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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-c**와 이사틴을 축합하여 indole-2-oxo-3-(2′-oxo-2′*H*-benzopyran-6′-yl-semicarbazone **3a-c**를 얻었다. 무수 $ZnCl_2$의 촉매하에서 무수 1,4-dioxane용매하에서 Thiaglycollic acid처리를 한 화합물 **3a-c**는 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3,2-(4*H*)-thiazolidin-1-yl]-2,4-dioxo-urea **4a-c**를 제공했고, 화합물 **3a-c**는 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3-chloro-2,4-dioxo-azetidin-1-yl]-urea를 얻기 위해 chloroacetyl chloride를 처리했다. 새로이 합성된 모든 화합물은 스펙트럼과 분석자료를 기초로 확인되었다. 합성된 화합물 **3a-c**, **4a-c**와 **5a-c**은 gram-양성과 gram-음성 박테리아에 대해 항균 활성도를 검사했고, 상당한 항균 활성도를 보였다.

주제어: 6-아미노-쿠마린, 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 thiaglycollic acid in dry 1,4-dioxane in presence of catalytic amount of anhydrous $ZnCl_2$ affords 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3,2-(4*H*)-thiazolidin-1-yl]-2,4-dioxo-urea **4a-c**, compound **3a-c** were also treated with chloroacetyl chloride to afford 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3-chloro-2,4-dioxo-azetidin-1-yl]-urea. **5a-c**. 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[1], fungicides,[2] anti-inflammatory,[3] and antitumour agents.[4,5] 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.[6] It has been reported that if the indole[7] ring is joined to other heterocyclic compounds through a spiro-carbon atom, the resulting compounds show increased spectrum of biological activities.[8-16] Spiro nuclei

have also drawn considerable attention of the chemist because of their antiseptic,[17] analgesic[18] and broad-spectrum antimicrobial activities.[19] Hence, introduction of spiro nucleus to such vital molecules will enhance their biological activity.[20]

By observing the importance of the above heterocycles, we planned to synthesize spiro-indolothiazolidin-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-c** which was subsequently converted into its acid hydrazide **2a-c**.The IR spectrum of **1c** 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 δ 1.40 and as a quartet at δ 4.35 for methylene protons and a peak at δ 8.30 for -NH group which is D_2O exchangeable. IR spectrum in KBr of compound **2c** 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-c** as a triplet and quartet indicating the formation of its acid hydrazide.

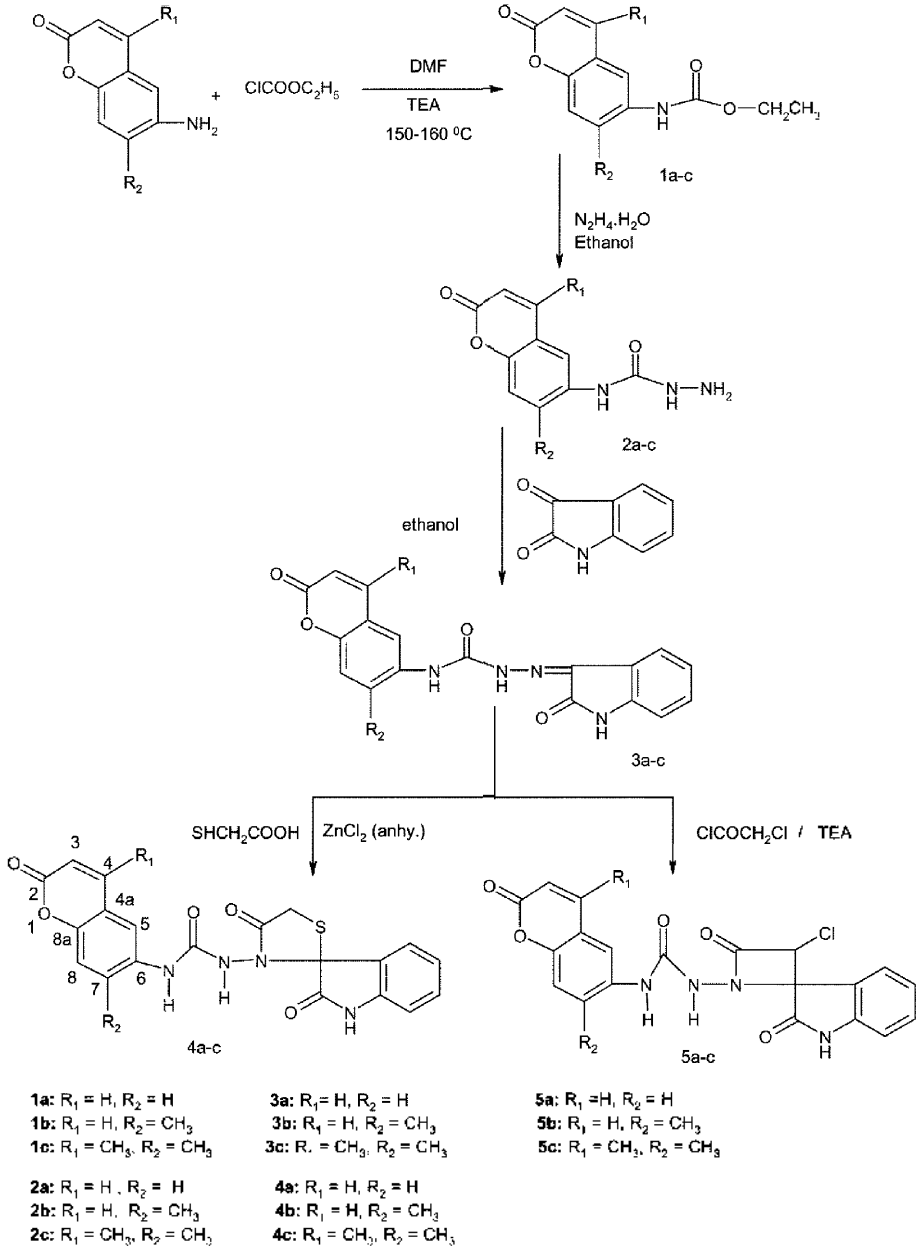
Compound **2a-c** was treated with isatin in ethanol to give indole-2-oxo-3-(2′-oxo-2′*H*-benzopyran-6′-yl-semicarbazone **3a-c**.The IR spectrum of compound **3c** in KBr showed bands at 3456cm^{-1} for -NH stretching, at 2950 cm^{-1} for -CH aromatics stretching, at 1723 cm^{-1} for carbonyl carbon. ^1H NMR spectrum in $CDCl_3$ shows peak at 12.5 ppm for -NH of indole nucleus which is D_2O exchangeable, along with other peaks. Compound **3a-c** separately on refluxing with thiaglycollic acid in dry 1,4-dioxane and with chloroacetylchloride gave 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3,2-(4*H*)-thiazolidin-1-yl]-2,4-dioxo-urea **4a-c**, and 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3-chloro-2,4-dioxo-azetidin-1-yl]-urea **5a-c** respectively. The IR spectrum of compound **4c** in KBr showed bands at 3430cm^{-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_2O exchangeable. In its ^{13}C 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 182.3 for carbonyl of thiazolidinone ring, etc. Similarly compound **5c** 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_3-H and peak at 5.09 ppm for -CH-Cl. In its ^{13}C 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 **5c** shows M+ at 452 and M+2 at 454 indicating the presence of chlorine atom. It also gives positive Beilstein and Lassignes 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 aurignosa* and *Escherichia coli* by cup plate method.[21] The results indicated that compound **3a-c**, **4a-c** and **5a-c** showed total inhibition of bacterial growth at 100 μgml $^{-1}$ 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-c** and **5a-c** it has been observed that presence of methyl group in coumarin ring increases the activity.[22]

CONCLUSION

In conclusion we here report the synthesis of



Scheme 1.

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_{max} in cm^{-1}) were recorded on Perkin Elmer FTIR and NMR (^1H and ^{13}C) was recorded on Jeol 300 MHz using TMS as

Table 1. In vitro anti-bacterial spectrum of coumarin derivative

Compound	Concentration μg/mL	Zone of Inhibition (%)			
		S. aureus	*B. Subtilis*	*P. aurignosa*	*E. coli*
3a	100	55	58	55	58
	50	45	50	45	50
3b	100	58	62	58	62
	50	45	45	45	50
3c	100	62	62	62	66
	50	50	41	54	45
4a	100	75	71	58	62
	50	50	55	50	50
4b	100	79	83	62	66
	50	58	62	54	54
4c	100	87	87	75	79
	50	62	66	62	66
5a	100	75	79	79	83
	50	70	71	67	75
5b	100	79	83	83	87
	50	70	70	75	79
5c	100	83	87	87	91
	50	75	75	75	79
Norfloxacin	100	100	100	100	100
	50	100	100	100	100

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 F_{254}, Merck), visualizing the sports 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-c

To a solution solution 6-amino-coumarin (0.01 mole) in DMF (60 mL), was added chloroethylformate (0.015 moles) and triethyl 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-c**

(1a): yield (62%); m.p 176-178 °C; IR (KBr, cm^{-1}): 3405(NH), 1730(COOEt), 1720(CO); ^{1}H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 4.20Hz, CH_2-CH_3), 4.26 (q, 2H, J = 6.60Hz, CH_2-CH_3), 6.37(d, 1H, J = 9Hz, C_3-H), 7.21(d, 1H, J = 9Hz, C_8-H), 7.23(d, 1H, J = 9Hz, C_7-H), 7.29(s, 1H, C_5-H), 7.75(d, 1H, J = 9Hz, C_4-H), 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 °C; IR (KBr, cm^{-1}): 3435 (NH), 1735(COOEt), 1725(CO); ^{1}H NMR ($CDCl_3$): δ 1.35 (t, 3H, J = 4.20Hz, CH_2-CH_3), 2.25 (s, 3H, CH_3), 4.30 (q, 2H, J = 6.60Hz, CH_2-CH_3), 6.26 (d, 1H, J = 9Hz, C_3-H), 7.15 (s, 1H, C_8-H), 7.30(s, 1H, C_5-H), 7.72(d, 1H, J = 9Hz, C_4-H), 8.32 (s, 1H, NH, D_2O-exchangable); 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 °C; IR (KBr, cm^{-1}): 3450 (NH), 2950 (CH-arom.), 1723 (>CO), 1730 (COOEt); ^{1}H NMR ($CDCl_3$): δ 1.40 (t, 3H, J = 4.20Hz, CH_2-CH_3), 2.30 (s, 3H, CH_3), 2.40 (s, 3H,

CH_3), 4.35 (q, 2H, J = 6.60Hz, CH_2-CH_3), 6.23 (s, 1H, C_3-H), 7.10 (s, 1H, C_8-H.), 7.25 (s,1H, C_5-H), 8.30(s, 1H, NH, D_2O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 64.36% (64.15%), H; 5.79% (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 2a-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); ^{1}H NMR ($CDCl_3$): δ 3.45 (s, 2H, NH_2, D_2O-exchangable), 4.15 (s, 1H, NH, D_2O-exchangable), 6.26(d, 1H, J = 9Hz, C_3-H), 7.21(d, 1H, J = 9Hz, C_8-H), 7.30(d, 1H, J = 9Hz, C_7-H), 7.39(s, 1H, C_5-H), 7.78(d, 1H, J = 9Hz, C_4-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); ^{1}H NMR ($CDCl_3$): δ 2.42 (s, 3H, CH_3), 3.55 (s, 2H, NH_2, D_2O-exchangable), 4.12 (s, 1H, NH, D_2O-exchangable), 6.25(d, 1H, J = 9Hz, C_3-H), 7.12(s, 1H, C_8-H), 7.28(s, 1H, C_5-H), 7.76(d, 1H, J = 9Hz, C_4-H), 8.28 (s, 1H, NH, D_2O-exchangable); Elemental analysis [Cal. (Obs.)]: C; 56.65% (56.47%), H; 4.75% (4.71%), N; 18.02% (17.97%).

(2c): Yield; (65%); m.p 200 °C; IR (KBr, cm^{-1}): 3500 (NH), 3100-3300 (NH_2), 1720 (>CO), 1688 (NHCO); ^{1}H NMR ($CDCl_3$): δ 2.20 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 3.60 (s, 2H, NH_2, D_2O-exchangable), 4.15 (s, 1H, NH, D_2O-exchangable), 6.20 (s, 1H, C_3-H), 6.85 (s, 1H, C_8-H), 7.20 (s,1H, C_5-H), 8.20 (s, 1H, NH, D_2O 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(32).

General procedure for indole-2-oxo-3-(2′-oxo-2′*H*-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); ^{1}H NMR ($CDCl_3$): δ 6.23(d, 1H, J = 9Hz, C_3-H), 7.25(d, 1H, J = 9Hz, C_8-H), 7.32(d, 1H, J = 9Hz, C_7-H), 7.50(s, 1H, C_5-H), 7.70-7.75 (m, 4H, aromatic-H), 7.76(d, 1H, J = 9Hz, C_4-H), 8.11(s, 1H, NH), 11.18(s, 1H, NH, D_2O-exchangable), 12.23(s,1H, NH-indole, $D_2$0-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); ^{1}H NMR ($CDCl_3$): δ 2.26 (s, 3H, CH_3), 6.24 (d, 1H, J = 9Hz, C_3-H), 7.30(s, 1H, C_8-H), 7.50(s, 1H, C_5-H), 7.70-7.75 (m, 4H, aromatic-H), 7.80(d, 1H, J = 9Hz, C_4-H), 8.15(s, 1H, NH), 11.20(s, 1H, NH, D_2O-exchangable), 12.30(s,1H, NH-indole, $D_2$0-exchangable); Elemental analysis [Cal. (Obs.)]: C; 62.98% (62.79%), H; 3.89% (3.83%), N; 15.46% (15.51%).

(3c): yield, (65%); m.p 198-200 °C; IR (KBr, cm^{-1}): 3456 (NH), 2950 (CH-arom.), 1723 (coumarin), 1675 (NHCO); ^{1}H NMR ($CDCl_3$): δ 2.31(s, 3H, CH_3), 2.50 (s, 3H, CH_3), 6.21 (s, 1H, C_3-H), 7.21 (s, 1H, C_8-H), 7.60(s, 1H, C_5-H), 7.65-7.85(m, 4H, aromatic-H), 8.20 (s, 1H, NH, $D_2$0-exchangable), 11.50 (s, 1H, NH, $D_2$0-exchangable), 12.50 (s, 1H, NH-indole, $D_2$0-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(45).

General procedure for synthesis of 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*, 2*H*)-3,2-(4*H*)-thiazolidin-1-yl]-2,4-dioxo-urea 4a-c

Compound **3a-c** (0.01 mole) and thiaglycollic acid (0.01 mole) was refluxed in presence of catalytic amount of anhydrous $ZnCl_2$ 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, washes with water and purified by recrystallization from methanol to give comp. **4a-c**.

(4a): yield, (72%); m.p 225°C; IR (KBr, cm^{-1}): 3433 (NH), 1725 (CO), 1640(NHCO); ^1H NMR ($CDCl_3$): δ 4.09(s, 2H, S-CH_2), 6.25(d, 1H, *J* = 9Hz, C_3-H), 7.30(d, 1H, *J* = 9Hz, C_8-H), 7.33(d, 1H, *J* = 9Hz, C_7-H), 7.50(m, 4H, aromatic-H), 7.65(s, 1H, C_5-H), 7.72(d, 1H, *J* = 9Hz, C_4-H), 8.18(s, 1H, NH, D_2O exchangeable), 11.26 (s, 1H, NH, D_2O exchangable), 12.32 (s, 1H, NH-indole, D_2O exchangable); Elemental analysis [Cal. (Obs.)]: C; 56.87% (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), 1635(NHCO); ^1H NMR ($CDCl_3$): δ 2.40 (s, 3H, CH_3), 4.12(s, 2H, S-CH_2), 6.26(d, 1H, *J* = 9Hz, C_3-H), 7.30(s, 1H, C_8-H), 7.50(m, 4H, aromatic-H), 7.70(s, 1H, C_5-H), 7.78(d, 1H, *J* = 9Hz, C_4-H), 8.15(s, 1H, NH, D_2O exchangeable), 11.30 (s, 1H, NH, D_2O exchangable), 12.45 (s, 1H, NH-indole, D_2O exchangable); ^{13}C NMR (300 MHz, $CDCl_3$): δ 18.0(CH_3), 32.4(S-CH_2), 44.2 (spiro-carbon), 111.5(C_3), 114-145(8- aromatic carbons), 128.1(C_{4a}), 151.0(C_{8a}), 151.2(C_4), 155.2 (NHCONH), 160.6(C_2), 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 238 °C; IR (KBr, cm^{-1}): 3430 (NH), 2921 (CH-arom.), 1721 (>CO), 1689(carbonyl of amide), 1610, 1550, 1400; ^1H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.10(s, 2H, S-CH_2), 6.23 (s, 1H, C_3-H), 7.20-7.80 (m, 6H, arom-H), 8.10 (s, 1H, NH, D_2O exchangeable), 11.50 (s, 1H, NH, D_2O-exchangable), 12.50 (s, 1H, NH-indole, D_2O exchangeable); ^{13}C NMR (300 MHz, $CDCl_3$): δ 17.2(CH_3), 19.6(CH_3), 33.4(S-CH_2), 45.6(spiro-carbon), 113.5(C_3), 115-145(8-aromatic carbons), 126.1(C_{4a}), 150.0(C_{8a}), 153.2(C_4), 156.2(NHCONH), 159.6(C_2), 175.5(CO, indole), 182.3(CO, thiazolidine); Elemental analysis [Cal. (Obs.)]: C; 58.66% (58.51%), H; 4.03% (3.98%), N; 12.44% (12.39%), S; 7.12% (7.23%); MS, m/z (%): M^+ 450(55), 421(30), 376(20), 215(100), 172(65), 145(15), 90(10).

General procedure for 3-(2′-oxo-2′*H*-benzopyran-6′-yl)-spiro-3*H*-[indole-(1*H*,2*H*)-3-chloro-2,4-dioxo-azetidin-1-yl]-urea 5a-c

To a solution of **4c** (0.01 moles) in 1, 4 dioxane, chloroacetyl chloride (0.01 moles) and triethylamine (0.01 moles) was added drop wise with constant stirring. The reaction mixture was then refluxed on water bath and excess of dioxane was distilled out and resulting mixture was poured in ice cold HCl, filtered, dried and recrystallized from ethanol to give the desired product.

(5a): yield, (62%); m.p 228-230 °C; IR (KBr, cm^{-1}): 3425 (NH), 2935(CH-arom.), 1721 (>CO), 1610; ^1H NMR ($CDCl_3$): ä 5.10 (s,1H, CH-Cl), 6.23(d, 1H, *J* = 9Hz, C_3-H), 7.28(d, 1H, *J* = 9Hz, C_8-H), 7.35(d, 1H, *J* = 9Hz, C_7-H), 7.50(m, 4H, aromatic-H), 7.73(s, 1H, C_5-H), 7.71(d, 1H, *J* = 9Hz, C_4-H), 8.28 (s, 1H, NH, D_2O-exchangable), 11.26 (s, 1H, NH, D_20-exchangable), 12.38 (s, 1H, NH-indole, D_20-exchangable); Elemental analysis [Cal. (Obs.)]: C; 56.55% (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 ($CDCl_3$): δ 2.28 (s, 3H, CH_3), 5.10 (s,1H, CH-Cl), 6.26(d, 1H, *J* = 9Hz, C_3-H), 7.30(s, 1H, C_8-H), 7.55(m, 4H, aromatic-H), 7.71(s, 1H, C_5-H), 7.74(d, 1H, *J* = 9Hz, C_4-H), 8.35 (s, 1H, NH, D_2O-exchangable), 11.32 (s, 1H, NH, D_20-exchangable), 12.45 (s, 1H, NH-indole, D_20-exchangable); ^{13}C NMR (300 MHz, $CDCl_3$): δ 19.2(CH_3), 40.6 (spiro-carbon), 63.1(CHCl), 112.5(C_3), 110.0-140.0 (8-aromatic-C), 125.3(C_{4a}), 151.0(C_{8a}), 155.0(C_4), 155.0(NHCONH), 161.0(C_2), 169.3(CO, ß-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 ($CDCl_3$): δ 2.30 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 5.09 (s,1H, CH-Cl), 6.23 (s, 1H, C_3-H), 7.20-7.80 (m, 6H, arom-H), 8.30 (s, 1H, NH, D_20-exchangable), 11.30 (s, 1H, NH, D_20-exchangable), 12.50 (s, 1H, NH-indole, D_2O-exchangable);

^{13}C NMR (300 MHz, $CDCl_3$): δ 17.1(CH_3), 18.8(CH_3), 38.6(spiro-carbon), 60.1(CHCl), 111.5(C_3) 110.0-140.0(8-aromatic-C), 125.3(C_{4a}), 151.0(C_{8a}), 154.0(C_4), 157.0(NHCONH), 160.0(C_2), 168.3(CO, ß-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^{+2} 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[21] against four bacterial strains at two concentrations using DMF as solvent. Antibacterial activity of test compounds was evaluated against gram-positive *S. aurea, Bacillus Subtilis* and gram-negative *P. aurigmosa, E. coli* bacterial strains using norfloxicin 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 (0.35%), potassium dihydrogen phosphate (0.13%) and potassium monohydrogen phosphate (0.13%). 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 0.02 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 norfloxicin 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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