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Pyridine clubbed coumarin analogues: Their synthesis and biological studies as antimicrobials and antioxidants

Navin B Patel[a], Nilesh B Chauhan[a], Sabir S Pathan[a], Vatsal M Patel*[b] & Bhupendra M Mistry[c]

[a] Department of Chemistry, Veer Narmad South Gujarat University, Surat 395 007, India

[b] Department of Chemistry, Jamanaben Narottambhai Motiram Patel Science College, Bharthana (Vesu), Surat 395 017, India

[c] Department of Food Science and Biotechnology, College of Life and Biotechnology, Dongguk University, Biomedical Campus, 32 Dongguk-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do, Republic of Korea

E-mail: patelvatsal1904@gmail.com; drnavinbpatel@gmail.com

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The major aim of this study is to develop the new class of coumarin candidate clubbed with dihydropyridine-3-carbonitrile with an improved potency as an antimicrobial and antioxidant agent. The key intermediate 6-nitro-4-methyl coumarin-yl chloro acetate 5 have been linked to the 6-(4-fluorophenyl)-2-oxo-4-phenyl-1,2-dihydro pyridine-3-carbonitrile IIa-j derivative to afford 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-(3-cyano-6-(4-fluoro phenyl)-4-(substituted-phenyl) pyridin-2-yl-oxy) acetates 7a-j *via* efficient organic transformations. All the new derivatives have been characterized by spectral studies (IR, ^{1}H and ^{13}C NMR and mass spectroscopy). *In vitro* antimicrobial activity have been carried out using the broth microdilution method and antioxidant potency using DPPH bioassays. Bioassay results reveal that compound 7e are equipotent against *E. coli* with MIC value 50 µg/ mL compared to standard drug ciprofoloxacin. A final analogue 7c with 4-chlorophenyl substituent indicated better antifungal potency against *C. albicans* with MIC value 100 µg/ mL compared to standard drug griseofulvin. In addition, newly synthesized analogues have been found to be significant scavengers of DPPH radical with IC_{50} values of 32.11 µg/mL. It has been observed that the potent antibacterial candidate has proved to possess significant antioxidant activity. The presence of chlorine and hydroxy group on phenyl ring plays an important role for the potency in above mentioned biological assay.

Keywords: Coumarin, cynopyridine, antibacterial, antifungal, antioxidant

The increasing resistance of pathogenic microorganisms to conventional drugs has become a serious problem in healthcare. This requires the urgent need to replace existing therapeutics with better alternatives. Heterocyclic hybridization approach to design new bioactive molecule is an emerging tool in drug discovery because it allows integrating two or more pharmacophore into one molecular scaffold with an improved pharmacological activities. Among a large variety of heterocyclic compounds, coumarin, a condensed heterocycle of benzene and pyran-2-one, recognized as an important pharmacophore. It is widely reported that natural[1] and synthetic coumarin[2] analogous shows a significant pharmacological effect. Novobiocin and Chlorobiocin are well known antimicrobials containing a coumarin nucleus[3]. Presence of coumarin nucleus in numerous categories of therapeutic agents such as antibacterial[4], antifungal[5], antitubercular[6], antiviral[7], antimalarial[8], analgesic[9], antiparasitic[10], antitumor[11], anti-inflammatory[12], antioxidants[13], anticoagulants[14], antidiabetics[15], *etc*. has made it a valuable lead for the development of new therapeutic agents. Antimicrobial activity of nitro coumarins has been reviewed extensively[16,17] and it has been observed that when the nitro group has been incorporated with coumarin, its antimicrobial activities is enhanced. The derivatives contain pyridine core are versatile and used as active pharmaceutical ingredients[18]. Among the varied pyridine derivatives, 3-cyanopyridines comprise a very interesting class of compounds because of their significant and versatile biological activities[19,20], *viz*, neurotropic activity[21], anti-inflammatory, analgesic[22], antioxidant[23], Tyrosine kinase inhibitors[24], *etc*.

With this consideration and continuation[25] of our ongoing interest in the synthesis of the pyridine clubbed coumarin derivatives, we have been prompted to synthesize possibly more potent pharmacologically active compounds. The present article deals with the design and synthesis of coumarin clubbed pyridine-3-carbonitrile analogous. We have condensed chloro acetate of 4-methyl-7-

hydroxy -6- nitrocoumarin with hydoxy pyridine intermediates. The synthesized compounds were assigned on the basis of IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. The *in vitro* antimicrobial evaluation of these derivatives were obtained by broth microdilution method and antioxidant activity have been evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

Results and Discussion

Chemistry

Scheme I represents the synthetic pathway followed to generate key intermediates (5) and IIa-j along with the final coumarine clubbed pyridine derivatives (7a–j). According to Scheme I, synthesis of 4-methyl-7-hydroxy-coumarin (3) by Pechmann condensation followed by nitration with cerric ammonium nitrate and then reacting with chloroacetyl chloride yielding 4-methyl-3-nitro-2-oxo-2*H*-chromen-7-yl 2-chloroacetate (5). For the synthesis of final compounds (Figure 1), the key intermediate (5) was condensed with 6-(4-fluorophenyl)-2-oxo-4-substituted-phenyl-1,2-dihydropyridine-3-carbonitriles (IIa-j) which are obtained *via* the multicomponent reaction using 4-fluoroacetophenone, substituted benzaldehyde and ethylcyanoacetate in the presence of ammonium acetate in ethanol. The structure of key intermediates and compounds 7a-j were characterized and confirmed utilizing spectroscopic techniques such as IR, ^{1}H and ^{13}C NMR and mass spectrometry. Absorption bands at 3037, 2912 cm^{-1} for the IR of 7a-j confirmed the presence of C-H stretching for aromatics and -CH_3 group, whereas C-N banding observed at 2226 cm^{-1}. The >C=O band indicated a sharp peak nearby 1714 and 1687 cm^{-1} along with sharp >C=N- stretching at 1624 cm^{-1}. The various characteristic bands nearer to 1161 for aryl-F and 1510, 1347 indicate the presence of -NO_2 group in the final compounds. ^{1}H NMR spectrum of compound showed singlet at 8.58 for –CH confirmed the neighboring -NO_2 group while -CH_2O showed singlet at 5.18 and –CH_3 at 3.23 δ_{ppm}

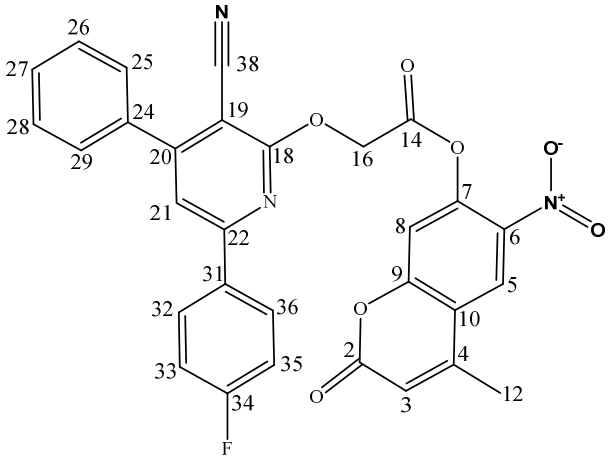
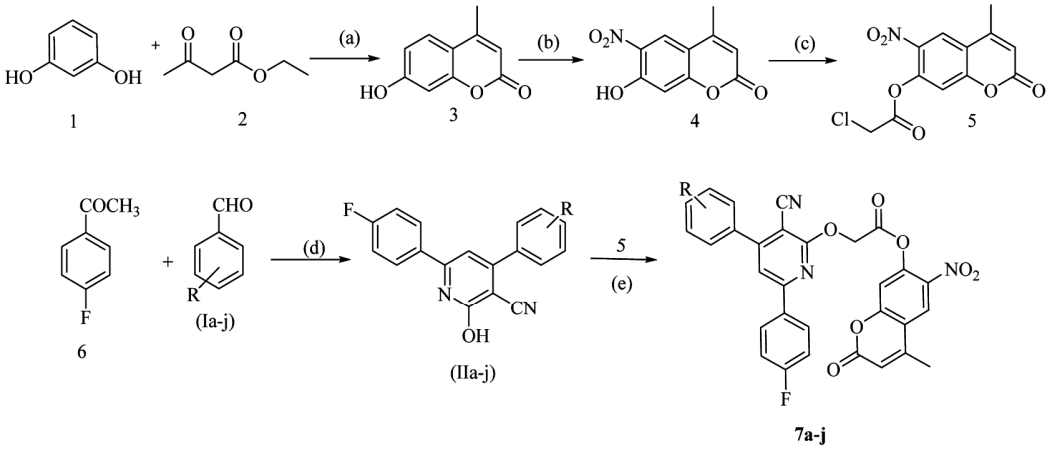


Figure 1 — Numbering of carbon atoms in the representative structure of the final compound (4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-((3-cyano-6-(4-fluorophenyl)-4-phenylpyridin-2-yl)oxy)acetate)



Reaction conditions: (a) Cooled (5°C – 10°C), conc. H_2SO_4; (b) cerric ammonium nitrate (CAN), 30% H_2O_2 + 5 mL H_2O, stirred; (c) α-chloroacetyl chloride, CH_2Cl_2, triethyl amine 1 h stirred; (d) Ethylcyanoacetate, ammonium acetate and ethanol, 6-8 h reflux; (e) DMF, K_2CO_3, 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-chloroacetate (5), 8-9 h refluxed.

Where, R = -H, 2-Cl, 4-Cl, 2-OH, 4-OH, 4-F, 4-CH_3, 4-C_3H_7, 3-Br, 3-OPh.

Scheme I

respectively. The ^{13}C NMR data observed for compounds 7a-j further confirmed the correct formation of the synthesized structure. ^{13}C NMR spectrum showed peaks at 160.63 and 165.06 for two different C=O, one peak at 19.40 for -CH_3 of coumarin, 114.21 was obtained for –C≡N group, which confirming the structure of final compounds. The high-resolution mass spectral data of the final derivatives exhibited molecular ion peaks at their respective molecular weights, which confirmed their formation.

Biology

Antimicrobial evaluation

Antibacterial and antifungal activities of 7a-j were evaluated (Table I). Among all the synthesized compounds, 7e showed better antibacterial activity against *E. coli* and 7c demonstrates the best antifungal activity against *C. albicans*. All the other compounds had moderate to poor activity as antifungal agents with higher MIC values. Both electron withdrawing and donating groups at *ortho*-, *meta*-, and *para*-position on the phenyl ring of 6-(4-fluorophenyl)-2-hydroxy-4-phenylnicotinonitrile were introduced to explore the SAR. From the wet lab results of the final derivatives, it can be observed that most compounds exhibited relatively moderate antibacterial activities. 4-Cl and 4-OH derivatives displayed better activity as antibacterial agent. This observation suggests that, the substitution position on the phenyl ring attached to the 3-cyno pyridine appeared to have influence on the antibacterial activity. The compound 7c with 4-Cl substituted showed the same antibacterial potency against *S. aureus*, *S. payogens* and *E. coli* with MIC value 62.5 µg/mL, whereas the change the position of same substituent showed poor antimicrobial potency. The compound 7c found to be equipotent antifungal agent (MIC = 100 µg/mL) compared to standard drug nystatin. Introduction of electron donation –OH group on same phenyl ring on *para* position decrease the antifungal potency but enhanced the antibacterial activity against *E. coli.* with MIC value 50 µg/mL when compared with ciprofloxacin. The position of –OH group on phenyl ring plays an important role. Other compounds had very high MIC values and seem to be poor to moderately active.

Antioxidant evaluation

The antioxidant activity by DPPH radical scavenging from ten coumarin derivatives is showed in Table II. 3-Cynopyridine moiety was incorporated to our compounds with different substituent on one of the aromatic ring. The compound 7c which was found to be better antimicrobial showed a null activity. Interestingly, incorporation from hydoxy substituent at 4-position on the aromatic ring drastically enhance the biological activity (compound 7e, IC_{50} 32.11 µg/mL), although incorporation same substituent on 2-position not affect the biological activity (compound 7d, IC_{50} 66.20 µg/mL). Interestingly, a change in the position of hydoxy atom in the phenyl ring of compound 7d to obtain compounds 7e has positive antioxidant potency.

Table I — Antibacterial and Antifungal activity of compounds 7a-j

Compd	MIC (µg/mL)						
	Antibacterial activity				Antifungal activity		
	S.aureus MTCC 96	*S. pyogenes* MTCC 442	*E. coli* MTCC 443	*P. aeruginosa* MTCC 1688	*C. albicans* MTCC 227	*A. niger* MTCC 282	*A. clavatus* MTCC 1323
7a	100	100	100	200	500	500	500
7b	250	250	250	125	500	1000	1000
7c	62.5	62.5	62.5	100	100	500	500
7d	125	200	100	250	1000	500	1000
7e	62.5	100	50	100	250	1000	1000
7f	250	100	125	250	500	1000	>1000
7g	125	250	250	250	1000	500	1000
7h	62.5	200	62.5	125	500	1000	500
7i	250	200	100	100	500	1000	1000
7j	200	100	200	200	>1000	>1000	>1000
Ciprofloxacin	25	25	50	50	–	–	–
Nystatin	–	–	–	–	100	100	100

Table II — Screening results of DPPH radical scavenging activity of 7a–j

Compd	IC_{50} μg/mL ± SD
7a	52.97 ± 0.673
7b	49.57 ± 0.487
7c	79.12 ± 1.912
7d	66.20 ± 0.995
7e	32.11 ± 0.123
7f	81.02 ± 1.438
7g	37.08 ± 0.308
7h	54.63 ± 0.812
7i	41.67 ± 0.287
7j	49.32 ± 0.451
Ascorbic Acid	36.22 ± 0.469

These results suggest explore more substituent on that aromatic ring to obtain a better structure-activity relationship. Introduction of methyl group at 4-position showed better result compared to other derivatives (compound 7e). These results can be comparable to that of control ascorbic acid while other compounds have very poor antioxidant power.

Experimental Section

The starting materials were purchased from Sigma-Aldrich with the highest purity and used without further purification. Analytical thin layer chromatography (TLC) was performed with Merck silica gel plates and visualized with UV irradiation (254 nm) or iodine. Melting points were determined with open capillary method on 'Equiptronics' digital melting point apparatus, model no. EQ-730 and were uncorrected. IR spectra were recorded on a Perkin Elmer spectrophotometer (KBr pellets) instrument. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II 400MHz and 100MHz respectively 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 Expression CMS from Advion, USA using ESI as ion source (mobile phase 0.1% Formic acid in 80:20, Methanol: Water).

Chemistry

4-Methyl-7-hydroxy-coumarin, 3

4-Methyl -7-hydroxy-coumarin **3** has been prepared as described in the literature[26]: White solid. Yield: 85%. m.p.183°C to 185°C. IR (KBr): 3493(-OH), 3098, 2817 (-CH_3, asym, sym), 1735 (>C=O str), 1148 cm^{-1} (-C-O-C); ^{1}H NMR (DMSO-d_6) δ (ppm): 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).

7-Hydroxy-4-methyl-6-nitro-2*H*-chromen-2-one, 4

7-Hydroxy-4-methyl-6-nitro-2*H*-chromen-2-one **4** has been prepared as described in the literature[27]. Yellow solid. Yield: 72%. m.p.193-195°C (as reported). IR (KBr): 3490 (-OH), 3094, 2827 (-CH_3, asym, sym), 1745 (>C=O str), 1190 (-C-O-C), 1536, 1357 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d_6) δ (ppm):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).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-chloroacetate 5:

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-chloro-acetate **5** has been prepared as described in the literature[28]. Brownish yellow crystals. Yield: 69%, m.p.109°C - 112°C. IR (KBr): 3003, 2799 (-CH_3, asym, sym), 1751, 1657 (>C=O str), 1190 (-C-O-C), 1571, 1346 (-NO_2), 831 cm^{-1} (-C-Cl); ^{1}H NMR (DMSO-d_6): δ 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).

General method for the synthesis of 6-(4-fluorophenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile, IIa-j

4-Fluoroacetophenone **6** (0.01 mol) and benzaldehyde (0.01 mol) were mixed in ethanol and stirred for 15 minutes, then ethylcyanoacetate (0.01 mol) was added drop wise followed by the addition of ammonium acetate (0.08 mol) in 40mL ethanol. The reaction mixture was refluxed in a round bottom flask for 6-7 h. The reaction mass was cooled and poured on to crushed ice water, the obtained precipitates were filtered off, dried and the desired compound IIa recrystallized by ethyl alcohol. Yield: 65%, m.p.: 246-248°C. IR (KBr): Compound **IIg**: 3330 (-OH), 2232 (-CN), 1608 cm^{-1} (C=N); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 12.91 (s, 1H, -OH), 7.24-8.25 (m, 9H, aromatic), 2.38 (s, 3H, -CH_3). The other compounds IIb-j was prepared by the same method using various substituted benzaldehydes.

General method for the synthesis of 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-(3-cyano-6-(4-fluoro phenyl)-4-(phenyl)pyridin-2-yl-oxy) acetate, 7a-j

To a solution of 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-chloroacetate **5** (0.01 mol) and 6-(4-

fluorophenyl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile **IIa** (0.01 mol) in DMF, K_2CO_3 (0.24 mol) were added under stirring and were refluxed for 8-9 h. After the completion of the reaction (TLC monitored by using ethylacetate : *n*-hexane; 1:1), the mixture was poured on to crush ice, solids that are separated out was filtered, washed with saturated solution of $NaHCO_3$ and recrystallized from ethanol. Yield: 61%, m.p.: 182-184°C. The other compounds **7b-j** were prepared by the same method.

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-phenylpyridin-2-yl)oxy) acetate 7a: Pale yellow solid. Yield: 78%, m.p.: 182-184°C, Mol. formula $C_{30}H_{18}FN_3O_7$. IR (KBr): 3037, 2912 (-CH_3), 2227 (-CN) 1714, 1687 (-C=O), 1624 (C=N), 1161 (aryl-F), 1510, 1347 cm^{-1} (-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.58 (s, 1H, -CH), 6.37-8.24 (m, 12H, aromatic), 5.18 (s, 2H, -CH_2O), 2.40 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.63 (C-2),112.29 (C-3), 152.41 (C-4), 120.58 (C-5), 137.42 (C-6), 135.58 (C-7), 115.80 (C-8), 159.71 (C-9), 117.50 (C-10), 19.40 (C-12), 191.75 (C-14), 68.90 (C-16), 165.06 (C-18), 92.10 (C-19), 156.06 (C-20), 108.51 (C-21), 157.23 (C-22), 140.02 (C-24), 126.78 (C-25, C-29), 130.15 (C-26, C-28, C-27), 133.62 (C-31), 130.62 (C-32, C-36), 116.05 (C-33, C-35), 161.52 (C-34), 114.21 (C-38), *m/z*: 551.11 (M^+).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-2-chlorophenyl pyridine-2-yl)oxy) acetate, 7b: Yellow solid. Yield: 69%, m.p.: 178-180°C, Mol. formula $C_{30}H_{17}ClFN_3O_7$. IR (KBr): 3045, 2924 (-CH_3), 2225 (-CN), 1723, 1679 (-C=O), 1631 (C=N), 1159 (aryl-F), 1509, 1339 (-NO_2), 765 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.54 (s, 1H, -CH), 6.32-8.24 (m, 12H, aromatic), 5.09 (s, 2H, -CH_2O), 2.41 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.63 (C-2),112.29 (C-3), 152.41 (C-4), 120.58 (C-5), 137.42 (C-6), 135.58 (C-7), 115.80 (C-8), 159.71 (C-9), 117.50 (C-10), 19.40 (C-12), 192.75 (C-14), 68.79 (C-16), 164.81 (C-18),93.10 (C-19), 156.06 (C-20), 108.51 (C-21), 157.23 (C-22), 133.02 (C-24), 132.65 (C-25), 132.15 (C-26), 128.69 (C-27),130.54 (C-28), 129.45 (C-29), 134.62 (C-31), 130.62 (C-32, C-36), 116.05 (C-33, C-35), 163.52 (C-34), 113.89 (C-38), *m/z*: 567.08 (M^+), 569.08 (M+2).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-4-chlorophenyl pyridine-2-yl)oxy) acetate, 7c: Yellow solid. Yield: 76%, m.p.: 210-212°C, Mol. formula $C_{30}H_{17}ClFN_3O_7$. IR (KBr): 3052, 2934 (-CH_3), 2224 (-CN), 1731, 1677 (-C=O), 1629 (C=N), 1147 (aryl-F), 1511, 1345 (-NO_2), 771 cm^{-1} (C-Cl); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.54 (s, 1H, -CH), 6.32-8.24 (m, 12H, aromatic), 5.09 (s, 2H, -CH_2O), 2.36 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): 160.63 (C-2),112.29 (C-3), 152.41 (C-4), 120.58 (C-5), 137.42 (C-6), 135.58 (C-7), 115.80 (C-8), 159.71 (C-9), 117.50 (C-10), 19.40 (C-12), 192.75 (C-14), 68.79 (C-16), 164.81 (C-18),93.10 (C-19), 156.06 (C-20), 108.51 (C-21), 157.23 (C-22), 133.02 (C-24), 129.65 (C-25), 132.15 (C-26), 132.69 (C-27),130.54 (C-28), 129.45 (C-29), 134.62 (C-31), 130.62 (C-32, C-36), 116.05 (C-33, C-35), 163.52 (C-34) 114.3 (C-38), *m/z*: 567.08 (M^+), 569.08 (M+2).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(2-hydroxy phenyl) pyridin-2-yl)oxy) acetate, 7d: Yellow solid. Yield: 73%, m.p.: 156-158°C, Mol. formula $C_{30}H_{18}FN_3O_8$. IR (KBr): 3487(-OH), 3051, 2922 (-CH_3), 2216 (-CN), 1718, 1678 (-C=O), 1622 (C=N) 1521, 1350 (-NO_2), 1174 cm^{-1} (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 9.41 (s, 1H, -OH), 8.59 (s, 1H, -CH), 6.32-8.29 (m, 11H, aromatic), 5.18 (s, 2H, -CH_2O), 2.39 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.63 (C-2),112.22 (C-3), 152.48 (C-4), 120.61 (C-5), 137.54 (C-6), 135.62 (C-7), 115.88 (C-8), 159.75 (C-9), 117.57 (C-10), 19.45 (C-12), 191.71 (C-14), 68.90 (C-16), 165.17 (C-18),79.14 (C-19), 156.13 (C-20), 108.56 (C-21), 157.24 (C-22), 128.25 (C-24), 130.57 (C-25), 121.82 (C-26), 131.32 (C-27), 117.48 (C-28), 155.63 (C-29), 133.60 (C-31), 130.59 (C-32, C-36), 116.14 (C-33, C-35), 161.50 (C-34), 114.51 (C-38); *m/z*: 567.11 (M^+).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(4-hydroxy phenyl) pyri din-2-yl)oxy) acetate, 7e: Light yellow solid. Yield: 81%, m.p.: 169-171°C, Mol. formula $C_{30}H_{18}N_3FO_8$. IR (KBr): 3481(-OH), 3057, 2925 (-CH_3), 2219 (-CN), 1716, 1679 (-C=O), 1624 (C=N) 1516, 1348 (-NO_2), 1175 cm^{-1} (C-F); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 10.26 (s, 1H, -OH), 8.59 (s, 1H, -CH), 6.39-8.25 (m, 11H, aromatic), 5.19 (s, 2H, -CH_2O), 2.40 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.60 (C-2),112.27 (C-3), 152.51 (C-4), 120.65 (C-5), 137.50 (C-6), 135.64 (C-7), 115.85 (C-8), 159.79 (C-9), 117.54 (C-10), 19.41 (C-

12), 191.78 (C-14), 68.92 (C-16), 165.11 (C-18),79.15 (C-19), 156.13 (C-20), 108.52 (C-21), 157.27 (C-22), 131.56 (C-24), 130.08 (C-25, C-29), 116.5 (C-26, C-28), 159.02 (C-27), 133.62 (C-31), 130.62 (C-32, C-36), 116.05 (C-33, C-35), 161.52 (C-34), 114.07 (C-38); *m/z*: 567.11 (M^+).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(4-flouro) pyridin-2-yl)oxy) acetate, 7f: Yellow solid. Yield: 86%, m.p.: 223-225°C, Mol. formula $C_{31}H_{17}F_2N_3O_7$. IR (KBr): 3029, 2933 (-CH_3), 2225 (-CN) 1723, 1678 (-C=O), 1645 (C=N), 1159 (aryl-F), 1504, 1351 cm^{-1} (-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.49 (s, 1H, -CH), 6.36-8.23 (m, 11H, aromatic), 4.95 (s, 2H, -CH_2O), 2.41 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.51 (C-2),112.19 (C-3), 152.48 (C-4), 120.64 (C-5), 137.48 (C-6), 135.67 (C-7), 115.90 (C-8), 159.69 (C-9), 117.48 (C-10), 19.38 (C-12), 191.68 (C-14), 69.03 (C-16), 165.20 (C-18),79.09 (C-19), 156.13 (C-20), 108.54 (C-21), 157.22 (C-22), 136.50 (C-24), 126.27 (C-25, C-29), 129.91 (C-26, C-28), 162.12 (C-27), 133.54 (C-32, C-34), 163.59 (C-33, C-37), 116.10 (C-34, C-36), 161.40 (C-35), 114.51(C-39); *m/z*: 661.13 (M^+).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(*p*-tolyl) pyridin-2-yl)oxy) acetate, 7g: Yellow solid. Yield: 81%, m.p.: 201-204°C, Mol. formula $C_{31}H_{20}FN_3O_7$. IR (KBr): 3034, 2928 (-CH_3), 2220 (-CN) 1714, 1687 (-C=O), 1624 (C=N), 1161 (aryl-F), 1510, 1347 cm^{-1} (-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.57 (s, 1H, -CH), 6.34-8.25 (m, 11H, aromatic), 5.18 (s, 2H, -CH_2O), 2.36 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.56 (C-2),112.23 (C-3), 152.50 (C-4), 120.65 (C-5), 137.50 (C-6), 135.63 (C-7), 115.87 (C-8), 159.72 (C-9), 117.51 (C-10), 19.40 (C-12), 191.72 (C-14), 68.98 (C-16), 165.17 (C-18),79.11 (C-19), 156.18 (C-20), 108.51 (C-21), 157.21 (C-22), 136.47 (C-24), 126.31 (C-25, C-29), 129.95 (C-26, C-28), 133.41 (C-27), 21.83 (C-30), 133.55 (C-31), 130.61 (C-32, C-36), 116.05 (C-33, C-35), 161.44 (C-34), 114.46 (C-38); *m/z*: 657.15 (M^+).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(4-propyl phenyl) pyridin-2-yl)oxy) acetate, 7h: Dark yellow solid. Yield: 74%, m.p.: 218-220°C, Mol. formula $C_{33}H_{28}FN_3O_8$. IR (KBr): 3035, 2928 (-CH_3), 2224 (-CN), 1712, 1684 (-C=O), 1641 (C=N), 1165 (aryl-F), 1515, 1349 cm^{-1} (-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, TMS): δ 8.58 (s, 1H, -CH), 6.37-8.29 (m, 11H, aromatic), 5.19 (s, 2H, -CH_2O), 2.37 (s, 3H, -CH_3), 2.98 (t, 2H, -CH_2CH$_2$CH$_3$), 1.67 (m, 2H, -CH$_2$CH_2CH$_3$), 0.97 (t, 2H, -CH$_2$CH$_2$CH_3); ^{13}C NMR (100MHz, DMSO-d_6 TMS): δ 160.63 (C-2), 112.21 (C-3), 152.44 (C-4), 120.59 (C-5), 137.52 (C-6), 135.61 (C-7), 115.83 (C-8), 159.70 (C-9), 117.58 (C-10), 19.42 (C-12), 191.80 (C-14), 68.91 (C-16), 165.15 (C-18),79.11 (C-19), 156.16 (C-20), 108.51 (C-21), 157.24 (C-22), 136.38 (C-24), 127.27 (C-25, C-29), 129.39 (C-26, C-28), 143.22 (C-27), 37.41 (C-30a), 24.12 (C-30b), 13.68 (C-30c),133.61 (C-31), 130.37 (C-32, C-36), 116.13 (C-33, C-35), 161.61 (C-34), 114.28 (C-38); *m/z*: 685.19 (M^+).

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(3-bromo) pyridin-2-yl)oxy) acetate, 7i: Pale Yellow solid. Yield: 70%, m.p.: 203-205°C, Mol. formula $C_{30}H_{17}BrFN_3O_7$. IR (KBr): 3040, 2931 (-CH_3), 2230 (-CN), 1714, 1687 (-C=O), 1642 (C=N), 1161 (aryl-F), 1514, 1351 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d_6): δ 8.54 (s, 1H, -CH), 6.41-8.31 (m, 11H, aromatic), 5.18 (s, 2H, -CH_2O), 2.40 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS): δ 161.56 (C-2), 112.19 (C-3), 152.49 (C-4), 120.66 (C-5), 137.48 (C-6), 135.69 (C-7), 115.90 (C-8), 159.69 (C-9), 117.56 (C-10), 19.39 (C-12), 191.68 (C-14), 68.99 (C-16), 165.20 (C-18), 79.09 (C-19), 156.20 (C-20), 108.48 (C-21), 157.19 (C-22), 136.44 (C-24), 126.29 (C-25, C-29), 122.24 (C-26), 126.61 (C-27), 131.59 (C-28), 133.53 (C-32), 130.69 (C-33, C-37), 116.49 (C-34, C-36), 161.47(C-35), 114.48 (C-39); *m/z*: 721.05 (M^+), (M+1) 722.05, (M+2) 723.05.

4-Methyl-6-nitro-2-oxo-2*H*-chromen-7-yl-2-((3-cyano-4-(4-fluorophenyl)-6-(3-phenoxy) pyridin-2-yl)oxy) acetate, 7j: Dark Yellow solid. Yield: 66%, m.p.: 209-211°C, Mol. formula $C_{36}H_{22}FN_3O_8$. IR (KBr): 3044, 2926 (-CH_3), 2227 (-CN), 1709, 1691 (-C=O), 1647 (C=N), 1157 (aryl-F), 1519, 1347 cm^{-1} (-NO_2); ^{1}H NMR (DMSO-d_6): δ 8.53 (s, 1H, -CH), 6.40-8.01 (m, 16H, aromatic), 5.20 (s, 2H, -CH_2O), 2.39 (s, 3H, -CH_3); ^{13}C NMR (100MHz, DMSO-d_6, TMS) δ 161.60 (C-2), 112.21 (C-3), 152.54 (C-4), 120.70 (C-5), 137.98 (C-6), 135.72 (C-7), 115.89 (C-8), 159.78 (C-9), 117.99 (C-10), 19.47 (C-12), 191.96 (C-14), 68.77 (C-16), 165.01 (C-18), 79.14 (C-19), 156.14 (C-20), 108.52 (C-21), 157.14 (C-22), 136.56 (C-24), 126.24 (C-25, C-29), 156.45 (C-26), 115.61 (C-27), 131.45 (C-28), 133.69 (C-31), 130.74 (C-32, C-36), 116.32 (C-33, C-35), 161.56(C-34), 114.61

(C-38), 158.09 (C-41), 118.78 (C-42, C-46), 129.05 (C-43, C-45) 121.91 (C-44); *m/z*:643.14 (M^+).

Biological assay

Antimicrobial assay

The broth micro dilution method has been employed to determine the MICs of synthesized compounds as described in the literature[29,30]. Dimethylsulfoxide (DMSO) was used as diluent to get desired concentration of drugs to test upon standard bacterial strains. A solvent control of a 1:10 dilution of the DMSO was used to dissolve the antimicrobial agent being tested. This 1:10 solution is prepared by adding 0.1 mL of solvent to 0.9 mL of the appropriate diluent. The active candidates found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 100 μM, 50 μM, 25 μM, 12.5 μM, 6.25 μM, 3.125 μM and 1.5625 μM concentrations. The highest dilution showing at least 99% inhibition was considered the minimum inhibitory concentration (MIC). The test mixture should contain 108 organism /mL. The control tube containing not any antibiotic was instantly subcultured [before inoculation] by spreading a loopful evenly over quarter of a plate of medium suitable for the growth of test organisms. The culture tubes were then incubated for 24 h at 37°C, and the growth was observed visually and spectrophotometrically. All experiments were done in triplicate manner in order to attain the results. After that, 10 μg mL^{-1} suspensions were further inoculated on appropriate media and development was noted after 24 and 48 h. The lowest concentration preventing appearance of turbidity was considered as minimum inhibitory concentration (MIC, μg mL^{-1}) *i.e.*, the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. Solvent had no influence on strain growth and the result of this was greatly affected by the size of inoculum. To evaluate the antimicrobial potency of the final derivatives, they were screened against different strains *viz*, two gram-positive bacteria *Staphylococcus aureus* (MTCC-96) and *Streptococcus pyogenes* (MTCC-442), two gram-negative bacteria *Escherichia coli* (MTCC-443) and *Pseudomonas aeruginosa* (MTCC-1688), and fungi, *Candida albicans* (MTCC-227), *Aspergillus niger* (MTCC-282), and *Aspergillus clavatus* (MTCC-1323), and compared with standard drugs, ciprofloxacin and griseofulvin.

Antioxidant assay (DPPH method)

Reduction of 2,2-diphenyl-1-picrylhydrazyl (free radical) is the base of the DPPH antioxidant bioassay. It has an odd electron that shows a maximum absorption band of 517 nm (deep violet colour) in ethanol. The DPPH bioassay is the widely used and acceptable method for evaluating the free radical scavenging action of the tested compounds. Such substances donate a hydrogen atom when mixed with the DPPH, thereby introducing its reduced congener, diphenylpicrylhydrazine (non-radical) with the loss of violet color. In the present study, DPPH bioassay was adopted to screen the berberine-based compounds for their *in vitro* antioxidant profiles. The results of this bioassay investigation were introduced in the form of the percentage of radical scavenging antioxidant activity (RSA%) of each substance. The investigation of the DPPH radical scavenging activity was operated according to the methodology described by Brand-Williams *et al.*[31] with some modifications[32]. A stable free radical, DPPH, was allowed to react with test compounds in methanol as 20 μg/ mL (100, 10, 1 and 0.1) quantities of title compounds were mixed up with 180 μg/mL of DPPH in methanol. Titled compounds donated hydrogen during the mixing thereby introduced the reduction of DPPH and hence a change in the color was observed from deep violet to light yellow at 517 nm after 25 min of reaction in a UV–visible spectrophotometer (Perkin-Elmer). The blank reading was also performed using the mixture of methanol (20 μg/mL) and sample (180 μg/mL of DPPH). Ascorbic acid served as a control drug in this assay, and its solution was prepared by mixing methanol (20 μg/mL) and DPPH radical solution (180 μg/mL). The results of this bioassay, RSA% was determined according to[33] as described in the below equation.

$$\textbf{\% Scavenging} = \frac{\text{Absorbance of blank} - \text{Absorbance of test}}{\text{Absorbance of blank}} \times 100$$

Conclusion

A new series of 4-methyl-6-nitro-2-oxo-2*H*-chromen-7-yl 2-((3-cyano-6-(4-fluorophenyl)-4-(substituted-phenyl)pyridin-2-yl)oxy)acetate has been efficiently formulated *via* coupling 4-methyl-6-nitro-

2-oxo-2*H*-chromen-7-yl 2-chloroacetate with 3-cynopyridine derivatives. All the synthesized compounds were characterized by spectral techniques. Final compounds were evaluated for their *in vitro* antimicrobial activity by broth microdilution method and antioxidant activity using DPPH bioassays. The presence of chloro and hydoxy group on phenyl ring on the chalcone system was essential to exert antibacterial, antifungal and antioxidant effect. A minimum inhibitory concentration of **7a-j** towards bacterial and fungal strains was studied and the derivatives **7e** displayed remarkable potency against *E. coli* with MIC values 50 µg/mL and **7c** showed equal potency against *C. albicans* compared to standard drugs ciprofloxacin and nystatin respectively. It has been observed that the potent antibacterial candidate proved to possess significant antioxidant activity. The present of hydoxy group on phenyl ring plays an important role for the potency in above mentioned biological assay.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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Conflict of interest

The authors declare that they have no conflict of interest.

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