SYNTHESIS, CHARACTERIZATION, SAR STUDY AND ANTI-INFLAMMATORY ACTIVITY OF 2, 4- THIAZOLIDINEDIONE DERIVATIVES OF 7-FLAVONOLS

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Graphical abstract:

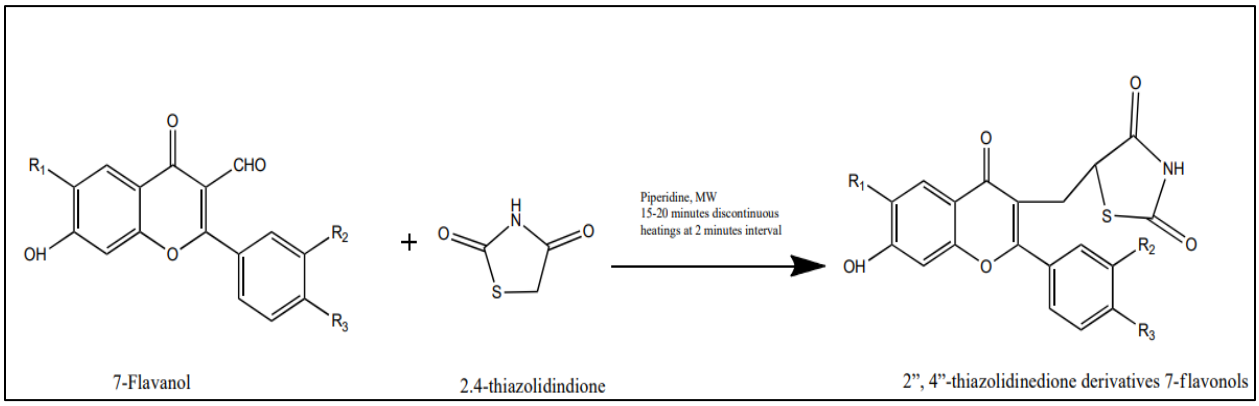


Fig. 1 Graphical abstract

Abstract

An analysis of the biological effects of flavanols revealed that although several natural 3-flavonols were used to treat conditions like inflammation, neither their synthetic derivatives nor information on natural 7-flavonols had been documented up to this point. The Phyto flavanols must also undergo a challenging and time-consuming process to generate, standardize, validate, and, if necessary, undergo chemical transfusion. Using an efficient method, diverse 3-formyl, 7-flavonols, and thiazolidinediones were converted into the compounds of various 2", 4"-thiazolidinedione derivatives of 7-flavonols through a technique MEC. Although different nitro- and acetyl-derivatives of flavanols have not yet been synthesized, under microwave circumstances, the reactions for both substituents on position 7 of the benzene ring A of the flavone ring performed well, and the analytical results were good. Through the use of Co-TLC, max, IR, HPTLC, 1HNMR, CHN analysis, and mass spectral studies, the re-crystallized products were identified. The fingerprint profiles produced by HPTLC differed from those produced by a standard Co-TLC approach in that they had a single conspicuous peak and matched Rf values. Using the in vitro protein denaturation technique and the in vivo paw edema model, all the produced compounds were evaluated for their ability to reduce inflammation The results showed that all compounds, except the unsubstituted 2', 4' -thiazolidinedione derivatives of 7-flavonols, produced an equal amount of or more powerful action as the standard.

Keywords: 2’, 4’ -thiazolidinedione derivatives of 7-flavonols; 3-formyl, 7-flavonols; HPTLC profiles; *In vitro* and *in vivo* anti-inflammatory activities.

Introduction

The synthetic method is one of the advanced techniques in the contemporary drug creation process. There have been a lot of studies done on various synthetic flavonoids as a result of the diverse biological/pharmacological activities of flavonoids (Furniss et al. 2005) and the fact that they can treat or prevent a variety of diseases (Theja et al. 2011). Why synthetic flavonoids? They are produced at a far higher rate than natural organic compounds, have better

potential (Gupta et al. 1999), provide a higher rate of return on investment, and have proven to be effective in times of crisis (Jain et al.2010).

As these flavonoids are of most vital consideration in health and human dietary elements aspect works of literature, a knowledge search on their structure and activity may become a crucial component of the study (Sohel et al. 2006). Regarding the flavanols' structure-activity approach, (Vaya et al.) make the following claims: According to Lawrence (2006), 6-hydroxy flavone is one of the cytochromes P4502C9 enzyme's non-competitive inhibitors. Taxifolin and quercetin's B rings include C3', C4'-catechol, which inhibits lipid peroxidation (Mageriino et al.2002), -responsible for its antioxidant effect (Sichel et al.); reduction in the anti-oxidant potential of hesperidin occurs by -CH3 substitution on C4 OH group (Samadhiya et al. 2012) or if OH groups are in meta position of a ring (Pant et al. 2007); the number of OH moieties is an apparent requirement for good antioxidant activity in flavanols (Mavandadi et al (Lotito et al. 2006). Baicalein has modest activity, having pyrogallol moiety on a ring (Lew et al.2002).

Since there are only a relatively limited number of scientific studies on the pharmacological effects of 7 flavonols and their derivatives, the current effort was designed to prioritize their synthetic derivatives.

Thiazole derivatives and the subtype 2,4-thiazolidinedione class have established their adaptability in the pharmaceutical industry (Samadhiya et al. 2012). The latter may be free of the hypersensitivity responses caused by the hydantoin ring since it is a hydantoin bio-isostere (Lew et al. 2002). (Pant et al. 2007). Despite several publications concerning the effectiveness of TZDs over the past three decades, clinical inadequacy has been noted due to PK issues, unfavorable side effects, or limited efficacy (Gupta et al. 1999). The present work was anticipated to be valuable in overcoming the undesirable effects through the synthesis (Molteni et al. 2005) of a

more naturally behaving, widely accessible, and least toxic nucleus, such as a hetero-aryl substituent like flavonol 2,4-thiazolidinedione ring substituent (Vivek). This was due to the exceptional synthetic efficiency (Niraldo et al. 2009).

The vast majority of pro- and anti-inflammatory mediators that control inflammation include histamine, PGE2, LTs, 5-HT, ILs, TNF-, prostacyclin's, bradykinin, ROS, growth factors, lysosomal contents of neutrophils, adipokines, etc. (Jain et al. 2010, 2010). (Lim et al. 2009). The development of modern anti-inflammatory drugs is based on (Seidler et al. 2002) early in vitro findings that involve powerful inhibition of the arachidonic acid pathways and certain cytokine cascades. Among the several screening approaches, one of the most popular ones is based on the suppression of edema following the injection of a phlogiston agent and the activity of HSF-1, which prevents albumin denaturation.

In the context of the aforementioned and observed studies, it was decided to synthesize various 2', 4'-thiazolidinedione derivatives of 7-flavonols for in vitro and in vivo anti-inflammatory activities, and the results were studied by comparing to Indomethacin, the reference drug.

Results and Discussion

Complete conversions and yields of the corresponding synthesized compounds of over 93% were achieved for all the tested analogs within 2 minutes of microwave heating, even under low-energy microwave settings (90-160 W intensity). The references state that raising the temperature would increase the yield of the nitro derivatives of flavonoids, but doing so would undoubtedly lengthen the heating process, which is not a positive factor.

Characterization of compound IA: 3-(5" -(thiazolidine-2",4"-dionyl) methyl)7-flavonol

Irradiation time in microwaves 2'30 s; MF: $C_{19}H_{11}N_2O_8S$, MW: 411, % yield: 81.2, M.Pt:107 ºC, ,IR (Nujol) 3550-3200 cm^{-1} $R_f$data 0.53, λmax 235 nm intermolecular -OH ,2700

cm^{-1} -C-H stretching , 1639 cm^{-1}, C=C-C=O, ketones; CH_2-C=O, primary alcohol; 1600 cm^{-1} C-C, benzene nucleus; 1435-1405 cm^{-1}, acyclic; 1010 cm^{-1}, C-O stretching, 500 cm^{-1}, for Sulphur;^{1}HNMR (D_2O, δ ppm): 2.58 (d, 2H, -CH_2, J=3.1), 3.07 (d, 1H, -CH in ring, J=3.6), 5.0 (m, 1H,-OH), 6.42 (m, 2H, ArH, J=7.2), 7.8-8.10 (m, 5H, ArH, H-2", H-3", H-4", H-5", H-6"), 8.5 (s, 1H, H-5, J=7.0), 8.7 (s,1H, >NH, J=7.6); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R); 34.68 (R_3-C-OH); 46.63 [R-C (=O)-R]; 54.10 (=C-N-); 127.43 (=C-S- in ring); 128-129 (aryl =CH carbons); 130.14 (Ar-C); 141.07 (R_2-C=C-R_2); 147.17 (conjugated diene carbon); 165.95 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); MS (m/z): 388.87 M+; CHN analysis: Calcd.(Found)- C-68.85 (71.23); H-11.07 (12.79);N-2.23 (4.32); O-12.74 (14.12); S-5.11 (6.77).

Characterization of compound Ib: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)6-nitro,7-flavonol:**

Irradiation time in microwaves2'30 s; MF: $C_{19}H_{10}N_3O_{10}S$; MW: 456; % yield: 81.6; M.Pt: 121 ºC; R_f data 0.66; λmax 222 nm; IR (Nujol) 3600-2600 cm^{-1} -OH; 2700 cm^{-1}-C-H 1639 cm^{-1}, C=C-C=O, , CH_2-C=O, acyclic; ketones; 1600 cm^{-1} C-C, benzene nucleus; C-O stretching, Aryl; 1010 cm^{-1}, C-O, primary alcohol; 500 cm^{-1}, for Sulphur; ^{1}HNMR (D_2O, δ ppm): 2.92 (d,2H, -CH_2, J=3.6), 3.15 (d, 1H, -CH in ring, J=3.2), 4.7 (m, 1H, -OH), 6.53 (m, 2H, ArH, J=7.1), 7.50-7.80 (m, 5H, ArH, H-2", H-3", H-4", H-5", H-6"), 8.0 (s, 1H, H-5,J=7.4), 8.7 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.33(R-CH_2-R); 34.63 (R_3-C-OH); 46.71[R-C (=O)-R]; 54.16 (=C-N-); 127.48 (=C-S- in ring); 1550-1510 cm^{-1} -NO_2 asymmetric stretching & aromatic nitro group; 1435-1405 cm^{-1}1320-1210 cm^{-1}, 128-129 (aryl =CH carbons); 130.14 (Ar-C); 133.862 (=6C-NO_2); 141.13 (R_2-C=C-R_2); 147.25 (conjugated diene carbon); 165.95 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); MS (m/z): 411.24 M+; CHN analysis: Calcd.(Found)- C-64.25 (68.10); H-10.18 (12.65); N-4.16 (5.02); O-16.64 (18.45); S-4.76 (5.62).

Characterization of compound Ic: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)6-acetyl,7-flavonol:**

Irradiation time in microwaves 2'30 s; MF: $C_{19}H_9N_4O_{12}S$; MW: 501; % yield: 94.7; M.Pt: 142 ºC; R_f data 0.54; λmax 230 nm; IR (Nujol) 3600-2600 cm^{-1} -OH; 2700 cm^{-1}-C-H; 1639 cm^{-1}, C=C-C=O, ; 946 cm^{-1} C-H out of plane pending; 500 cm^{-1}, CH_2-C=O, 1010 cm^{-1} C-O, primary alcohol ^{1}HNMR (D_2O, δ ppm): for Sulphur;ketones1600 cm^{-1} C-C, benzene nucleus; 1435-1405 cm^{-1}, 2.49 (s,3H,>CO-CH_3, J=3.2), 3.1 (d,2H, -CH_2, J=3.2), 3.35 (d, 1H, -CH in ring, , J=3.6), 5.43 (m, 1H, -OH), 6.22 (m, 1H, ArH, , J=5.4), 7.10-7.20 (m, 5H, ArH, H-2", H-3", H-4", H-5", H-6"), 8.4 (s, 1H, H-5, J=6.9), 8.7 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R); 28.41 (-CH_3 in acetyl group); 34.64 (R_3-C-OH); 40.94 (-C(=O)-CH_3, an acetyl carbon);46.75 [R-C(=O)-R]; 54.14 (=C-N-); 127.47 (=C-S- in ring); 128-129 (aryl =CH carbons); 141.12 (R_2-C=C-R_2); 147.20 (conjugated diene carbon); 165.90 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); MS (m/z): 409.54 M+; CHN analysis: Calcd.(Found)- C-68.12 (71.12); H-10.68 (12.85); N-2.09(4.16); O-14.33(15.64); S-4.79(5.99).

Characterization of compound Id: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)4'-nitro,7-flavonol:**

Irradiation time in microwaves 2'30 s; MF: $C_{19}H_{12}NO_5S$; MW: 366; % yield: 87.6; M.Pt: 96 ºC; R_f data 0.62; λmax 214 nm; IR (Nujol) 3600-2600 cm^{-1} -OH; benzene nucleus; 1550-1510 cm^{-1} -NO_2 asymmetric stretching & aromatic nitro group; 2700 cm^{-1}-C-H;), 4.62 (m, 1H, -OH), 1639 cm^{-1}, C=C-C=O, ketones; 1600 cm^{-1} C-C, 1010 cm^{-1} C-O, primary alcohol; 500 cm^{-1}, for Sulphur; ^{1}HNMR (D_2O, δ ppm): 2.60 (d,2H, -CH_2, J=2.8), 3.07 (d, 1H, -CH in ring, , J=3.86.39 (m, 2H, ArH, , J=6.2), 7.90-8.10 (m, 4H, ArH, H-2", H-3", H-5", H-6"), 8.7 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R);121.0&127.3 (aryl =CH carbons); 147.60 (=4'C-NO_2); 165.95 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); MS (m/z): 410.24 M+; CHN analysis: Calcd.(Found)- C-64.25(68.10); H-10.18(12.65); N-4.16(5.02); O-16.64(18.45); S-4.76(5.62).

Characterization of compound Ie: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)6,4'-dinitro,7-flavonol:**

Irradiation time in microwaves 2'30 s; MF: $C_{19}H_{11}N_2O_8S$; MW: 411; % yield: 82.9; M.Pt: 108 ºC; R_f data 0.48; λmax 226 nm; IR (Nujol) 3600-2600 cm^{-1} -OH; 3250-2500 cm^{-1} OH (Intra molecular with C=O, NO_2 etc.,) ; 1550-1510 cm^{-1} -NO_2 asymmetric stretching & aromatic nitro group 1320-1210 cm^{-1}, C-O, Aryl; 2700 cm^{-1}-C-H; 1639 cm^{-1}, C=C-C=O, ketones1600 cm^{-1} C-C, benzene nucleus; 1010 cm^{-1}, C-O, primary alcohol; 500 cm^{-1}, for Sulphur; ^{1}HNMR (D_2O, δ ppm): 2.5 (d,2H, -CH_2, J=3.0), 3.15 (d, 1H, -CH in ring, , J=3.7), 4.86 (m, 1H, -OH), 6.65 (m, 2H, ArH, J=6.0), 7.80-8.05 (m, 4H, ArH, H-2", H-3", H-5", H-6"), 8.5 (s, 1H, H-5, J=7.1), 8.7 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R); 34.71 (R_3-C-OH); 46.7 [R-C (=O)-R]; 54.15 (=C-N-); 127.47 (=C-S- in ring); 128-129 (aryl =CH carbons); 133.862 (=6C-NO_2); 141.11 (R_2-C=C-R_2); 147.60 (=4'C-NO_2);165.95 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon). MS (m/z): 454.53M+; CHN analysis: Calculated. (Found)- C-60.22(62.35); H-9.41(11.41); N-5.85(6.53); O-20.06(22.96); S-4.47(6.34).

Characterization of compound If: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)6-acetyl,4'-nitro,7-flavonol:**

Irradiation time in microwaves 2'30 s; MF: $C_{19}H_{10}N_3O_{10}S$; MW: 456; % yield: 92.3; M.Pt: 127 ºC; R_f data 0.59; λmax 213 nm; IR (Nujol3600-2600 cm^{-1} -OH; 3250-2500 cm^{-1} –OH (Intra molecular with C=O, NO_2 etc.,) ; 2700 cm^{-1}-C-H; C-O, Aryl; 1010 cm^{-1}, C-O, primary alcohol; 500 cm^{-1}, for Sulphur; ^{1}HNMR (D_2O, δ ppm): 1639 cm^{-1}, C=C-C=O, ketones, 1600 cm^{-1} C-C, benzene nucleus; 1550-1510 cm^{-1} -NO_2 asymmetric stretching & aromatic nitro group; 1435-1405 cm^{-1}, CH_2-C=O, acyclic; 1320-1210 cm^{-1}, 2.65(s,3H,>CO-CH_3, J=2.9), 2.9 (d,2H, -CH_2, J=3.7), 3.90 (d, 1H, -CH in ring, J=4.1), 5.14 (m, 1H, -OH), 6.53 (m, 2H, ArH, , J=7.1), 7.85-7.95 (m, 4H, ArH, H-2", H-3", H-5", H-6"), 8.45 (s, 1H, H-5, , J=8.0), 8.6 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm23.28 (R-CH_2-R); 28.39 (-CH_3 in acetyl group); 34.58 (R_3-C-OH);37.96(-C(=O)-CH_3,an acetyl

carbon);46.71 [R-C(=O)-R]; 54.10 (=C-N-); 127.93 (=C-S- in ring); 124-127 (aryl =CH carbons); 141.08 (R_2-C=C-R_2); 145.31(=4'C-NO_2);147.16(conjugated diene carbon); 165.90 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); 176.77 (-C=O); MS (m/z): 420.28 M+; CHN analysis: Calc.(Found)- C-63.83(65.21); H-9.87(10.85) N-3.92(5.42); O-17.90(19.32); S-4.48(6.24).

Characterization of compound Ig: 3-(5''-(thiazolidine-2",4"-dionyl) methyl)3'4'- dinitro, 7-flavonol:

Irradiation time in microwaves 2'30 s; MF: $C_{21}H_{14}NO_7S$; MW: 408; % yield: 80.9; M.Pt: 99 ºC; R_f data 0.56; λmax 232 nm; IR (Nujol) 3600-2600 cm^{-1} –OH; 3250-2500 cm^{-1} OH (Intra molecular with C=O, NO_2 etc.,) ; C=C-C=O, ketones1600 cm^{-1} C-C, benzene nucleus; 1550-1510 cm^{-1} 2700 cm^{-1}-C-H; 1639 cm^{-1}, -NO_2 asymmetric stretching & aromatic nitro group; 1435-1405 cm^{-1}, CH2-C=O, acyclic; 1320-1210 cm^{-1}, C-O, Aryl; 1010 cm^{-1}, C-O, primary alcohol; 500 cm^{-1}, for Sulphur;^{1}HNMR (D_2O, δ ppm): 2.40 (d,2H, -CH_2, J=2.9), 3.10 (d, 1H, -CH in ring, , J=3.6), 5.0 (m, 1H, -OH), 6.31 (m, 2H, ArH, , J=6.8), 7.40-7.80 (m, 3H, ArH, H-2", H-5", H-6"), 8.5 (s, 1H, H-5, , J=7.5), 8.6 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R); 34.70 (R_3-C-OH); 46.64 [R-C (=O)-R]; 54.11 (=C-N-); 127.47 (=C-S- in ring); 128-129 (aryl =CH carbons); 141.11 (R_2-C=C-R_2); 143.4 (=3'C-NO_2);147.60 (=4'C-NO_2);165.98 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); 176.77 (-C=O); MS (m/z): 457.32 M+; CHN analysis: Calc.(Found)- C-60.22(62.35); H-9.41(11.41); N-5.85(6.53); O-20.06 (22.96); S-4.47(6.34).

Characterization of compound Ih: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)3'4'6- trinitro, 7-Flavonol:**

Irradiation time in microwaves 2'30 s; MF: $C_{21}H_{13}N_2O_9S$; MW: 421; % yield: 77.3; M.Pt: 116 ºC; R_f data 0.62; λmax 225 nm; IR (Nujol) 3600-2600 cm^{-1} OH; 3250-2500 cm^{-1} OH (intramolecular with C=O, NO2, etc.,) ; 2700 cm^{-1}-C-H; 1639 cm^{-1}, C=C-C=O, ketones 1600 cm^{-1} C-C, benzene nucleus; 1550-1510 cm^{-1} -NO_2 asymmetric stretching & aromatic nitro group;

1435-1405 cm^{-1}, CH2-C=O, acyclic; 1320-1210 cm^{-1}, C-O, Aryl; 1010 cm^{-1}, C-O, primary alcohol; 500 cm^{-1}, for Sulphur; ^{1}HNMR (D_2O, δ ppm): 2.37 (d,2H, -CH_2, , J=3.2), 3.21 (d, 1H, -CH in ring, J=3.8), 4.7 (m, 1H, -OH), 6.64 (m, 2H, ArH, J=7.1), 7.40-7.80 (m, 3H, ArH, H-2", H-5", H-6"), 8.2 (s, 1H, H-5, J=7.9), 8.6 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R); 25.79 (R_3-CH); 34.65 (R_3-C-OH); 46.72 [R-C (=O)-R]; 54.11 (=C-N-); 108.54 (=C in ring); 127.49 (=C-S- in ring); 124-128 (aryl =CH carbons); 130. 19 (=6C-NO_2); 141.12 (R_2-C=C-R_2); 143.6 (=3'C-NO_2);147.2 (=4'C-NO_2);165.85 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); 176.43 (-C=O); MS (m/z): 502 M+; CHN analysis: Calc.(Found)- (found): C-56.67 (59.41); H-8.72 (11.65);N-7.34 (9.855); O-23.07(25.96); S-4.20(5.52).

Characterization of compound Ii: **3-(5''-(thiazolidine-2",4"-dionyl) methyl)6-acetyl, 3'4'-dinitro, 7-flavonol:**

Duration of microwave irradiation 2'30 s; MF: $C_{21}H_{12}N_3O_{11}S$; MW: 498; % yield: 94.9; M.Pt:134 ºC; R_f data 0.69; λmax 236 nm; IR (Nujol) 3600-2600 cm^{-1} OH; 3250-2500 cm^{-1} OH (intramolecular with C=O, NO_2 etc.,) ; 2700 cm^{-1}-C-H; 1639 cm^{-1}, C=C-C=O, ketones, 1600 cm^{-1} C-C, benzene nucleus; 1550-1510 cm^{-1} -NO_2 asymmetric stretching & aromatic nitro group; 1435-1405 cm^{-1}, CH_2-C=O, acyclic; 1320-1210 cm^{-1}, C-O, Aryl; 1010 cm^{-1}, C-O, primary alcohol; 500 cm^{-1}, for sulfur; ^{1}HNMR (D_2O, δ ppm): 2.61 (d, 2H, -CH_2, J=2.7), 3.0 (d, 1H, -CH in ring, J=3.6), 5.44 (m, 1H, -OH), 6.0 (m, 2H, ArH, , J=6.7), 7.20-7.30 (m, 4H, ArH, H-2", H-5", H-6"), 8.7 (s,1H, >NH); ^{13}CNMR (D_2O, δ ppm): 23.3(R-CH_2-R); 28.39 (-CH_3 in acetyl group); 34.58 (R_3-C-OH);37.96(-C(=O)-CH_3,anacetyl carbon); 121.0&127.3 (aryl =CH carbons); 143.6 (=3'C-NO_2); 147.60 (=4'C-NO_2); 165.95 (HN-C(=O)-S- or R-C(=O)-NR_2 carbon); MS (m/z): 497.61 M+; CHN analysis: Calc.(Found)- C-60.05(62.14); H-9.15(11.10); N-5.53(6.23); O-21.05(23.42); S-4.22 (6.16).

The compounds (Ia-iNMR)'s data revealed the presence of singlet ArH protons at 6.50 ppm, doublet ArH protons at 6.8-6.9 ppm, singlet, 3H,>CO-CH3 at 2.25 ppm, and multiplet ArH

protons with 4-5 hydrogens at the peaks between δ 7.2-7.5. A distinctive singlet at δ 5.14 showed that a phenolic proton integrating for one proton was present at C7-OH. In the downfield area of the spectrum, or at around δ 7.40-7.80, it was discovered that the aromatic protons displayed multiplets.

The occurrence of five aromatic multiplets at δ 7.80–8.10 integrating for five protons, respectively, was explained by the 1HNMR data of compounds (Ia–i). Compounds (Ia-i) also displayed typical doublet integrating for two protons of the methylene(C3-CH2) bridge between the B-ring of 7-flavonol and thiazolidinedione ring, respectively, at around 2.58.1H NMR displayed a broad singlet peak in δ 10.28-12.31 ppm to confirm NH proton of thiazolidinedione in final compounds. This strong de-shielding action on the NH proton was caused by electron-withdrawing carbonyl groups. The compounds of (Ia-i) showed signals in their proton NMR spectra at δ 8.6–8.7 ppm for >NH proton and 8.10 ppm for arylidene =C–H. The spectra also revealed two multiplets at δ 6.65 and δ 6.62 integrating for one proton each that may be ascribed to C5-H and C8-H, respectively, and a doublet of one proton at 3.0-3.15 assignable to -CH in the ring. Due to their attachment to electron-withdrawing atoms, the final two protons' downfield absorptions are caused. Collectively, these findings showed that the named ingredients were all part of a successful and beneficial mixture.

In the ^{13}CNMR spectra of compounds (Ia-i), five characteristic signals appeared at δ 23.3(R-CH_2-R), δ 128-129 (aryl =CH carbons); δ 141.12 (R_2-C=C-R_2); δ 147.20 (conjugated diene carbon); δ 28.39 (-CH_3 in acetyl group) respectively. In ^{13}CNMR spectral studies, absorptions in the range, δ 28.41may be assigned to methyl carbon in the acetyl group at the C-4 position; δ 34.64 for a tertiary phenolic alcohol carbon at C-7; δ 40.94 to an oxo carbon of acetyl group at C-4 position; δ 54.14 may be assigned to (=C-N-) in TZD ring; δ 127.47 for (=C-S- in a ring) in TZD ring

attachment; δ 133.862, δ143.4, δ147.60 may be assigned for (=6C-NO_2) of ring A of the 7-flavonol nucleus;(=3'C-NO_2) and (=4'C-NO_2) of attached phenyl ring at C-2 position of 7-flavonols respectively; and δ 165.90 assigned to (HN-C(=O)-S- or R-C(=O)-NR_2 carbon) at C-2" of TZD ring. The molecular ion peaks M+ corresponded to the calculated molecular mass of all synthesized compounds. The results of the elemental analysis were found to be satisfactory.

Table 1. HPTLC Rf and Area details of the synthesized 2'', 4'' -thiazolidinedione derivatives of 7-flavonols (Ia-i)

Sl. No.	The compounds' names (Ia-i)	R_f values	Height of the peak (AU)	Area (AU)	Area percentage
01.	3-(5"-(thiazolidine-2",4"-dionyl) methyl) 7-flavonol **(Ia)**	0.59	354.8	8135.0	54.78
02.	3-(5''-(thiazolidine-2",4"-dionyl) methyl) 6-nitro,7-flavonol **(Ib)**	0.60	357.7	6621.9	43.73
03.	3-(5''-(thiazolidine-2",4"-dionyl) methyl) 6-acetyl,7-flavonol **(Ic)**	0.59	351.7	6178.0	30.39
04.	3-(5''-(thiazolidine-2",4"-dionyl) methyl) 4'-nitro,7-flavonol **(Id)**	0.62	351.4	8058.8	49.7
05.	3-(5''-(thiazolidine-2",4"-dionyl) methyl) 6,4'-dinitro,7-flavonol **(Ie)**	0.61	328.6	6238.8	11.52
06.	3-(5''-(thiazolidine-2",4"-dionyl) methyl) 6-acetyl,4'-nitro,7-flavonol **(If)**	0.50	338.9	6405.8	33.61
07.	3-(5''-(thiazolidine-2",4"-dionyl) methyl)3'4'-dinitro, 7-flavonol **(Ig)**	0.49	344.6	8523.4	46.67
08.	3-(5''-(thiazolidine-2",4"-dionyl) methyl)3'4'6-trinitro, 7-F**(Ih)**	0.52	362.4	6185.4	9.14
09.	3-(5''-(thiazolidine-2",4"-dionyl) methyl)6-acetyl, 3'4'- dinitro, 7-flavonol **(Ii)**	0.66	197.0	6019.7	38.14

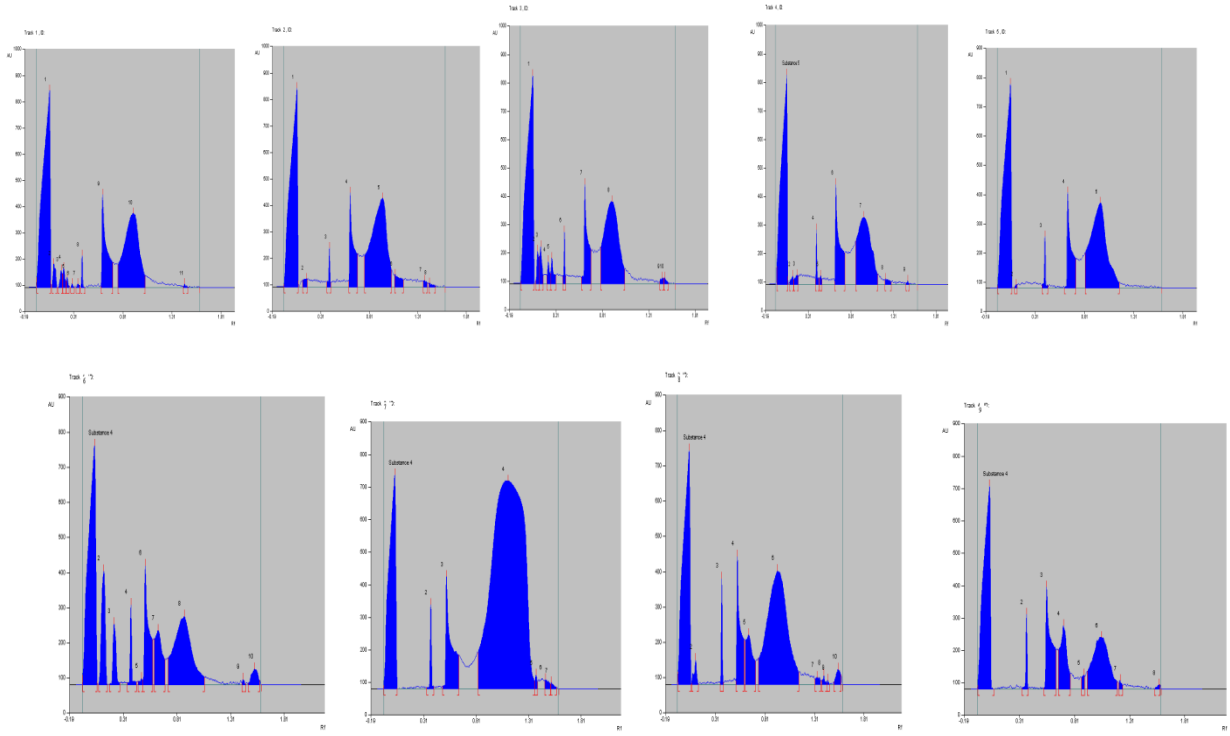


Fig.2 HPTLC chromatograms of 2'', 4'' -thiazolidindione derivatives of 7-flavonols (Ia-i)

Table 2. Fixation of dose based on Acute toxicity study (OECD guidelines)

Sl. No.	Dose levels (mg/kg)	Weight of the animal (in gms)	Signs of toxicity	Weight of the animal after the study (in g)	Onset of toxicity	Duration of study in days
01.	2000	210±04	No	212±10	No	14
02.	300	225±05	No	216±15	No	14
03.	50	220±10	No	221±10	No	14

Five animals were used in each dose level for the sighting research (5, 50, 300, and 2000 mg/kg), and all dose levels were up to 300 mg/kg/b.w. orally administered to animals were proven to be safe. Based on the 14th day of observation, 300 mg/kg was chosen as the dosage for the in vivo anti-inflammatory investigation.

Table 3. *In vitro* anti-inflammatory activity of the synthesized 2",4"-thiazolidinedione derivatives of 7-flavonols by protein denaturation method

Sl. No.	Samples	Dose (µg/ml)	Absorbance at 660 nm	% inhibition
01.	Solvent Control	-	2.181	nil
02.	Standard, Diclofenac	500	0.151	93
03.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl)7-flavanol **(Ia)**	500	1.194	44
04.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl) 6-nitro, 7-flavanol **(Ib)**	500	0.456	80
05.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl) 6-acetyl, 7-flavanol **(Ic)**	500	0.745	66
06.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl) 4'-nitro, 7-flavanol **(Id)**	500	0.245	89
07.	3-(5''-(thiazolidine-2'',4''-vinyl) methyl) 6,4'-dinitro, 7-flavanol **(Ie)**	500	0.229	90
08.	3-(5''-(thiazolidine-2'',4''-vinyl) methyl) 6-acetyl, 4'- nitro, 7-flavanol **(If)**	500	0.412	81
09.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl) 3',4'-dinitro, 7-flavanol **(Ig)**	500	0.802	63
10.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl) 6,3',4'-trinitro, 7-flavanol **(Ih)**	500	0.144	94
11.	3-(5''-(thiazolidine-2'',4''-dionyl) methyl) 6 – acetyl, 3',4'- dinitro, 7-flavanol **(Ii)**	500	0.200	91

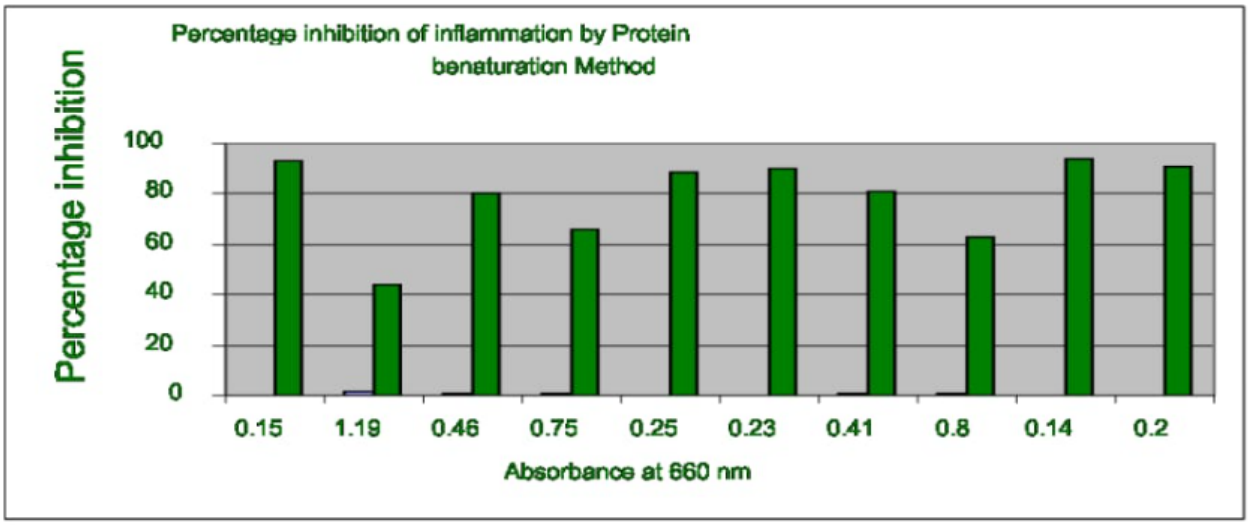


Fig. 3 Anti-inflammatory activity of 2", 4"-thiazolidinedione derivatives 7-flavonols by protein denaturation method

The same in vitro approach was taken into consideration for the investigation of 2', 4'-thiazolidinediones of 7-flavonols since NSAIDs have demonstrated dose-dependent anti-inflammation capacity against thermally induced protein denaturation. Egg albumin was chosen for the investigation because it exhibits sensitivity to oral anti-inflammatory drugs during the acute phase. When compared to the control, the conventional Indomethacin exhibited a 93 percent inhibition while the nitro derivatives of the test chemicals (500 g/ml) demonstrated a substantial percent inhibition (94 percent). Nitro derivatives showed the highest level of inhibition at 94%.

Table 4. Rats' paw edema caused by carrageenan was reduced by a percentage when 2", 4" thiazolidinedione derivatives of 7- flavanols were taken.

Treatment (mg/Kg oral route)	**OEDEMA (ml) [Time after carrageenan 30 µg/paw (h)]**							
	0.5	**1**	**2**	**3**	**4**	**6**	**12**	**24**
Control	1.4±0.07	1.5±0.06	1.8±0.06	1.9±0.14	1.9±0.14	1.8±0.03	1.7±0.04	1.6±0.13
Standard, Indomethacin (20)	0.41±0.02 (71.4 %)	0.4±0.02* (73.3 %)	0.47±0.02** (74.0 %)	0.42±0.02** (78.0 %)	0.38±0.03*** (80.0 %)	0.15±0.02*** (91.7 %)	0.19±0.02*** (88.8 %)	0.19±0.01*** (88.1 %)
Sample **IIId** (30)	1.04±0.04 (25.7 %)	1.03±0.02 (31.3 %)	1.21±0.06* (32.8 %)	1.06±0.03* (44.2 %)	0.78±0.03** (59.0 %)	0.65±0.02** (64.0 %)	0.65±0.02** (61.8 %)	0.69±0.01** (57.0 %)
Sample **IIIe** (30)	0.55±0.08 (60.7 %)	0.54±0.05* (64.0 %)	0.61±0.06** (66.1 %)	0.61±0.06** (68.0 %)	0.51±0.06*** (73.2 %)	0.34±0.10*** (81.1 %)	0.41±0.08*** (76.0 %)	0.45±0.16*** (72.0 %)
Sample **IIIf** (30)	0.67±0.02 (52.1 %)	0.64±0.05* (57.3 %)	0.7±0.05** (61.1 %)	0.7±0.02** (63.2 %)	0.6±0.02*** (68.4 %)	0.34±0.04*** (81.1 %)	0.39±0.06*** (77.1 %)	0.54±0.02*** (66.3 %)
Sample **IIIg** (30)	0.76±0.02	0.75±0.02	0.9±0.02*	0.82±0.04**	0.78±0.04**	0.45±0.05***	0.51±0.03***	0.62±0.03***

	(45.7 %)	(50.0 %)	(50.0 %)	(57.0 %)	(59.0 %)	(75.0 %)	(70.0 %)	(61.3.0 %)
Sample **IIIh** (30)	0.7±0.02 (50.0 %)	0.6±0.02 * (60.0 %)	0.65±0.03** (64.0 %)	0.44±0.01** (77.0 %)	0.42±0.01*** (78.0 %)	0.23±0.05*** (87.2 %)	0.37±0.03*** (78.2 %)	0.38±0.03*** (76.3 %)
Sample **IIIi** (30)	0.7±0.02 (50.0 %)	0.64±0.05 (57.3 %)	0.72±0.07* (60.0 %)	0.74±0.03* (61.1 %)	0.61±0.01** (68.0 %)	0.5±0.02** (72.2 %)	0.51±0.02** (70.0 %)	0.60±0.02** (62.5 %)

Samples **Id**: 3-(5’’-(thiazolidine-2’’,4’’-dionyl) methyl) 4’-nitro, 7-flavanol; **Ie**: 3-(5’’-(thiazolidine-2’’,4’’-dionyl) methyl) 6,4’-dinitro, 7-flavanol; **If**: 3-(5’’-(thiazolidine-2’’,4’’-dionyl) methyl) 6-acetyl, 4’- nitro, 7-flavanol; **Ig**: 3-(5’’-(thiazolidine-2’’,4’’-dionyl) methyl) 3’,4’- dinitro, 7-flavanol; **Ih**: 3-(5’’-(thiazolidine-2’’,4’’-dionyl) methyl) 6,3’,4’- trinitro, 7-flavanol; **Ii**: 3-(5’’-(thiazolidine-2’’,4’’-dionyl) methyl) 6 –acetyl, 3’,4’- dinitro, 7-flavanol. Values are expressed as mean± SD; Values are from triplicate readings; and are statistically significant at $p<0.05$*, $p<0.01$**, $p<0.001$***, when compared to the standard, Indomethacin

The carrageenan-induced anti-inflammatory investigation of the aforementioned compounds' results showed that all synthetic TZDs of 7 flavonols had effects (*P 0.05, **P 0.01, and ***P 0.001) in contrast to the reference drug and the control. The reduction in paw volume caused by the dinitro compounds was represented as (**P0.05) and (***P0.001) as that of ordinary Indomethacin.

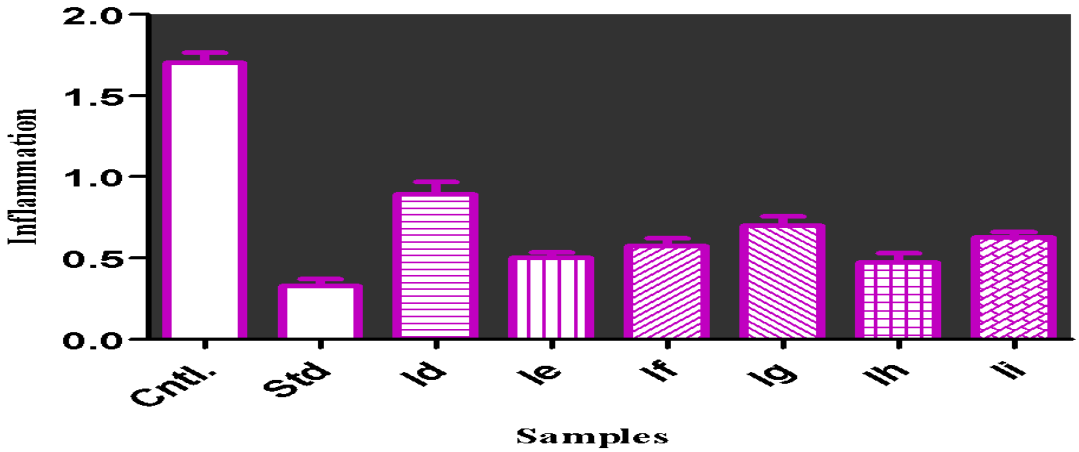


Fig. 4 Anti-inflammatory activity of 2”, 4”-thiazolidinedione derivatives 7-flavonols in carrageenan-induced paw edema in rat method

The percentages of compounds of 2" 4"-thiazolidinedione derivatives of 7-flavonols that inhibited the protein denaturation of egg albumin were computed using the Newbuild formula

and are displayed in Fig. 4 as Ib (80%), Id (89%), Ie (90%), Ih (94%) and Ii (91%) as well as standard, Indomethacin (93 percent).

When compared to the control, the test chemicals' nitro derivatives (500 g/ml) demonstrated substantial action (91 percent) and the typical Indomethacin (93 percent) inhibition. Only the nitro derivatives (b, d, e, g, and hand I of 7-flavonol-based compounds showed the maximum inhibition of 91 percent, as opposed to the other acetylated and unsubstituted derivatives (a, c, and f). The activity's order is determined by the SAR research of substituent groups that remove electrons: $-NO_2$> $-COCH_3$>un substitution.

The greatest percentage of suppression of inflammation after six hours by compounds of 2, 4, and 6-thiazolidinedione derivatives of flavonols were detected as Id (64%) Ie (81%) If (81%) Ig (75%) Ih (87%) Ii (72%) compared to standard (92 percent). In comparison to the control, a statistically significant value of $P < 0.05$ was seen (Table 5 and Figure 5). All these values started dropping to 50% of the previously reported activity levels during the 12th hour or later, showing that the animals were progressively ridding themselves of any accumulated doses of test material and returning to a normal state.

The following changes occur when egg protein is heated, such as the activation of phagocytes, the production of -OH radicals and H2O2 species, the dissociation of DNA's double-strand structure, and finally denaturation of albumin.

The anti-inflammatory effects of the thiazolidinedione derivatives of 7-flavonols (Ia-i) were significantly equivalent to those of the gold standard, indomethacin. This is because these TZDs for 7 flavonols should have had an action mechanism. There was a gradual decrease in the inflammation of the paw volume at 1-2 hours due to the inhibition of first-phase bradykinins, histamine, or serotonin release. Alternatively, lysosomal enzymes (COX-1, COX-2) may be

inhibited or the lysosomal membranes stabilized to stop the conversion of arachidonic acid to prostaglandin, which causes inflammation (as there existed an increase in the percentage inhibition of inflammation at 2-4 hrs.). Since the 1960s, thiazolidinediones (glitazones) have been used successfully to treat adult-onset diabetes mellitus. I) Insulin resistance and hyperinsulinemia are reduced by thiazolidinediones. ii) Plasma triglycerides, blood glucose, and glycosylated hemoglobin; iii) they do not by themselves result in hypoglycemia; and iv) they enhance metabolic state by raising plasma levels of HDL and LDL.

Experimental Section

Materials and methods

Materials

A modern LG domestic microwave oven (Model No. MS-2342AE), Schimadzu UV spectrophotometer (Model No. UV- 2400 PC), Schimadzu 8201 PC-FTIR spectrophotometer, and a Bruker 400 Spectrophotometer in $CDCl_3$ at 400 and 75 MHz and a CHN-Rapid analyzer were the instruments, used in the synthetic processes as well as for the synthesized compounds' spectral characterization. Analytical grade chemicals and solvents were in use. The purity of the substances upon reaction and their completeness was checked out by melting point determination and CO-TLC methods.

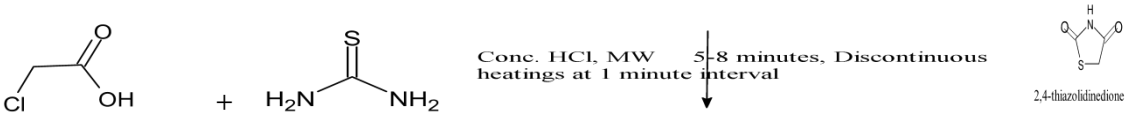
Methods

Procedure for synthesizing 2, 4- thiazolidinediones

Prepare a solution of chloroacetic acid (0.6 mol, 56.70 g) dissolved in distilled water (60 ml) and a solution of thiourea (0.6 mol, 45.67 g) solvated in distilled water (60 ml). Placed these two solutions in a beaker (250 ml capacity), stirred, and cooled well for 15 minutes to get a white

precipitate; then added concentrated hydrochloric acid (60 ml) dropwise to it. The ice cubes on the lid of the beaker is to absorb the excess microwave irradiation and a glass tube inside, is to avoid the bumping of the contents. A microwave oven, with a provision of a magnetic stirrer, thermometer, water condenser, and a heating sink, is made used for automatic microwave-enhanced reactions.

The mixture was then microwave irradiated in a discontinuous manner for 4 minutes (at intervals of 30 seconds each). The reaction was examined by TLC, and the resultants were then cooled to get a cluster of white needles. It was re-crystallized from ethanol. Scheme to synthesize 2, 4- thiazolidinediones represented in figure 5.

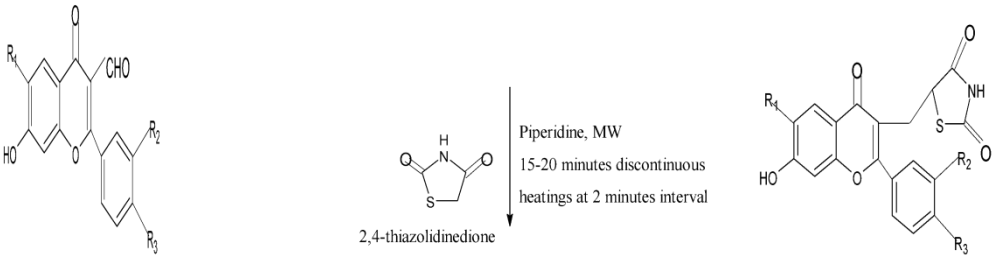


Chloroacetic acid Thiourea 2", 4"- thiazolidinedione

Fig.5 Scheme to synthesize 2, 4- thiazolidinediones

Procedure for synthesizing 2", 4"- thiazolidinedione derivatives of 7-flavonols

A mixture of 2, 4- thiazolidinedione (0.01 mol) and aromatic aldehydes (0.01 mol) were taken in a beaker (250 ml) containing ethanol (10 ml). The ice cubes on the lid of the beaker are to absorb the excess microwave irradiation and a glass tube inside, is to avoid bumping. Scheme to synthesize 2'', 4''- thiazolidindiones of 7-flavanols represented in figure 6.



3-formyl, 7-flavonols 2, 4- thiazolidindione 2, 4- thiazolidindione drivatives of 7-flavonols

Fig.6 Scheme to synthesize 2'', 4''- thiazolidindiones of 7-flavanols

Table 5. The list of substituents in various derivatives of 2'', 4''- thiazolidindione of 7-flavonols

S. No.	Name of the compounds (Ia-i)	R^1	R^2	R^3	R^4	R^5	R^6
1.	2'', 4''- thiazolidindione (TZD) moiety attached 7-flavonols **(Ia)**	H	H	H	H	H	H
2.	5-nitro, TZD derivative of 7-flavonol **(Ib)**	$-NO_2$	H	H	$-NO_2$	H	H
3.	6-acetyl, TZD derivative of 7-flavonol **(Ic)**	$-COCH_3$	H	H	$-COCH_3$	H	H
4.	4'-nitro, TZD derivative of 7-flavonol **(Id)**	H	H	$-NO_2$	H	H	$-NO_2$
5.	4',5-dintro, TZD derivative of 7-flavonol **(Ie)**	$-NO_2$	H	$-NO_2$	$-NO_2$	H	$-NO_2$
6.	4'-nitro, 6-acetyl, TZD derivative of 7-flavonol **(If)**	$-COCH_3$	H	$-NO_2$	$-COCH_3$	H	$-NO_2$
7.	3'4'-dintro, TZD derivative of 7-flavonol **(Ig)**	H	$-NO_2$	$-NO_2$	H	$-NO_2$	$-NO_2$
8.	3'4'5,-trinitro TZD derivative of 7-flavonol **(Ih)**	$-NO_2$	NO_2	$-NO_2$	$-NO_2$	$-NO_2$	$-NO_2$
9.	3'4'-dinitro, 6-acetyl, TZD derivative of 7-flavonol **(Ii)**	$-COCH_3$	NO_2	$-NO_2$	$-COCH_3$	$-NO_2$	$-NO_2$

A microwave oven, with a provision of a magnetic stirrer, thermometer, water condenser, and a heating sink, is made use for automatic microwave enhanced reactions. The mixture was then microwave irradiated in a discontinuous manner for 5 minutes (at an interval of 30 seconds each) and added pyridine (1 ml) was. After 5 hrs, the separated particles were filtered and subjected to washing with ethanol: water (1:1), and a cold mixture. After re-crystallizing the resultants from ethanol (95 %), tabulated the data of their % yield, the crude products (Ia-i) were re-crystallized from ethanol (95 %). The yield and physical data of the synthesized products were given in Table 2.

HPTLC Finger Print profiles, Development technique (Kulkarni et al. 2006**)**

Make	: Camag (Switzerland)
Sample Applicator	: Linomat IV
Scanner	: Camag TLC Scanner II
Development Chamber	: 10X10, Camag Twin-trough chamber
Source of radiations for detection	: Deuterium and tungsten lamp
Detection nm	: 254 and 366 nm
Documentation Installation	: Digi store-2, Video Scan Software 3.15 version
Syringe	: Hamilton (USA)
Stationary phase	: Pre coated Silica gel 60 F_{254}
Aluminum foil	: Merck, Germany
Syringe size	: 100 µl syringe
Sample spotting rate	: 10 s μl^{-1}
Applied quantity	: 2 µl
Mode of development	: Ascending
Mobile phase preparation	: Benzene: Ethyl acetate: Formic acid 40:10:5 (v/v/v)
Spraying reagent	: Sodium borohydride in alcohol (1 %) followed with ethanolic aluminium chloride
Preparation of Solution	: Sample in 10 ml of methanol (1 µgµL^{-1}).

Anti-inflammatory activity

Animal Selection and Acclimatization

Our in vivo tests have received permission from the institutional animal ethics committee under reference number KU/IAEC/Ph.D./065. Male and female Wistar Albino rats weighing between 150 and 200 grams were bought from the Amrita Institute in Kerala and kept in our college's animal house for seven days while being fed a high-quality rodent pellet diet, given access to water whenever they wanted, kept at a temperature between 21 and 25 degrees Celsius, and allowed to fast for at least 12 hours at night. The medicine was given out via syringes and feeding tubes. OECD 423 recommendations for acute toxicity studies

The test specimens were administered orally in CMC suspension. Following administration, individual animals were monitored frequently during the first 30 minutes,

carefully for the following 4 hours, intermittently for the first 24 hours, and then daily for the following 14 days.

***In vitro,* anti-inflammatory activity by Protein Denaturation Method**

In separate sample tubes, add egg albumin to 1 ml of the solvent control (distilled water), standard (Indomethacin, 500 µg/ml), and test samples (500 µg/ml) (1 ml, 1 mM). To denaturize albumin, the reaction mixture was heated for 10 minutes in a water bath at 70 °C. Cooled the supernatant and used a spectrophotometer to determine the turbidity at 660 nm.

% Inhibition of protein denaturation = [$A_{(Control)} - A_{(Sample)}/A_{(Control)}$] X 100, where A- Absorbance

***In vivo* anti-inflammatory by Carrageenan induced Paw Oedema in Rats**

Dose

Carrageenan: 0.1 ml of a 1 percent w/v solution injected beneath the plantar area;

(20 mg/kg b. w.) of the standard (Indomethacin) and test solution A 10 ml stock solution containing 4 mg/ml was made and administered orally (0.5 ml/100 g/b.w. of the animal);

Solvent management Carboxy methyl cellulose at 0.3 w/v.

Experimental Procedure (Kulkarni et al. 1999)

The Wistar rats were weighed and counted, and then a color indication was made on both of their hind feet just beyond the tibia-tarsal junction. The experiment was conducted with six rats in each of the eight groups using the mercury displacement method, and the initial paw volume of each rat's left and right hind paw was recorded. Group I received the vehicle of 1 ml, and Group II received the regular dose of indomethacin in the amount of 1 ml. In contrast, Group III to Group VIII received the test samples in the amount of 1 ml administered orally. Upon the oral administration of the standard and test samples for 30 minutes, 0.1 ml of carrageenan was

subcutaneously injected into each of the eight groups' left paws, while the right paw served as a reference. One, two, three, four, six, twelve, and twenty-four hours after the carrageenan challenge, the paw volume of both hind legs of all groups was measured plethysmometrically. The results were tabulated in Table-5 and Figure -5**.**

Statistical analysis

Dunnett's multiple comparison tests and Analysis of Variance (ANOVA) were used in its application.

Conclusion

It was found that our thiazolidinedione derivative compounds could very well contribute to inhibiting the initiation and extension of the inflammatory process by IL-6, a central mediator for inflammation and subsequently for diabetes because our anti-inflammatory study also extended for 24 hours to ensure that the animals attain a normal state. In the context of this research, it was decided to broaden the current study to include anti-diabetic properties.

Acknowledgement

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Conflicts of Interest: Nil

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