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Original Article

Microwave and Conventional Study of Coumarin-Oxadiazole Adducts and their Anti-Microbial Evaluation

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Abstract

Introduction: Nowadays, researchers are progressively concentrated to generate economical, affordable and also greener synthesis approach for the synthesis of various heterocycles. On look at the beauty of coumarin molecules and oxazoles, it seems to be lead molecules in the anti-microbial area.

Aim: With the target to identify efficient molecules, we studied 2-oxo-2*H*-chromen-4-yl-2-((5-substituted aryl-1,3,4-oxadiazol-2-yl) thio)acetate derivatives using two synthetic protocol/methods, i.e. conventional synthesis and microwave-based synthesis.

Materials and methods: Two simultaneous methods, i.e. conventional and microwave synthesis have been used for the synthesis of 2-oxo-2H-chromen-4-yl-2-((5-substituted aryl-1,3,4-oxadiazol-2-yl)thio)acetate (6a-l) derivatives. The desired molecules were synthesized by conventional and microwave synthesis and a comparative study was carried out to identify an easy route for industrial applications. The confirmations of the compounds were carried out by spectroscopic techniques such as IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analysis.

Results: All synthesized compounds were evaluated for their in-vitro antibacterial activity against gram-positive bacteria (*Staphylococcus aureus*, *Staphylococcus pyogenes*), gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*), and antifungal activity (*Candida albicans, Aspergillus niger*).

Conclusions: All conventional synthesis of final coumarin derivatives were completed within 4-6 h. While that of microwave-based reaction took comparatively more reaction time. Surprisingly, the compounds 6f and 6g could not be synthesized by microwave radiation even after 32 minutes of irradiation. As to the medicinal application part, microbial evaluation of synthesized analogues showed that the compounds 6b, 6e, 6d, and 6j were found more potent in comparison to the reference drug.

Keywords

1,3,4-oxadiazole, antimicrobial activity, coumarin, microwave synthesis

INTRODUCTION

Currently, researchers are oriented to generate potent compounds by applying principles of the "Green Chemistry" approaches. It is at the frontiers of this interdisciplinary science and positive side to reduce the environmental issues in global view by developing a technology base that is fundamentally non-toxic to living organisms and the environment. Microwave (MW) irradiation is an efficient technique for the synthesis of heterocyclic compounds using mild reaction conditions and reagent activation in the organic chemistry.[1] It has been a progressive area because of its

capability to reduce reaction time, atom-economy, high yields, having environmental and economic advantages, and the simplified work-up processes.[2]

Many of the heterocyclic compounds have been studied to date and of these coumarin and oxadiazole have been found to be the most efficient in enhancing the biological significance. Literature survey shows that combination of both these moieties showed extraordinary medicinal importance. Coumarins and several heterocyclic ring containing coumarins are associated with diverse bioactivity such as anti-inflammatory, antiviral and antitumor, antiproliferative, cytotoxic effects against Hep2 cells and many more.[3,4] Oxadiazole have been successfully tested against several diseases and therefore received special attention in pharmaceutical chemistry due to its diverse medicinal potential like antibacterial, antifungal, analgesic, anti-inflammatory, antiviral, anticancer, antihypertensive, anticonvulsant, and antidiabetic properties.[5,6] Literature survey showed that coupling of both these moieties was of extraordinary medicinal importance.[7]

On the basis of the above discussion, we have reported an efficient synthesis of 2-oxo-2*H*-chromen-4-yl-2-((5-substitutedaryl-1,3,4-oxadiazol-2-yl)thio)acetate **(6a-l)** derivatives by four step processes and biological application was carried by their anti-microbial study against various strains of bacteria and fungi.

MATERIALS AND METHODS

Materials

The required chemicals and solvents for the synthesis were purchased from Merck Ltd., SD fine chemicals, LOBA Chemicals and HIMEDIA. Most of the reactions were carried out by standard techniques for exclusion of moisture. Open-end capillary method was used to determine the melting points of the synthesized derivatives and the results were reported and were uncorrected. TLC (Thin Layer Chromatography) was used for reaction monitoring using ethyl acetate: hexane as mobile phase and visualized in UV. IR spectra of all compounds were recorded on a Bruker FT-IR alpha-t (ATR). The ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Spectrophotometer-400 MHz using DMSO-d_6 as solvent and TMS as the internal reference. Mass spectra were recorded on a Schimadzu LC-MS 2010 spectrometer. Elemental analysis was carried out by a Perkin-Elmer 2400 CHN analyzer. MW-assisted reactions were carried out in a domestic microwave oven (LG MC3286BRU) at 180 W.

Method of Synthesis

Procedure for the synthesis of 2-oxo-2*H*-chromen-4-yl 2-chloroacetate (2).

A solution of coumarin **1** (0.01 mole) in *N*,*N*-dimethylformamide (DMF) and triethylamine (TEA) (0.001 mole) was stirred at room temperature for 10 minutes. Chloro acetyl chloride (CAC) (0.015 mole) was added in a dropwise manner to the reaction mixture at 0-5°C. The reaction mixture was stirred for 4 h at room temperature and poured onto crushed ice. The solid separated was filtered and dried using vacuum dryer. The completion of the reaction was monitored using TLC using n-hexane: ethyl acetate (7:3) as a mobile phase.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.63 (m, 1H), 7.43 (m, 1H), 7.19 (m, 1H), 5.86 (s, 1H), 4.40 (s, 2H); ^{13}C NMR (101.1 MHz, DMSO-d_6) 164.74, 162.56, 161.94, 152.87, 133.52, 125.68, 124.26, 117.30, 115.47, 99.56, 40.64.

General procedure for the synthesis of substituted benzohydrazide (4a-l)

A solution of hydrazine hydrate (0.05 mole) in methanol was added to a solution of substituted methyl benzoate 3a-l (0.01 mole) in methanol containing round bottom flask (RBF) and was refluxed for 12 h. Completion of the reaction was monitored out by TLC (mobile phase: hexane: ethyl acetate). After completion of the reaction, methanol was distilled off in vacuum and then cooled to room temperature. It was poured onto crushed ice and separated solid product was filtered out and washed with cold water. Dried product was recrystallized using ethanol to afford analytically pure products.

General procedure for the synthesis of 5-substitutedphenyl-1,3,4-oxadiazole-2-thiol (5a-l)

In a 100 ml RBF, ethanolic KOH solution [Ethanol (4 ml): KOH (0.01 mole)] and various benzohydrazide derivatives (4a-l) (0.01 mol) was stirred at RT till solution becomes clear. Carbon disulfide (CS_2) (0.02 mole) was added to the reaction mixture and was refluxed for 14 h. The ethanol was distilled off in vacuo and then cooled to room temperature. The reaction mass was poured into water and was acidified with diluted HCl till the precipitates were separated. The separated solid was washed with cold water and dried to get the desired product. The completion of the reaction was monitored by TLC using ethyl acetate: hexane (6:4) as a mobile phase.

General procedure for the synthesis of 2-oxo-2H-chromen-4-yl-2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)acetate (6a-l)

In an inert atmosphere (N_2), 2-oxo-2*H*-chromen-4-yl-2-chloroacetate **(2)** (0.01 mole) and various 5-substituted-phenyl-1,3,4-oxadiazole-2-thiol (5a-l) (0.01 mol) were mixed in 100 ml RBF in acetone. To this reaction mixture, dry K_2CO_3 powder (0.02 mole) was added to neutralize the HCl, liberated during the progress of the reaction. The reaction mixture was stirred at RT for 4-6 h. It was poured onto crushed ice, filtered, washed with cold water and dried it. The obtained dried product was crystallized by using ethanol. The completion of the reaction was confirmed by using TLC with mobile phase n-hexane: ethyl acetate (7:3).

Purification of the final product was carried out by silica gel column chromatography (60-120 mesh) by using ethyl acetate and n-hexane as mobile phase **(Fig. 1)**. All the synthesized newer compounds were completely soluble in polar solvents (mostly in methanol, DMF and DMSO).

All the compounds of the series **6a-l** were synthesized according to the above cited method and characterization was carried out by spectroscopic techniques.

Analytical Data of Representative Compounds

2-oxo-2H-chromen-4-yl 2-((5-phenyl-1,3,4-oxadiazol-2-yl)thio)acetate (6a)

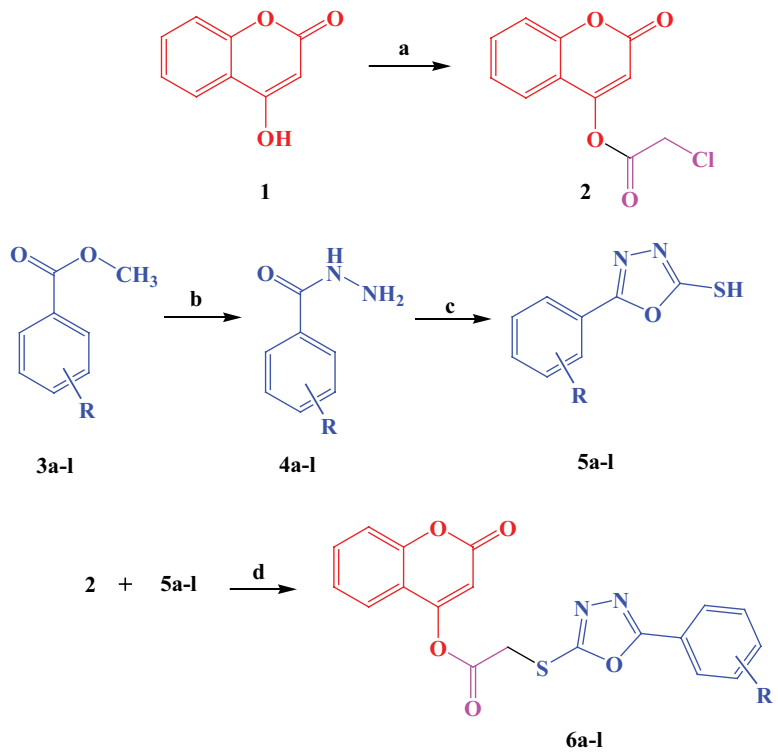
Yield: 81%; mp 284°C; IR (ATR, cm^{-1}): 2862 (-C-H stretching of methylene group), 1736 (>C=O stretching of esteric carbonyl group), 1708 (>C=O stretching of lactone carbonyl group), 1361 (C-N stretching of carbon nitrogen linkage), 1211, 1061 (C-O-C stretching of oxadiazole ring), 1162 (C-O stretching of ester); ^{1}H NMR (400 MHz, DMSO-$d_{6,}$ δppm) 8.23 (m, 5H), 7.81 (m, 4H), 7.11 (s, 1H), 4.13 (s, 2H); ^{13}C NMR (101 MHz, DMSO-$d_{6,}$ δppm) 177.60, 170.22, 167.28, 165.15, 161.23, 140.28, 132.61, 130.74, 129.85, 128.35, 127.45, 126.18, 122.87, 116.18, 103.56, 81.33, 36.21; MS: *m/z* 381 (M^+); Elemental analysis of $C_{19}H_{12}N_2O_5S$: Calculated= C, 55.99; H, 3.18; N, 7.36; O, 21.03; S, 8.43 and Experimental= C, 59.97; H, 3.13; N, 7.32; O, 21.01; S, 8.45.

2-oxo-2H-chromen-4-yl 2-((5-(4-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6b)

Yield: 74%; mp 245°C; IR (ATR, cm^{-1}): 2887 (-C-H stretching of methylene group), 1746 (>C=O stretching of esteric carbonyl group), 1704 (>C=O stretching of lactone carbonyl group), 1471, 1313 (C-NO_2 stretching), 1328 (C-N stretching of carbon nitrogen linkage), 1286, 1051 (C-O-C stretching of oxadiazole ring), 1226 (C-O stretching of ester); ^{1}H NMR (400 MHz, DMSO-$d_{6,}$ δppm) 7.86 (m, 4H), 7.56 (m, 4H), 7.12 (s, 1H), 4.26 (s, 2H); ^{13}C NMR (101 MHz, DMSO-$d_{6,}$ δppm) 176.62, 173.28, 164.17, 162.63, 160.92, 153.81, 150.15, 147.69, 136.43, 129.64, 127.51, 124.47, 122.17, 115.20, 113.36, 47.81, 37.10; MS: *m/z* 426 (M^+); Elemental analysis of $C_{19}H_{11}N_3O_7S$: Calculated= C, 53.65; H, 2.61; N, 9.88; O, 26.33; S, 7.54 and Experimental= C, 53.62; H, 2.58; N, 9.75; O, 26.37; S, 7.56.

2-oxo-2H-chromen-4-yl 2-((5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6c)

Yield: 78%; mp 235°C; IR (ATR, cm^{-1}): 2919 (-C-H stretching methylene group), 1739 (>C=O stretching esteric carbonyl group), 1702 (>C=O stretching lactone carbonyl group), 1482, 1351 (C-NO_2 stretching), 1361 (C-N stretching carbon nitrogen linkage), 1271, 1021 (C-O-C stretching oxadiazole ring), 1286 (C-O stretching ester); ^{1}H NMR (400 MHz, DMSO-$d_{6,}$ δ, ppm) 8.11 (m, 4H), 7.47 (m, 4H), 7.26 (s, 1H), 4.87 (s, 2H); ^{13}C NMR (101 MHz, DMSO-$d_{6,}$ δ, ppm) 177.18, 174.27, 170.35, 167.21, 162.96, 148.18, 145.32, 142.65, 139.43, 136.21, 132.63, 128.16, 126.92,



Reaction Conditions: (a) DMF, $ClCH_2COCl$, $N(CH_2CH_3)_3$, Stirring, 4h; (b) MeOH, NH_2NH_2, Reflux, 12h; (c) EtOH, CS_2, HCl, Reflux, 14h; (d) CH_3COCH_3, K_2CO_3, Stir, 4-6h.

Figure 1. Reaction scheme. Synthetic pathway of compounds (6a-l).

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125.67, 120.23, 115.42, 103.26, 50.15, 38.30; MS: *m/z* 426 (M^+); Elemental analysis of $C_{19}H_{11}N_3O_7S$: Calculated= C, 53.65; H, 2.61; N, 9.88; O, 26.33; S, 7.54 and Found= C, 53.68; H, 2.64; N, 9.91; O, 26.28; S, 7.49.

2-oxo-2H-chromen-4-yl 2-((5-(2-nitrophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6d)

Yield: 80%; mp 260°C; IR (ATR, cm^{-1}): 2965 (-C-H stretching methylene group), 1741 (>C=O stretching esteric carbonyl group), 1710 (>C=O stretching lactone carbonyl group), 1457, 1321 (C-NO_2 stretching), 1344 (C-N stretching carbon nitrogen linkage), 1200, 1071 (C-O-C stretching oxadiazole ring), 1187 (C-O stretching ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.76 (m, 4H), 7.58 (m, 4H), 7.21 (s, 1H), 4.11 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 176.25, 172.31, 170.52, 168.54, 152.30, 150.11, 149.22, 144.87, 134.53, 132.18, 129.23, 126.81, 123.77, 122.56, 115.12, 112.27, 47.51, 36.31; MS: *m/z* 426 (M^+); Elemental analysis of $C_{19}H_{11}N_3O_7S$: Calculated= C, 53.65; H, 2.61; N, 9.88; O, 26.33; S, 7.54 and Found= C, 53.59; H, 2.67; N, 9.83; O, 26.38; S, 7.58

2-oxo-2H-chromen-4-yl 2-((5-(4-fluorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6e)

Yield: 78%; mp 243°C; IR (ATR, cm^{-1}): 2911 (-C-H str. methylene group), 1715 (>C=O str. esteric carbonyl group), 1687 (>C=O str. lactone carbonyl group), 1380 (C-N str. carbon nitrogen linkage), 1242, 1030 (C-O-C str. oxadiazole ring), 1218 (C-O str. ester), 1126 (C-F, str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.81 (m, 4H), 7.68 (m, 4H), 7.13 (s, 1H), 4.12 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 177.30, 171.53, 167.39, 164.21, 156.19, 153.36, 151.47, 130.56, 128.83, 124.18, 122.76, 121.21, 115.13, 103.26, 86.63, 45.11, 35.21; MS: *m/z* 399 (M^+); Elemental analysis of $C_{19}H_{11}FN_2O_5S$: Calculated= C, 57.29; H, 2.78; F, 4.77; N, 7.03; O, 20.08; S, 8.05 and Found= C, 57.24; H, 2.73; F, 4.81; N, 7.07; O, 20.05; S, 8.03.

2-oxo-2H-chromen-4-yl 2-((5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6f)

Yield: 81%; mp 218°C; IR (ATR, cm^{-1}): 2972 (-C-H stretching of methylene group), 1761 (>C=O stretching of esteric carbonyl group), 1606 (>C=O stretching of lactone carbonyl group), 1394 (C-N stretching of carbon nitrogen linkage), 1273, 1056 (C-O-C stretching of oxadiazole ring), 1188 (C-O stretching of ester), 688 (C-Cl stretching); ^{1}H NMR (400 MHz, DMSO-d_6, δppm) 7.97 (m, 4H), 7.65 (m, 4H), 7.13 (s, 1H), 4.90 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d6, δppm) 176.88, 165.52, 163.84, 162.35, 153.44, 136.45, 132.41, 129.51, 128.05, 125.72, 122.52, 122.50, 115.76, 100.79, 47.78, 39.47, 38.85; MS: m/z 415 (M^+); Elemental analysis of $C_{19}H_{11}ClN_2O_5S$: Calculated= C, 55.01; H, 2.67; Cl, 8.55; N, 6.75; O, 19.28; S, 7.73 and Experimental= C, 55.12; H, 2.60; Cl, 8.53; N, 6.71; O, 19.24; S, 7.78.

2-oxo-2H-chromen-4-yl 2-((5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6g)

Yield: 79%; mp 246°C; IR (ATR, cm^{-1}): 2982 (-C-H str. methylene group), 1746 (>C=O str. esteric carbonyl group), 1653 (>C=O str. lactone carbonyl group), 1358 (C-N str. carbon nitrogen linkage), 1291, 1073 (C-O-C str. oxadiazole ring), 1165 (C-O str. ester), 658 (C-Cl str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.81 (m, 4H), 7.59 (m, 4H), 7.21 (s, 1H), 4.84 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 178.19, 166.75, 164.18, 161.65, 152.19, 137.03, 132.51, 131.16, 130.21, 129.18, 127.23, 125.18, 122.61, 121.30, 117.14, 101.86, 47.43, 40.80, 37.13; MS: *m/z* 415 (M^+); Elemental analysis of $C_{19}H_{11}ClN_2O_5S$: Calculated= C, 55.01; H, 2.67; Cl, 8.55; N, 6.75; O, 19.28; S, 7.73 and Found= C, 55.23; H, 2.65; Cl, 8.51; N, 6.77; O, 19.31; S, 7.68.

2-oxo-2H-chromen-4-yl 2-((5-(3-(trifluoromethyl) phenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6h)

Yield: 84%; mp 219°C; IR (ATR, cm^{-1}): 2876 (-C-H str. methylene group), 1746 (>C=O str. esteric carbonyl group), 1713 (>C=O str. lactone carbonyl group), 1364 (C-N str. carbon nitrogen linkage), 1291, 1068 (C-O-C str. oxadiazole ring), 1203 (C-O str. ester), 1016 (C-F str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 8.14 (m, 4H), 7.62 (m, 4H), 7.25 (s, 1H), 4.63 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 177.63, 165.54, 163.13, 162.41, 154.18, 153.67, 140.22, 137.19, 132.28, 130.79, 129.15, 127.76, 125.81, 124.53, 123.16, 113.47, 100.86, 50.74, 39.43, 38.61; MS: *m/z* 449 (M^+); Elemental analysis of $C_{20}H_{11}F_3N_2O_5S$: Calculated= C, 55.57; H, 2.47; F, 12.71; N, 6.25; O, 17.84; S, 7.15 and Found= C, 55.61; H, 2.44; F, 12.67; N, 6.27; O, 17.89; S, 7.19.

2-oxo-2H-chromen-4-yl 2-((5-(4-bromophenyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6i)

Yield: 78%; mp 198°C; IR (ATR, cm^{-1}): 2975 (-C-H str. methylene group), 1767 (>C=O str. esteric carbonyl group), 1712 (>C=O str. lactone carbonyl group), 1329 (C-N str. carbon nitrogen linkage), 1268, 1049 (C-O-C str. oxadiazole ring), 1193 (C-O str. ester), 567 (C-Br str.); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 8.07 (m, 4H), 8.63 (m, 4H), 7.18 (s, 1H), 4.47 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 176.67, 165.18, 163.56, 162.47, 160.15, 138.41, 135.97, 129.68, 128.46, 123.18, 124.76, 122.68, 115.18, 103.27, 81.63, 50.18, 36.13; MS: *m/z* 460 (M^+); Elemental analysis of $C_{19}H_{11}BrN_2O_5S$: Calculated= C, 49.69; H, 2.41; Br, 17.40; N, 6.10; O, 17.42; S, 6.98 and Found= C, 49.65; H, 2.38; Br, 17.43; N, 6.15; O, 17.49; S, 6.86.

2-oxo-2H-chromen-4-yl 2-((5-(p-tolyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6j)

Yield: 75%; mp 232°C; IR (ATR, cm^{-1}): 3129 (C-H str. -CH_3 group), 2890 (-C-H str. methylene group), 1751 (>C=O str. esteric carbonyl group), 1690 (>C=O str. lactone carbonyl group), 1318 (C-N str. carbon nitrogen linkage), 1237, 1081 (C-O-C str. oxadiazole ring), 1173 (C-O str. ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.87 (m, 4H), 7.65 (m,

4H), 7.08 (s, 1H), 4.35 (s, 2H), 3.14 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 175.38, 166.13, 163.46, 162.35, 161.67, 140.31, 134.18, 127.41, 125.20, 124.83, 123.15, 122.69, 120.45, 118.34, 81.15, 37.35, 28.17; MS: *m/z* 395 (M^+); Elemental analysis of $C_{20}H_{14}N_2O_5S$: Calculated= C, 60.91; H, 3.58; N, 7.10; O, 20.28; S, 8.13 and Found= C, 60.85; H, 3.64; N, 7.13; O, 20.24; S, 8.08.

2-oxo-2H-chromen-4-yl 2-((5-(m-tolyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6k)

Yield: 79%; mp 167°C; IR (ATR, cm^{-1}): 3185 (C-H str. -CH_3 group), 2910 (-C-H str. methylene group), 1785 (>C=O str. esteric carbonyl group), 1689 (>C=O str. lactone carbonyl group), 1346 (C-N str. carbon nitrogen linkage), 1248, 1078 (C-O-C str. oxadiazole ring), 1098 (C-O str. ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.75 (m, 4H), 7.47 (m, 4H), 7.28 (s, 1H), 4.68 (s, 2H), 3.38 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 176.11, 173.63, 171.48, 163.45, 161.18, 141.47, 139.51, 135.48, 132.61, 130.35, 129.13, 127.67, 125.11, 124.10, 122.45, 119.21, 118.67, 95.82, 35.30, 29.18; MS: *m/z* 395 (M^+); Elemental analysis of $C_{20}H_{14}N_2O_5S$: Calculated= C, 60.91; H, 3.58; N, 7.10; O, 20.28; S, 8.13 and Found= C, 60.93; H, 3.57; N, 7.08; O, 20.31; S, 8.15.

2-oxo-2H-chromen-4-yl 2-((5-(o-tolyl)-1,3,4-oxadiazol-2-yl)thio)acetate (6l)

Yield: 84%; mp 255°C; IR (ATR, cm^{-1}): 3032 (C-H str. -CH_3 group), 2878 (-C-H str. methylene group), 1767 (>C=O str. esteric carbonyl group), 1670 (>C=O str. lactone carbonyl group), 1369 (C-N str. carbon nitrogen linkage), 1243, 1041 (C-O-C str. oxadiazole ring), 1141 (C-O str. ester); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) 7.94 (m, 4H), 7.68 (m, 4H), 7.12 (s, 1H), 4.45 (s, 2H), 3.52 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6, δ, ppm) 166.35, 165.79, 163.87, 161.71, 152.68, 142.45, 140.28, 139.17, 132.65, 130.37, 129.61, 128.16, 125.36, 123.81, 122.67, 119.21, 117.15, 90.38, 36.11, 28.35; MS: *m/z* 395 (M^+); Elemental analysis of $C_{20}H_{14}N_2O_5S$: Calculated= C, 60.91; H, 3.58; N, 7.10; O, 20.28; S, 8.13 and Found= C, 60.87; H, 3.59; N, 7.14; O, 20.26; S, 8.10.

Antimicrobial screening methodology

The newly synthesized entities **(6a-l)** were screened for their antimicrobial assay against a broad panel of gram-positive bacteria i.e. *S. aureus* (ATCC No. 25923), *E. faecalis* (ATCC No. 29212) and gram-negative bacteria i.e. *E. coli* (ATCC No. 25922), *P. aeruginosa* (ATCC No. 27853), and fungi *C. albicans* (ATCC No. 10231), *A. niger* (ATCC No. 1015). Antifungal and antibacterial evaluations were carried out by micro dilution/broth titer method.[8] The stock solution (DMSO: 1000 µg/ml) for each compound was prepared and antimicrobial assay was carried out by serial dilution and preparing the sets consecutively from 1000, 500, 200, 100, 50, 25 and 12.5 µg ml^{-1}. The tubes along with the control were then kept for incubation at 37°C for 24 h. Suspensions were further inoculated on an appropriate media and growth was noted after 48 h. The obtained results (MIC) in µg/ml was noted by observing the highest dilutions (low turbidity) were recorded and compared with the MIC value of standard drugs using ampicillin for antibacterial activity and griseofulvin for antifungal activity.

RESULTS AND DISCUSSION

Chemistry: synthetic and microwave based approach

The key intermediate 2-oxo-2*H*-chromen-4-yl-2-chloroacetate **(2)** was synthesized in excellent yields by condensation of 4-hydroxy-2*H*-chromen-2-one and chloroacetylchloride under basic condition by stirring at RT. To introduce the oxadiazole ring to coumarin moiety, intermediate **5a-l** was accomplished by hydrazide formation of molecules **3a-l** followed by reaction with CS_2 in acidic condition under refluxed for 14 h. Novel molecules **6a-l** were synthesized by coupling of previously prepared intermediate **2** and **5a-l** using dry K_2CO_3 as a catalyst and acetone as a solvent in handsome yields (around 80%) as shown in **Fig. 1**.

To make the process economical as well as ecofriendly, we have tried green chemistry approach for the synthesis of compounds **6a-l** by microwave irradiation method. **Table 1** summarized the comparison study between conventional and microwave processes.

The desired molecules **(6a-l)** were synthesized from intermediate **2** and **5a-l** with yields ranging from 74% to 84% when synthesized by conventional method and 0-70% when synthesized using microwave irradiation. Literature study shows that microwave-assisted synthesis led to improved yields and less reaction time, but in our case reverse statistics were observed. Comparative analysis of percentage yields and reaction time for all **6a-l** derivatives by both

Table 1. Conventional and microwave based optimization of compounds **(6a-6l)**

Compounds	% of yield		Reaction time	
	Conventional	MW	Conventional (h)	MW (min.)
6a	81	56	4	15
6b	74	49	4.5	20
6c	78	59	4	15
6d	80	60	5	25
6e	78	45	6	22
6f	81	-	5	32
6g	79	-	5	32
6h	84	63	5.5	23
6i	78	34	6	22
6j	75	44	4.5	18
6k	79	50	4	15
6l	84	70	5.5	15

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conventional method and microwave-assisted method was carried out to find out if microwave-assisted synthesis of coumarin derivatives adds any advantage or not.

All conventional synthesis of final coumarin derivatives were completed within 4-6 h, while that of microwave based reaction took comparatively more reaction time. **Table 1** shows that compounds **6f** and **6g** could not be synthesized by microwave radiation even after 32 minutes of irradiation.

Spectroscopic analysis

Successful formation of our key products coumarin derivatives **6a-l** has been confirmed by spectroscopic analysis such as ^{1}H NMR, ^{13}C NMR and mass analysis, which are further supported by elemental analysis and IR spectral studies.

In the IR spectrum, the structure of **6a-l** showed a characteristic absorption band at ~1755 cm^{-1} due to esteric carbonyl stricture and for lactone ring, it was found at ~1740 cm^{-1}. Moreover, the absence of primary amine stretching value at 3300 cm^{-1} show that the desired adduct **5a-l** has been formed. Disappearance of the –S-H stretching frequency and appearance of C–S value indicated that C–S–C linkage was formed between coumarin and oxadiazole ring .

The ^{1}H NMR spectral data of **6a-l** showed a characteristic value at ~4.0 δppm due to presence of methylene group near to esteric oxygen atom. Compounds **6j**, **6k** and **6l** showed singlets at ~3.5 δppm, which confirms the presence of a methyl group. Remaining aromatic and substituents protons were in good agreements with theoretical values.

The ^{13}C NMR spectra helped us to identify the formation of the final adducts. The characteristic value at ~35 δppm showed the presence of methylene group and C-3 (carbon number 3) of coumarin ring exhibited peak at ~99 δppm. The aromatic ring carbon and heterocyclic ring carbons were in decent covenants with the theoretical values.

Biological evaluation

Antibacterial screening

All the newly synthesized moieties **(6a-l)** were evaluated for their in vitro antibacterial activity against gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus pyogenes*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) by conventional broth microdilution method using ampicillin as a standard drug for antibacterial activity at different concentrations of 1000, 500, 200, 100, 50, 25 and 12.5 μg ml^{-1} as shown in **Table 2**. Compound **6b** (-4-NO_2-C_6H_4) has excellent activity against *E. coli* and *S. aureus*. It is noteworthy that compound **6b** (-4-NO_2-C_6H_4) showed the greatest inhibition at MIC= 12.5 μg ml^{-1}, while compound **6e** (-4-F-C_6H_4) showed inhibition at MIC= 12.5 μg ml^{-1} against *P. aeruginosa* and *S. pyogenes*. These data revealed that compound **6e** (-4-F-C_6H_4) was highly active against both organisms. Compounds **6b** (-4-NO_2-C_6H_4) and **6e** (-4-F-C_6H_4) showed very good activity at MIC= 50 μg ml^{-1}. Compound **6e** (-4-F-C_6H_4) displayed very good activity against *P. aeruginosa* while compound **6b** (-4-NO_2-C_6H_4) showed

Table 2. In vitro results of antibacterial and antifungal screening of compounds **6a-l**

No.	-R	Minimum inhibitory concentration (MIC) in μg ml^{-1}					
		Bacteria				Fungi	
		E.c.	P.a.	S.a.	S p.	C.a.	A.n.
6a	-H	500	250	500	250	>1000	500
6b	-4-NO_2	12.5	50	12.5	50	>1000	500
6c	-3-NO_2	100	250	100	250	100	100
6d	-2-NO_2	125	250	50	500	12.5	50
6e	-4-F	50	12.5	250	12.5	500	1000
6f	-4-Cl	100	250	100	500	500	>1000
6g	-3-Cl	125	>1000	500	500	250	100
6h	-3-CF_3	250	500	250	250	>1000	500
6i	-4-Br	250	100	100	500	500	250
6j	-4-CH_3	125	500	1000	500	50	12.5
6k	-3-CH_3	500	100	>1000	500	500	>1000
6l	-2-CH_3	250	250	>1000	500	>1000	500
Ampicillin		100	100	250	100	-	-
Griseofulvin		-	-	-	-	500	100

E.c.: *Escherichia coli MTCC 443; P.a.: Pseudomonas aeruginosa MTCC 1688; S.a.: Staphylococcus aureus MTCC 96; S.p.: Staphylococcus pyogenes MTCC 442; C.a.: Candida albicans MTCC 227; A.n.: Aspergillus niger MTCC 282.*

very good activity against *E. coli*. Moreover, compound **6d** (-2-NO_2-C_6H_4) exhibited very good activity against *S. aureus*. Compounds **6c** (-3-NO_2-C_6H_4) and **6f** (-4-Cl-C_6H_4) displayed good activity against *E. coli* and *S. aureus* while compound **6i** (-4-Br-C_6H_4) showed good activity against *P. aeruginosa* and *S. aureus* at MIC=100 **µg ml**$^{-1}$. The remaining compounds of the series possessed feeble antibacterial activity. On the other hand, the presence of similar functional groups at the *para* position resulted in minor increase in antibacterial activity as compared to **6b** (-4-NO_2-C_6H_4) and **6e** (-4-F-C_6H_4).

Antifungal screening

Minimum inhibition concentration (MIC) values of antifungal activity were observed against *Candida albicans,* and *Aspergillus niger* by conventional broth micro dilution method in **Table 2** using griseofulvin as a standard drug. Antifungal activity displayed that compound **6j** (-4-CH_3-C_6H_4) showed very good activity against *A. niger* at 12.5 µg ml^{-1} MIC. It was confirmed in the above table that compound **6g** (-3-Cl-C_6H_4) exhibited slightly reduced activity against *C. albicans*, while compound **6c** (-3-NO_2-C_6H_4) possessed good activity against *A. niger* and *C. albicans,* respectively. A couple of molecules i.e. **6d** (-2-NO_3-C_6H_4) and **6j** (-4-CH_3-C_6H_4) showed excellent activity against *C. albicans* and *A. niger* with fourfold greater MIC (12.5-50 µg ml^{-1}) than the reference drug. The remaining compounds of the series exhibited feeble antifungal activity.

CONCLUSIONS

In this short communication, we have described the comparative study for the synthesis of 2-oxo-2*H*-chromen-4-yl 2-((5-substitutedaryl-1,3,4-oxadiazol-2-yl)thio)acetate **(6a-l)** derivatives using conventional and microwave approaches. All the synthesized compounds were screened for their anti-microbial and anti-fungal study. It was concluded that electron withdrawing group containing scaffolds i.e. **6b, 6e, 6d,** and **6j** showed the best response when compared to the reference drugs, while 6d and 6j molecules exhibited extraordinary anti-fungal activity.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author contribution

Laboratory synthesis was carried out by Piyush Dholairya and the remaining biological work and writing were carried out by Khushal Kapadiya.

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Микроволновое и традиционное исследование аддуктов кумарин-оксадиазол и их антимикробная оценка

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Резюме

Введение: В настоящее время учёные продолжают сосредоточиваться на создании экономичного, доступного и экологически чистого синтеза различных гетероциклов. В форме молекул кумарина и оксазолов молекулы свинца, по-видимому, находятся в антимикробной области.

Цель: Для установления эффективных молекул мы протестировали производные 2-оксо-2H-хромен-4-ил-2 - ((5-арилзамещенный-1,3,4-оксадиазол-2-ил) тио) ацетата с использованием двух синтетических протоколов / методов, т.е. традиционный синтез и синтез на основе микроволнового излучения.

Материалы и методы: Два одновременных метода – т.е. традиционный синтез и синтез на основе микроволнового излучения были использованы для синтеза (6a-l) производного 2-оксо-2H-хромен-4-ил-2 - ((5-арилзамещенный-1,3,4-оксадиазол-2-ил) тио) ацетат. Желаемые молекулы были синтезированы с помощью традиционного и микроволнового синтеза, и было проведено сравнительное исследование, чтобы определить простой путь для промышленного применения. Подтверждение ингредиентов проводили с помощью спектроскопических методов, таких как ИК, ^{1}H ЯМР, ^{13}C ЯМР, масс-спектральный и элементный анализ.

Результаты: Все синтезированные ингредиенты были оценены на предмет антибактериальной активности in vitro против грамположительных бактерий (*Staphylococcus aureus, Staphylococcus pyogenes*), грамотрицательных бактерий (*Escherichia coli, Pseudomonas aeruginosa*) и противогрибковой активности (*Candida albicans, Aspergillus niger*)

Заключение: Все традиционные синтезы конечных производных кумарина были завершены в течение 4-6 часов, в то время как синтез с использованием микроволн длился относительно дольше. Удивительно, но ингредиенты 6f и 6g не могут быть синтезированы микроволновым излучением даже после 32 минут облучения. Что касается части введения лекарственного средства, микробиологическая оценка синтезированных аналогов показала, что ингредиенты 6b, 6e, 6d и 6j были более эффективными по сравнению с референтным лекарство.

Ключевые слова

1,3,4-оксадиазол, антимикробное действие, кумарин, микроволновый синтез