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SYNTHESIS AND BIOLOGICAL EVALUATION OF COUMARIN CLUBBED OXAZINES

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Keywords:

Coumarin, Oxazine, Antimicrobial, Antitubercular, Antioxidant activity

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ABSTRACT: Coumarin segment have been reported broad spectrum of different biological activities with the oxazine derivatives. In view that we have synthesized newer coumarin clubbed 4-(4-fluorophenyl)-6-substituted phenyl-2*H*-1,3-oxazin-2-amine and screened for their biological studies. 4-(4-fluorophenyl)-6-substituted phenyl-2*H*-1,3-oxazin-2-amine IIa-j condensed with 4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl chloro acetate 5 to afford 4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-(4-(4-sustitutedphenyl) oxazine - 2- yl - amino) acetates 2a-j. The newer compounds were characterized by IR, NMR and mass spectral studies and were screened for their antimicrobial, antitubercular and antioxidant activities. Compound 2a (-C_6H_5) 62.5 µg/ml, 2d (2-OH) 100 µg/ml against *S. aureus*, 2a (-C_6H_5) 50 µg/ml, 2f (4-F) and 2j (3-OPh) 100 µg/ml against *S. pyogenes* as compared to chloramphenicol and the other compound 2c (4-Cl) showed excellent potency 25 µg/ml against *E. coli* and 12.5 µg/ml against *P. aeruginosa* while compound 2e (4-OH) exhibited comparable activity of 62.5 µg/ml and 100 µg/ml against *E. coli* and *P. areuginosa* respectively. Furthermore 2i (3-Br) also showed 100 µg/ml moderate potency against *E. coli* compared to the standard drugs chloramphenicol and ciprofloxacin. The study of antifungal activity indicates that 2i (3-Br) showed remarkable potency of 250 µg/ml against *C. albicans* and compounds 2a (-C_6H_5), 2b (2-Cl), 2c (4-Cl), 2f (4-F) and 2j (3-OPh) exhibited comparable activity of 500 µg/ml against *C. albicans* when compared to the standard drug Griseofulvin.

INTRODUCTION: Coumarin is a versatile compound which exhibits excellent activity as antibacterial [1], as a continuous work on the synthesis of bioactive coumarin-containing analogs [2] herein this article we have reported the synthesis of coumarin based heterocyclic compounds having a wide range of pharmacological activities such as antimalarial [3], antioxidant [4], antiplatelet and antithrombotic [5], antifungal [6], herbicidal [7], antiviral [8], anticoagulant [9], anti-inflammatory [10], antitumor [11], anti-oxidant activity [12] and anti cancer [13].

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In addition, 4 & 7 - hydroxy and nitro-coumarins are also potent antimicrobial [14] and antioxidant and very important for the synthesis of other coumarin derivatives [15]. Antimicrobial activities of 4-methyl-7-hydroxycoumarin are enhanced by nitration. It was then reacted with chloro acetyl chloride to give chloro acetate derivative of 4-methyl-6-nitro-7-hydroxycoumarin.

Oxazines as well as 2-amino oxazines are well known moiety of heterocycles having a wide variety of biological activities and their uses as medicines are well established. Oxazines derivatives are reported to exhibit diverse biological activities as antitubercular [16], anticoagulant [17], antimicrobial [18], antioxidant-anticancer [19] and antifungal agents [20]. In view of these finding and in continuation of our work on

the synthesis of novel heterocyclic systems exhibiting good biological activity, we have coupled chloro acetate derivative of 4-methyl-6-nitro-7-hydroxycoumarin with a series of substituted amino oxazines to synthesize newer coumarin analogous and evaluated their antimicrobial, anti tuberculosis and anti oxidant activities.

MATERIALS AND METHODS: All solvents, chemicals and reagents were purchased from Sigma-Aldrich with the highest purity and used without further purification. Melting points were determined with open capillary method on 'Equiptronics' digital melting point apparatus, model no. EQ-730 and melting points are uncorrected. IR spectra were recorded on a Perkin Elmer spectrophotometer (KBr pellets) instrument. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II 400MHz NMR Spectrometer using DMSO- d_6 as solvent and TMS as internal standard. All chemical shifts were reported as δ values (ppm). Mass spectra were recorded using water, Q-TOF Micromass (ESI – MS). Analytical thin-layer chromatography (TLC) was performed with Merck silica gel plates and visualized with UV irradiation (254 nm) or iodine.

RESULTS AND DISCUSSION:

Chemistry: The synthetic protocol for the lead molecule 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-chloroacetate 5 and final compounds 2a-j is depicted in scheme. Synthesis of 4-methyl-7-hydroxy-coumarin 3 was carried out by Pechmann condensation [21] followed by nitration with cerric ammonium nitrate (CAN), water, hydrogen peroxide [22] and then reacting with chloroacetyl chloride to get 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-chloroacetate 5 [23]. 2-amino oxazines IIIa-j were prepared by cyclo-addition reaction between substituted chalcones and urea [24]. These substituted amino oxazines are condensed with compound (5) to synthesize desired analogs 2a-j.

Synthesis of 4-methyl -7-hydroxy-coumarin. (3): To a cooled conc. H_2SO_4 (75 ml), previously prepared mixture of resorcinol 1 (0.01 mol) in ethylacetoacetate 2 (0.01 mol) was added drop wise with maintaining the temperature below 10 ^{0}C. After completion of addition stirring was continued for 1 h. The reaction mixture was poured on to crushed ice. The product was filtered and washed with distilled water to remove access of acid. The product was dissolved in cold 10% NaOH solution and re-precipitated by 10% aqueous HCl till the solution becomes acidic. The product was washed with cold water till filtrate become neutral, dried and crystallized from ethanol using activated charcoal to get white solid 3, Yield: 85%, mp: 183°C to 185°C.

Synthesis of 7-hydroxy-4-methyl-6-nitro-2H-chromen - 2-one (4): The compound 3 (0.01 mol) was charged in two portions to a solution of cerric ammonium nitrate(CAN) (0.01 mol) containing 30% hydrogen peroxide (1 ml) in water (5ml). The resulting mixture was heated at 50 - 60 ^{0}C with stirring for 1h. The completion of the reaction was monitored by TLC (ethylacetate:n-hexane 7:3), then reaction mixture was cooled, diluted with water (20 ml) and extracted with chloroform (3×30 ml). Solvent was removed under reduced pressure to offer crude product which was further purified by column chromatography using silica gel 60-120 mesh and ethyl acetate in hexane as eluent to get desired pure product 4. Yield: 72%, mp: 193 - 195°C (as reported).

Synthesis of 4-methyl-6-nitro-2-oxo-2H-chromen -7-yl 2-chloroacetate. (5): To a solution of 7-hydroxy-4-methyl-6-nitro - 2*H*- chromen-2-one 4 (0.01mol) and α-chloro acetyl chloride (0.012 mol) in dichloromethylene (30 ml), triethyl amine (0.0102 mol) was added drop wise and the mixture was stirred for 1h. The completion of the reaction was monitored by TLC (dichloro methylene: methanol, 9:1). The reaction mixture was then washed with 1 M HCl solution (100 ml) followed by 1 M NaOH solution (100 ml × 3). The dichloromethylene layer was separated, dried over $MgSO_4$ and evaporated. The residue was crystallized from ethyl acetate-hexane as brownish yellow crystals 5. Yield: 69%, mp: 109^{o}C -111^{o}C.

Synthesis of (E)-1-(4-fluorophenyl)-3-phenyl prop-2-en-1-one (IIa): p-Fluoro acetophenone 6 (0.01 mol) and benzaldehyde Ia (0.01 mol) were dissolved in 15 ml ethanol. NaOH solution (0.02 mol) in ethanol was added slowly and the mixture stirred at 20 ^{o}C for 2h until the entire mixture becomes very thick so the stirring is no longer

effective. The progress of the reaction was monitored by TLC (toluene: acetone, 8:2). Then the reaction mixture was poured slowly on to 400 ml of water with stirring and kept in refrigerator for 24 h. The precipitate obtained was filtered, washed and recrystallized from ethanol. The other compounds IIb-j were prepared by the same method using substituted benzaldehydes I b-j.

Synthesis of 4-(4-fluorophenyl)-6-phenyl-2H-1,3-oxazin-2-amine (IIIa): A mixture of (*E*)-1-(4-fluorophenyl)-3-phenylprop-2-en-1-one IIa (0.01 mol), urea (0.01 mol) were dissolved in ethanolic NaOH (10 mL) was refluxed about 2-3h. The completion of the reaction was monitored by TLC using a mixture of ethylacetate:n-hexane (3:7). This was then poured on to 400 ml of cold water with continuous stirring for 1h and then kept in refrigerator for 24h. The precipitate 4-(4-fluorophenyl)-6-phenyl-2*H*-1,3-oxazin - 2 - amine IIIa obtained was filtered, washed and recrystallized.

The other compounds IIIb-j were prepared by the same method using substituted (*E*)-1-(4-fluoro phenyl)-3-phenylprop-2-en-1-one IIb-j.

Synthesis of 4-methyl-6-nitro-2-oxo-2H-chromen -7yl-2-(4-(4-fluorophenyl) - 6 - phenyl - 2H-1, 3-oxazin-2-yl amino)acetate (2a): Compound (5) (0.01mol), 4-(4-fluorophenyl)-6-phenyl - 2H - 1, 3-thiazin-2-amine IIIa (0.01mol) and K_2CO_3 (0.024 mol) in DMF was stirred for 10-15 min and refluxed for 8-9h under nitrogen.

TABLE 1: CHARACTERIZATION DATA OF COMPOUNDS 2 a-j

Com. No.	R	Mol. Formula	M.P.	Yield
			^{0}C	%
2a	-C_6H_5	$C_{28}H_{20}FN_3O_7$	125-127	68
2b	2-Cl C_6H_4	$C_{28}H_{19}ClFN_3O_7$	116-118	69
2c	4-Cl C_6H_4	$C_{28}H_{19}ClFN_3O_7$	125-127	74
2d	2-OH C_6H_4	$C_{28}H_{20}ClFN_3O_8$	135-137	65
2e	4-OH C_6H_4	$C_{28}H_{20}ClFN_3O_8$	141-143	69
2f	4-F C_6H_4	$C_{28}H_{19}F_2N_3O_7$	112-114	61
2g	4-$CH_3C_6H_4$	$C_{29}H_{22}FN_3O_7$	120-122	65
2h	4-C_3H_7 C_6H_4	$C_{31}H_{26}FN_3O_7$	129-131	63
2i	3-Br C_6H_4	$C_{28}H_{19}BrFN_3O_7$	132-134	61
2j	3-OPh C_6H_4	$C_{34}H_{24}FN_3O_8$	119-121	69

The completion of the reaction was monitored by TLC on silica gel using a mixture of ethylacetate:toluene (3:7). After the completion of the reaction, mixture was poured on to crushed ice and precipitate was filtered, washed with saturated solution of $NaHCO_3$, dried and recrystallized from EtOH. The other compounds 2b-j were prepared by the same method using IV b-j.

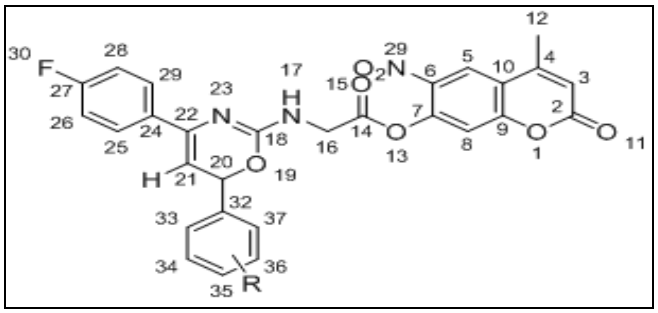
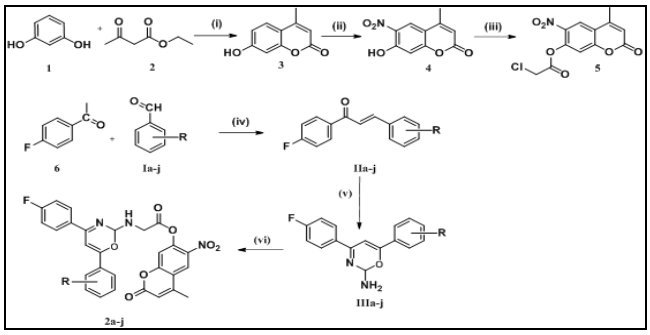


FIG. 1: GENERAL STRUCTURE OF COUMARIN CLUBBED OXAZINE



SCHEME

(i) Cooled (5°C - 10°C), conc. H_2SO_4
(ii) CAN, 30% H_2O_2 + 5ml H_2O, stirred
(iii) α-chloroacetyl chloride, CH_2Cl_2, triethyl amine 1h stirred
(iv) NaOH, EtOH, 2 -3 h stirred
(v) Urea, ethanolic NaOH, 2-3h refluxed
(vi) 4-methyl-6-nitro-2-oxo-2H-chromen-7-yl 2-chloroacetate, DMF, K_2CO_3, 8-9 h refluxed

Spectral Characterization of the Compounds:

4-Methyl-6-nitro-2-oxo-2H - chromen-7yl- 2 - (4-(4-fluorophenyl)-6-phenyl - 2H - 1, 3-oxazin - 2-yl-amino)acetates(2a): Light brown solid, yield: 68%, mp: 125-127°C, M.F.: $C_{28}H_{20}FN_3O_7$. IR (KBr) ν cm^{-1}: 3243 (-NH), 1747, 1664 (-C=O), 1594 (-C=N), 3059, 2845 (-CH_3), 15348, 1349 (-NO_2), 1263, 1047 (-C-O-C-), 1165 (C-F); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ: 8.58 (s, 1H, -CH), 6.36-7.78 (m, 12H, aromatic), 5.34 (s, 1H, -CH), 3.73 (s, 2H, -CH_2NH), 3.24 (s, 1H, -CH_2NH), 2.42 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS) δ: 160.12 (C-2),113.92 (C-3), 152.51 (C-4), 120.74 (C-5), 139.46 (C-6), 135.63 (C-7), 115.75 (C-8), 159.14 (C-9), 118.66 (C-10), 19.43(C-12), 168.17 (C-14), 40.96 (C-16), 111.14 (C-18),157.34 (C-20), 74.14 (C-21), 160.04 (C-22), 134.11 (C-24), 130.12 (C-25, C-29), 115.54 (C-26, C-28), 165.16 (C-27), 131.24 (C-32), 127.95 (C-33, C-37), 128.83 (C-34, C-36), 126.31 (C-35),; m/z: 529.13 (M^+).

4-Methyl-6-nitro-2-oxo-2H-chromen - 7yl - 2-(4-(4-fluorophenyl) - 6 - 2- chlorophenyl - 2H - 1, 3-oxazin-2-yl-amino)acetates(2b): Cream yellow solid, yield: 69%, mp: 116-118°C, M.F.: $C_{28}H_{19}ClFN_3O_7$ IR (KBr) ν cm^{-1}: 3238 (-NH), 1740, 1669 (-C=O), 1599 (-C=N-), 3067, 2840 (-CH_3), 1538, 1358 (-NO_2), 1258, 1057 (-C-O-C-), 1170 (C-F), 758 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ: 8.54 (s, 1H, -CH), 6.30-7.77 (m, 11H, aromatic), 5.34 (s, 1H, -CH), 3.70 (s, 2H, -CH_2NH), 3.23 (s, 1H, -CH_2NH), 2.41 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS) δ: 160.12 (C-2),113.94 (C-3), 152.51 (C-4), 120.72 (C-5), 139.44 (C-6), 135.64 (C-7), 115.77 (C-8), 159.12 (C-9), 118.67 (C-10), 19.41 (C-12), 168.17 (C-14), 40.96 (C-16), 111.13 (C-18),157.37 (C-20), 74.16 (C-21), 160.14 (C-22), 134.13 (C-24), 130.16 (C-25, C-29), 115.52 (C-26, C-28), 165.12 (C-27), 131.51 (C-32), 133.10 (C-33), 131.56 (C-37), 127.80 (C-34), 131.25 (C-36), 128.35 (C-35),; m/z: 563.09 (M^+), 565.09 (M+2).

4-Methyl-6-nitro-2-oxo-2H-chromen -7yl – 2 -(4-(4-fluorophenyl) - 6 - 4 -chlorophenyl - 2H - 1, 3-oxazin-2-yl-amino)acetates (2c): Cream yellow solid, yield: 69%, mp: 116-118°C, M.F.: $C_{28}H_{19}ClFN_3O_7$ IR (KBr) ν cm^{-1}: 3245 (-NH), 1736, 1663 (-C=O), 1585 (-C=N-), 3059, 2836 (-CH_3), 1545, 1351 (-NO_2), 1249, 1061 (-C-O-C-), 1168(C-F), 769 (C-Cl); ^{1}H NMR (400 MHz, DMSO-d6, TMS) δ: 8.58 (s, 1H, -CH), 6.38-7.78 (m, 11H, aromatic), 5.36 (s, 1H, -CH), 3.70 (s, 2H, -CH_2NH), 3.20 (s, 1H, -CH_2NH), 2.40 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.11 (C-2),113.94 (C-3), 152.52 (C-4), 120.71 (C-5), 139.42 (C-6), 135.63 (C-7), 115.77 (C-8), 159.12 (C-9), 118.65 (C-10), 19.40 (C-12), 168.12 (C-14), 40.90 (C-16), 111.10 (C-18),157.34 (C-20), 74.11 (C-21), 160.02 (C-22), 134.11 (C-24), 130.11 (C-25, C-29), 115.55 (C-26, C-28), 165.07 (C-27), 128.42 (C-32), 129.34 (C-33, C-37), 128.84 (C-34, C-36), 133.62 (C-35); m/z: 563.09 (M^+), 565.09(M+2).

4-Methyl-6-nitro-2-oxo-2H-chromen - 7yl- 2- (4-(4-fluorophenyl)- 6 - 2 -hydroxyphenyl - 2H-1, 3-oxazin - 2-yl-amino) acetates (2d): Dark yellow solid, yield: 65%, mp: 135-137°C, M.F.: $C_{28}H_{20}FN_3O_8$ IR (KBr) ν cm^{-1}: 3378 (-OH), 3238 (-NH), 1740, 1661(-C=O), 1597 (-C=N-), 3064, 2830 (-CH_3), 1531, 1357 (-NO_2), 1251, 1053(-C-O-C-), 1174 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ : 9.34 (s, 1H, -OH), 8.57 (s, 1H, -CH), 6.32-7.77 (m, 11H, aromatic), 5.35 (s, 1H, -CH), 3.70 (s, 2H, -CH_2NH), 3.21 (s, 1H, -CH_2NH), 2.44 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.13 (C-2),113.94 (C-3), 152.54 (C-4), 120.72 (C-5), 139.42 (C-6), 135.65 (C-7), 115.78 (C-8), 159.14 (C-9), 118.65 (C-10), 19.44 (C-12), 168.15 (C-14), 40.98 (C-16), 111.15 (C-18),157.36 (C-20), 74.12 (C-21), 160.05 (C-22), 134.15 (C-24), 130.16 (C-25, C-29), 115.54 (C-26, C-28), 165.11 (C-27), 110.24 (C-32), 155.82 (C-33), 123.91 (C-37), 118.03 (C-34), 121.52 (C-36), 129.36 (C-35); m/z: 545.47 (M^+).

4-Methyl-6-nitro-2-oxo-2H- chromen -7yl - 2 -(4-(4-fluorophenyl)-6-4-hydroxy phenyl - 2H -1, 3 -oxazin-2-yl-amino)acetates (2e): Yellow solid, yield: 69%, mp: 141-143°C, M.F.: $C_{28}H_{20}FN_3O_8$ IR (KBr) ν cm^{-1}: 3375 (-OH), 3236 (-NH), 1740, 1664 (-C=O), 1595 (-C=N-), 3064, 2833 (-CH_3), 1530, 1357 (-NO_2), 1251, 1055 (-C-O-C-), 1172 (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ : 9.37 (s,1H, -OH), 8.56 (s, 1H, -CH), 6.37-7.78 (m, 11H, aromatic), 5.38 (s, 1H, -CH), 3.73 (s, 2H, -CH_2NH), 3.24 (s, 1H, -CH_2NH), 2.42 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.13 (C-2),113.91 (C-3), 152.55 (C-4), 120.70 (C-5), 139.41 (C-6), 135.67 (C-7), 115.73 (C-8), 159.10 (C-9), 118.54 (C-10), 19.38 (C-12), 168.13 (C-14), 40.91 (C-16), 111.06 (C-18),157.31 (C-20), 74.16 (C-21), 160.01 (C-22), 134.12 (C-24), 130.11 (C-25, C-29), 115.52 (C-26, C-28), 165.06 (C-27), 122.64 (C-32), 129.71 (C-33, C-37), 115.54 (C-34, C-36), 156.73 (C-35).; m/z: 545.47 (M^+)

4-Methyl-6-nitro-2-oxo-2H-chromen - 7yl - 2- (4-(4-fluorophenyl)- 6- 4 -fluoro phenyl -2H - 1, 3-oxazin-2-yl-amino)acetates (2f): Pale yellow solid, yield: 61%, mp: 102-104°C., M.F.: $C_{28}H_{19}F_2N_3O_7$; IR (KBr) ν cm^{-1}: 3238 (-NH), 1742, 1663 (-C=O), 1598 (-C=N), 3064, 2832 (-CH_3), 1535, 1353 (-NO_2), 1251, 1055 (-C-O-C), 1176 (C-F); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.57 (s, 1H, -CH), 6.37-7.76 (m, 11H, aromatic), 5.38 (s, 1H, -CH), 3.73 (s, 2H, -CH_2NH), 3.22 (s, 1H, -CH_2NH), 2.43 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.12 (C-2),113.91 (C-3), 152.54 (C-4), 120.71 (C-5), 139.44 (C-6), 135.65 (C-7), 115.75 (C-8), 159.13 (C-9), 118.64 (C-10), 19.44 (C-12), 168.19 (C-14), 40.94 (C-16), 111.15 (C-18),157.32 (C-20),

74.13 (C-21), 160.08 (C-22), 134.17 (C-24), 130.14 (C-25, C-29), 115.54 (C-26, C-28), 165.11 (C-27), 123.64 (C-32), 131.71 (C-33, C-37), 116.04 (C-34, C-36), 161.54 (C-35); m/z: 547.12 (M^+).

4-Methyl-6-nitro-2-oxo-2H-chromen - 7yl - 2 -(4-(4-fluorophenyl)-6-4-tolyl- 2 H- 1, 3- oxazin-2-yl-amino)acetates (2g): Pale yellow solid, yield: 65%, mp: 110-112°C., M.F.: $C_{29}H_{22}FN_3O_7$; IR (KBr) ν cm^{-1}: 3234 (-NH), 1745, 1661(-C=O), 1596 (-C=N-), 3074, 2834 (-CH_3), 1534, 1357 (-NO_2), 1251, 1058 (-C-O-C-), 1179 (C-F); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.59 (s, 1H, -CH), 6.38-7.79 (m, 11H, aromatic), 5.35 (s, 1H, -CH), 3.71 (s, 2H, -CH_2NH),3.21 (s, 1H, -CH_2NH),2.42(s, 3H, -CH_3), 2.38 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.10 (C-2),113.93 (C-3), 152.52 (C-4), 120.76 (C-5), 139.48 (C-6), 135.63 (C-7), 115.74 (C-8), 159.14 (C-9), 118.66 (C-10), 19.43 (C-12), 168.15 (C-14), 40.96 (C-16), 111.13 (C-18),157.36 (C-20), 74.15 (C-21), 160.05 (C-22), 134.12 (C-24), 130.14 (C-25, C-29), 115.54 (C-26, C-28), 165.13 (C-27), 127.02 (C-32), 127.44 (C-33, C-37), 128.93 (C-34, C-36), 136.35 (C-35), 24.44 (C-38).; m/z: 543.14(M^+), 544.15(M+1).

4-Methyl-6-nitro-2-oxo-2H-chromen - 7yl - 2- (4-(4-fluorophenyl)-6-4-propyl - 2 H- 1, 3-oxazin-2-yl-amino)acetates (2h): Light yellow solid, yield: 63%, mp: 129-131°C., M.F.: $C_{31}H_{26}FN_3O_7$; IR (KBr) ν cm^{-1}: 3228 (-NH), 1735, 1654 (-C=O), 1598 (-C=N), 3071, 2832 (-CH_3), 1521, 1352 (-NO_2), 1256, 1049 (-C-O-C-), 1176 (C-F); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.59 (s, 1H, -CH), 6.36-7.78 (m, 11H, aromatic), 5.37 (s, 1H, -CH), 3.69 (s, 2H, -CH_2NH), 3.24 (s, 1H, -CH_2NH),2.41(s, 3H, -CH_3), 2.97 (t, 2H, -$CH_2CH_2CH_3$), 1.67 (m, 2H, -$CH_2CH_2CH_3$), 0.96 (t, 3H, -$CH_2CH_2CH_3$); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.13 (C-2),113.94 (C-3), 152.57 (C-4), 120.73 (C-5), 139.51 (C-6), 135.59 (C-7), 115.73 (C-8), 159.23 (C-9), 118.54 (C-10), 19.42 (C-12), 168.25 (C-14), 40.96 (C-16), 111.13 (C-18),157.36 (C-20), 74.15 (C-21), 160.05 (C-22), 134.12 (C-24), 130.14 (C-25, C-29), 115.54 (C-26, C-28), 165.13 (C-27), 127.02 (C-32), 127.44 (C-33, C-37), 128.93 (C-34, C-36), 139.35 (C-35), 34.34 (C-38), 24.12 (C-39), 13.34 (C-40); m/z: 571.55(M^+).

4-Methyl-6-nitro-2-oxo-2H-chromen - 7yl -2 -(4-(4-fluorophenyl) - 6 - 3 -bromo phenyl - 2H - 1, 3-oxazin-2-yl-amino)acetates (2i): Dark yellow solid, yield: 61%, mp: 132-134°C., M.F.: $C_{28}H_{19}BrFN_3O_7$; IR (KBr) ν cm^{-1}: 3225 (-NH), 1733, 1652 (-C=O), 1599 (-C=N), 3071, 2831 (-CH_3), 1522, 1352 (-NO_2), 1257, 1048 (-C-O-C), 1177 (C-F); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.58 (s, 1H, -CH), 6.37-7.72 (m, 11H, aromatic), 5.36 (s, 1H, -CH), 3.70 (s, 2H, -CH_2NH), 3.23 (s, 1H, -CH_2NH), 2.41 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.12 (C-2),113.91 (C-3), 152.51 (C-4), 120.74 (C-5), 139.44 (C-6), 135.63 (C-7), 115.74 (C-8), 159.15 (C-9), 118.64 (C-10), 19.41 (C-12), 168.16 (C-14), 40.97 (C-16), 111.14 (C-18),157.34 (C-20), 74.17 (C-21), 160.05 (C-22), 134.18 (C-24), 130.18 (C-25, C-29), 115.51 (C-26, C-28), 165.15 (C-27), 131.37 (C-32), 130.05 (C-33), 124.16 (C-34), 129.88 (C-35), 129.12 (C-36), 127.52 (C-37).; m/z: 607.04 (M^+),

4-Methyl-6-nitro-2-oxo-2H-chromen - 7 yl- 2- (4-(4-fluorophenyl)-6-3-phenoxy phenyl - 2H - 1, 3-oxazin-2-yl-amino) acetates (2j): Yellow solid, yield: 69%, mp: 109-111°C., M.F.: $C_{34}H_{24}FN_3O_8$; IR (KBr) ν cm^{-1}: 3232 (-NH), 1734, 1655 (-C=O), 1597 (-C=N-), 307, 2833 (-CH_3), 1525, 1354 (-NO_2), 1256, 1045 (-C-O-C-), 1173 (C-F); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.56 (s, 1H, -CH), 6.38-7.77 (m, 16H, aromatic), 5.36 (s, 1H, -CH), 3.70 (s, 2H, -CH_2NH), 3.24 (s, 1H, -CH_2NH), 2.40 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ: 160.11 (C-2),113.93 (C-3), 152.51 (C-4), 120.73 (C-5), 139.45 (C-6), 135.64 (C-7), 115.77 (C-8), 159.13 (C-9), 118.68 (C-10), 19.42(C-12), 168.18 (C-14), 40.95 (C-16), 111.12 (C-18),157.35 (C-20), 74.13 (C-21), 160.04 (C-22), 134.14 (C-24), 130.15 (C-25, C-29), 115.57 (C-26, C-28), 165.10 (C-27), 130.64 (C-32), 127.01 (C-33), 142.01 (C-34), 127.42 (C-35), 127.03 (C-36), 126.21 (C-37), 160.51 (C-39), 118.21 (C-40, C-44), 130.02 (C-41, C-43) 122.86 (C-42); m/z: 621.15 (M^+)

Biological Studies:

Antimicrobial Studies:

Antituberculer Activity: We have used the MIC to evaluate the anti-tuberculosis activity [25]. Each synthesized compound was diluted obtaining 2000 μg/ml concentration, as a stock solution and then many dilution made from it *i.e.* 500 μg/ml, 250 μg/ml, 125 μg/ml, 100 μg/ml, 50 μg/ml, 25 μg/ml, 12.5 μg/ml, 6.250 μg/ml, 3.125 μg/ml, 1.5625 μg/ml.

Reading Result: The highest dilution showing at least 99 % inhibition of colonies is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 10^8 organism / ml.

TABLE 2: ANTIBACTERIAL AND ANTIFUNGAL DATA OF COMPOUNDS 2 a-j

Compound Number	MIC (µg/mL)						
	Antibacterial activity				Antifungal activity		
	S. aureus	*S. pyogenes*	*E.coli*	*P. aeruginosa*	*C.albicans*	*A.niger*	*A.clavatus*
	MTCC 96	MTCC 442	MTCC 443	MTCC 1688	MTCC227	MTCC282	MTCC1323
2a	62.5	50	250	250	500	>1000	>1000
2b	125	200	250	200	500	250	250
2c	125	200	25	12.5	500	1000	1000
2d	100	125	200	125	>1000	500	500
2e	250	250	62.5	100	1000	1000	500
2f	125	100	500	500	500	1000	1000
2g	100	125	200	250	1000	500	1000
2h	125	125	250	250	>1000	1000	1000
2i	250	250	100	250	250	1000	1000
2j	100	100	500	500	500	1000	1000
Chloramphenicol	50	50	50	50	-	-	-
Ciprofloxacin	25	25	50	50	-	-	-
Norfloxacin	10	10	10	10	-	-	-
Nystatin	-	-	-	-	100	100	100
Griseofulvin	-	-	-	-	500	100	100

TABLE 3: *MYCOBACTERIUM TUBERCULOSIS* SCREENING RESULTS OF COUMARIN DERIVATIVES 2 a-j

Compound Number	*$H_{37}Rv$* MTCC 200	% Inhibition
2a	>1000	99
2b	100	98
2c	500	99
2d	1000	99
2e	>1000	99
2f	500	98
2g	100	98
2h	500	99
2i	1000	99
2j	500	98
Rifampicin		0.25 µg/mL
Isoniazid		0.20 µg/mL

TABLE 4: SCREENING RESULTS OF DPPH AND ABTS RADICAL SCAVENGING ACTIVITY OF 2 a–j

Compound Number	DPPH	ABTS
	IC_{50} µg/mL ± SD	IC_{50} µg/mL ± SD
2a	64.08 ± 1.313	67.82 ± 0.94
2b	137.58 ± 1.538	563.02 ± 0.590
2c	63.66 ± 1.829	268.55 ± 0.362
2d	95.79 ± 0.717	384.04 ± 4.025
2e	64.68 ± 0.752	87.63 ± 0.149
2f	64.35 ± 0.966	59.74 ± 0.355
2g	99.94 ± 0.582	373.98 ± 5.869
2h	59.78 ± 1.153	265.47 ± 3.576
2i	56.19 ± 0.509	61.64 ± 0.757
2j	124.70 ± 1.436	548.83 ± 0.801
Ascorbic Acid	36.22 ± 0.469	22.64 ± 0.260

Antioxidant activity: *In vitro* antioxidant activity of all compounds 2 a-j were carried out by the 2,-diplenyl1-1-picrylhydrazyl (DPPH) and 2,2′-Azinobis (3-ethylbenzthiazoline - 6 - sulfonate) (ABTS) cation radical assay according to the literature [26]. DPPH radical scavenging activity evaluation is a rapid and the best technique for screening antioxidant activities of the antioxidants. The values of IC_{50} of all compounds are higher than the IC_{50} value of ascorbic acid, which is shown in the following table.

RESULTS AND DISCUSSION:

Chemistry: 4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-chloroacetate 5 was synthesized from 7-hydroxy-4-methyl-6-nitro-2*H*-chromen - 2 - one 4. Compound 5 on coupling with 4-(4-Fluorophenyl)-6-substitutedphenyl-2*H*-1,3-oxazin-2-amine IV a-j to yield 4-methyl-6-nitro-2-oxo-2*H*-chroman-7yl-2-(4-(4-fluorophenyl)-6-substitutedphenyl - 2*H* - 1, 3-oxazin-2-ylamino) acetates 2a-j were obtained form 4-(4-fluorophenyl)-6-substitutedphenyl - 2*H*- 1, 3-oxazin-2-amine IV a-j Scheme.

Characterizations of intermediate and final compounds have been described in **Table 1**. The structures of compounds were confirmed by their spectral analysis. The characteristic band of 3242 cm^{-1} of -NH, while two bands for -C=O appeared at 1715 and 1671 cm^{-1} and 1523, 1351 cm^{-1} for -NO_2 in IR spectrum confirmed the structure of compounds. ^{1}H NMR spectrum of compound showed singlet at 8.55 of -CH confirmed the neighboring -NO_2 group while -CH_2NH showed two singlet at 3.71 and 3.23 respectively. ^{13}C NMR spectrum of compounds showed at 160.10 and 168.20 for two different C=O and one peak at 19.40 for CH_3 of coumarin. Peaks at 43.90, 81.10 and 111.30 were obtained for -CHNH, -CH of oxazine (C-18) respectively, while peaks observed at 150.20 and 164.70 for oxazine ring which confirming the structure of compounds.

Biology:

Antimicrobial Activities:

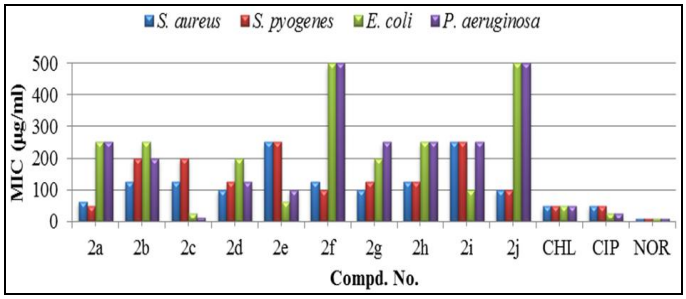


FIG. 1: MINIMUM INHIBITORY CONCENTRATIONS FOR ANTIBACTERIAL ACTIVITY OF 2a-j

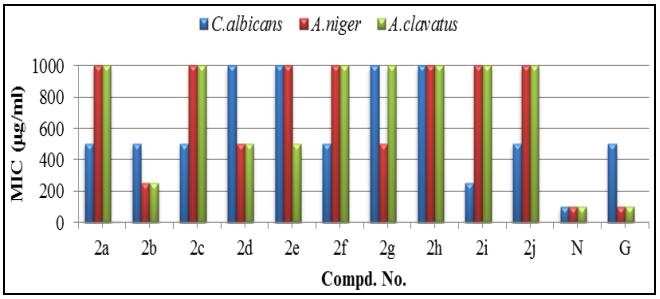


FIG. 2: MINIMUM INHIBITORY CONCENTRATIONS FOR ANTIFUNGAL ACTIVITY OF 2a-j

The minimum inhibitory concentrations (MIC) of 2a-j were evaluated against various bacterial and fungal species. The results of this activity are mentioned in **Table 2**.

Compound 2c with –Cl substitution at position 4 had excellent activity with 25 µg/ml while compound 2c, it showed excellent activity 12.5 µg/ml against *P. aeruginosa* which is comparable to chloramphenicol and ciprofloxacin while other compounds showed poor activity against *S. aureus* and *S. pyogenes* when compared with chloramphenicol, ciprofloxacin and norfloxacin. Other compounds 2i and 2j having substituent 3-Br and 3-OC_6H_5 respectively exhibited significant activity with MIC value 250 µg/ml. Compounds 2a (-C_6H_5), 2b (2-Cl), 2c (4-Cl), 2f (4-F) and 2j (3-OPh) exhibited comparable activity of 500 µg/ml against *C. albicans* as compared to the standard drug griseofulvin.

The minimum inhibitory concentrations (MIC) of 2a-j were tested for antituberculer activities. The results of this activity are mentioned in **Table 3**. Antituberculer activity results showed that, compound 2b and 2g containing 2-chloro and 3-bromo group demonstrated better activity 100 µg/ml with 99% inhibition against *M. tuberculosis* $H_{37}Rv$.

Antituberculer activities

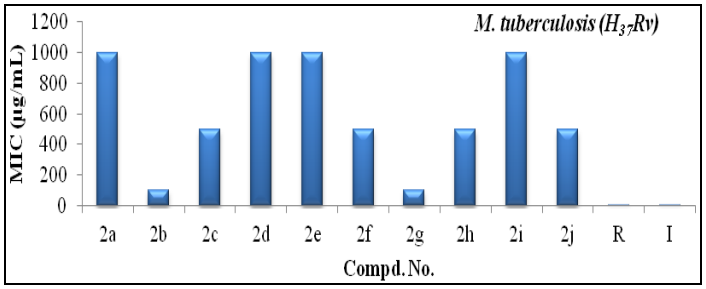


FIG. 3: ANTITUBERCULER ACTIVITIES OF 2a-j

Antioxidant activities

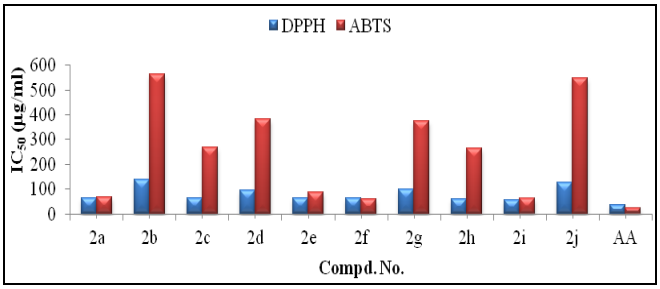


FIG. 4: ANTIOXIDANT POWER IC_{50} µg/mL ± SD of 2a-j

From the antioxidant inspections molecules 2i and 2h bearing 3-Br and 4-C_3H_7 respectively were appeared to have high radical scavenging efficacies as 56.19 ± 0.509 µg/ml ± SD and 59.78 ± 1.153

µg/ml ± SD of IC_{50} values in DPPH and 2f and 2i containing 59.74 ± 0.355 and 65.64 ± 0.757 µg/ml ± SD of IC_{50} values in ABTS bioassay, respectively and can be comparable to that of control ascorbic acid while other compounds have moderate to poor antioxidant power against scavenging DPPH and ABTS, results were summarized in **Table 4**.

CONCLUSION: The novel compound 2c had excellent activity with 25 µg/ml against *E. coli* and 12.5µg/ml against *P. aeruginosa* which is comparable to the standard drugs chloramphenicol and ciprofloxacin while other compounds showed poor activity against *S. aureus* and *S. pyogenes* when compared with chloramphenicol, ciprofloxacin and norfloxacin. Candidate compound 2b and 2g demonstrated better antiTB activity 100 µg/ml with 99% inhibition and 2i (56.19 ± 0.509) against DPPH and 2f (59.74 ± 0.355) against ABTS radical scavenging activity are highly potent amongst all compounds. While other compounds exhibited very less reactivity.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interest.

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