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Research Article

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Synthesis and Biological Evaluation of 4-Methyl-6-Nitro-2-oxo-2H-Chromen-7-yl 2-(4-(4-Sustitutedphenyl) Thiazol-2-ylamino) Acetates

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ABSTRACT

Coumarin segment shows remarkable broad spectrum of different biological activities with the thiazole derivative. In view that we have synthesized newer coumarin based thiazole and screened for their biological studies. 4-Phenyl-thiazol-2-ylamine (2a-j) condensed with 4-Methyl-6-nitro-2-oxo-2H-chromen-7-yl chloroacetate (VI) to afford 4-Methyl-6-nitro-2-oxo-2H-chromen-7-yl 2-(4-(4-sustitutedphenyl)thiazol-2-ylamino)acetates (3a-j). The newly synthesized compounds were characterized by IR, NMR and mass spectral studies and were screened for their antimicrobial, antitubercular and antioxidant activities. Compound 3a, 3e and 3g showed moderate antibacterial activity (100 µg/ml) against S. aureus and compound 3d showed good antibacterial activity (62.5 µg/mL) while compound 3a, 3c, 3g and 3j showed moderate activity (100 µg/mL) against S. pyogenus compared to chloramphenicol and ciprofloxacin. Compound 3a (100 µg/mL) exhibited moderate activity against E. coli and Compound 3b and 3i (100 µg/mL) exhibited moderate activity against P. aeruginosa compared to chloramphenicol and ciprofloxacin. Compounds 3g and 3j showed excellent activity (125 µg/ml) against C. albicans and compound 3g and 3j showed moderate activity against A. niger as compared to standard drug griseofulvin. While other compounds are less active against A. niger and A. clavatus compared to the standard drugs. The newly compound 3c (50.00 µg/mL), 3b (50.11 µg/mL), 3d (50.15 µg/mL), 3a (50.23 µg/mL) displayed higher potency amongst all synthesized compounds compared to ascorbic acid. The compound 3d (62.5 µg/mL) exhibited higher potency as antituberculosis amongst all newly synthesized compounds compared to the activity of rifampicin and isoniazid.

Keywords: Coumarin; Thaizole; Antimicrobial; Antitubercular; Antioxidant activity

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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, anticancer [4], antiplatelet, antithrombotic [5], antifungal [6], herbicidal [7], antiviral [8], anticoagulant [9], anti-inflammatory [10], antitumor [11], anti-oxidant activity [12] and cytotoxic [13]. In addition, 4 - and 7–hydroxy and nitro-coumarins are also antimicrobial [14] and antioxidant active and very important for the synthesis of other coumarin derivatives [15]. When it may be nitrated its antimicrobial activities is enhanced. It was then reacted with chloro acetyl chlorideto give chloro acetate derivative [16] of 4-Methyl-6-nitro-7-hydroxycoumarin. Thiazoles are a well-known group of heterocyclic compounds having a wide variety of biological activities, and their application as medicines are well established. Thiazole derivatives are reported to exhibit diverse biological activities as antimicrobial [17], antioxidant [18], antitubercular [19], anticonvulsant [20] and anticancer agents [21]. 2-aminothiazole and its derivatives are very useful because of its enormous activities in a wide spectrum due to various substituted thiazole moiety. 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 thiazoles to synthesize newer coumarin analogous and evaluated their antimicrobial, anti TB and anti-oxidant.

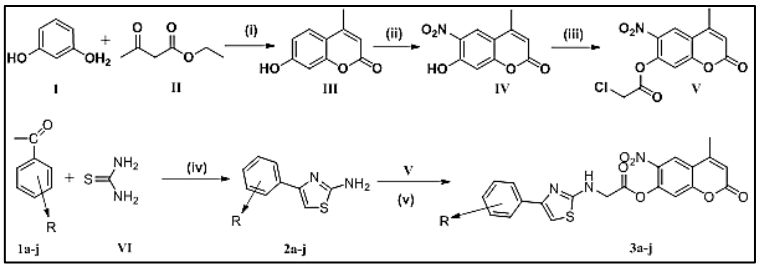
MATERIALS AND METHODS

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 are uncorrected. IR spectra were recorded on a PerkinElmer 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-2H-chromen-7-yl 2-chloroacetate (V) and final compounds (3a-j) is depicted in schemes. Synthesis of 4-Methyl-7-hydroxy-coumarin (III) was carried out by Pechmann condensation [22] followed by nitration with CAN, water, hydrogen peroxide [23] and then reacting with chloroacetyl chloride to get 4-Methyl-6-nitro-2-oxo-2H-chromen-7-yl 2-chloroacetate(V). 2-amino thiazoles (2a-j) was prepared by cycloaddition reaction between substituted acetophenones and thiourea [24]. These substituted amino thiazoles are condensed with compound (V) to synthesize desired final analoges (3a-j) scheme 1.



Scheme 1:(i) Cooled 5°C - 10°C, concentrated H_2SO_4; (ii) CAN, water, hydrogen peroxide, 50°C –60°C (iii) α-chloroacetyl chloride, CH_2Cl_2, triethyl amine; (iv) I_2, heating with stirring 8 h; (v) DMF, K_2CO_3, 6-8 h stirring

R= a.-H, **b.** 2-Cl, **c.** 4-Cl, **d.** 2,4-di Cl, **e.** 4-OH, **f.** 4-F, **g.** 4-CH_3, **h.** 4-C_3H_7, **i.** 3-Br, **j.** 3-OMe

The synthesized compounds were confirmed by the IR, NMR and mass spectra. IR spectrum of compound (V) shows absorption bands at 1751 for >C=O of ester and 1657 for ->C=O of lactone ring. Absorption band observed at 1190 indicating presence of -C-O-C, where -NO_2 gives band at 1571, 1346, while 3003, 2799 confirmed the -CH_3 and 831 for -C-Cl. ^{1}H NMR spectrum displayed singlet at δ 2.48 ppm due to -CH_3 group, singlet observed at 4.47 ppm due to -$COCH_2$ group, three proton of aromatic ring observed as multiplet at 6.37-8.38 ppm. The synthesized amino derivatives of substituted thiazoles were confirmed by the absorption bands at 3456 for -NH_2 and 1634 for -C=N, while ^{1}H NMR spectrum showed singlet at δ 6.78 ppm due to 2H of -NH_2 and six aromatic protons showed multiplet at δ 6.93-7.67 ppm. Final compound 3a showed bands at 3388 due to the presence of –NH and 1738 for -C=O, C=N of thiazole gave band at 1643, while ^{1}H NMR two singlet peaks were observed at δ 2.37 for 3 protons of -CH_3 and 4.45 for 2H of -$COCH_2$ while multiplet founded at 7.03-8.66 for 9H of aromatic rings. ^{13}C NMR spectrum showed signals at δ 169.87(C-14), 164.73(C-18), 160.02(C-2), 158.31(C-9), 152.01(C-4), 150.02(C-20), 141.26(C-6), 134.98(C-7), 131.68(C-23), 120.72(C-5), 118.70(C-10), 115.67(C-8), 112.36(C-3), 102.22(C-21), 43.36(C-16), 19.38(C-12) confirmed different carbons in 3a. Molecular ion peak at m/z: 437.30 (M+) in mass spectrum of 3a gave the confirmation of final compound 3a.

Synthesis of 4-Methyl -7-Hydroxy-Coumarin (III)

0.01 mol resorcinol**(I)** was added to 0.01 mol of ethylacetoacetate (II) by stirring to make a homogeneous solution and this solution was added drop by drop in to the flat bottom flask containing previously cooled (5°C – 10°C) H_2SO_4 (75 mL) by maintaining the temperature of the reaction mass below 10°C. After completion of addition stirring was continued for 30 min. The reaction mixture was poured on to crushed ice. The product was filtered and washed several time with distilled water. The product was dissolved in cold 10% NaOH solution and re-precipitated by 10% aqueous hydrochloric acid till the solution becomes acidic to litmus paper. The product was washed with cold water till filtrate become neutral, it was crystallized from ethanol using activated charcoal to get white solid, Yields: 85%, mp: 183°C to 185°C. IR (KBr): 3423 (-OH), 1733 (-CO), 1555 (-C=C)

cm-^{1}; ^{1}H NMR (300MHz, $CDCl_3$): 10.35 (s, 1H, Phenolic -OH), 7.48 (d, 1H, aromatic) 6.76 (d,1H,aromatic), 6.69 (s, 1H,aromatic), 2.37 (s, 3H, -CH_3).

Synthesis of Mono nitro derivative of 4-Methyl -7-Hydroxy-Coumarin (IV)

The substrate (III) (0.01 mol) was charged in two portions to a solution of Cerric ammonium nitrate (CAN) (0.01 mol) in water (5 mL) to which 30% hydrogen peroxide (1 mL) was added. The reaction mixture was heated at 50–60°C with continuous vigorous stirring for 1 h. the progress of reaction was monitored by TLC (Ethyl acetate: Toluene, 3:7). After the completion of the reaction, the reaction mixture was cooled, diluted with water (20 mL), and extracted with chloroform (3 × 30 mL). The solvent was removed from the concentrated extract and the product (IV) was purified by column chromatography. Yield 75%, m.p. 196°C.IR (KBr) cm^{-1}: 3490(-OH), 3094,2827(-CH_3, asym, sym), 1745 (>C=O str), 1190 (-C-O-C), 1536, 1358 (-NO_2);^{1}H NMR (400 MHz, DMSO-d_6, TMS): 13.26 (s, 1H, Phenolic -OH), 8.44 (s, 1H, aromatic), 7.09 (s,1H,aromatic), 6.35 (s, 1H,aromatic), 2.37(s, 3H, -CH_3).

Synthesis of 4-Methyl-6-Nitro-2-Oxo-2H-Chromen-7-yl 2-Chloroacetate (V)

To a solution of compound (IV) (0.01 mol) and α-chloroacetyl chloride (0.012 mol) in di chloro methane [CH_2Cl_2](30 mL), triethyl amine (9.6 mL, 0.0765 mol) was added drop wise and the mixture was stirred for one hour. The completion of the reaction was monitored by TLC (CH_2Cl_2:MeOH, 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 CH_2Cl_2 layer was then dried ($MgSO_4$) filtered and evaporated. The residue was crystallized from ethyl acetate-hexane as brownish yellow crystals, yields: 69%, mp: 109°C to 112°C. IR (KBr) cm^{-1}: 3003, 2799(-CH_3, asym, sym), 1751, 1657 (>C=O str), 1190 (-C-O-C), 1571, 1346 (-NO_2), 831 (-C-Cl);^{1}H NMR (400 MHz, DMSO-d_6, TMS): 8.38 (s, 1H,aromatic), 7.27 (s,1H,aromatic), 6.37 (s, 1H, aromatic), 4.47 (s, 2H, -$COCH_2$), 2.48 (s, 3H, -CH_3).

Synthesis of 2-Amino-4-Substituted Thiazoles (2a-j)

Thiourea (0.04 mol) and I_2 (0.02 mol) were triturated and mixed with acetophenone (0.02 mol). The mixture was heated on a water bath with occasional stirring for 8 hours. The completion of the reaction was monitored by TLC. The obtained solid was triturated with ethanol to remove unreacted acetophenone, washed with aqueous sodium thiosulfate to remove excess iodine and then with water. The crude product was dissolved in hot water, filtered to remove the sulphur, and 4-phenylthiazol-2-amine (2a) was precipitated by addition of NH_4OH till the solution became slight basic.

4-phenylthiazol-2-amine (2a):

White crystal, Yield 63%, mp: 146-148°C. IR (KBr) cm^{-1}: 3453 (-NH_2), 1635 (-C=N), ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 6.35 (s, 2H, -NH_2), 7.40-7.80 (m, 6H, Ar-H).

4-p-tolylthiazol-2-amine (2b):

White crystal, Yield 69%, mp: 131-134°C. IR (KBr) cm^{-1}: 3449 (-NH_2), 1634 (-C=N), 2929 (-CH_3), 1H NMR (400 MHz, DMSO-d_6, TMS): δ 2.34 (s, 3H, -CH_3), 6.34 (s, 2H, -NH_2), 7.37-7.79 (m, 5H, Ar-H).

4-o-tolylthiazol-2-amine (2c):

White crystal, Yield 62%, mp: 130-133°C. IR (KBr) cm^{-1}: 3447 (-NH_2), 1637 (-C=N), 2925 (-CH_3), 1H NMR (400 MHz, DMSO-d_6, TMS): δ 2.36 (s, 3H, -CH_3), 6.79 (s, 2H, -NH_2), 7.02-7.69 (m, 5H, Ar-H).

Synthesis of 4-Methyl-6-nitro-2-oxo-2H-chromen-7-yl 2(4-4-sustituted phenyl) thiazol-2-ylamino) Acetates (3a-j)

All the reactions were carried out under nitrogen atmosphere. In a round bottom containing compound (V) (0.01 mol) and 4-phenylthiazol-2-amine (2a) (0.01 mol), DMF and K_2CO_3 (2.4 equivalent) were added under with constant stirring. Reaction Mixture was refluxed for 8-9 h. The completion of the reaction was monitored by TLC on silica gel using ethylacetate: toluene (1:3). After the completion of the reaction, mixture was poured over crushed ice, solids that are separated out was filtered, washed with saturated solution of $NaHCO_3$ and dried. The crude product was purified by column chromatography using silica gel 100-200 mesh and gradient (0-80%) ethyl acetate in hexane as eluent. The precipitate obtained was filtered, washed and recrystallized from suitable solvent (Figure 1).

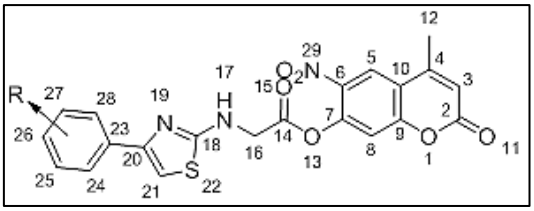


Figure 1: Synthesis of 4-methyl-6-nitro-2-oxo-2H-chromen-7-yl 2(4-4-sustituted phenyl) thiazol-2-ylamino)acetates(3a-j)

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-phenylthiazol-2-ylamino) acetate (3a):
Yellowish brown solid, yield: 49%, mp: 168-169°C, M.F.: $C_{21}H_{15}N_3O_6S$.
IR (KBr) cm^{-1}: 3388 (-NH), 1738 (-C=O), 1643 (-C=N), 2912, 2840 ($-CH_3$), 1509, 1377 ($-NO_2$); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 7.03-8.66 (m, 9H, aromatic), 5.92 **(**s, 1H, -NH**),** 4.45 (s, 2H, $=COCH_2$), 2.37 (s, 3H, $-CH_3$);^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 169.87(C-14), 164.73(C-18), 160.02(C-2), 158.31(C-9), 152.01(C-4), 150.02(C-20), 141.26(C-6), 134.98(C-7), 131.68(C-23), 129.83(C-25,C-27), 128.70(C-26), 125.66(C-24,C-28), 120.72(C-5), 118.70(C-10), 115.67(C-8), 112.36(C-3), 102.22(C-21), 43.36(C-16), 19.38(C-12); m/z: 437.40 (M^+).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-(2-chlorophenyl) thiazol-2-ylamino) acetate (3b):
Redish yellow solid, yield: 79%, mp: 207-208°C, M.F.: $C_{21}H_{14}ClN_3O_6S$.
IR (KBr) cm^{-1}: 3328 (-NH), 1748 (-C=O), 1625 (C=N of thiazole), 2899, 2959 ($-CH_3$), 786 (aryl-Cl), 1514, 1337 ($-NO_2$);^{1}H NMR (400 MHz, DMSO-d6, TMS): δ 7.02-8.65 (m, 8H, aromatic), 5.93 (s, 1H, -NH), 4.44 (s, 2H, $=COCH_2$), 2.37 (s, 3H, $-CH_3$);^{13}C NMR (100MHz, DMSO-d_6, TMS): δ 169.88(C-14), 164.75(C-18), 160.03(C-2), 158.34(C-9), 152.05(C-4), 147.15(C-20), 141.26(C-6), 134.95(C-7), 133.13(C-25), 130.60 (C-27), 130.50(C-26), 130.45(C-24),128.62(C-23), 126.63(C-28), 120.73(C-5), 118.72(C-10), 115.65(C-8), 112.34(C-3), 102.25(C-21), 43.35(C-16), 19.33(C-12); m/z: 471.87(M^+),473.07(M+2).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-(4-chlorophenyl) thiazol-2-yl amino) acetate (3c):
Brown yellow solid, yield: 78%, mp: 231-233 °C, M.F.: $C_{21}H_{14}ClN_3O_6S$.
IR (KBr) cm^{-1}: 3326 (-NH), 1749 (-C=O), 1623 (C=N of thiazole), 2895, 2958 ($-CH_3$), 785(aryl-Cl) 1514, 1331 ($-NO_2$), ^{1}H NMR (400 MHz, DMSO-d6, TMS): δ 2.35(s, 3H, $-CH_3$), 3.89 (s, 2H, $-COCH_2$), 7.31-8.10 (m, 7H, Ar-H), ^{13}C NMR (100MHz, DMSO-d_6, TMS): δ 168.04(C-14), 164.75(C-18), 160.03(C-2), 158.34(C-9), 152.05(C-4), 148.55(C-20), 141.26(C-6), 134.95(C-7), 136.63(C-25), 135.60 (C-27), 136.32(C-26), 128.69(C-24),136.56(C-23), 126.63(C-28), 120.73(C-5), 118.72(C-10), 115.65(C-8), 112.34(C-3), 115.35(C-21), 43.57(C-16), 19.33(C-12); m/z: 471.03(M^+),473.07(M+2).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-(2,4-dichlorophenyl) thiazol-2-ylamino) acetate (3d):
Pale yellow solid, yield: 65%, mp: 233-235 °C., M.F.:$C_{21}H_{13}Cl_2N_3O_6S$.
IR (KBr) cm^{-1}: 3326 (-NH), 1749 (-C=O), 1623 (>C=N of thiazole), 2895, 2958 ($-CH_3$), 795(aryl-Cl), 1514, 1331 ($-NO_2$), ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.32(s, 3H, $-CH_3$), 3.88 (s, 2H, $-COCH_2$), 7.31-8.10 (m, 8H, Ar-H), ^{13}C NMR (400MHz, DMSO-d_6, TMS): δ 168.04(C-14), 164.75(C-18), 160.08(C-2), 158.34(C-9), 152.05(C-4), 148.95(C-20), 141.26(C-6), 134.95(C-7), 130.97 (C-25), 135.60 (C-27), 135.70(C-26), 133.61(C-24),136.56(C-23), 130.34(C-28), 120.73(C-5), 118.72(C-10), 115.65(C-8), 112.34(C-3), 115.35(C-21), 43.07(C-16), 19.43(C-12); m/z: 506.32 (M^+),508.18 (M+2),510.15 (M+4).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-(4-hydroxyphenyl) thiazol-2- ylamino) acetate (3e):
Brown solid, yield: 72%, mp: 135-137°C, M.F.: $C_{21}H_{15}N_3O_7S$.
IR (KBr) cm^{-1}: 3456 (-OH), 3327 (-NH), 1745 (-C=O), 1628 (C=N of thiazole), 2899, 2955 ($-CH_3$), 1511, 1334 ($-NO_2$);^{1}H NMR (400 MHz, DMSO-d6, TMS): δ 9.61 (s, 1H, -OH), 7.03-8.66 (m, 8H, aromatic), 5.91 (s, 1H, -NH), 4.46 (s, 2H, $-COCH_2$), 2.35 (s, 3H, $-CH_3$); ^{13}C NMR (100MHz, DMSO-d_6, TMS): 168.04(C-14), 164.75(C-18), 160.08(C-2), 158.38(C-9), 152.07(C-4), 148.91(C-20), 139.82(C-6), 134.93(C-7), 116.55 (C-25&C-27), 158.21(C-26), 133.60(C-24), 136.52(C-23), 130.33(C-28), 120.73(C-5), 118.70C-10), 115.64(C-8), 112.54(C-3), 115.42(C-21), 43.02(C-16), 19.32(C-12); m/z: 453.43 (M^+).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl 2-(4-(4-fluorophenyl) thiazol-2ylamino) acetate (3f):
Dark yellow solid, yield: 68%, mp: 197-199 °C, M.F.: $C_{21}H_{14}ClN_3O_6S$.
IR (KBr) cm^{-1}: 3319 (-NH), 1745 (-C=O), 1609 (C=N of thiazole), 2865, 2941 ($-CH_3$), 1177(aryl-F) 1514, 1331 ($-NO_2$), ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.34(s, 3H, $-CH_3$), 4.44 (s, 2H, $-COCH_2$), 7.08-8.54 (m, 8H, Ar-H);^{13}C NMR (100MHz, DMSO-d_6, TMS): δ 168.54 (C-1), 43.69 (C-2), 23.56 (C-19), 148.69 (C-21), 115.36 (C-22), 136.56 (C-24), 128.69 (C-25), 128.86 (C-29), 131.62 (C-26), 131.61 (C-28), 126.24 (C-27),

26.38 (-CH_3). 168.01(C-14), 164.72(C-18), 160.07(C-2), 158.32(C-9), 152.07(C-4), 148.90(C-20), 131.83(C-6), 134.90(C-7), 116.15 (C-25 & C-27), 162.9(C-26), 131.60(C-24), 136.52(C-23), 131.63(C-28), 120.73(C-5), 118.70(C-10), 115.64(C-8), 112.54(C-3), 115.42(C-21), 43.02(C-16), 19.36(C-12); m/z: 471.87 (M^+).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-p-tolylthiazol-2-ylamino) acetate (3g):
Yellow solid, yield: 63%, mp: 139-141°C, M.F.: $C_{22}H_{17}N_3O_6S$.
IR (KBr) cm^{-1}: 3319 (-NH), 1745 (-C=O), 1609 (C=N of thiazole), 2865, 2941 (-CH_3), 1177(aryl-F) 1514, 1331 (-NO_2);^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.34(s, 3H, -$CH_3$of pyrone ring of coumarin), 2.33(s, 3H, -CH_3 of aromatic ring), 4.19 (s, 2H, -$COCH_2$), 7.24-8.55 (m, 8H, Ar-H);^{13}C NMR (100MHz, DMSO-d_6, TMS): δ 169.87(C-14), 164.71(C-18), 160.01(C-2), 158.31(C-9), 152.0(C-4), 150.03(C-20), 141.26(C-6), 134.98(C-7), 131.63(C-23), 129.83(C-25,C-27), 131.70(C-26),129.45(C-24,C-28), 120.71(C-5), 118.71(C-10), 115.67(C-8), 112.36(C-3), 102.22(C-21), 43.36(C-16), 21.16(C-30), 19.38(C-12).; m/z: 451.08(M^+).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl 2-(4-(4-propylphenyl) thiazol-2-ylamino) acetate (3h):
Yellow solid, yield: 63%, mp: 139-141°C, M.F.: $C_{24}H_{21}N_3O_6S$.
IR (KBr) cm^{-1}: 3319 (-NH), 1745 (-C=O), 1609 (C=N of thiazole), 2865, 2941 (-CH_3), 1177(aryl-F) 1514, 1331 (-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.34(s, 3H, -CH_3 of pyrrone ring of coumarin), 4.18 (s, 2H, -$COCH_2$), 2.5,1.51 & 0.81(s, 2H, s, 2H & s, 3H, of -CH-CH_2-CH_3respectively), 7.33-8.54 (m, 8H, Ar-H);^{13}C NMR: (100MHz, DMSO-d_6, TMS): δ169.87(C-14), 164.73(C-18), 160.02(C-2), 158.31(C-9), 152.01(C-4), 150.02(C-20), 141.26(C-6), 134.98(C-7), 131.68(C-23), 130.14(C-25,C-27), 141.70(C-26), 125.68(C-24,C-28), 120.72(C-5), 118.70(C-10), 115.67(C-8), 112.36(C-3), 102.22(C-21), 43.36(C-16), 37.9 (C-30), 24.1 (C-31), 13.6(C-32), 19.38(C-12); m/z: 479.12 (M^+).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl-2-(4-(3-bromophenyl) thiazol-2-ylamino acetate (3i):
Light yellow solid, yield: 64%, mp: 236-239°C, M.F.: $C_{21}H_{14}BrN_3O_6S$.
IR (KBr) cm^{-1}: 3326 (-NH), 1749 (-C=O), 1623 (C=N of thiazole), 2895, 2958 (-CH_3), 1257(aryl-Br), 1514, 1331 (-NO_2), ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 2.50(s, 3H, -CH_3), 3.99 (s, 2H, -$COCH_2$), 7.11-8.51 (m, 8H, Ar-H);^{13}C NMR (100MHz, DMSO-d6, TMS): δ 169.87(C-14), 164.72(C-18), 160.05(C-2), 158.34(C-9), 152.02(C-4), 164.12(C-20), 141.26(C-6), 134.98(C-7), 131.68(C-23), 122.83(C-25),131.39(C-27), 128.70(C-26), 122.66(C-24), 126.9(C-28), 120.72(C-5), 118.70(C-10), 115.67(C-8), 112.39(C-3), 104.22(C-21), 43.16(C-16), 19.39(C-12).m/z: 514.38(M^+),516.27(M+2).

4-methyl-6-nitro-2-oxo-2H-chromen-7-yl 2-(4-(3-methoxyphenyl) thiazol-2-ylamino) acetate (3j):
Pale yellow solid, yield: 72%, mp: 205-207°C, $C_{22}H_{17}N_3O_7S$.
IR (KBr) cm^{-1}: 3385 (-NH), 1737 (-C=O), 1641 (-C=N), 2911, 2840 (-CH_3), 2816 (-OCH_3) 1507, 1378 (-NO_2);^{1}H NMR (400 MHz, DMSO-d_6, TMS): **):**δ 7.08-8.59 (m, 8H, aromatic), 5.96 **(**s, 1H, -NH**),** 4.42 (s, 2H, =$COCH_2$), 2.39 (s, 3H, -CH_3), 3.81(-OCH_3);^{13}C NMR (100MHz, DMSO-d_6, TMS): δ169.82(C-14), 164.73(C-18), 160.02(C-2), 158.31(C-9), 152.01(C-4), 150.01(C-20), 141.25(C-6), 133.98(C-7), 133.68(C-23), 159.83(C-25), 132.2(C-27), 114.70(C-26), 113.66(C-24), 119.4(C-28), 120.71(C-5), 118.71(C-10), 115.65(C-8), 112.35(C-3), 105.22(C-21), 55.6(C-31,-OCH_3)43.16(C-16), 19.37(C-12).m/z: 467.04(M^+).

Table 1: Antibacterial activity of 3(a-j)

Antibacterial activity					
Minimal concentration in µg/ml					
Sr. No	Code	Gram Positive		Gram Negative	
		S. aureus	**S. *pyogenus***	***E. coli***	**P. *aeruginosa***
		MTCC 96	MTCC 442	MTCC 443	MTCC 1688
1	**3a**	100	100	100	250
2	**3b**	250	250	125	100
3	**3c**	250	100	500	200
4	**3d**	200	62.5	250	125
5	**3e**	100	200	250	200
6	**3f**	200	200	200	250
7	**3g**	100	100	200	200
8	**3h**	250	250	200	250
10	**3i**	250	125	125	100
9	**3j**	125	100	250	200
Ciprofloxacin		50	50	25	25
Chlormphenicol		50	50	50	50

Table 2: Antifungal activity of 3(a-j)

Sr. No.	Code	Minimal concentration in µg/mL		
		C. *albicans*	**A. *niger***	**A. *clavatus***
		MTCC 227	MTCC 282	MTCC 1323
1	**3a**	500	500	500
2	**3b**	200	250	1000
3	**3c**	250	200	>1000
4	**3d**	500	200	500
5	**3e**	200	250	1000
6	**3f**	250	125	250
7	**3g**	125	100	1000
8	**3h**	250	200	1000
9	**3i**	250	1000	1000
10	**3j**	125	100	1000
Nystatin		100	100	100
Griseofulvin		500	100	100

Table 3: Screening results of DPPH radical scavenging activity of coumarin derivatives (3a–j)

No.	IC_{50}µg/mL ± SD
3a	50.23 ± 0.418
3b	50.11 ± 0.771
3c	50.00 ± 1.077
3d	50.15 ± 1.827
3e	55.78 ± 1.717
3f	53.62 ± 0.910
3g	61.46 ± 0.721
3h	58.44 ± 1.734
3i	63.24 ± 0.975
3j	60.05 ± 0.579
Ascorbic Acid	36.22 ± 0.469

Table 4: *Mycobacterium tuberculosis* screening results of coumarin derivatives (3a-j) against H37Rv (Acid Fast Bacilli) MTCC – 200 (DMSO as diluent)

No	MIC
3a	1000
3b	500
3c	500
3d	62.5
3e	100
3f	250
3g	500
3h	250
3i	100
3j	250
Rifampicin Isoniazid	**0.25 µg/mL 0.20 µg/mL**

DISCUSSION

Antibacterial Activity

The compound 3d showed good activity (62.5 µg/mL) against *S. pyogenus* compared to chloramphenicol (50 µg/mL) and ciprofloxacin (50 µg/mL). 3a(phenyl substituent), 3c(4-chloro substituent), 3g and 3j showed moderate activity (100 µg/ml) against *S. pyogenus* compared to chloramphenicol and ciprofloxacin. Compound 3j (100 µg/mL) and (125 µg/mL) exhibited comparable activity against *S. pyogenus* and *S. aureus* respectively compared to chloramphenicol and ciprofloxacin. Compound 3a (100 µg/mL) exhibit good activity against *E. coli* and Compound 3b and 3i (100 µg/mL) exhibit moderate activity against *P. aeruginosa* compared to chloramphenicol and ciprofloxacin (50 µg/mL) (Table 1).

Anti-Fungal

Compound 3g (4-Methylsubstituent) and 3j(3-methoxysubstituent) (100µg/mL) showed excellent activity against *A. niger* compared to standard drugs griseofulvin and nystatin. Compound 3g(4-Methyl substituent) and 3j(3-methoxy substituent) (125 µg/mL) showed good activity against *C. albicans* compared to standard drug

griseofulvin and nystatin. While other compounds are less active against *A. niger* and *A. clavatus* compared to the standard drugs.

Antioxidant Activity

In vitro antioxidant activity of all newly synthesized compounds 3a-j were carried out by the 2,-diplenyl-1-picrylhydrazyl (DPPH) assay according to the literature [25]. 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 Table 2. Compound 3c (50.00 µg/mL), 3b (50.11 µg/mL), 3d (50.15 µg/mL), 3a (50.2 ± 0.469).

Anti-tubercular Activity

We have used the minimal inhibition concentration to evaluate the anti-tuberculosis activity [26]. It is one of the non-automated *in vitro* bacterial susceptibility tests. Following common standard strain is used for screening of antituberculer activities. Mycobacterium tuberculosis $H_{37}Rv$ [Acid Fast Bacilli] MTCC – 200 DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon standard bacterial strains. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms. It is carried out in bottle. The compound 3d (2,4-dichlorophenyl substituent) exhibited higher potency(62.5 µg/mL) (Tables 3 and 4) amongst all newly synthesized compounds compared to standard drugs rifampicin and isoniazid.

CONCLUSION

The newly synthesized compound **3d** showed excellent antibacterial activity against *S. pyogenus* compared to standard drug that is because of the presence of two chlorine atoms as substituent. In antifungal activity compound **3g** and **3j** exhibited excellent activity against *A. niger* compare to standard drugs because of presence of methyl and methoxy groups in para position which give rise ortho / para resonance. The results of antioxidant and antitubercular activities displayed moderate activity but certain modification in the synthesized compounds may give rise to both of the activities.

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