99 (16.92%), MS, m/z (%): M⁺ 247(100), 216(30), 188(40), 160(25), 133(22).

**General procedure for indole-2-oxo-3-(2-oxo-2H-benzopyran-6-yl)-semicarbazone 3a-c.** To the suspension of coumarin-6-yl-carbamic acid hydrazide (0.01 mole) in ethanol (30 mL) was added isatin (0.01 mole) and catalytic amount of glacial acetic acid (3-4 drops) and the reaction mixture was refluxed on water bath for three hrs. The mixture was then cooled and poured into crushed ice the product separated was filtered, washed with water dried and recrystallized from ethanol.

**(3a):** yield; (70%); m.p 200-202°C; IR (KBr, cm⁻¹): 3423 (NH), 2950 (CH-arom.), 1721 (-CO), 1670 (NHCO); ¹H NMR (CDCl₃): δ 6.23(d, 1H, J = 9Hz, C=H), 7.25(d, 1H, J = 9Hz, C=H), 7.32(d, 1H, J = 9Hz, C=H), 7.50(s, 1H, C=H), 7.70-7.75 (m, 4H, aromatic-H), 7.76(d, 1H, J = 9Hz, C=H), 8.11(s, 1H, NH), 11.18(s, 1H, NH, D,O-exchangable), 12.23(s, 1H, NH-indole, D,O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 62.07% (62.17%), H; 3.47% (3.50%), N; 16.08% (16.18%).

**(3b):** yield; (68%); m.p 205-207°C; IR (KBr, cm⁻¹): 3432 (NH), 2945 (CH-arom.), 1721 (-CO), 1675 (NHCO); ¹H NMR (CDCl₃): δ 2.26 (s, 3H, CH₃), 6.24 (d, 1H, J = 9Hz, C=H), 7.30(s, 1H, C=H), 7.50(s, 1H, C=H), 7.70-7.75 (m, 4H, aromatic-H), 7.80(d, 1H, J = 9Hz, C=H), 8.15(s, 1H, NH), 11.20(s, 1H, NH, D,O-exchangable), 12.30(s, 1H, NH-indole, D,O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 62.98% (62.79%), H; 3.89% (3.83%), N; 15.46% (15.51%).

**(3e):** yield; (65%); m.p 198-200°C; IR (KBr, cm⁻¹): 3456 (NH), 2950 (CH-arom.), 1675 (NHCO); ¹H NMR (CDCl₃): δ 2.31(s, 3H, CH₃), 2.50 (s, 3H, CH₃), 6.21 (s, 1H, C=H), 7.21 (s, 1H, C=H), 7.60(s, 1H, C=H), 7.65-7.85(m, 4H, aromatic-H), 8.20 (s, 1H, NH, D,O-exchangable), 11.50 (s, 1H, NH, D,O-exchangable), 12.50 (s, 1H, NH-indole, D,O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 63.83% (63.65%), H; 4.28% (4.33%), N; 14.89% (14.80%); MS, m/z (%): M⁺ 376(100), 231(25), 188(15), 145(5), 161(15), 133(20), 77(20).

**General procedure for synthesis of 3-[2-(2-oxo-2H-benzopyran-6-yl)-spiro-3H-indole-(1H, 2H)-3,2-(4H)-thiazolidin-1-yl]-2,4-dioxo-urea 3a-c.** Compound 3a-c (0.01 mole) and thioglycolic acid (0.01 mole) was refluxed in presence of catalytic amount of anhydrous ZnCl₂ in dry 1, 4-dioxane (25 mL) for 6 hrs. After the completion of reaction,
excess of I, 4 dioxane was evaporated under rotor evaporator to give solid, the solid obtained was filtered, washed with water and purified by recrystallization from methanol to give compd. 4a-c.

(4a): yield, (72%); m.p 225°C; IR (KBr, cm⁻¹): 3433 (NH), 1725 (CO), 1640(NHCO); ¹H NMR (CDCl₃): δ 4.09(s, 2H, -S-CH₂); 6.25(d, 1H, J = 9Hz, C-H); 7.30(d, 1H, J = 9Hz, C-H); 7.33(d, 1H, J = 9Hz, C-H), 7.50(m, 4H, aromatic-H), 7.65(s, 1H, C-H); 7.72(d, 1H, J = 9Hz, C-H); 8.18(s, 1H, NH, D-O exchangeable), 11.26(s, 1H, NH, D-O exchangeable), 12.32(s, 1H, NH-indole, D-O exchangeable); Elemental analysis [Cal. (Obs.): C; 56.58% (56.95%), H; 3.34% (3.27%), N; 13.26% (13.30%), S; 7.59% (7.66%).

(4b): yield, (68%); m.p 243-245°C; IR (KBr, cm⁻¹): 3435 (NH), 1719 (CO), 1650(NHCO); ¹H NMR (CDCl₃): δ 2.40(s, 3H, CH₃), 4.12(s, 2H, -S-CH₂), 6.26(d, 1H, J = 9Hz, C-H); 7.30(s, 1H, C-H), 7.50(m, 4H, aromatic-H), 7.70(s, 1H, C-H), 7.78(d, 1H, J = 9Hz, C-H), 8.15(s, 1H, NH, D-O exchangeable), 11.30(s, 1H, NH, D-O exchangeable), 12.45(s, 1H, NH-indole, D-O exchangeable); ¹³C NMR (300 MHz, CDCl₃): δ 18.0(CH₃), 32.4(-S-CH₂), 44.2(spiro-carbon), 111.5(C), 114-145(8 aromatic carbons), 128.1(C), 151.0(C), 151.2(C), 155.2(NHCONH), 160.6(C), 176.5(CO, indole), 180.3(CO, thiazolidine); Elemental analysis [Cal. (Obs.): C; 57.79% (57.58%), H; 3.70% (3.65%), N; 12.84% (12.90%), S; 7.35% (7.41%).

(4c): yield, (76%); m.p 228° C; IR (KBr, cm⁻¹): 3450 (NH), 2928(CH-arom.), 1721 (=CO), 1610; ¹H NMR (CDCl₃): δ 5.10(s, 1H, CH₂), 6.23(d, 1H, J = 9Hz, C-H); 7.28(d, 1H, J = 9Hz, C-H), 7.35(d, 1H, J = 9Hz, C-H), 7.50(m, 4H, aromatic-H), 7.73(s, 1H, C-H), 7.71(d, 1H, J = 9Hz, C-H), 8.28(s, 1H, NH, D-O-exchangeable), 11.26(s, 1H, NH, D-O-exchangeable), 12.38(s, 1H, NH-indole, D-O-exchangeable); Elemental analysis [Cal. (Obs.): C; 56.59% (56.62%), H; 3.08% (3.14%), N; 13.19% (13.23%).

(5a): yield, (62%); m.p 228-230°C; IR (KBr, cm⁻¹): 3425 (NH), 2935(CH-arom.), 1721 (=CO), 1610; ¹H NMR (CDCl₃): δ 5.10(s, 1H, CH₂), 6.23(d, 1H, J = 9Hz, C-H), 7.28(d, 1H, J = 9Hz, C-H), 7.35(d, 1H, J = 9Hz, C-H), 7.50(m, 4H, aromatic-H), 7.73(s, 1H, C-H), 7.71(d, 1H, J = 9Hz, C-H), 8.28(s, 1H, NH, D-O-exchangeable), 11.26(s, 1H, NH, D-O-exchangeable), 12.38(s, 1H, NH-indole, D-O-exchangeable); Elemental analysis [Cal. (Obs.): C; 56.59% (56.62%), H; 3.08% (3.14%), N; 13.19% (13.23%)

(5b): yield, (60%); m.p 238-240° C; IR (KBr, cm⁻¹): 3450 (NH), 2928(CH-arom.), 1723 (=CO), 1610, 1550; ¹H NMR (CDCl₃): δ 5.10(s, 1H, CH₂), 6.23(d, 1H, J = 9Hz, C-H), 7.30(s, 1H, C-H), 7.55(m, 4H, aromatic-H), 7.71(s, 1H, C-H), 7.74(d, 1H, J = 9Hz, C-H), 8.35(s, 1H, NH, D-O-exchangeable), 11.32(s, 1H, NH, D-O-exchangeable), 12.45(s, 1H, NH-indole, D-O-exchangeable); ¹³C NMR (300 MHz, CDCl₃): δ 19.2(CH₂), 40.6(spiro-carbon), 63.1(CH₃), 110.0-140.0(8 aromatic-C), 123.5(C), 151.0(C), 155.0(C), 155.0(NHCONH), 161.0(C), 169.3(CO, _β-Lactam), 175.6(CO, indole); Elemental analysis [Cal. (Obs.): C; 57.48% (57.29%), H; 3.45% (3.42%), N; 12.77% (12.74%)

(5c): yield, (58%); m.p 230-235° C; IR (KBr, cm⁻¹): 3450 (NH), 2930(CH-arom.), 1720 (=CO), 1610, 1550, 1400; ¹H NMR (CDCl₃): δ 2.30(s, 3H, CH₃), 6.23(s, 1H, CH₂), 7.20-7.80(m, 6H, aromatic-H), 8.30(s, 1H, NH, D-O-exchangeable), 11.30(s, 1H, NH, D-O-exchangeable), 12.50(s, 1H, NH-indole, D-O-exchangeable);
$^{13}$C NMR (300 MHz, CDCl$_3$, δ 17.1 (CH$_3$), 18.8 (CH$_2$), 38.6 (spiro carbon), 60.1 (CH$_2$), 111.5 (C$_2$), 110.0-140.0 (aromatic C), 125.2 (C$_6$), 151.0 (C$_5$), 154.0 (C$_3$), 157.0 (NHCONH), 160.0 (C$_4$), 168.3 (CO, β-Lactam), 174.6 (CO, indole). Elemental analysis [Cal. (Obs.)]: C, 58.35% (58.24%); H, 3.78% (3.76%); N, 12.37% (12.40%); MS, m/z (%): M$^+$ 452(100), M$^+$ 454(33), 376(70), 188(32), 160(25), 145(10), 132(15), 43(20).

**In vitro anti-bacterial assay**

Various coumarin derivatives synthesized during present investigation have been subjected for their antibacterial screening by cup plate method against four bacterial strains at two concentrations using DMSO as solvent. Antibacterial activity of test compounds was evaluated against gram-positive *S. aureus*, *Bacillus Stabitelli* and gram-negative *P. aeruginosa*, *E. coli* bacterial strains using norfloxacin as standard by cup plate method. Dimethyl formamide was used as solvent control. The bacteria were sub-cultured in a medium containing peptone (0.5%), yeast extract (0.15%), sodium chloride (0.3%), potassium dihydrogen phosphate (0.13%) and potassium monohydrogen phosphate (0.15%). Nutrient agar which served as the basal medium was prepared by dissolving bacteriological peptone (0.6%), yeast (0.3%), beef extract (0.13%) and agar (2.1%) in distilled water. The solution was sterilized for 20 min at 15 lbs. pressure in an autoclave. The basal medium (25-30 ml) (with glucose solution to hasten the bacterial growth) with bacterial culture was poured in sterile petri dishes. After the solidification medium holes of 9 mm diameter were bored to form cups with the help of a sterile cork borer. To this cup 0.2 ml of the solution of the test compound was added by sterilized pipettes. The Petri dishes were kept in a cold room to facilitate the diffusion of the solvent for about 2 h. The plates were then incubated at 37 °C for 24 h. The extent of inhibition was measured by the width of the inhibition zone in mm. Minimum inhibitory concentration (MIC) of the test solution was determined by diluting the test solution of required concentration. The zones of inhibition of norfloxacin were taken as 100% and the observed zones of inhibition of newly synthesized compounds have been expressed as related to the standard.

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**REFERENCES**


