

Synthesis and antimicrobial activity of spiro[chromeno[4,3-*d*][1,2,3]thiadiazole-4,1'-cyclohexane, spiro[chromeno[4,3-*d*][1,2,3]selenadiazole-4,1'-cyclohexane and spiro[chroman-2,1'-cyclohexan]-4-one-5-spiro-4-acetyl-2-(acetylamino)- Δ^2 -1,3,4-thiadiazoline compounds

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Abstract

The synthesis of some new heterocyclic compounds containing spiro- benzopyrans, thiadiazoles, -selenadiazole and -thiadiazolines as sub-units in the molecule is achieved using a high- yielding synthetic protocol. The products were tested for antibacterial and antifungal activity.

Keywords: Antimicrobial activity, semicarbazones, spiro benzopyrans, 1,2,3-thiadiazoles, 1,2,3-selenadiazoles

Introduction

Resistance to antimicrobial agents is now recognized as a major global public health problem, so that the discovery of new antibacterial and antifungal compounds has become increasingly critical to fighting infectious disease.

Benzopyrans belong to an important structural class, which have valuable and diverse biological properties.¹ Synthetic benzopyran derivatives including the K⁺ channel opener Cromakalim and the aldose reductase inhibitor Sorbinil exemplify the pharmacological importance of this heterocyclic sub-structure.² Benzopyrans are found in many natural products, in particular the flavonoids and the cannabinoids.³ Chroman-4-ones are extensively used as synthetic intermediates. They have been used to synthesize various heterocyclic compounds which have wide ranges of pharmacological activities,⁴ for example Khellin is a coronary vasodilator.^{5,6} Chroman-4-one 2-carboxylic acids are spasmolytic agents, and disodium chromoglycate acts an anti-allergenic drug.⁷ Therefore, benzopyrans were considered a good biological properties.¹ Synthetic benzopyran derivatives including the K⁺ channel opener Cromakalim and the aldose reductase inhibitor Sorbinil exemplify the pharmacological importance of this heterocyclic sub-structure.² Benzopyrans are found in many

natural products, in particular the flavonoids and the cannabinoids.³ Chroman-4-ones are extensively used as synthetic intermediates. They have been used to synthesize various heterocyclic compounds which have wide ranges of pharmacological activities,⁴ for example Khellin is a coronary vasodilator.^{5,6} Chroman-4-one 2-carboxylic acids are spasmolytic agents, and disodium chromoglycate acts as an anti-allergenic drug.⁷ Therefore, benzopyrans were considered a good template to incorporate in novel 1,2,3-thiadiazole and 1,2,3-selenadiazole derivatives.

A simple 1,2,3-thiadiazole derivative is as potent as penicillin G in an *in vitro* inhibition zone assay.⁸ Several 1,2,3-thiadiazoles were synthesized by the method of Hurd and Mori⁹ as collagen-induced inhibitors of platelet aggregation.¹⁰ 1,2,3-Selenadiazoles have generated wide interest owing to their ready conversion into selenium heterocycles¹¹ and reactive intermediates.¹² Selenium-containing heterocycles exhibit varied biological activities,¹³⁻¹⁶ which include anti-inflammatory agents, immuno-modifiers, cytokine inducers and enzyme inhibitors. Their chemotherapeutic activity has been reviewed.¹⁷

Oxidation of aldehyde thiosemicarbazones with NaOH/K₃[Fe(CN)₆] gives 1,3,4-thiadiazolines,¹⁸ and the synthesis of 5 α -cholestan-3-spiro-(3,4-acetyl-2-acetylamino)- Δ^2 -1,3,4-thiadiazoline¹⁹ from 5 α -cholestan-3-one thiosemicarbazone have been reported. A previously reported method of conversion of unsubstituted aldehyde thiosemicarbazones into 1,3,4-thiadiazolines involved the acetylation of thiosemicarbazones.²⁰⁻²² Spiro-1,3,4-thiadiazolines were shown to be potent medicinally active compounds.²³ Activities associated with chromone and the spiro thiadiazoline nucleus are a continuation of our work²⁴⁻²⁸ and the molecules **2a-g**, **3a-g**, **4a-g**, **5a-g** and **6a-g** could provide starting materials for the development of new classes of antimicrobial molecules.

Results and Discussion

Chemistry

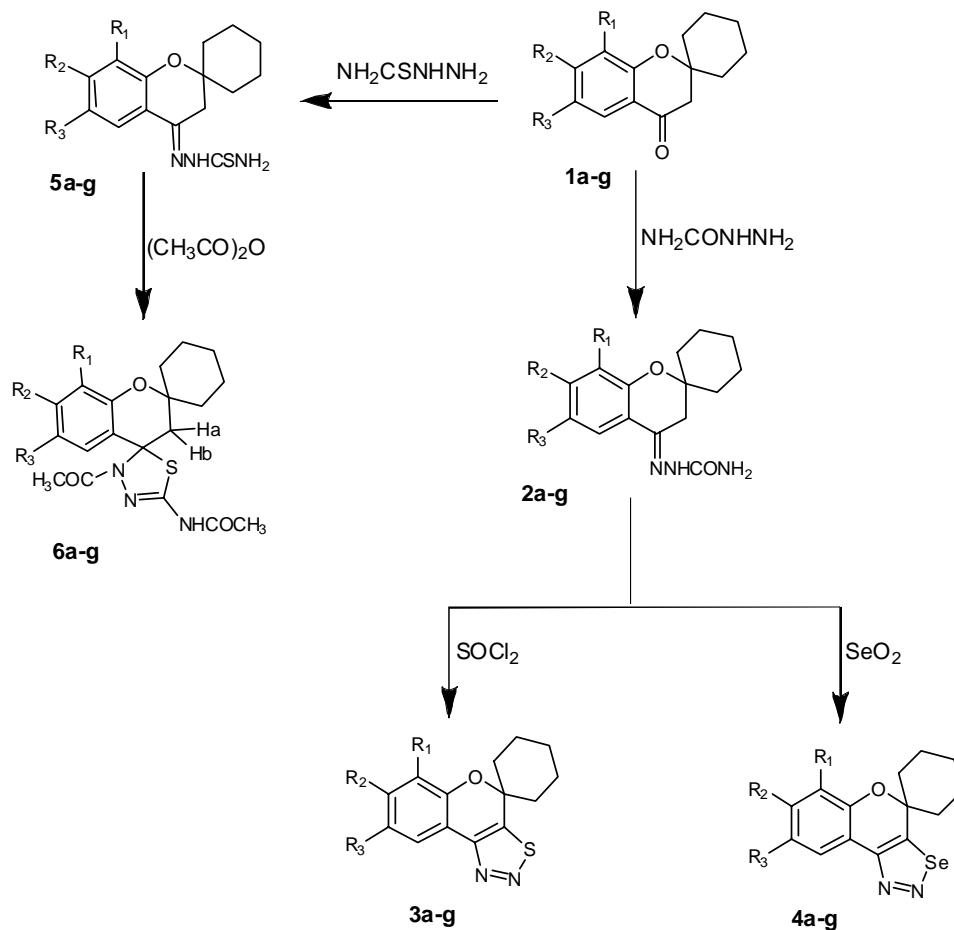
The spiro[chroman-2,1'-cyclohexan]-4-ones **1a-g** were converted into their semicarbazone derivatives **2a-g** by reacting **1a-g** with semicarbazide hydrochloride. Compounds **2a-g** upon treatment with SeO₂ in glacial acetic acid gave the corresponding spiro(chromeno-[4,3-*d*][1,2,3]-selenadiazole-4,1'-cyclohexanes) **3a-g**, whereas the same semicarbazones when treated with thionyl chloride gave the spiro(chromeno[4,3-*d*][1,2,3]thiadiazole-4,1'-cyclohexanes) **4a-g**. The thiosemicarbazones **5a-g** when heated with acetic anhydride gave (spiro-chroman-2,1'-cyclohexan-4-one)-5-spiro-4-acetyl-2-(acetylamino)- Δ^2 -1,3,4-thiadiazolines, **6a-g** (see Scheme 1). The synthesized compounds were characterized by their analytical and spectroscopic data.

Antimicrobial activity

The antimicrobial activity of the synthesized compounds **2a-g**, **3a-g**, **4a-g**, **5a-g** and **6a-g** was determined *in vitro* against a variety of bacteria and fungi. The tests were carried out using the agar spot method. For this study, test cultures of the bacterial strains *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecium* were each inoculated into

Tryptone Soya Broth. Similarly, test cultures of the fungal strains, *Candida kruseii*, *Candida albicans*, and *Candida glabrata* were inoculated into Sabouraud Dextrose Broth.

The plates with test cultures were spotted serially with the extracts of the fermented cultures. Standard antibiotics for each culture were also spotted onto the respective plates as positive controls, and also for comparative study of the extract with an antibiotic. The compounds for antimicrobial studies were taken as extracts at 20 $\mu\text{g/ml}$ in DMSO, and each standard antibiotic at 20 $\mu\text{g/ml}$ in DMSO. The plates were incubated in an incubator at 37°C overnight, and the zones of inhibition were measured in mm.



Substituents

	a	b	c	d	e	f	g
R_1	H	H	H	H	Cl	H	H
R_2	H	H	H	H	H	CH_3	H
R_3	Cl	H	Br	CH_3	Cl	Cl	F

Scheme 1

All compounds were assayed for antimicrobial activity. From the data presented in Table 1, the preliminary screening results for the compounds **2a-g**, **3a-g**, **4a-g**, **5a-g** and **6a-g** established that only compounds **2d**, **2e**, **6e**, **3g**, **4b**, **4c** and **4d** showed some activity against one or two organisms, while the remaining derivatives were either inactive or weakly active against all the bacterial as well as fungal strains. In summary, their overall activity profiles were found to be moderate or poor.

Table 1. Antimicrobial activities of compounds **2a-g**, **3a-g**, **4a-g**, **5a-g** and **6a-g**. Zones of inhibition. Sample loaded: 4 µl each (concentrations 20µg/ml)

(A) Antifungal Activity

Sample	<i>Candida albicans</i>	<i>Candida Krusei</i> GO3	<i>Candida Glabrata</i> HO5	<i>Aspergillus</i> <i>fumigatus</i>
2d	-	(4)C	-	-
2e	(3)C	-	-	-

(B) Antibacterial Activity

Sample	<i>Staphylococcus</i> <i>Aureus</i> 209P	<i>Escherichia</i> <i>Coli</i> 20732	<i>Enterococcus</i> <i>faecium</i> R-2	<i>Pseudomonas</i> <i>Aeruginosa</i> M-35	MRSA
6e	-	-	-	-	(3)VH
3g	-	-	-	-	(5)H
4b	(5)C	-	-	-	-
4c	(5)H	-	(5)H	-	-
4d	(5)C	-	-	-	-

(C) Activity data for standard antibiotics used

Test culture	Antibiotics (20ug/ml)	Zone Size (mm)
<i>Escherichia coli</i> 20732	Oxacillin + Vancomycin	0 + 0
<i>Pseudomonas aeruginosa</i> ATCC 23053	Oxacillin + Vancomycin +	0 + 0 + 0
<i>Staphylococcus aureus</i> 209P	Oxacillin	7C
MRSA ATCC 33501	Oxacillin + Vancomycin	0 + 4C
Test culture	Antibiotics (20ug/ml)	Zone Size (mm)
<i>Candida albicans</i> ATCC 14503	Ampicillin + flucazolidone	6C + 0
<i>Candida krusel</i> GO6	Ampicillin + flucazolidone	8C + 0
<i>Aspergillus fumigatus</i> ATCC 16424	Ampicillin + flucazolidone	8C + 0
<i>Candida glabrata</i> H04	Ampicillin + flucazolidone	6C + 0

Zone of inhibition (in mm): C = clear zone, H = hazy zone, VH = very hazy zone, - = no activity or less activity.

Experimental Section

General. Monitoring of the reactions and checking of purity of the products were done using pre-coated silica gel plates and visualization using an iodine / UV lamp. Melting points were taken in open capillaries and are uncorrected. IR spectra were recorded on Perkin-Elmer 1420 spectrophotometer. ^1H NMR spectra were recorded on a Varian 300 MHz spectrometer in CDCl_3 or DMSO as solvent with TMS as internal standard. Chemical shifts are in δ (ppm). Mass spectra were recorded on an HP 1100 LC/MSD instrument (positive and negative APCI ion source, 50-200 V, nitrogen). Elemental analysis was performed on a Perkin-Elmer analyzer. Compounds **1a–g** were prepared by reported procedures.¹⁸

(Spiro[chroman-2,1'-cyclohexan]-4-one)semicarbazone **2a**

Spiro[chroman-2,1'-cyclohexan]-4-one **1a** (2.5g, 10 mmol) was reacted with semicarbazide hydrochloride (1.34g, 12 mmol) and sodium acetate (1.23g, 15 mmol) in absolute ethanol (25mL) under reflux for 1 h. The solid products given upon cooling were separated by filtration, dried, and crystallized from ethanol to give 1.78g of **2a**. Compounds **2b–g** were prepared similarly, with the physical and analytical data given below.

2^a. ($R_1 = R_2 = \text{H}$, $R_3 = \text{Cl}$). Yield 58%, Yellow solid mp 195°C. IR (KBr): 3470, 2932, 2856, 1673, 1567, 1467, 1423, 1276 cm^{-1} ; ^1H -NMR (DMSO- d_6): δ 1.28-1.72 (m, 10H), 2.73 (s, 2H), 6.68 (brs, 2H, NH_2), 6.85 (d, 1H, $J = 8.7$ Hz), 7.20 (dd, 1H, $J = 8.4, 2.4$ Hz), 8.19 (d, 1H, $J = 2.4$ Hz), 9.50 (s, 1H, NH). Anal.: C, 58.38; H, 5.65; N, 13.25. Calcd for $\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{O}_2$: C, 58.54; H, 5.89; N, 13.65%.

2^b. ($R_1 = R_2 = R_3 = \text{H}$). Yield 52%, Yellow solid mp 201°C. IR (KBr): 3471, 3264, 3210, 2931, 1716, 1687, 1584, 1457, 1229 cm^{-1} . ^1H NMR (DMSO- d_6) δ 1.28-1.90 (m, 10H), 2.73 (s, 2H), 6.54 (brs, 2H, NH_2), 6.81 (dd, 1H, $J = 8.1, 1.2$ Hz), 6.87 (t, 1H, $J = 7.8$ Hz), 7.20 (t, 1H, $J = 7.8$ Hz), 8.08 (dd, 1H, $J = 7.8, 1.5$ Hz), 9.44 (s, 1H, NH). Anal. Calcd for $\text{C}_{15}\text{H}_{19}\text{N}_3\text{O}_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 65.70; H, 6.70; N, 15.20%.

2^c. ($R_1 = R_2 = \text{H}$, $R_3 = \text{Br}$). Yield 54%, Yellow solid mp 178°C. IR (KBr): 3477, 3197, 2931, 2857, 1682, 1467, 1417, 1278 cm^{-1} ; ^1H NMR (DMSO- d_6) : δ 1.22-1.90 (m, 10H), 2.73 (s, 2H), 6.70 (brs, 2H, NH_2), 6.79 (d, 1H, $J = 9.0$ Hz), 7.32 (dd, 1H, $J = 9.0, 2.4$ Hz), 8.30 (d, 1H, $J = 2.7$ Hz), 9.48 (s, 1H, NH). Anal Calcd for $\text{C}_{15}\text{H}_{18}\text{BrN}_3\text{O}_2$: C, 51.15; H, 5.15; N, 11.93. Found: C, 51.04; H, 5.08; N, 11.84%.

2^d. ($R_1 = R_2 = \text{H}$, $R_3 = \text{CH}_3$). Yield 59%, Yellow solid mp 217°C. IR (KBr): 3461, 3180, 2929, 2859, 1702, 1579, 1489, 1227 cm^{-1} ; ^1H NMR (DMSO- d_6) : δ 1.28-1.76 (m, 10H), 2.23 (s, 3H), 2.69 (s, 2H), 6.55 (brs, 2H, NH_2), 6.70 (d, 1H, $J = 8.1$ Hz), 6.99 (dd, 1H, $J = 8.1, 1.5$ Hz), 7.89 (d, 1H, $J = 1.2$ Hz), 9.39 (s, 1H, NH). Anal Calcd for $\text{C}_{16}\text{H}_{21}\text{N}_3\text{O}_2$: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.60; H, 7.20; N, 14.50%.

2^e. ($R_1 = \text{Cl}$, $R_2 = \text{H}$, $R_3 = \text{Cl}$). Yield 57%, Yellow solid mp 218°C. IR (KBr): 3433, 2931, 2859, 1694, 1615, 1447, 1242 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.18-1.90 (m, 10H), 2.77 (s, 2H), 6.82 (brs,

2H, NH₂), 7.50 (d, 1H, $J = 2.4$ Hz), 8.27 (d, 1H, $J = 2.4$ Hz), 9.58 (s, 1H, NH). Anal Calcd for C₁₅H₁₇Cl₂N₃O₂: C, 52.64; H, 5.01; N, 12.28. Found: C, 52.55; H, 5.00; N, 12.40%.

2f. (R₁ = H, R₂ = CH₃, R₃ = Cl). Yield 64%, Yellow solid mp 210°C. IR (KBr): 3461, 3158, 2930, 2859, 1695, 1586, 1446, 1400, 1237 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.28-1.82 (m, 10H), 2.71 (s, 2H), 2.26 (s, 3H), 6.68 (brs, 2H, NH₂), 6.86 (s, 1H), 8.17 (s, 1H), 9.45 (s, 1H, NH); MS : m/z: [M+1] 322.47, [M+3] 324.65. Anal Calcd for C₁₆H₂₀ClN₃O₂: C, 59.72; H, 6.26; N, 13.06. Found: C, 59.80; H, 6.30; N, 13.08%.

2g. (R₁ = R₂ = H, R₃ = F). Yield 52%, Yellow solid mp 199°C. IR (KBr): 3478, 3206, 2933, 2860, 1693, 1578, 1485, 1442, 1115 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.20-1.80 (m, 10H), 2.73 (s, 2H), 6.68 (brs, 2H, NH₂), 6.82-6.90 (m, 1H), 6.96-7.10 (m, 1H), 7.99 (dd, 1H, $J = 9.6, 3.0$ Hz), 9.53 (s, 1H, NH). Anal Calcd for C₁₅H₁₈FN₃O₂: C, 61.84; H, 6.23; N, 14.42. Found: C, 61.70; H, 6.10; N, 14.30%.

Spiro-(chromeno[4,3-*d*][1,2,3]thiadiazole-4,1'-cyclohexane) 3a

The compound **2a** (0.6g, 1.94 mmol) was added portionwise to an excess of freshly distilled thionyl chloride (6mL) at 0°C. The reaction mixture was then allowed to attain room temperature. After 1 h methylene chloride (15mL) was added and the resulting mixture was decomposed with saturated sodium carbonate. The methylene chloride layer was washed thoroughly with water and dried over anhydrous Na₂SO₄. Evaporation of solvent gave syrupy substance, which was purified by column chromatography to get pure 0.35g of **3a**. Compounds **3b-g** were prepared similarly. Physical and analytical data of **3a-g** are given below.

3^a. (R₁ = R₂ = H, R₃ = Cl). Yield 61%, Off-white solid mp 191°C. IR (KBr): 3479, 3132, 2932, 1673, 1472, 1420, 1279 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.38-1.58 (m, 3H), 1.60-1.96 (m, 5H), 2.08-2.18 (m, 2H), 7.20 (d, 1H, $J = 9.0$ Hz), 7.45 (d, 1H, $J = 9.0$ Hz), 8.03 (d, 1H, $J = 2.4$ Hz). Anal. Calcd for C₁₄H₁₃ClN₂OS: C, 57.43; H, 4.48; N, 9.57. Found: C, 57.40; H, 4.30; N, 9.45%.

3b. (R₁ = R₂ = R₃ = H). Yield 56%, Brown solid mp 198°C. IR (KBr): 3429, 2928, 1611, 1480, 1226, 944, 766 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.38-1.96 (m, 8H), 2.20-2.28 (m, 2H), 7.05 (dd, 1H, $J = 8.4, 0.9$ Hz), 7.10 (t, 1H, $J = 7.2$ Hz), 7.31 (t, 1H, $J = 8.1$ Hz), 8.11 (dd, 1H, $J = 7.2, 1.5$ Hz). Anal. Calcd for C₁₄H₁₄N₂OS: C, 65.09; H, 5.46; N, 10.84. Found: C, 64.90; H, 5.20; N, 10.48%.

3c. (R₁ = R₂ = H, R₃ = Br). Yield 59%, Off-white solid mp 260°C. IR (KBr): 3045, 1618, 1514, 1487, 1143, 682 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.32-1.58 (m, 3H), 1.62-1.92 (m, 5H), 2.12-2.22 (m, 2H), 7.15 (d, 1H, $J = 8.4$ Hz), 7.38 (dd, 1H, $J = 8.7, 2.4$ Hz), 8.00 (d, 1H, $J = 2.4$ Hz). Anal. Calcd for C₁₄H₁₃BrN₂OS: C, 49.86; H, 3.89; N, 8.31. Found: C, 49.45; H, 3.60; N, 8.10%.

3d. (R₁ = R₂ = H, R₃ = CH₃). Yield 54%, Off-white solid mp 187°C. IR (KBr): 3255, 3166, 2924, 1596, 1505, 1480, 1280, 1109, 974 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.38-1.58 (m, 3H), 1.60-1.92 (m, 5H), 2.06-2.16 (m, 2H), 2.34 (s, 3H), 7.02 (d, 1H, $J = 8.4$ Hz), 7.18 (d, 1H, $J = 8.4$ Hz), 7.85 (s, 1H). Anal. Calcd for C₁₅H₁₆N₂OS: C, 66.15; H, 5.92; N, 10.29. Found: C, 66.02; H, 5.58; N, 9.95%.

3e. (R₁ = Cl, R₂ = H, R₃ = Cl). Yield 58%, Dark brown solid mp 165°C. IR (KBr): 3045, 1618, 1514, 1487, 1143, 682 cm⁻¹; ¹H NMR (DMSO-d₆): δ 1.34-1.60 (m, 3H), 1.62-1.94 (m, 5H), 2.14-2.26 (m, 2H), 7.61 (d, 1H, $J = 2.1$ Hz), 7.96 (d, 1H, $J = 2.1$ Hz). Anal. Calcd for C₁₄H₁₂Cl₂N₂OS: C, 51.39; H, 3.70; N, 8.56. Found: C, 51.18; H, 3.60; N, 8.30%.

3f. ($R_1 = H$, $R_2 = CH_3$, $R_3 = Cl$). Yield 54%, Yellowish white solid mp 111°C. IR (KBr): 2938, 1471, 1446, 1233, 1106, 916, 791 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.40-1.58 (m, 3H), 1.60-1.94 (m, 5H), 2.08-2.18 (m, 2H), 2.36 (s, 3H), 7.21 (s, 1H), 7.99 (s, 1H); ^{13}C NMR ($CDCl_3$): 20.40, 21.43, 24.66, 37.29, 79.74, 116.32, 119.80, 124.16, 127.57, 138.77, 149.89, 153.06, 161.12. Anal. Calcd for $C_{15}H_{15}ClN_2OS$: C, 58.72; H, 4.93; N, 9.13. Found: C, 58.45; H, 4.60; N, 9.10%.

3g. ($R_1 = R_2 = H$, $R_3 = F$). Yield 56%, Off white solid mp 80°C. IR (KBr): 2934, 1486, 1436, 1271, 1247, 878 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.40-1.58 (m, 3H), 1.60-1.94 (m, 5H), 2.08-2.18 (m, 2H), 7.14-7.29 (m, 2H), 7.76-7.84 (m, 1H). Anal. Calcd for $C_{14}H_{13}FN_2OS$: C, 60.85; H, 4.74; N, 10.14. Found: C, 60.70; H, 4.55; N, 9.98%.

Spiro-(chromeno[4,3-*d*][1,2,3]selenadiazole-4,1'-cyclohexane) 4a

Compound **2a** (0.6g, 1.94 mmol) was dissolved in glacial acetic acid (10mL) and warmed to 60°C with stirring. To this selenium dioxide (0.215g, 1.94 mmol) was added portion wise during a period of 30 min and the stirring was continued at 60°C for 2-3 h till the evolution of gas ceased. After completion of the reaction, it was filtered to remove the deposited selenium. The filtrate was poured over crushed ice and the solid obtained was filtered, washed thoroughly with cold water and sodium carbonate and again with water. It was then purified by column chromatography to get pure 0.397g of **4a**. Compounds **4b-f** were prepared similarly. Physical and analytical data of **4a-g** are given below.

4a. ($R_1 = R_2 = H$, $R_3 = Cl$). Yield 60%, Brown solid mp 83°C. IR (KBr): 2938, 1475, 1308, 1262, 1229, 948, 748 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.36-1.58 (m, 3H), 1.62-1.92 (m, 5H), 2.12-2.22 (m, 2H), 7.09 (d, 1H, $J = 8.7$ Hz), 7.50 (dd, 1H, $J = 8.4, 2.7$ Hz), 8.12 (d, 1H, $J = 2.1$ Hz). Anal. Calcd for $C_{14}H_{13}ClN_2OSe$: C, 49.50; H, 3.86; N, 8.25. Found: C, 49.40; H, 3.70; N, 8.12%.

4b. ($R_1 = R_2 = R_3 = H$). Yield 62%, Brown solid mp 113°C. IR (KBr): 2924, 2926, 1481, 1305, 1228, 944, 765 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.32-1.94 (m, 8H), 2.29-2.39 (m, 2H), 7.03 (dd, 1H, $J = 8.1, 1.5$ Hz), 7.08 (t, 1H, $J = 7.2$ Hz), 7.28 (t, 1H, $J = 7.8$ Hz), 8.12 (dd, 1H, $J = 7.2, 1.2$ Hz). Anal. Calcd for $C_{14}H_{14}N_2OSe$: C, 55.09; H, 4.62; N, 9.18. Found: C, 54.90; H, 4.46; N, 9.04%.

4c. ($R_1 = R_2 = H$, $R_3 = Br$). Yield 64%, Brown solid mp 105°C. IR (KBr): 2938, 1475, 1308, 1262, 1229, 948, 748 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.36-1.58 (m, 3H), 1.62-1.92 (m, 5H), 2.12-2.22 (m, 2H), 7.09 (d, 1H, $J = 8.7$ Hz), 7.50 (dd, 1H, $J = 8.4, 2.7$ Hz), 8.12 (d, 1H, $J = 2.1$ Hz). Anal. Calcd for $C_{14}H_{13}BrN_2OSe$: C, 43.77; H, 3.41; N, 7.29. Found: C, 43.45; H, 3.30; N, 7.10%.

4d. ($R_1 = R_2 = H$, $R_3 = CH_3$). Yield 59%, Brown solid mp 161°C. IR (KBr): 2931, 1494, 1299, 1231, 944, 819 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.36-1.58 (m, 3H), 1.62-1.94 (m, 5H), 2.12-2.22 (m, 2H), 2.33 (s, 3H), 6.99 (d, 1H, $J = 8.1$ Hz), 7.14 (d, 1H, $J = 8.1$ Hz), 7.85 (s, 1H). Anal. Calcd for $C_{15}H_{16}N_2OSe$: C, 56.43; H, 5.05; N, 8.77. Found: C, 56.40; H, 5.02; N, 8.75%.

4e. ($R_1 = Cl$, $R_2 = H$, $R_3 = Cl$). Yield 57%, Brown solid mp 135°C. IR (KBr): 2925, 1458, 1262, 1241, 1120, 763 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.36-1.64 (m, 3H), 1.66-1.98 (m, 5H), 2.19-2.30 (m, 2H), 7.69 (d, 1H, $J = 2.4$ Hz), 8.02 (d, 1H, $J = 2.4$ Hz). Anal. Calcd for $C_{14}H_{12}Cl_2N_2OSe$: C, 44.95; H, 3.23; N, 7.49. Found: C, 44.90; H, 3.10; N, 7.30%.

4f. ($R_1 = H$, $R_2 = CH_3$, $R_3 = Cl$). Yield 58%, Brown solid mp 126°C. IR (KBr): 2936, 1481, 1308, 1233, 1094, 877, 762 cm^{-1} ; 1H NMR (DMSO- d_6) : δ 1.36-1.58 (m, 3H), 1.60-1.92 (m, 5H), 2.12-

2.22 (m, 2H), 2.35 (s, 3H), 7.16 (s, 1H), 7.97 (s, 1H). ^{13}C NMR (DMSO- d_6): 19.64, 20.98, 23.81, 36.96, 81.63, 117.20, 119.75, 123.47, 126.06, 137.52, 149.43, 151.55, 159.00. Anal. Calcd for $\text{C}_{15}\text{H}_{15}\text{ClN}_2\text{OSe}$: C, 50.94; H, 4.27; N, 7.92. Found: C, 50.55; H, 4.12; N, 7.70%.

4g. ($\text{R}_1 = \text{R}_2 = \text{H}$, $\text{R}_3 = \text{F}$). Yield 65%, Brown solid mp 92°C . IR (KBr): 3045, 1618, 1514, 1487, 1143, 682 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.35–1.45 (m, 3H), 1.60–1.92 (m, 5H), 2.14–2.20 (m, 2H), 7.10–7.26 (m, 2H), 7.76–7.82 (m, 1H). Anal. Calcd for $\text{C}_{14}\text{H}_{13}\text{FN}_2\text{OSe}$: C, 52.02; H, 4.05; N, 8.67. Found: C, 51.90; H, 4.00; N, 8.60%.

Spiro[chroman-2,1'-cyclohexan]-4-one thiosemicarbazone **5a**

To a solution of spiro [chroman-2, 1'-cyclohexan]-4-one **1a** (1.5g, 6 mmol) in ethanol (25mL) a few drops of conc. HCl and the ethanolic solution of thiosemicarbazide (0.547g, 6 mmol) was added dropwise with constant stirring. The reaction mixture was refluxed for 3 h on a water-bath. After cooling, the solid product was filtered off and recrystallized from ethanol to get 1.16g of **5a**. Compounds **5b-g** were prepared similarly. Physical and analytical data of **5a-g** are given below.

5a. ($\text{R}_1 = \text{R}_2 = \text{H}$, $\text{R}_3 = \text{Cl}$). Yield 60%, Yellow solid mp 230°C . IR (KBr): 3431, 3285, 2929, 1613, 1582, 1506, 1441, 1226 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.26–1.58 (m, 5H), 1.58–1.72 (m, 3H), 1.84–1.94 (m, 2H), 2.58 (s, 2H), 6.47 (brs, 1H, NH), 6.84 (d, 1H, $J = 8.7\text{ Hz}$), 7.21 (d, 1H, $J = 8.7\text{ Hz}$), 7.35 (brs, 1H, NH), 7.21 (d, 1H, $J = 8.7\text{ Hz}$), 7.90 (d, 1H, $J = 3.0\text{ Hz}$), 8.82 (brs, 1H, NH). Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{ClN}_3\text{OS}$: C, 55.63; H, 5.60; N, 12.98. Found: C, 55.50; H, 5.65; N, 13.12%.

5b. ($\text{R}_1 = \text{R}_2 = \text{R}_3 = \text{H}$). Yield 54%, Yellow solid mp 198°C . IR (KBr): 3413, 3243, 3147, 2935, 1604, 1594, 1486, 1278 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.24–1.56 (m, 5H), 1.61–1.76 (m, 3H), 1.86–1.96 (m, 2H), 2.60 (s, 2H), 6.43 (brs, 1H, NH), 6.87–6.95 (m, 2H), 7.28 (t, 1H, $J = 6.9\text{ Hz}$), 7.35 (brs, 1H, NH), 7.85 (d, 1H, $J = 7.8\text{ Hz}$), 8.78 (brs, 1H, NH). Anal. Calcd for $\text{C}_{15}\text{H}_{19}\text{N}_3\text{OS}$: C, 62.25; H, 6.62; N, 14.52. Found: C, 62.60; H, 6.30; N, 14.40

5c. ($\text{R}_1 = \text{R}_2 = \text{H}$, $\text{R}_3 = \text{Br}$). Yield 55%, Yellow solid mp 185°C . IR (KBr): 3375, 3233, 3154, 2929, 1594, 1502, 1462, 1279 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.26–1.58 (m, 5H), 1.58–1.72 (m, 3H), 1.84–1.94 (m, 2H), 2.58 (s, 2H), 6.45 (brs, 1H, NH), 6.80 (d, 1H, $J = 8.7\text{ Hz}$), 7.34 (brs, 1H, NH), 7.35 (d, 1H, $J = 8.7\text{ Hz}$), 7.94 (d, 1H, $J = 2.4\text{ Hz}$), 8.78 (brs, 1H, NH). Anal. Calcd for $\text{C}_{15}\text{H}_{18}\text{BrN}_3\text{OS}$: C, 48.92; H, 4.93; N, 11.41. Found: C, 48.80; H, 4.59; N, 11.61%.

5d. ($\text{R}_1 = \text{R}_2 = \text{H}$, $\text{R}_3 = \text{CH}_3$). Yield 56%, Yellow solid mp 165°C . IR (KBr): 3404, 3257, 2929, 1593, 1513, 1489, 1291, 1228 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.22–1.54 (m, 5H), 1.58–1.72 (m, 3H), 1.84–1.94 (m, 2H), 2.28 (s, 3H), 2.57 (s, 2H), 6.44 (brs, 1H, NH), 6.79 (d, 1H, $J = 8.4\text{ Hz}$), 7.09 (dd, 1H, $J = 8.7, 2.1\text{ Hz}$), 7.36 (brs, 1H, NH), 7.62 (d, 1H, $J = 1.5\text{ Hz}$), 8.75 (brs, 1H, NH). Anal. Calcd for $\text{C}_{16}\text{H}_{21}\text{N}_3\text{OS}$: C, 63.33; H, 6.98; N, 13.85. Found: C, 63.08; H, 6.60; N, 13.56%.

5e. ($\text{R}_1 = \text{Cl}$, $\text{R}_2 = \text{H}$, $\text{R}_3 = \text{Cl}$). Yield 59%, Yellow solid mp 207°C . IR (KBr): 3421, 3259, 3153, 2933, 1644, 1600, 1485, 1267 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.22–1.46 (m, 3H), 1.49–1.59 (m, 2H), 1.62–1.79 (m, 3H), 1.90–1.99 (m, 2H), 2.62 (s, 2H), 6.51 (brs, 1H, NH), 7.31 (brs, 1H, NH), 7.36 (d, 1H, $J = 2.4\text{ Hz}$), 7.73 (d, 1H, $J = 2.4\text{ Hz}$), 8.84 (brs, 1H, NH). Anal. Calcd for $\text{C}_{15}\text{H}_{17}\text{Cl}_2\text{N}_3\text{OS}$: C, 50.28; H, 4.78; N, 11.73. Found: C, 50.12; H, 4.48; N, 11.60%.

5f. ($\text{R}_1 = \text{H}$, $\text{R}_2 = \text{CH}_3$, $\text{R}_3 = \text{Cl}$). Yield 58%, Yellow solid mp 237°C . IR (KBr): 3409, 3265, 3160, 2931, 1594, 1516, 1400, 1236 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.26–1.55 (m, 5H), 1.58–1.72 (m, 3H),

1.84-1.93 (m, 2H), 2.32 (s, 3H), 2.55 (s, 2H), 6.42 (brs, 1H, NH), 6.79 (s, 1H), 7.33 (brs, 1H, NH), 7.77 (s, 1H), 8.76 (brs, 1H, NH); Mass Spectra : [M+1] 338.02, [M+3] 340.02. Anal. Calcd for C₁₆H₂₀CIN₃OS: C, 56.88; H, 5.97; N, 12.44. Found: C, 57.04; H, 5.90; N, 12.50%.

5g. (R₁ = R₂ = H, R₃ = F). Yield 62%, Yellow solid mp 189°C. IR (KBr): 3429, 3282, 3160, 1630, 1594, 1494, 1215. cm⁻¹; ¹H NMR (DMSO-d₆) : δ 1.72-1.79 (m, 10H), 2.28 (s, 3H), 2.88 (s, 2H), 6.85 (dd, 1H, *J* = 8.7, 4.8 Hz), 7.06 (dd, 1H *J* = 8.4, 3.6 Hz), 7.17 (brs, 1H, NH), 7.53 (brs, 1H, NH), 8.11 (dd, 1H, *J* = 8.4, 3.6 Hz), 8.63 (s, 1H, NH). Anal. Calcd for C₁₅H₁₈FN₃OS: C, 58.61; H, 5.90; N, 13.67. Found: C, 58.55; H, 5.80; N, 13.50%.

Spiro[chroman-2,1'-cyclohexan]-4-one-5-spiro-4-acetyl-2-(acetylamino)-Δ²-1,3,4-thia-diazolines 6a

Compound **5a** (0.6g, 1.85 mmol) was treated with freshly distilled acetic anhydride (6mL) and the mixture was refluxed for 6-7 h on a water bath (90-100°C). The resulting contents were poured over crushed ice with vigorous stirring to give a pale yellow product, which was separated by filtration and purified by column chromatography to get pure 0.41g of **6a**. Compounds **6b-g** were prepared similarly. Physical and analytical data of **6a-g** are given below.

6^a. (R₁ = R₂ = H, R₃ = Cl). Yield 54%, Off-white solid mp 143°C. IR (KBr): 2933, 1698, 1640, 1615, 1479, 1410, 1292, 1236, 755. cm⁻¹ ¹H NMR (DMSO-d₆) : δ 1.24-1.36 (m, 3H), 1.42-1.68 (m, 5H), 1.76-1.88 (m, 2H), 2.04 (s, 3H), 2.22 (s, 3H), 2.45 (d, 1H, *J* = 14.1 Hz), 3.29 (d, 1H, *J* = 14.1 Hz), (6.71 (d, 1H, *J* = 8.7 Hz), 7.05 (dd, 1H, *J* = 8.7, 2.4 Hz), 7.29 (d, 1H, *J* = 2.4 Hz), 9.24 (s, 1H, due to NH). Anal. Calcd for C₁₉H₂₂CIN₃O₃S: C, 55.94; H, 5.44; N, 10.30. Found: C, 55.70; H, 5.40; N, 10.20%.

6b. (R₁ = R₂ = R₃ = H). Yield 61%, Off-white solid mp 157°C. IR (KBr): 3175, 1699, 1622, 1582, 1508, 1248, 1234, 770. cm⁻¹; ¹H NMR (DMSO-d₆) : δ 1.22-1.38 (m, 3H), 1.42-1.68 (m, 5H), 1.76-1.90 (m, 2H), 1.99 (s, 3H), 2.22 (s, 3H), 2.46 (d, 1H, *J* = 13.8 Hz), 3.32 (d, 1H, *J* = 14.1 Hz), 6.75 (dd, 1H, *J* = 7.8, 1.2 Hz), 6.86 (t, 1H, *J* = 7.8 Hz), 7.10 (t, 1H, *J* = 7.8 Hz), 7.33 (dd, 1H, *J* = 7.8, 1.5 Hz), 9.40 (s, 1H, due to NH). Anal. Calcd for C₁₉H₂₃N₃O₃S: C, 61.10; H, 6.21; N, 11.25. Found: C, 61.30; H, 6.15; N, 11.20%.

6c. (R₁ = R₂ = H, R₃ = Br). Yield 55%, Off-white solid mp 171°C. IR (KBr): 2935, 1692, 1612, 1583, 1482, 1247, 1229, 946, 760. cm⁻¹; ¹H NMR (DMSO-d₆) : δ 1.22-1.38 (m, 3H), 1.42-1.68 (m, 5H), 1.76-1.90 (m, 2H), 2.07 (s, 3H), 2.22 (s, 3H), 2.45 (d, 1H, *J* = 14.1 Hz), 3.30 (d, 1H, *J* = 14.1 Hz), 6.66 (d, 1H, *J* = 9.0 Hz), 7.19 (dd, 1H, *J* = 9.0, 2.4 Hz), 7.43 (d, 1H, *J* = 2.4 Hz), 8.88 (s, 1H, due to NH). Anal. Calcd for C₁₉H₂₂BrN₃O₃S: C, 50.45; H, 4.90; N, 9.29. Found: C, 50.28; H, 4.80; N, 9.55%.

6d. (R₁ = R₂ = H, R₃ = CH₃). Yield 61%, Off-white solid mp 108 °C. IR (KBr): 2932, 1699, 1673, 1642, 1617, 1495, 1405, 1235, 752. cm⁻¹; ¹H NMR (DMSO-d₆) : δ 1.24-1.36 (m, 3H), 1.42-1.68 (m, 5H), 1.76-1.88 (m, 2H), 2.03 (s, 3H), 2.22 (s, 6H), 2.44 (d, 1H, *J* = 14.4 Hz), 3.30 (d, 1H, *J* = 14.4 Hz), 6.66 (d, 1H, *J* = 8.4 Hz), 6.90 (dd, 1H, *J* = 8.1, 1.8 Hz), 7.10 (d, 1H, *J* = 1.5 Hz), 9.15 (s, 1H, due to NH). Anal. Calcd for C₂₀H₂₅N₃O₃S: C, 61.99; H, 6.50; N, 10.84. Found: C, 61.68; H, 6.40; N, 10.70%.

6e. ($R_1 = \text{Cl}$, $R_2 = \text{H}$, $R_3 = \text{Cl}$). Yield 65%, Off-white solid mp 140 °C. IR (KBr): 2933, 1698, 1643, 1619, 1455, 1404, 1293, 1245. cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.24-1.34 (m, 3H), 1.36-1.65 (m, 5H), 1.66-1.98 (m, 2H), 2.05 (s, 3H), 2.23 (s, 3H), 2.45 (d, 1H, $J = 14.1$ Hz), 3.31 (d, 1H, $J = 14.1$ Hz), 7.20 (d, 1H, $J = 2.4$ Hz), 7.21 (d, 1H, $J = 2.4$ Hz), 9.36 (s, 1H, due to NH). Anal. Calcd for $\text{C}_{19}\text{H}_{21}\text{Cl}_2\text{N}_3\text{O}_3\text{S}$: C, 51.59; H, 4.78; N, 9.50. Found: C, 51.20; H, 4.50; N, 9.30%.

6f. ($R_1 = \text{H}$, $R_2 = \text{CH}_3$, $R_3 = \text{Cl}$). Yield 65%, Off-white solid mp 224 °C. IR (KBr): 2930, 1694, 1639, 1620, 1490, 1408, 1243 cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.24-1.34 (m, 3H), 1.40-1.68 (m, 5H), 1.77-1.87 (m, 2H), 2.05 (s, 3H), 2.21 (s, 3H), 2.26 (s, 3H), 2.43 (d, 1H, $J = 14.1$ Hz), 3.29 (d, 1H, $J = 14.1$ Hz), 6.66 (s, 1H), 7.28 (s, 1H), 9.08 (s, 1H, due to NH); Mass Spectra : $[\text{M}+1]$ 422.79, $[\text{M}+3]$ 444.22. Anal. Calcd for $\text{C}_{20}\text{H}_{24}\text{ClN}_3\text{O}_3\text{S}$: C, 56.93; H, 5.73; N, 9.96. Found: C, 56.80; H, 5.40; N, 9.70%.

6g. ($R_1 = R_2 = \text{H}$, $R_3 = \text{F}$). Yield 64%, Off-white solid mp 199 °C. IR (KBr): 2935, 1689, 1641, 1625, 1486, 1247, 1235, 948. cm^{-1} ; ^1H NMR (DMSO- d_6): δ 1.20-1.80 (m, 10H), 2.05 (s, 3H), 2.12 (s, 3H), 2.51 (d, 1H, $J = 14.1$ Hz), 3.08 (d, 1H, $J = 14.1$ Hz), 6.77 (m, 1H), 6.96 (m, 1H), 7.03 (m, 1H), 11.70 (s, 1H, due to NH). Anal. Calcd for $\text{C}_{19}\text{H}_{22}\text{FN}_3\text{O}_3\text{S}$: C, 58.30; H, 5.66; N, 10.73. Found: C, 58.24; H, 5.45; N, 10.60%.

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