IJPSR (2023), Volume 14, Issue 7 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 13 November 2022; received in revised form, 24 December 2022; accepted, 01 May 2023; published 01 July 2023

SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL TETRAZOLE ANALOGUES OF FLAVONES AS ANTIMICROBIAL AGENTS

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Keywords:

Flavones, Tetrazoles, Synthesis, Antibacterial, Antifungal activity

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ABSTRACT: A series of flavones derivatives 11(a-j) have been synthesized and evaluated for their *in-vitro* antibacterial and antifungal activities. The antibacterial screening data showed that compounds with 4-methoxyphenyl (11c), 4-fluorophenyl (11d), and 2, 5-fluorophenyl (11h) substituent's on tetrazole ring, showed the maximum activity against the organisms used. The antifungal screening revealed that a compound with 4-nitropheny (11g) on tetrazole ring exhibited the highest activity against *A. niger* and a compound with 4-methoxyphenyl (11c) group exhibited good activity against *C. albicans*.

INTRODUCTION: Flavones and their derivatives were considered potent antitumor activity, associated with their ability to induce apoptosis [1]. The drugs baicalein, 3, 7-dihydroxyflavone and chrysin, considered antitumor agents, act as inducers of apoptosis in tumor cells through caspases-dependent pathways [2]. The introduction of prenyl side chains on flavones increases in growth inhibitory activities towards tumor cell lines [3], due to the effect related to the activation of caspase for some of the prenylated derivatives [4]. The alkylation/heteroarylation on flavones improved the antitumor activity [5]. Similarly, tetrazole and its derivatives can act as a pharmacophore for the carboxylate group, increasing their utility. Angiotensin II blocker often contain tetrazoles as Losartan and candesartan.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.14(7).3444-51

This article can be accessed online on www.ijpsr.com

DOI link: http://doi.org/10.13040/IJPSR.0975-8232.14(7).3444-51

A well-known tetrazole is MTT, which is dimethyl thiazolyl diphenyl tetrazolium salt. This tetrazole is used in an MTT assay to quantify the respiratory activity of live cells in cell culture, although it kills cells in the process [6]. Tetrazole derivatives have potential pharmacological activities such as antihypertensive [7], antimicrobial [8], corrosion inhibitor [9], anti-inflammatory [10], anticancer [11], antioxidant [12], analgesic [13], antiviral [14], protein arginine deiminase inhibitor[15], anti-allergic [16], dual selective serotonin and norepinephrine reuptake inhibitors [17] and HIV inhibitors [18]. They are used as catalysts in the synthesis of phosphonates.

Given the biological profile of flavones and tetrazole derivatives and our work on synthesizing new heterocyclic compounds [19-22], we designed a set of hybrid molecules with different heterocyclic scaffolds, such as flavone and tetrazole intending to increase their antimicrobial activities. In the present study, we report the synthesis of new series of 2-phenyl-3-(1-aryl-1*H*-1, 2, 3, 4-tetraazol-5-yl)-4*H*-4-chromenone 11(a-j) and evaluation of their *in-vitro* antimicrobial activities.

MATERIALS AND METHODS: All reagents are commercial grade and were used as supplied. Reactions were monitored by thin-layer chromatography (TLC) on pre-coated silica gel F254 plates from Merck and compounds visualized by exposure to UV light. Chromatographic columns 70–230 mesh silica gel for separations were used. IR spectra were recorded using KBr disk on a Perkin–Elmer FTIR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are reported in δ ppm units concerning TMS as an internal standard, and coupling constants (*J*) are reported in Hz units. Mass spectra were recorded on a VG micro mass 7070H spectrometer.

Preparation of Diethyl 2-benzoylmalonate (3): To a mixture of $MgCl_2$ (0.01 mol) in dry CH_3CN (25 mL), diethyl melonate 1 (0.01 mol) was added and the flask was immersed in an ice bath, and triethylamine (0.02 mol) was added and stirred the mixture for 15 min at 0 ^{o}C and benzoyl chloride 2 (0.01 mol) was added, the resulting mixture was stirred 1 h at 0 ^{o}C and then 12 h at room temperature and then the reaction mixture was quenched with 5M hydrochloric acid and the product was extracted with diethyl ether and subjected to vacuum distillation to get compound 3 as yellow oil in 67% of yield. IR (KBr)ν_{max}: 3078 (CH-Ar), 2982 (CH-Ali), 1751 (C=O), 1739 (C=O), 1694 (C=O), 1239 (C-O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.13 (t, 6H, $2CH_3$), 3.45 (s, 1H, CH), 4.10 (q, 4H, $2CH_2$), 7.40-7.60 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 20.1, 64.2, 69.1, 120.7, 126.2, 132.3, 140.6, 164.9, 188.7; MS: *m/z* 264 (M^+).

Preparation of Diethyl 2-[chloro (phenyl) Methylene] Malonate (4): To a cold and stirred mixture of compound 3 (0.01 mol) in $POCl_3$ (0.04 mol), tributylamine (0.01 mol) was added dropwise under stirring. After the complete addition, the mixture was heated in an oil bath at 110 ^{o}C with stirring for 6 h. The excess $POCl_3$ was removed with a rotary evaporator under reduced pressure. The residue obtained was cooled to room temperature, hexane (15 mL) was added and extracted with diethyl ether, and the organic layer was washed with cold aq. 10% HCl and then aq. 5% NaOH and concentrate with a rotary evaporator to get compound 4 as yellow oil in 54% yield. IR (KBr)ν_{max}: 3071 (CH-Ar), 2982 (CH-Ali), 1752 (C=O), 1739 (C=O), 1609 (C=C), 1241 (C-O), 696 (C-Cl) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.05 (t, 3H, CH_3), 1.35 (t, 3H, CH_3), 4.00 (q, 2H, CH_2), 4.40 (q, 2H, CH_2), 7.20-7.30 (m, 5H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 17.2, 65.3, 124.3, 125.5, 127.5, 129.0, 133.1, 139.7, 162.7, 171.2; MS: *m/z* 282 (M^+).

Preparation of Diethyl 2-[phenoxy (phenyl) Methylene] Malonate (5): The mixture of compound 4 (0.01 mol), phenol (0.015 mol), and potassium carbonate (0.012 mol) in dimethylformamide (100 mL) was heated under stirring in an oil bath at 140 ^{o}C for 12 h. After completion of the reaction removed, the solvent and the residue was dissolved in ether and NaOH (5%), extracted compound with ether and washed the layers with NaOH and then water, dried with anhydrous Na_2SO_4 and concentrated on getting the compound 5 as yellow solid in 57% of yield; IR (KBr)ν_{max}: 3042 (CH-Ar), 2979 (CH-Ali), 1759 (C=O), 1742 (C=O), 1611 (C=C), 1241 (C-O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.06 (t, 3H, CH_3), 1.35 (t, 3H, CH_3), 4.03 (q, 2H, CH_2), 4.32 (q, 2H, CH_2), 6.95-7.15 (m, 6H, ArH), 7.40-7.50 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 16.2, 64.5, 101.5, 117.8, 122.5, 126.4, 128.6, 129.0, 130.8, 131.2, 161.7, 166.2, 169.2, 170.1; MS: *m/z* 340 (M^+).

Preparation of 2-[phenoxy (phenyl) Methylene] Malonic Acid (7): To a solution of compound 5 (0.01 mol) in ethanol (25 mL), potassium hydroxide (0.02 mol) was added and refluxed under stirring for 2 h, cooled the mixture to room temperature and filtered with suction to get the di-potassium salt 6 and dissolved it in acetic acid and 5.5% (w/w) solution of HCl gas in acetic acid was added with stirring and external cooling. The solution is stirred 30 min, filtered and concentrated on a rotary evaporator to give compound 7 a yellow solid in 62% of yield. IR (KBr)ν_{max}: 3269 (OH), 3031 (CH-Ar), 1722 (C=O), 1718 (C=O), 1607 (C=C), 1241 (C-O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.52 (d, *J* = 8.3 Hz, 2H, ArH), 7.02 (d, *J* = 8.1 Hz, 2H, ArH), 7.15-7.20 (m, 1H, ArH), 7.30-7.35 (m, 3H, ArH), 8.51 (d, *J* = 8.6 Hz, 2H, ArH), 10.42 (s, 2H, COOH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 102.1, 118.7, 122.3, 124.1, 126.3, 131.2, 132.4,

133.1, 160.1, 168.3, 172.1, 178.8; MS: *m/z* 284 (M^+).

Preparation of 4-oxo-2-phenyl-4*H*-3-Chromecarboxylic Acid (8): To a cold concentrated sulphuric acid (100 mL), compound 7 (0.01 mol) was added in portions with stirring over a period of 1.5 h. The stirring was continued for 3 h at room temperature. The mixture is poured onto crushed ice, and the crude product was collected by filtration and recrystallized from ethyl acetate to get pure compound 8 as a yellow solid in 56% of yield; IR (KBr)ν_{max}: 3282 (COOH), 3049 (CH-Ar), 2981 (CH-Ali), 1710 (C=O), 1697 (C=O), 1607 (C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.55-7.65 (m, 6H, ArH), 8.21 (d, *J* = 8.6 Hz, 2H, ArH), 8.63 (d, *J* = 8.5 Hz, 1H, ArH), 10.78 (s, 1H, COOH); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 110.5, 120.4, 125.1, 126.3, 128.7, 129.1, 129.8, 130.4, 135.1, 135.6, 155.7, 172.0, 175.6, 180.1; MS: *m/z* 266 (M^+).

General Procedure for the synthesis of 2-Phenyl-4-oxo-4*H*-3-chromenearylcarboxamide (10 A-J): To a cold solution of compound **8** (0.01 mol) in toluene (15 mL), pyridine (0.2 mL) was added and further cooled the solution to 0-5 ^{o}C and then thionylchloride (0.05 mol) was added dropwise for a period of 15 min, allowed the temperature to reach to 30 ^{o}C and then stirred for 2 h, after the completion of the reaction, the mixture was cooled to 0 ^{o}C and corresponding arylamine (0.015 mol) was added and allowed to reach 30 ^{o}C and maintained for 5 h. After completion of the reaction, the solvent was removed, water (50 mL) was added and extracted with ethyl acetate, dried the organic layer over sodium sulphate; the solvent was removed and dried the residue under vacuum at 40 ^{o}C for 8 h to get corresponding compound 10a-j.

***N*3, 2 – diphenyl – 4 – oxo - 4*H* - 3 –chromenecarboxamide (10a):** IR (KBr)ν_{max}: 3369 (N-H), 3071 (CH-Ar), 1718 (C=O), 1699 (C=O), 1611 (C=C) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.79 (s, 1H, NH), 7.30-7.35 (m, 5H, ArH), 7.40-7.65 (m, 6H, ArH), 7.90-7.95 (d, *J* = 8.3 Hz, 2H, ArH), 8.40-8,45 (d, *J* = 8.2 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 110.8, 118.0, 119.0, 120.4, 121.2, 123.5, 124.8, 126.3, 127.9, 128.9, 131.6, 132.8, 139.1, 129.7, 154.1, 165.1, 176.7, 179.0; MS: *m/z* 342 (M^++1).

General Procedure for the synthesis of 2-Phenyl-3-(1-aryl-1*H*-1, 2, 3, 4-tetraazol-5-yl)-4*H*-4-chromenone (11 a-j): To a stirred solution of corresponding compound 10 (0.01 mol) in carbon tetrachloride (60 mL) was added phosphorous pentachloride (0.01 mol) under nitrogen atmosphere. The reaction mixture was heated to reflux and maintained for 4 hrs. After completion of the reaction, the solvent was distilled off completely under reduced pressure. The residue was cooled to 0-5 ^{o}C, added DMF (40 mL) under a nitrogen atmosphere and stirred for 10-15 minutes to get clear solution. A suspension of sodium azide (0.015 mol) in DMF (40 mL) was cooled to 0-5 ^{o}C. The iminoyl chloride solution in DMF was taken into the addition funnel and added to the suspension of sodium azide in DMF at 0-5 ^{o}C during 1 hr. After the addition, cooling was removed and stirred at 25-30 ^{o}C for overnight. The reaction mass was cooled to 0-5 ^{o}C and added water (50 mL) slowly during 20-30 minutes and maintained for 30-45 minutes at same temperature. The separated precipitate was flittered and washed with water to get the corresponding compound 11 (a-j).

2-phenyl-3-(1-phenyl-1*H*-1, 2, 3, 4-tetraazol-5-yl)-4*H*-4-chromenone (11a): IR (KBr)ν_{max}: 3061 (CH-Ar), 1697 (C=O), 1629 (C=N), 1611 (C=C), 1322 (N=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.40-7.60 (m, 7H, ArH), 7.90-7.95 (m, 4H, ArH), 8.10-8.15 (d, J = 8.6 Hz, 2H, ArH), 8.40-8.45 (d, *J* = 8.8 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.1, 118.9, 123.0, 123.7, 124.6, 125.2, 126.1, 129.5, 130.5, 131.1, 133.1, 134.6, 135.0, 135.9, 142.2, 153.7, 168.6, 172.7; MS: *m/z* 367 (M^++1).

3-[1-(4-methylphenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone(11b): IR (KBr) ν_{max}: 3048 (CH-Ar), 2978 (CH-Ali), 1695 (C=O), 1627 (C=N), 1610 (C=C), 1325 (N=N) cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.47 (s, 3H, CH_3), 7.40-7.60 (m, 8H, ArH), 7.90-7.95 (m, 4H, ArH), 8.40-8.45 (d, *J* = 8.8 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 22.2, 113.2, 118.6, 123.9, 124.4, 125.3, 126.3, 127.3, 129.7, 130.3, 131.1,

134.8, 135.1, 136.8, 137.3, 142.2, 153.5, 168.4, 172.5; MS: *m/z* 380 (M^+).

3-[1-(4-methoxyphenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11c): IR (KBr) ν_{max}: 3052 (CH-Ar), 1699 (C=O), 1624 (C=N), 1610 (C=C), 1321 (N=N), 1078 (C-O-C) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 3.92 (s, 3H, OCH_3), 7.40-7.60 (m, 8H, ArH), 7.90-8.00 (m, 4H, ArH), 8.40-8.45 (d, *J* = 8.8 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 58.2, 113.2, 117.9, 118.6, 123.0, 124.4, 125.3, 126.5, 127.3, 128.1, 129.5, 131.2, 134.7, 135.3, 142.2, 153.0, 158.8, 168.2, 172.0; MS: *m/z* 397 (M^++1).

3-[1-(4-fluorophenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11d): IR (KBr) ν_{max}: 3074 (CH-Ar), 1693 (C=O), 1627 (C=N), 1611 (C=C), 1410 (C-F), 1321 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.40-7.60 (m, 10H, ArH), 7.90-7.95 (d, *J* = 8.7 Hz, 2H, ArH), 8.40-8.45 (d, *J* = 8.6 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.6, 118.9, 119.5, 123.5, 124.8, 125.3, 126.3, 127.7, 129.7, 131.3, 134.1, 135.5, 136.0, 142.0, 153.4, 165.1, 168.5, 172.2; MS: *m/z* 384 (M^+).

3-[1-(4-bromophenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11e): IR (KBr) ν_{max}: 3078 (CH-Ar), 1701 (C=O), 1624 (C=N), 1613 (C=C), 1322 (N=N), 686 (C-Br) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.40-7.60 (m, 8H, ArH), 7.95-8.00 (m, *4*H, ArH), 8.40-8.45 (d, *J* = 8.5 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.0, 118.4, 122.3, 123.5, 124.8, 125.1, 126.7, 128.3, 129.4, 131.6, 134.1, 135.8, 136.1, 137.8, 142.1, 153.8, 168.3, 172.1; MS: *m/z* 446 (M^++1).

3-[1-(3-nitrophenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11f): IR (KBr) ν_{max}: 3057 (CH-Ar), 1693 (C=O), 1627 (C=N), 1612 (C=C), 1370 (NO_2), 1320 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.40-7.60 (m, 6H, ArH), 7.90-7.95 (m, 4H, ArH), 8.40-8.45 (m, *2*H, ArH), 8.95 (s, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 112.9, 113.7, 118.3, 119.3, 123.2, 124.0, 124.7, 125.4, 126.8, 129.6, 131.8, 133.8, 134.6, 135.4, 140.1, 142.9, 152.3, 153.7, 168.5, 172.4; MS: *m/z* 411 (M^+).

3-[1-(4-nitrophenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11g): IR (KBr) ν_{max}: 3063 (CH-Ar), 1695 (C=O), 1631 (C=N), 1617 (C=C), 1379 (NO_2), 1325 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 7.40-7.60 (m, 8H, ArH), 7.90-7.95 (d, *J* = 8.6 Hz, 2H, ArH), 8.40-8.45 (m, 3H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 112.7, 118.0, 123.5, 124.4, 125.1, 126.0, 129.9, 127.3, 129.9, 131.5, 134.1, 135.5, 140.8, 142.8, 144.9, 153.3, 168.4, 172.3; MS: *m/z* 412 (M^++1).

3-[1-(2,5-difluorophenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl] - 2 - phenyl - 4*H*-4 - chromenone (11h): IR (KBr)ν_{max}: 3039 (CH-Ar), 1696 (C=O), 1625 (C=N), 1617 (C=C), 1412 (C-F), 1328 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 6.68 (d, *J* = 8.8 Hz, 1H, ArH), 7.40-7.60 (m, 7H, ArH), 7.90-7.95 (m, 3H, ArH), 8.40-8.45 (d, *J* = 8.8 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 109.1, 112.7, 113.7, 118.0, 121.5, 122.0, 123.9, 124.6, 125.4, 126.1, 129.6, 131.1, 134.3, 135.2, 142.7, 153.5, 164.3, 166.2, 168.2, 172.4; MS: *m/z* 402 (M^+).

3-[1-(3-hydroxyphenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11i): IR (KBr) ν_{max}: 3342 (O-H), 3087 (CH-Ar), 1696 (C=O), 1629 (C=N), 1613 (C=C), 1328 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 5.58 (s, 1H, OH), 6.70-6.75 (d, *J* = 8.4 Hz, 1H, ArH), 7.00-7.05 (d, *J* = 8.6 Hz, 1H, ArH), 7.40-7.60 (m, 7H, ArH), 7.95-8.00 (m, 3H, ArH), 8.40-8.45 (d, *J* = 8.8 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 109.1, 112.4, 113.0, 118.8, 119.2, 123.4, 124.3, 125.1, 126.3, 129.7, 131.2, 133.0, 134.6, 135.8, 138.0, 142.4, 153.8, 162.0, 168.1, 172.4; MS: *m/z* 382 (M^+).

3-[1-(4-hydroxyphenyl)-1*H*-1, 2, 3, 4-tetraazol-5-yl]-2-phenyl-4*H*-4-chromenone (11j): IR (KBr) ν_{max}: 3378 (O-H), 3071 (CH-Ar), 1694 (C=O), 1623 (C=N), 1617 (C=C), 1320 (N=N) cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz): δ 5.37 (s, 1H, OH), 7.00-7.10 (d, *J* = 8.8 Hz, 2H, ArH), 7.40-7.60 (m, 6H, ArH), 7.90-7.95 (m, 4H, ArH), 8.40-8.45 (d, *J* = 8.8 Hz, 1H, ArH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 113.4, 118.5, 120.0, 123.0, 124.7, 125.3, 126.5, 127.4, 128.4, 129.7, 131.1, 134.6, 135.4, 142.3, 153.7, 155.7, 168.6, 172.9, MS: *m/z* 382 (M^+).

Biological Evaluation:

Antibacterial Activity: All the newly synthesized compounds 11(a-j) were screened for their *in vitro*

antibacterial activity against Gram-positive organisms of Staphylococcus aureus, Bacillus subtilis and Gram-negative organisms *Escherchia coli*, *Pseudomonas aeruginosa*.

The zones of inhibition (mm) at 100 µg/mL concentration of the test compounds were determined using the cup-plate method [23].

The antibacterial screening data of compounds 11(a-j) showed that all compounds exhibited antibacterial activity against Gram-positive and Gram-negative organisms. Among these, compounds with 4-methoxyphenyl (11c), 4-fluorophenyl (11d) and 2,5-difluorophenyl (11h) substituent on tetrazole ring, showed the maximum activity against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* respectively, while the other compounds showed moderate to good activity against these organisms employed.

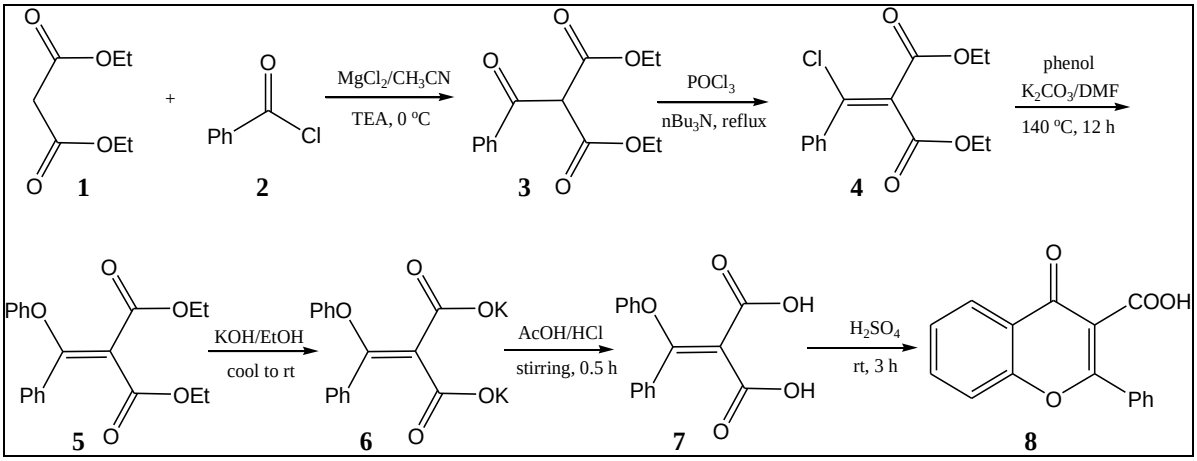
Antifungal Activity: The compounds 11(a-j) were also screened for their antifungal activity against *Aspergillus niger*, *Candida albicans* at 100 µg/mL using cup-plate method [24]. The antifungal activity of these compounds was compared with the standard reference Amphotericin B. The zones of inhibition formed were measured in mm and are presented in **Table 1**. The antifungal screening data of compounds 11(a-j) showed that all compounds exhibited antifungal activity against *A. niger*. Among these, compound with 4-nitropheny (11g) on tetrazole ring exhibited the highest activity against *A. niger*, while compound (11a) showed the least activity. These compounds except 11a and 11i, also showed activity against *C. albicans*. Among these, compound with 4-methoxyphenyl (11c) group on tetrazole ring exhibited good activity against *C. albicans*, while others showed moderate to good activity.

TABLE 1: ANTIMICROBIAL ACTIVITY OF COMPOUNDS 11A-J

Compound	Ar	Antibacterial Activity				Antifungal Activity	
		SA	***BS***	***EC***	***PA***	***AN***	***CA***
11a	phenyl	12	11	10	13	9	—
11b	4-methylphenyl	20	18	19	20	13	9
11c	4-methoxyphenyl	25	24	21	22	12	14
11d	4-fluorophenyl	28	26	24	22	10	10
11e	4-bromophenyl	19	17	18	17	12	13
11f	3-nitrophenyl	22	18	20	15	10	11
11g	4-nitrophenyl	23	16	18	19	17	10
11h	2,5-difluorophenyl	27	24	21	23	14	12
11i	3-hydroxyphenyl	23	20	16	17	12	—
11j	4-hydroxyphenyl	19	21	18	14	10	12
Ampicillin	—	38	32	33	30	—	—
Amphotericin B	—	—	—	—	—	18	16

SA=*Staphylococcus aureus*, BS=*Bacillus subtilis*, EC=*Escherchia coli*, PA=*Pseudomonas aeruginosa*, *AN*=*Aspergillus niger*, CA=*Candida albicans.*

RESULTS AND DISCUSSION: The synthesis of the title compounds commenced from diethylmelonate 1 which reacted with benzoyl-chloride 2 in the presence of magnesium chloride, triethylamine in dry acetonitrile at 0 ^{o}C with stirring for 12 h to yield diethyl-2-benzoylmalonate 3 as yellow oil in 67% of yield, which on chlorination with $POCl_3$ in the presence of tri-n-butylamine under reflux at 110 ^{o}C for 5-6 h to give diethyl-2-[chloro(phenyl) methylene] malonate 4 as yellow oil in 54% of yield. Compound 4 in DMF solvent was treated with phenol in the presence of K_2CO_3 and heated under stirring at 140 ^{o}C for 12 h, furnished the diethyl-2-[phenoxy (phenyl) methylene] malonate 5 as yellow solid in 57% of yield. Compound 5 undergo the ester hydrolysis with potassium hydroxide in ethyl alcohol at reflux with stirring for 2 h to give the di-salt 6. The salt was dissolved in acetic acid and solution of HCl gas in acetic acid is added with stirring and external cooling, further stirring at room temperature for 30 min to give 2-[phenoxy (phenyl) methylene] malonic acid 7 as yellow solid in 62% of yield. Compound 7 on cyclocondensation in the presence of cold concentrated sulphuric acid under stirring at room temperature for 3 h to give 4-oxo-2-phenyl-4*H*-3-chromenecarboxylic acid 8 as yellow solid in 56% of yield (Scheme 1). The structure was established by interpreting their IR, MS and NMR spectral data.



SCHEME 1

In the IR spectrum of compound 3, the absorption bands due to C=O of diethylmelonate appeared at 1751, 1739 and C=O of benzylchloride appeared at 1694, the absorption due to C-O of ester group appeared at 1239 cm^{-1}. Its ^{1}H NMR spectra, protons of both methyl groups and methylene protons signals appeared as a triplet at δ 1.13 and quartet at δ 4.10 ppm, respectively, the signals for protons of aromatic ring observed at δ 7.40-7.60 ppm. Its ^{13}C NMR specta, the signals of C=O of diethyl-melonate and benzoyl group appeared at δ 188.7 and 164.9 ppm, respectively, the methyl and methylene carbon appeared at δ 20.1 and 64.2 ppm respectively.

In the IR spectrum of 4, the absorption bands due to C=O of diethylmelonate appeared at 1752, 1739, the ester C-O at 1241 and the C-Cl appeared at 696 cm^{-1} Its ^{1}H NMR spectrum, the signals appeared for the protons of methyl group at δ 1.05 and 1.35 ppm as triplet for three protons in each, the methylene protons as quartet appeared at δ 4.00 and 4.40 ppm, the other aromatic protons appeared in the range of δ 7.20-7.30 ppm. In the CMR spectrum, the signals at δ 162.7, 171.2 ppm and at δ 133.1, 139.7 were assigned to the carbons of C=O and carbons of alkene group respectively.

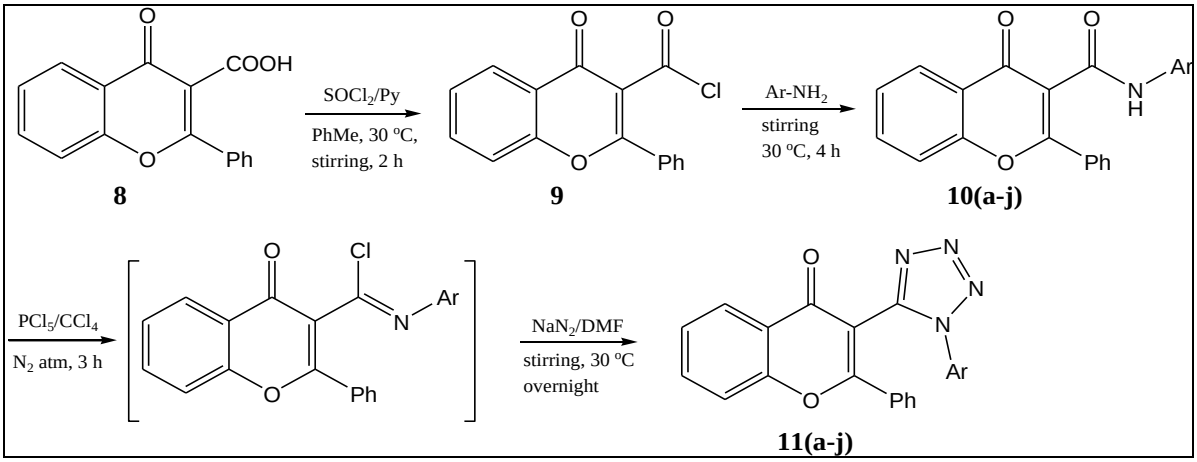
In the IR spectrum of compound 5, the absorption bands due to the C=O of diethylmelonate appeared at 1759 and 1742, and the other bands at 1611 (C=C) and 1241 (C-O) cm^{-1}. In its ^{1}H NMR spectrum, the signals corresponding to the ethyl group of ester appeared at δ 1.06 and 1.35 ppm as triplet corresponding to the methyl groups and at δ 4.03 and 4.32 ppm as a quartet for CH_2 group, the other aromatic protons signals appeared in the range of δ 6.95-7.15 and 7.40-7.50 ppm. In the CMR spectrum, the signals at δ 169.2, 170.1 ppm and at δ 130.8, 131.2 were assigned to the carbons of C=O and alkene group, respectively.

The IR spectrum of compound 7, the absorption bands due to C=O of melonic acid appeared at 1722, 1718 and O-H of acid appeared at 3269, the absorption due to C-O of acid group appeared at 1241 cm^{-1}. Its ^{1}H NMR spectra, the aromatic protons appeared in the range at δ 6.52, 7.02, 7.15-7.20, 7.30-7.35 and 8.51 ppm, the corboxyl proton appeared as singlet at δ 10.42 ppm. In the CMR spectrum, the signals at δ 172.1, 178.8 ppm and at δ 132.4, 133.1 were assigned to the carbons of C=O and alkene group, respectively.

In the IR spectrum of 8, the absorption bands due to C=O flavone appeared at 1710 and C=O of acid group appeared at 1697, the other bands at 3282 (OH) and 1607 (C=C) cm^{-1} Its ^{1}H NMR spectrum, the signals appeared for the aromatic protons in the range at δ 7.55-7.65, 8.21 and 8.63 ppm, the carboxylic acid proton appeared as singlet at δ 10.78 ppm. Its ^{13}C NMR specta, the flavone ring carbons (C2-C6) signals appeared at δ 180.1, 110.5, 175.6, 120.4, 155.7 ppm respectively, and the C=O of carboxyl carbon appeared at δ 172.0 ppm.

Further, compound 8 was treated with thionylchloride in the presence of pyridine in toluene at 30 ^{o}C under stirring for 2 h, to get the compound 9 and it was reacted with corresponding arylamine at 30 ^{o}C under stirring for 5 h to get corresponding compounds 2-phenyl-4-oxo-4*H*-3-chromene- arylcarboxamide 10(a-j) in 45-67% of yields. The corresponding compound 10 was

treated with phosphorous pentachloride in carbon tetrachloride under nitrogen atmosphere and then heated to reflux for 4 h to get iminoyl chloride as an intermediate which was further reacted with sodium azide in DMF under stirring at 25-30 ^{o}C for overnight to get corresponding 2-phenyl-3-(1-aryl-1*H*-1,2,3,4-tetraazol-5-yl)-4*H*-4-chromenone 11(a-j) in 40-60% of yields (Scheme 2). The structure of the synthesized compounds was established from the interpretation of their IR, MS and NMR spectral data.



SCHEME 2

In the IR spectrum of compound 10a, the absorption bands corresponding to carbonyl groups appeared at 1718 (C=O), 1699 (C=O), the amide N-H group at 3369 (N-H) cm^{-1}. Its ^{1}H NMR spectra, the aromatic protons appeared in the range at δ 7.30-7.35 and 7.40-765 ppm as two multiplets and at δ 7.90-7.95, 8.40-8.45 ppm as two doublets, the amide proton signal appeared as broad singlet at δ 5.79 ppm. Its ^{13}C NMR specta, the signals flavone ring carbons appeared at δ 179.0 (C_2), 118.0 (C_3), 176.7 (C_4), 126.3 (C_5) and 154.1 (C_6) ppm respectively, and the signals for carbon of amide carbonyl appeared at δ 165.1 ppm.

The IR spectrum of compound 11a, the prominent absorption bands appeared at 1697 (C=O), 1629 (C=N) and 1322 (N=N) cm^{-1}, the disappearance of the band of amide carbonyl group indicated cyclization. In its 1H NMR spectra, the aromatic protons appeared at δ 7.40-7.60, 7.90-7.95 ppm as two multiplets and at δ 8.10-8.15, 8.40-8.45 ppm as two doublets. Its ^{13}C NMR specta, the signals flavone ring carbons appeared at δ 168.6 (C_2), 113.1 (C_3), 172.7 (C_4), 124.6 (C_5) and 153.7 (C_6) ppm, respectively and the signals for the carbon of tetrazole ring appeared at δ 142.2 ppm.

CONCLUSION: In conclusion, a new series of flavones derivatives 11a-j have been synthesized and evaluated for their antimicrobial activities, which revealed that compounds that contain 4-methoxyphenyl (11c), 4-fluorophenyl (11d) and 2,5-difluorophenyl (11h) group on tetrazole ring showed significant antibacterial activity. The compound with 4-nitropheny (11g) exhibited the highest antifungal activity against *A. niger* and compound with 4-methoxyphenyl (11c) group exhibited good activity against *C. albicans*, while others showed moderate to good activity.

ACKNOWLEDGEMENT: The authors are thankful to the Director, Indian Institute of Chemical Technology, Hyderabad, India, for providing NMR and mass spectral data.

CONFLICTS OF INTEREST: This research work has no conflict of interest because I do all work individually and all funding resources are self-funding by me.

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How to cite this article:
Nagaraj A and Rao GN: Synthesis and biological evaluation of novel tetrazole analogues of flavones as antimicrobial agents. Int J Pharm Sci & Res 2023; 14(7): 3444-51. doi: 10.13040/IJPSR.0975-8232.14(7).3444-51.