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Synthesis, Stereochemistry and Antimicrobial Activity of Some Novel Flavanone-hydrazono-thiazolidin-4-ones from Flavanones

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The synthesis of four novel compounds, (i) *E*-2-{[2-(4-methoxyphenyl)chroman-*E*-4-ylidene]hydrazono}thiazolidin-4-one **(4a)**, (ii) *E*-2-{[2-(4-methoxyphenyl)chroman-*Z*-4-ylidene]hydrazono}thiazolidin-4-one **(5a)**, (iii) *E*-2-{[2-(phenyl)chroman-*E*-4-ylidene]hydrazono}-thiazolidin-4-one **(4b)** and (iv) *E*-2-{[2-(phenyl)chroman-*Z*-4-ylidene]hydrazono}thiazolidin-4-one **(5b)** from 4'-methoxy flavanone **(1a)** in (i) and (ii) and from flavanone **(1b)** in (iii) and (iv) *via* the reaction of respective thiosemicarbazones **(2a)** and **(2b)**, with chloroacetic acid and sodium acetate in acetic acid is described. Structural assignment, stereochemistry and biological assays are discussed.

Keywords: Flavanone, Thiosemicarbazone, Thiazolidin-4-ones, Antimicrobial activity.

INTRODUCTION

There has been considerable interest in the chemistry of thiazolidin-4-one ring systems, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activities [1-4]. (–)2-(5-carboxypentyl)thiazolidin-4-one (actithiazic acid) isolated from the culture broth of a strain of streptomycin shows highly specific *in vitro* activity against *Mycobacterium tuberculosis* [5,6]. Other substituted thiazolidin-4-ones exhibit diverse biological activities such as antimicrobial [7-11], antidiabetic [12-14], anticancer [15], antiviral/anti-HIV [16-18], anti-inflammatory and analgesic [19-21], antihistaminic [22-24], oxygenase inhibitory [25], calcium antagonist [26], K^+ channel inhibitory [27] and a promising agent for treating Alzheimer disease [28]. Thiazolidin-4-ones substituted at 2-position were proven to be biological potent and selective [29,30]. Also, flavonoids are naturally occurring antioxidant and have been implicated as novel antiviral and antitumor compounds [31]. In light of these findings and as part of our aim in search of biologically active compounds with sulphur and nitrogen containing heterocycles [32,33], we report here the synthesis of some novel flavanoidal hydrazono derivatives bearing thiazolidinones moiety in order to assess their pharmacological profile.

Although a large number of thiosemicarbazones [34] of a variety of aldehydes and ketones have been prepared and their reactions with α-chloroacetic acid or its functional derivatives in the presence of sodium acetate have been investigated [35,36], no report for the isomeric products (*E* and *Z*) of thiosemicarbazone and the corresponding hydrazono-thiazolidin-4-ones has so far been appeared in the literature. The present paper deals with the synthesis, stereochemistry and biological screening of four novel compounds, (i) *E*-2-{[2-(4-methoxyphenyl)chroman-*E*-4-ylidene]hydrazono}thiazolidin-4-one **(4a)**, (ii) *E*-2-{[2-(4-methoxyphenyl)chroman-*Z*-4-ylidene]-hydrazono}thiazolidin-4-one **(5a)**, (iii) *E*-2-{[2-(phenyl)-chroman-*E*-4-ylidene]hydrazono}thiazolidin-4-one **(4b)** and (iv) *E*-2-{[2-(phenyl)chroman-*Z*-4-ylidene]hydrazono}-thiazolidin-4-one **(5b)** from 4'-methoxy flavanone **(1a)** *via* *E*-(4'-methoxyflavanone thiosemicarbazone) **(2a)** in (i) and (ii) and from flavanone **(1b)** *via* *E*-(flavanone thiosemicarbazone) **(2b)** in (iii) and (iv), respectively, with chloroacetic acid and sodium acetate in acetic acid. Screening results of the compounds **2a**, **2b**, **4a** and **4b** are summarized for antimicrobial activity against four bacteria, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and one fungus *Candida albicans*.

EXPERIMENTAL

Reagents and solvents were of commercial grade and were used without further purification. Column chromatography was performed on silica gel (60-120 mesh LR, 25049). Melting points were determined on a Koffler hot-plate apparatus and are uncorrected. The FTIR spectra of the compounds were recorded on a Perkin-Elmer RX1 spectrophotometer using KBr disc techniques and the wave numbers (ν_{max}) is calculated in cm^{-1}. The ^1H NMR and ^{13}C NMR (δ ppm) spectra were recorded on a Varian Unity 400 spectrometer in $CDCl_3$ and DMSO-d_6

using tetramethylsilane as the internal standard. EI-MS was recorded on V.G. Micromass Model ZAB-IF apparatus at 70 eV ionization voltage. HRMS values were recorded in a quadrupole-time of flight mass spectrometer (Q to f2, Micromass, Manchester, U.K). Follow up of the reactions were made by TLC on silica gel 'G' (Merck) and the spots were visualized in exposure to iodine vapour and UV-lamp.

4'-Methoxyflavanone (1a): 4'-Methoxyflavanone **(1a)** was prepared by known method [37] with slightly modified procedure by condensing 2-hydroxy acetophenone with benzaldehyde (molar ratio, 1:1.25) in the presence of KOH.

To a mixture of 2-hydroxy acetophenone (5 g, 36.7 mmol) and anisaldehyde (6.24 g, 45.9 mmol) in alcohol (50 mL) was gradually added a warm solution of KOH (35 mL, 20 %) with stirring. After complete addition it was stirred 0.5 h more and kept tightly stoppered at room temperature for 7 days with occasional shaking. The colour of the mixture changed from yellow to red. The progress of the reaction was regularly monitored by TLC. The reaction mixture was then extracted with ethyl acetate, washed several times with water until the filtrate was neutral and dried over sodium sulphate. The solvent was distilled off under reduced pressure to give a gummy mass, which was chromatographed using ethyl acetate-petroleum ether (3:7 v/v) as eluent. Elution of the column yielded a yellow solid, which on crystallization from ethyl acetate afforded **(1a)** as light greenish yellow crystals in 44 % yield, m.p. = 87-88 °C [38], R_f = 0.76 (petroleum ether-benzene, 8.2 v/v). Anal. calcd. for $C_{16}H_{14}O_3$: C, 75.57; H, 5.55; O, 18.88 %; Found: C, 75.54; H, 5.54 %.

Flavanone (1b): The reaction described as above was repeated with benzaldehyde. The reaction mixture on usual work up and crystallization as above furnished white crystals of flavanone **(1b)**, (47 %), m.p. = 75-76 °C [39], R_f = 0.79 (petroleum ether-benzene, 8:2 v/v); Anal. calcd. for $C_{15}H_{12}O_2$: C, 80.34; H, 5.39; O, 14.27 % Found:. C, 80.37; H, 5.38 %

4'-Methoxyflavanone-*E*-4-thiosemicarbazone (2a) and 4'-methoxyflavanone-*Z*-4-thiosemicarbazone (3a): To a solution of thiosemicarbazide (2.24 g, 24.6 mmol) in freshly distilled acetic acid (20 mL) was added slowly 4'-methoxyflavanone **(1a)**, (5 g, 19 mmol) dissolved in absolute alcohol (50 mL). After complete addition, the reaction mixture was refluxed for 8 h with stirring on an oil bath at 80 °C. After completion of the reaction, the reaction mixture was then cooled, extracted with ethyl acetate, washed with water and kept over sodium sulfate. The reaction mixture was then concentrated under reduced pressure to give a yellowish gummy mass which purified by column chromatography over a silica gel column (benzene-ethyl acetate, 8.5:1.5 v/v). Elution of the column yielded a white solid which was on crystallization from benzene-chloroform furnished **2a** as white crystalline needles, 3.47 mg (54.0 %), m.p.= 218 °C, R_f = 0.58 (benzene-ethyl acetate, 8.5:1.5 v/v). IR (KBr, ν_{max}, cm^{-1}): 3477 (NH), 3355, 3190 (NH_2), 2827 (C-H), 1618 (C=N and phenyl), 1572, 1511 (phenyl), 1472 (C-H bend), 1156 (C=S), 1121 (C-O-C), 1434, 1275, 912, 876, 835, 758. The δ_H and δ_C values are given in Tables 1 and 2; *m/z* (%): 327 (M^+, 33.5), 328 [(M^+ + 1), 6.7], [(M^+ + 2), 2.01]; Anal. calcd. for $C_{17}H_{20}O_2N_3S$: C, 61.79; H, 6.10, N, 12.72; O, 9.68, S, 9.70 % Found: C, 61.77; H, 6.08 %, N, 12.70 %.

Further elution of the column yielded a dark brown-coloured solid, which on crystallization from chloroform-benzene gave **3a** as brown cubic crystals, 1.03 g (16 %), m.p. = 226 °C, R_f = 0.49 (benzene-ethyl acetate, 8:1.5 v/v); IR (KBr, ν_{max}, cm^{-1}): 3390 (NH), 3257, 3165 (NH_2), 2832 (C-H), 1613 (C=N and phenyl), 1577, 1511 (phenyl), 1470 (C-H bend), 1168 (C=S), 1115 (C-O-C), 1089 (C-N), 840, 748. The δ_H and δ_C values are given in Tables 1 and 2; *m/z* (%): 327 [(M^+) 87.1], 328 [(M^+ + 1), 18.76], 329[(M^+ + 2), 5.36]. Anal. calcd. for $C_{17}H_{20}O_2N_3S$: C, 61.79; H, 6.10, N, 12.72; O, 9.68, S, 9.70 % Found: C, 61.77; H, 6.08, N, 12.70 %.

Flavanone-*E*-4-thiosemicarbazone (2b) and flavanone-*Z*-4-thiosemicarbazone (3b): The reaction described above was repeated using flavanone (5 g, 2.3 mmol). The reaction mixture was worked up as usual and purified by column chromatography (benzene-ethyl acetate, 8:2 v/v) and crystallized from chloroform-benzene to give compound **2b** as white crystalline needles, 4.1 g (62 %), m.p. = 202-204 °C, R_f = 0.61 (benzene-ethyl acetate, 8.5:1.5, v/v). IR (KBr, ν_{max}, cm^{-1}): 3426 (NH), 3226, 3144 (NH_2), 2840 (C-H), 1599 (C=N and Phenyl), 1564, 1511 (Phenyl), 1473 (C-H bend), 1116 (C-O-C), 1153 (C=S), 1077 (C-N), 911, 886, 700; δ_H ($CDCl_3$); 5.17 (dd, 1H, H-2_{ax}, *J* = 12.36 Hz, *J* = 3.02 Hz), 2.83 (dd, 1H, H-3_{ax}, *J* = 16.48 Hz, *J* = 12.36 Hz), 3.1 (dd, 1H, H-3_{eq}, *J* = 16.48 Hz, *J* = 3.02 Hz), 8.72 (s, 1H, NH,), 6.41 (s, 2H, NH_2,), 7.92 (dd, 1H, Ar-H-5, *J* = 7.96 Hz, *J* = 1.65 Hz), 7.2 (ddd, 1H, Ar-H-6, *J* = 7.96 Hz, *J* = 7.14 Hz *J* = 0.94 Hz), 7.35 (ddd, 1H, Ar-H-7, *J* = 7.69 Hz, *J* = 7.14 Hz, *J* = 1.65 Hz), 7.05 (dd, 1H, Ar-H-8, *J* = 7.69 Hz, *J* = 0.94 Hz), 7.38-7.52 (br m, 5H, H-Ar′); δ_C ($CDCl_3$): 76.883 (C-2), 32.358 (C-3), 142.823 (C-4), 119.107 (C-4a), 124.359 (Ar-C-5), 121.984 (Ar-C-6), 131.2670 (Ar-C-7), 118.225 (Ar-C-8), 157.726 (C-8a), 138.861 (Ar′-C-1), 126.020 (Ar′- C-2, 6), 128.855 (Ar′-C-3, 5), 128.919 (C-Ar′-4), 179.111 (NH-(CS)-NH_2; *m/z* (%): 297 ($M^{+\bullet}$,61.64), 298[(M^+ + 1), 16.75], 299 [(M^+ + 2), 4.69]. Anal. calcd. for $C_{16}H_{15}ON_3S$: C, 64.62; H, 5.08, N, 14.13; O, 5.38, S, 10.78 % Found: C, 64.60; H, 5.07, N, 14.11 %.

Further elution of the column yielded a brown coloured solid which on crystallization from chloroform-benzene afforded **3b** as brown crystalline cubes, 1.31 g (21.0 %), m.p. = 212 °C, R_f = 0.48 (benzene-ethyl acetate, 8.5:1.5 v/v); IR (KBr, ν_{max}, cm^{-1}): 3450 (NH), 3300, 3150 (NH_2), 2850 (C–H), 1600 (C=N), 1580, 1560 (phenyl), 1165 (C=S), 1120 (C–O–C), 1070 (C–N), 1020, 990, 910, 880, 830 and 755; δ_H ($CDCl_3$); 5.96 (dd, 1H, H-2, *J* = 11.65, *J* =3.3); 4.02 (dd, 1H, H-3_{ax}, *J* =17. 85, *J* =11.65); 3.21 (dd, 1H, H-3_{eq}, *J* =17.85, *J* =3.3); 9.48 (s, 1H, NH,); 7.78 (s, 2H, NH_2); 7.36 (dd, 1H, Ar-H-5, *J* = 7.91, *J* = 1.7); 6.82 (ddd, 1H, Ar-H-6, *J* = 7.91, *J* = 7.17, *J* = 0.94) ;7.54 (ddd, 1H Ar-H-7, *J* = 7.17, *J* = 8.55, *J* = 1.7); 6.86 (dd, 1H, Ar-H-8, *J* = 8.55, *J* = 0.94); 7.08-7.33 (br m, 5H, H-Ar′); *m/z* (%): 297 (15.02). Anal. calcd. for $C_{16}H_{15}ON_3S$: C, 64.62; H, 5.08, N, 14.13; O, 5.38, S, 10.78 % Found: C, 64.61; H, 5.07, N, 14.11 %.

***E*-2-{[2-(4-Methoxyphenyl)chroman-*E*-4-ylidene]-hydrazono}thiazolidin-4-one (4a) and *E*-2-{[2-(4-methoxyphenyl)chroman-*Z*-4-ylidene]hydrazono}thiazolidin-4-one (5a):** A mixture of 4'-methoxyflavanone-*E*-4-thiosemicarbazone **(2a)**, (500 mg, 1.529 mmol), chloroacetic acid (180 mg, 1.911 mmol) and sodium acetate (188 mg, 2.293 mmol)

dissolved in acetic acid (20 mL) was refluxed on an oil bath at 110 °C for 10 h. The reaction mixture was then cooled, extracted with ethyl acetate, washed with water and kept over sodium sulfate. The solvent was distilled off under reduced pressure to give a brown-coloured gummy mass, which was chromatograhed over a silica gel column (benzene-ethyl acetate, 8.5:1.5 v/v). Elution of the column yielded a white solid which on crystallization from chloroform-benzene furnished **(4a)** as white crystalline solid, 310.0 mg (43.0 %), m.p. = 217 °C, R_f = 0.52 (benzene:ethyl acetate, 8.5:1.5 v/v). IR (KBr, ν_{max}, cm^{-1}): 3344 (NH), 2795 (C-H), 1710 (C=O), 1624 (C=N and phenyl), 1485, (S-CH_2), 1423 (C-N), 1610, 1574 (phenyl), 1126 (C-O-C), 1073, 1022, 901, 826, 755, 690. The δ_H and δ_C values in $CDCl_3$ are given in Tables 3 and 4; δ_H (DMSO-d_6): 5.19 (dd, 1H, H-2_{ax}, *J* = 11.72 Hz, *J* = 2.93), 2.88 (dd, 1H, H-3_{ax}, *J* = 16.85 Hz, *J* = 11.72 Hz), 3.42 (dd, 1H, H-3_{eq}, *J* = 16.85 Hz, *J* = 2.93 Hz), 3.76 (s, 2H, SC$\underline{H}_2$), 3.86 (s, 3H, OC$\underline{H}_3$),9.86 (s, 1H, N$\underline{H}$), 7.96 (dd, 1H, Ar-H-5, *J* = 1.71 Hz, *J* = 7.82 Hz), 7.32 (dd, 1H, Ar-H-7, *J* = 8.07 Hz, *J* = 8.55 Hz), 7.02 (brdd, 1H, Ar-H-6, *J* = 7.82 Hz, *J* =8.07 Hz), 6.98 (d, 1H, Ar-H-8, *J* = 8.55 Hz), 7.40 (d, 2H, Ar′-H-2,6, *J* = 8.79 Hz), 6.94 (d, 2H, Ar′-H-3,5, *J* = 8.79 Hz); δ_C (DMSO-d_6):32.657 (C-3), 32.839 (S-$\underline{C}H_2$), 55.12 (O$\underline{C}H_3$), 76.556 (C-2), 159.183 (Ar′-C-4), 113.827 (Ar′-C-3,5), 127.899 (Ar′-C-2,6), 131.781 (Ar′-C-1), 124.439 (Ar-C-5), 121.264 (Ar-C-6), 131.949 (Ar-C-7), 117.709 (Ar-C-8), 119.923 (C-4a), 157.194 (C-8a), 154.740 (C-4), 164.106 ($\underline{C}$=N),173.947 ($\underline{C}$O); *m/z* (%): 367 (M^+, 100), 336 [(M^+–1), 20.80], 368 [(M^++1), 22.11], 369 [(M^++2), 4.02]. Anal. calcd. for $C_{19}H_{20}O_3N_3S$: C, 61.60; H, 5.44, N, 11.34; O, 12.96, S, 8.66 % Found: C, 61.58; H, 5.43, N, 11.33 %.

Further elution of the column yielded a brown solid which on crystallization from chloroform-benzene afforded brown cubic shaped crystals of compound **5a**, 200 mg (28.0 %), m.p. = 222 °C, R_f = 0.12 (benzene-ethyl acetate 8.5:1.5 v/v); IR (KBr, ν_{max}, cm^{-1}): 3346 (NH), 2840, (C-H), 1700 (C=O), 1619 (C=N and phenyl), 1594, 1544 (phenyl), 1470 (S-CH_2), 1410 (C-N), 1125 (C-O-C), 1253, 1217, 1107, 1027, 826, 755. The δ_H and δ_C values are given in Tables 3 and 4; *m/z* (%): 367 (M^+, 100), 366 [(M^+–1), 17.42], 368 [(M^++1), 22.78)], 369 [(M^++2), 7.37]. Anal. calcd. for $C_{19}H_{20}O_3N_3S$: C, 61.60; H, 5.44, N, 11.34; O, 12.96, S, 8.66 % Found: C, 61.57; H, 5.43, N, 11.33 %.

***E*-2-{(2-(Phenyl)chroman-*E*-4-hydrazono}thiazolidin-4-one (4b) and *E*-2-{(2-(phenyl)chroman-*Z*-4-hydrazono}-thiazolidin-4-one (5b):** A mixture of flavanone-*E*-4-thiosemicarbazone **(2b)** (600 mg, 2.02 mmol), chloroacetic acid (286 mg, 3.03 mmol) and sodium acetate (330 mg, 4.04 mmol) dissolved in acetic acid (25 mL) was heated under refluxed at 110 °C on an oil bath for 8 h. The reaction mixture on usual work up followed by purification as above first furnished a white solid. This on crystallization from chloroform-benzene afforded **(4b)** as white crystals, 280 mg (48 %), m.p. = 215 °C, R_f = 0.54 (benzene-ethyl acetate, 8.5:1.5 v/v); IR (KBr, ν_{max}, cm^{-1}): 3387 cm^{-1} (NH), 2795 (C-H), 1705(C=O), 1626 (C=N and phenyl), 1590, 1566 (phenyl), 1500 (C-S), 1410 (C-N), 1136 (C-O-C), 1079, 1011, 875, 757, 694, 528; δ_H ($CDCl_3$): 5.15 (dd, 1H, H-2, *J* = 12.62 Hz, *J* = 2.83 Hz), 3.62 (dd, 1H, H-3_{eq}, *J* =17.14 Hz, *J* = 3.02 Hz), 2.85 (dd, 1H, H-3_{ax}, *J* =17.14 Hz, *J* = 12.62 Hz), 3.8 (s, 2H, S-$\underline{C}H_2$), 9.73 (s, 1H, NH), 8.14 (dd, 1H, Ar-H-5, *J* = 7.91 Hz, *J* = 1.71 Hz), 7.03 (ddd, 1H, Ar-H-6, *J* = 8.29 Hz, *J* = 7.91 Hz, *J* = 0.94 Hz), 7.34 (ddd, 1H, Ar-H-7, *J* = 8.29 Hz, *J* = 8.1 Hz, *J* = 1.7 Hz), 6.98 (dd, 1H, Ar-H-8, *J* = 8.1 Hz, *J* = 0.94 Hz), 7.36 to 7.48 (m, 5H, Ar′); δ_C ($CDCl_3$): 77.75 (C-2), 32.292 (C-3), 158.269 (C-4), 120.275 (C-4a), 125.705 (Ar-C-5), 121.92 (Ar-C-6), 132.757 (Ar-C-7), 118.288 (Ar-C-8), 157.34 (C-8a), 140.121(Ar′-C-1), 126.627 (Ar′-C-2,6), 129.086 (Ar′-C-3,5), 172.791 ($\underline{C}$=O), 34.099 (S-$\underline{C}H_2$), 128.835 (Ar′-C-4), 161.772 ($\underline{C}$=N) ; *m/z* (%): 337 (M^+, 100), 336 [(M^+–1), 14.74], 338 [(M^++1), 21.44], 339 [(M^++2), 7.37]. Anal. calcd. for $C_{18}H_{15}O_2N_3S$: C, 64.08; H, 4.48, N, 12.45; O, 9.48, S, 9.50 % Found: C, 64.09; H, 4.47, N 12.43 %.

Further elution of the column yielded a brownish solid compound which on crystallization as above gave compound **5b** as brown crystalline solid, 180 mg (30 %), m.p. = 219 °C, R_f = 0.16 (benzene-ethyl acetate, 8.5:1.5 v/v); IR (KBr, ν_{max}, cm^{-1}): 3364 (NH), 2836 (C-H), 1698 (C=O), 1618 (C=N and phenyl), 1593, 1533 (phenyl), 1464 (C-S), 1404(C-N), 1134 (C-O-C), 1111, 1038, 881, 826, 754, 697, 664, 622, 570, 511, 457; δ_H ($CDCl_3$): 3.9 (s, 2H, S-C$\underline{H}_2$), 5.78 (dd, 1H, H-2, *J* = 11.31 Hz, *J* = 3.9 Hz), 4.08 (dd, 1H, H-3_{eq}, *J* = 17.90 Hz, *J* = 11.31 Hz), 3.52 (dd, 1H, H-3_{ax}, *J* = 17.9 Hz, *J* = 3.77 Hz), 9.75 (s, 1H, NH), 7.36 (dd, 1H, Ar-H-5, *J* = 7.34 Hz, *J* = 1.7 Hz), 6.97 (ddd, 1H, Ar-H-6, *J* = 7.34 Hz, *J* = 7.15 Hz, *J* = 0.95 Hz), 7.44 (ddd, 1H, Ar-H-7, *J* = 7.15 Hz, *J* = 8.47 Hz, *J* = 1.7 Hz), 7.12 (dd, 1H, Ar-H-8, *J* = 8.47 Hz, *J* = 0.95 Hz), 7.23 – 7.33 (br m, 5H, Ar′); δ_C ($CDCl_3$): 62.469 (C-2), 39.395 (S-$\underline{C}H_2$-), 43.857 (C-3), 161.31 (C-4), 114.026 (C-4a), 128.891 (Ar-C-5), 120.057 (Ar-C-6), 133.674 (Ar-C-7), 117.567 (Ar-C-8), 158.15 (C-8a), 138.989 (Ar′-C-1), 129.188 (Ar′-C-2,6), 125.807 (Ar′-C-3,5), 128.89 (C-Ar′-4), 177.62 (C=N),187.18 ($\underline{C}$=O),43.857 (S-$\underline{C}H_2$); *m/z* (%): 337 (M^+, 100), 336 [(M^+–1), 15.41], 338 [(M^+ + 1), 23.45)], 339 [(M^+ + 2), 7.37]. Anal. calcd. for $C_{18}H_{15}O_2N_3S$: C, 64.08; H, 4.48, N, 12.45; O, 9.48, S, 9.50 % Found: C, 64.09; H, 4.47, N 12.43 %.

***in vitro* Antimicrobial activity:** The antimicrobial activity of the test compounds was assayed on nutrient agar medium [Hi-Media Lab. Pvt. Mumbai, India]. The antifungal activity was tested using Sabouraud dextrose agar medium [Hi-Media, Lab. India] by agar well diffusion method of Prez *et al.* [40] as adopted earlier by Ahmad and Beg [41]. Briefly 0.1 mL of the diluted inoculum (10^6 CFU/mL) of test organism was spread on NA/SDA (Nutrient agar/Sabouraud dextrose agar) plates. Wells of 8 mm diameter were punctured into the agar medium and filled separately with 100 mL of compound (250 mg/mL) solvent blank and an antibiotic (chloramphenicol, 100 mg/mL) to which the test bacteria were sensitive. Fluconozole at the concentration of 100 mg/mL was used as the control against *C. albicans*. The plates were incubated for 18 h at 37 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism.

RESULTS AND DISCUSSION

The compounds **4a** & **5a** and **4b** & **5b** have been synthesized in two steps (Fig. 1). The 4'-methoxyflavanone thiosemicarbazones (*E* and *Z*) **2a** and **3a** and flavanone

TABLE-1
^{1}H NMR DATA OF **2a** AND **3a** IN $CDCl_3$

	δ ppm [Integration, Multiplicity, *J* (Hz)]			
	2a		**3a**	
H-no		NOE		NOE
2_{ax}	5.1 (1H, dd,$J_{2ax,3ax}$ 12.24; $J_{2ax,\ 3eq}$ 3.2)		6.0 (1H, dd, $J_{2ax,\ 3eq}$3.3; $J_{2ax,\ 3ax}$11.54)	Ar'-2,6
3_{ax}	2.77 (1H, dd, $J_{3ax,3eq}$ 16.58; $J_{2ax,\ 3ax}$ 12.24)	H_{3eq},Ar′-2,6	3.93 (1H, dd, $J_{3ax,3e}$ 17.85; $J_{2ax,\ 3ax}$ 11.54)	$H_{3eq,}$ H-2 Ar-5
3_{eq}	3.04 (1H, dd, $J_{3ax,3eq}$ 16.58; $J_{2ax,\ 3eq}$ 3.2)	H_{3ax}, H-2, NH	3.32 (1H, dd, $J_{3ax,\ 3e17.85}$; $J_{2ax,\ 3eq}$ 3.3)	$H_{3ax,}$ Ar′-2,6 Ar-5
OCH_3	3.84 (3H, s)		3.79 (3H, s)	
NH	8.65 (1H, s)	H_{3eq}	9.65 (1H, s)	H-8
NH_2	6.35 (2H, s)		6.33 (2H, s)	
Ar-5	7.95 (1H, dd, $J_{Ar-5,6}$ 7.91; $J_{Ar-5,7}$1.7)	Ar-6	7.25 (1H, d, $J_{Ar,\ 5,6}$ 8.24)	
Ar-6	7.02 (1H, ddd, $J_{Ar-5,6}$ 7.91; $J_{Ar-6,7}$7.53; $J_{Ar-6,8}$1.13)		6.95 (1H, t, *J* 7.69)	
Ar-7	7.34 (1H, ddd, $J_{Ar-6,}$ 77.53; $J_{Ar-7,8}$8.85; $J_{Ar-5,7}$1.7)		7.39 (1H, t, *J* 7.69)	
Ar-8	6.97 (1H, dd, $J_{Ar-7,8}$ 8.85; $J_{Ar-6,8}$ 1.13)		7.06 (1H, d, $J_{Ar-7,8}$ 8.51)	
Ar′-2,6	7.39 (2H, d, $J_{Ar'-2,6,\ Ar'-3,5}$ 8.85)		7.39 (2H, d, $J_{Ar'-2,6,Ar'-3,5}$ 8.51)	
Ar′-3,5	6.97 (2H, d, $J_{Ar'-2,6,\ Ar'-3,5}$ 8.85)		6.85 (2H, d, $J_{Ar'-2,6,Ar'-3,5}$ 8.51)	

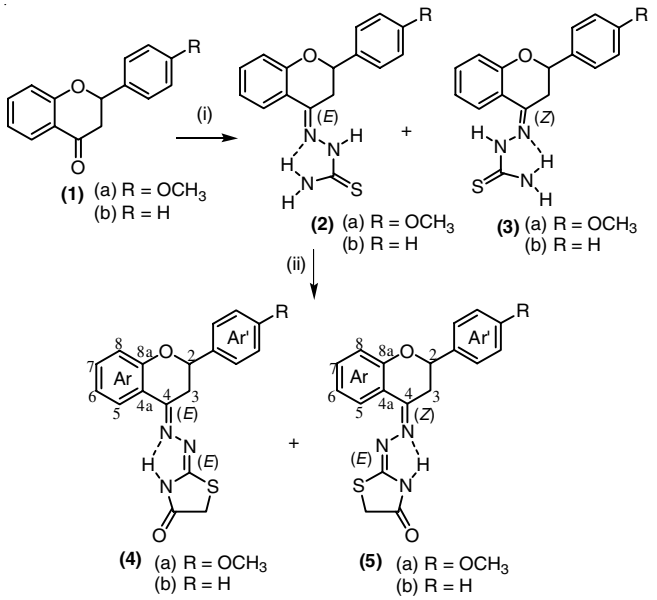

Fig. 1. Synthesis of **2** + **3** (i) **1**/$H_2N.NH.(C=S).NH_2$ (molar ratio, 1:1.25), AcOH, absolute alcohol, reflux, 8 h. **4** + **5** (ii) **2**/$Cl.CH_2COOH$/AcONa (molar ratio, 1:1.25:1.5), AcOH, reflux, 10 h

TABLE-2
^{13}C NMR DATA OF **(2a)** AND **(3a)** IN $CDCl_3$

Compd. No.	**2a**		**3a**	
	δ (ppm)	DEPT	δ (ppm)	DEPT
2	75.854	CH	61.779	CH
3	38.878	CH_2	43.443	CH_2
OCH_3	55.166	CH_3	55.255	CH_3
4	156.232[a]	C	157.604[a]	C
4a	120.075	C	114.665	C
Ar-5	125.477	CH	129.065	CH
Ar-6	121.213	CH	120.165	CH
Ar-7	131.129	CH	133.115	CH
Ar-8	117.435	CH	117.183	CH
8a	156.983[a]	C	158.863[a]	C
Ar'-1	131.683	C	133.229	C
Ar'-2,6	127.874	CH	126.841	CH
Ar'-3,5	113.726	CH	114.316	CH
Ar'-4	159.146	C	159.167	C
C=S	178.779	C	176.176	C

[a]Assignment may be reversed

thiosemicarbazones (*E* and *Z*) **2b** and **3b** as precursors were first prepared following a published but a modified procedure [34] by refluxing a solution of 4'-methoxyflavanone and flavanone separately with thiosemicarbazide (molar ratio, 1:1.25) in absolute alcohol in the presence of freshly distilled acetic acid for 8 h as yellow gummy mass. On crystallization it furnished compounds **2a** (54 %) and **2b** (62 %) as white crystals; and **3a** (16 %) and **3b** (21 %) as brown cubic crystals respectively. The *E*-forms of thiosemicarbazones **(2a** and **2b)** were then condensed with chloroacetic acid and sodium acetate (molar ratio, 1:1.25:1.5) in acetic acid to give compound **4a** (43 %) as white crystalline solid and compound **5a** (28 %) as brownish crystalline solid in the former and compound **4b** (48 %) and **5b** (30 %) in the latter of identical nature.

The structures of **2a**, **2b**, **3a** and **3b** have been confirmed by IR, HRMS, ^{1}H NMR, ^{13}C NMR and an additional NOE-experiments (spectral data of the representative compounds **2a** and **3a** are shown in Tables 1 and 2). HRMS spectra of these compounds showed characteristic [M^++1] peaks corresponding to their molecular weights. The assignments of all the signals to individual H or C-atoms have been performed on the basis of typical δ-values, *J*-constants and a NOE-experiment. The HRMS spectrum of **2a** was found to be super imposable with that of **3a** as the peaks are exactly identical in both but with difference in intensities. Both showed the same molecular ion peak at *m/z* 327.1046 corresponding with $[C_{17}H_{17}N_3O_2S]^{+\bullet}$. In ^{1}H NMR spectrum, the two singlets at δ 8.65 and 6.35 in **2a** and at δ 9.65 and 6.33 in **3a** were assigned to NH and NH_2 protons respectively. Two double doublets at δ 2.77 (*J* = 16.58 Hz, *J* = 12.24 Hz) and δ 3.04 (*J* = 16.58 Hz, *J* = 3.2 Hz) in **2a** and at δ 3.93 (*J* = 17.85 Hz, *J* = 11.84 Hz) and δ 3.32 (*J* = 17.85 Hz, *J* = 3.30 Hz) in **3a** were assigned to H-3_{ax} and H-3_{eq} respectively. A double doublet at δ 5.10 (*J* = 12.24 Hz, *J* = 3.2 Hz) in **2a** and δ 6.0 (*J* = 11.54 Hz, *J* = 3.3 Hz) in **3a** was assigned to H-2. The chemical shifts of aromatic protons at all positions were found to be almost identical except at position H-5 where there is a significance difference of δ 0.7. This is due to the different orientation of $NHC(S)NH_2$ group. When this group oriented towards C-3 protons and away from Ar-ring, H-3_{ax} and H-3_{eq} are somewhat shielded and appeared at upfield δ 2.77 (H-3_{ax} more shielded) due to close proximity

TABLE-3
^{1}H NMR SPECTRAL DATA OF **4a** AND **5a** IN $CDCl_3$

H-no	δ ppm [Integration, Multiplicity, *J* (Hz)]	
	4a	**5a**
2_{ax}	5.1(1H, dd, $J_{2ax,3ax}$ 12.62; $J_{2ax,3eq}$ 2.82)	5.72 (1H, dd, $J_{2ax,3eq}$ 3.76; $J_{2ax,3ax}$ 11.3)
3_{ax}	2.86 (1H, dd, $J_{3ax,3eq}$ 17.14; $J_{2ax,3ax}$ 12.62)	4.03 (1H, dd, $J_{3ax,3eq}$ 17.9; $J_{2ax,3ax}$ 11.3)
3_{eq}	3.58 (1H, dd, $J_{3ax,3eq}$ 17.14; $J_{2ax,3eq}$ 2.82)	3.52 (1H, dd, $J_{3ax,3eq}$ 17.9; $J_{2ax,\ 3eq}$ 3.76)
OCH_3	3.83 (3H, s)	3.77 (3H, s)
NH	Not appeared	9.70 (1H, s)
S-CH_2-	3.81 (2H, s)	3.88 (2H, s)
Ar-5	8.13 (1H, dd, $J_{Ar\text{-}5,6}$ 7.91; $J_{Ar\text{-}5,7}$ 1.7)	7.29 (1H, dd, $J_{Ar,\ 5,6}$ 7.91; $J_{Ar\text{-}5,7,}$ 1.7)
Ar-6	7.01 (1H, ddd, $J_{Ar\text{-}5,6}$ 7.91; $J_{Ar\text{-}6,7}$ 8.10; $J_{Ar\text{-}6,8}$ 0.94)	6.97 (1H, ddd, $J_{Ar\text{-}\ 5,6}$ 7.91; $J_{Ar\text{-}6,7,}$ 6.97; $J_{Ar\text{-}6,8}$ 0.94)
Ar-7	7.33 (1H, ddd, $J_{Ar\text{-}6,7}$ 8.1; $J_{Ar\text{-}7,8}$ 8.29; $J_{Ar\text{-}5,7}$ 1.7)	7.43 (1H, ddd, $J_{Ar\text{-}6,7,}$ 6.97; $J_{Ar\text{-}7,8}$ 8.29; $J_{Ar,\ 5,7}$ 1.7)
Ar-8	6.97 (1H, dd, $J_{Ar\text{-}7,8}$ 8.29; $J_{Ar\text{-}6,8}$ 0.94)	7.11 (1H, dd, $J_{Ar\text{-}7,8}$ 8.29; $J_{Ar\text{-}\ 6,8}$ 0.94)
Ar′-3,5	6.92 (2H, d, $J_{Ar'\text{-}2,6,\ Ar'\text{-}3,5}$ 8.85)	6.85 (2H, d, $J_{Ar'\text{-}2,6,\ Ar''\text{-}3,5}$ 8.85)
Ar′-2,6	7.38 (2H, d, $J_{Ar'\text{-}2,,6,\ Ar'\text{-}3,5}$ 8.85)	7.2 (2H, d, $J_{Ar'\text{-}2,6,\ Ar'\text{-}3,5}$ 8.85)

of the lone pair of electrons on nitrogen as in *E*-form **2a**. When this group is oriented towards Ar-ring and away from C-3 protons, the NH being in close proximity to Ar-ring, is deshielded and appeared at lower field δ 9.65 while H-5 proton is somewhat shielded and appeared at upfield δ 7.25 as in *Z*-form compound **3a**. In support of two geometrical isomers (*E* and *Z*) of thiosemicarbazone, some additional NOE-experiments were performed. Irradiation of NH activated H-3_{eq} in **2a** and H-8 in **3a** indicating that NH is near to H-3_{eq} in **2a** and H-8 in **3a**. Further, the irradiation of H-3_{eq} in **2a** activated H-3_{ax}, H-2_{ax} and NH whereas in **3a** activated H-3_{ax}, H-2', 6' and H-5 but not NH. These observations together with the variations in chemical shifts of protons at C-2, C-3 and C-5 in **2a** and **3a** are the clear evidence for the existence of two geometrical isomers (*E* and *Z*) of 4'-methoxyflavanone thiosemicarbazone. On the basis of above spectral findings, the preferred stereo structure deduced for **2a** and **3a** is shown in Fig. 2.

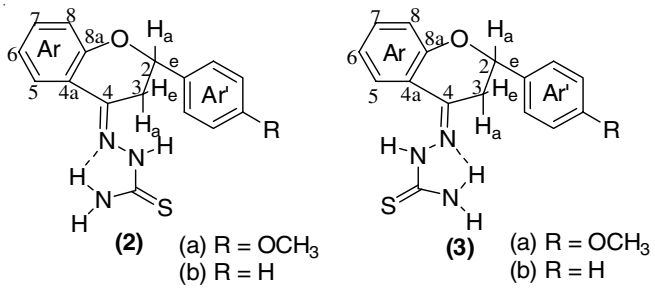

Fig. 2

The stereo structures of compounds **4a**, **5a**, **4b** and **5b** have been fully established by FTIR, HRMS, ^{1}H NMR and ^{13}C NMR spectra. The assignments of all the signals to individual H and C-atoms (Tables 3 and 4) have been performed on the basis of the typical chemical shift values, splitting constants, relative integrations, decoupling and by a comparison with the spectral data of previously established structures of the corresponding thiosemicarbazones (**2a** and **3a**) (Tables 1 and 2). FTIR spectra of compounds **4a** and **5a** displayed identical characteristic bands for NH, CH, C–O, C=N, phenyl, S–CH_2, C–N and C–O–C groups. The HRMS spectra of compound **4a** was found to be super imposable with that of compound **5a** as the peaks are exactly identical in both with only slight difference in intensities. Both showed the same molecular ion

TABLE-4
^{13}C NMR DATA OF **4a** AND **5a** IN $CDCl_3$

Compd. No.	**4a**		**5a**	
	δ (ppm)	INEPT	δ (ppm)	INEPT
2	77.20	CH	62.111	CH
3	32.853	CH_2	43.667	CH_2
OCH_3	55.334	CH_3	55.288	CH_3
4	157.182[a]	C	158.172[a]	C
4a	119.829	C	114.526	C
Ar-5	125.274	CH	128.883	CH
Ar-6	121.428	CH	120.065	CH
Ar-7	132.318	CH	133.643	CH
Ar-8	117.879	CH	117.582	CH
8a	157.952[a]	C	159.711[a]	C
Ar'-1	131.77	C	131.046	C
Ar'-2,6	127.695	CH	127.452	CH
Ar'-3,5	114.041	CH	114.462	CH
Ar'-4	159.688	C	161.371	C
S-$\underline{C}H_2$	33.439	CH_2	39.349	CH_2
C=N	Not appeared	C	177.523	C
C=O	172.177	C	187.256	C

[a]Assignment may be reversed.

peak *m/z* 367.0977 corresponding with $[C_{19}H_{17}N_3O_3S]^{+\bullet}$ as the base peak, which is equivalent to the sum of molecular weights of the thiosemicarbazone (327) and chloroacetic acid (94) minus one molecule each of HCl and water. This indicated the formation of thiazolidinone ring by the cyclocondensation of the thiosemicarbazone moiety with chloroacetic acid. In both ^{1}H NMR and ^{13}C NMR spectra of compounds **4a** and **5a**, the chemical shifts and splitting constants for protons/carbons, especially of H-5/C-5, H-2/C-2 and H-3/C-3 of the flavanone moiety were found comparable with that of compounds **2a** and **3a**, respectively. This suggested that with flavanone moiety of the hydrazono compounds, the stereo structure of **4a** corresponds with the *E*-isomer as in **2a** and that of **5a** with the *Z*-isomer as in **3a**. In ^{1}H NMR spectra a remarkable difference in the appearance of NH proton signal of thiazolidinone ring of two isomers **4a** and **5a** in $CDCl_3$ was observed. A peak appeared at δ 9.70 in the spectrum of **5a** in $CDCl_3$ solution while in **4a** spectrum, it could not been seen. However in **4a** in DMSO-d_6 solution, this signal appeared at low field δ 9.86. This showed there must be some difference in spatial orientation of the thiazolidinone ring of the two isomers **4a** and **5a**. The orientation of thiazolidinone ring (*E*) in the two isomers

TABLE-5
ANTIMICROBIAL ACTIVITY OF SOME OF THE SYNTHESIZED COMPOUNDS BY AGAR WELL DIFFUSION METHOD

Test compounds	Effective concentration (µg/well)	Antimicrobial activity in terms of zone of inhibition in mm				
		Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*
2a	200	14	16	21	24	–
2b	200	13	15	19	18	–
4a	200	–	–	16	22	–
4b	200	–	–	14	20	–
Chloramphenicol	100	25	20	24	30	–
Fluconozole	100	–	–	–	–	25

could be deduced from the ^{13}C NMR spectra. A peak appeared at δ 177.52 in the spectrum of **5a** in $CDCl_3$ solution for (C=N) while in **4a** spectrum, it could not been seen. However, this peak appeared in **4a** in DMSO-d_6 solution at δ 164.106. On the basis of above spectral findings, the orientation in the two parts of the molecule in **4a** and **5a** is deduced as (*E*,*E*) and (*Z*,*E*) respectively. The deduced streo structures of **4a** and **5a** are shown in Fig. 1.

Biological activity: The newly synthesized compounds were evaluated for their antimicrobial (antibacterial and antifungal) activity.

Antimicrobial activity: The *in vitro* antibacterial (*S. aureus* IOA-106, *B. subtilis* MTCC-121 laboratory isolate, *E. coli* U.P–2566 and *P. aeruginosa* IOA-110) and antifungal (*C. albicans* SC-5314 laboratory isolate) activities of the compounds **2a**, **2b**, **3a** and **3b** were evaluated by agar well diffusion method. The results for the antimicrobial study of the tested compounds against the test organisms are given in Table-5. Antimicrobial activity against "Gram-negative" bacteria was found in all compounds. Compounds **2a** and **2b** demonstrated overall broad-spectrum antimicrobial activity, *i.e.*, against both "Gram-positive" and "Gram-negative bacteria" while its thiazolidin-4-ones derivatives **(4a** and **4b)** showed activity only against "Gram-negative" bacteria but no significant activity was found for all tested compounds against fungus (*C. albicans*). Effective concentration of these active compounds was 200 mg/well. Further exploration requires detailed study on exact mode of interaction of these peculiar compounds with "Gram-negative" and "Gram-positive" bacteria. *in vivo* Protection and possible toxicity data on these compounds are to be generated further.

Conclusion

The aim of the present research work was to synthesize some novel flavanone-hydrazono-thiazolidin-4-ones and to evaluate their antitumor and antimicrobial activities. It has been achieved by the synthesis of four novel thiazolidin-4-ones **(4a**, **5a**, **4b** and **5b)**, all bearing a flavanone-hydrazone side chain. We have also investigated the stereochemistry of flavanone thiosemicarbazones **(2a**, **3a**, **2b**, **3b)** and the corresponding flavanone-hydrazono-thiazolidinones **(4a**, **5a**, **4b** and **5b)**. It was observed that the hydrazono-thiazolidinones prepared from *E*-flavanone thiosemicarbazones were found to exist in (*E, E*) and (*Z, E*) forms. The compounds **2a** and **2b** exhibited broad-spectrum antimicrobial activity. Further investigations for other biological assays are required to explore their potentialities in future.

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