Novel spiroheterocycles containing a 1,3,4-thiadiazole unit: Synthesis and spectroscopic characterization

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Abstract
We describe the preparation of a series of novel spiroheterocycles, namely 1,3,4-thiadiazole derivatives possessing an indane unit. These active heterocyclic compounds are prepared starting from thiocarbohydrazide and 2-indanone via an intermediate indan-2-thiocarbohydrazone which is used to afford the corresponding 2-(1-acetylhydrazino)-4H-acetyl-5-spiro(indano-2-yl)-1,3,4-thiadiazoline in an acidic medium. The 1,3,4-thiadiazole derivatives are obtained in good yields by reaction with aromatic carboxylic acids at reflux temperature in the presence of POCl₃ as a catalyst, their structure–activity relationships are discussed and the structures of the newly synthesized derivatives are confirmed by spectroscopic techniques.

Keywords
1,3,4-thiadiazoles, bioactivity, indanone, spiroheterocycles, structure–activity relationships

Introduction
Heterocyclic compounds are important structures in organic chemistry because of their roles in pharmaceuticals, materials science, and medicinal research (they are ubiquitous in drug molecules), while the presence of heteroatoms can modify many other molecular properties.¹ According to the literature, nitrogen-containing heterocyclic molecules are of great interest due to their biological and industrial significance.²,³ Heterocyclic derivatives

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Compounds with heterocyclic rings, including spiroheterocycles with five-membered rings at symmetrical positions such as 1,3,4-thiadiazole derivatives, have been studied extensively due to their interesting bioactivity profiles and anti-inflammatory activity with reduced ulcerogenic effects. Small molecules containing 5-membered heterocyclic moieties have received significant interest in the design of antitumor agents. For example, 1,3,4-thiadiazoles are important in a wide range of applications in various scientific fields, such as in the pharmaceutical industry, drug discovery, as scintillating materials, and in the dyestuff industry. Besides, thiadiazole rings are deficient in π electrons and do not react easily on carbon or nitrogen via electrophilic attack. However, electrophilic attack on an azine ring can occur in the presence of electron-donating substituents. Therefore, 1,3,4-thiadiazole derivatives have the ability to interfere with processes associated with DNA replication. As a result, they can inhibit the replication of both bacterial and cancer cells. Thiadiazole rings exhibit potent structural–activity relationships, which result in unique chemical phenomena and valuable biological properties.

Heterocyclic moieties have diverse activities depending on their molecular structure. For example, 1,3,4-thiadiazole moieties are present in antidiabetic, anticancer, anti-inflammatory, antispasmodic, antiviral, antihypertensive, and antibacterial drugs. Medicines based on a thiadiazole moiety include butazolamide and acetazolamide.

In light of the great importance of spirocyclic systems containing a 1,3,4-thiadiazole unit, the present study focuses on the synthesis of spiro compounds consisting of a 1,3,4-thiadiazoline moiety and different substituents with the potential to act as active pharmacophores. The desired compounds are obtained from thiocarbohydrazide 1 and 2-indanone via the hydrazone derivative 2 (Scheme 1). The adopted protocol has advantages of reduced environmental pollution, is high yielding and delivers high purity products. Also, the final products are obtained through an efficient, catalytic and solvent-free reactions.

**Results and discussion**

A series of new spiro derivatives 4a-h containing a 1,3,4-thiadiazole unit has been synthesized by refluxing a mixture of the hydrazide 3 in phosphorus oxychloride in the presence of aromatic carboxylic acids (Scheme 1). The nucleophilic addition reaction of hydrazine with carbon disulfide followed by attack of another hydrazine molecule and loss of hydrogen sulfide gave the hydrazide 1. The formation of thiocarbohydrazide 1 was confirmed from its physical and spectral data. Compound 1 then underwent a condensation reaction with 2-indanone in the presence of glacial acetic acid to form hydrazide 2. Next, acetic anhydride was employed to mediate the cyclization of 2 to give 2-(1-acetylhydrazino)-4H-acetyl-5-spiro(indan-2-yl)-1,3,4-thiadiazoline (3) through a thermal reaction according to Scheme 2.

In addition, in vitro anticancer activities of 1,3,4-thiadiazolo derivatives have been assessed against human tumor
cell lines and exhibit different cytotoxic effects. An efficient synthesis of dihydrothiadiazoles was accomplished via intramolecular cyclization by condensation of the 2-thiocarbohydrazide derivative with acetic anhydride in the presence of pyridine. The cyclization required higher reaction temperatures in order to form the nitrogen-containing heterocycles. The formation of indane-2-thiocarbohydrazide was confirmed from its physical and spectral data. In the FT-IR spectrum, compound showed the presence of a C=N stretching band at 1597 cm⁻¹ and the aromatic system was represented by a C=H stretching band at 3036 cm⁻¹. In the ¹H NMR spectrum, the signals at 9.30 ppm (s, NH-N=C), 2.26 ppm (s, CH₂ indane), and 7.13–7.22 ppm (aromatic hydrogens) confirmed the formation of compound.

The formation of spiro compound was proved by a clear shift in the UV spectrum at λ_max 346 and 258 nm. Also, in the FT-IR spectrum, the absence of the C=S stretching band at 1280 cm⁻¹ and the appearance of a C-S-C stretching band at 748 cm⁻¹ provided a clear indication that the reaction had proceeded toward formation of the spiro system. Spiro compound structure of 3 was also confirmed by the ¹H NMR spectrum, in particular by the appearance of a singlet at 2.50 ppm corresponding to the acetyl group (H₃C-C=O).

Compound 3 was used as the hydrazide source in reactions with substituted benzoic acids to prepare the target compounds 4a-h. These reactions occurred by conventional heating through a free-solvent process in the presence of catalytic amounts of phosphorous oxychloride (POCl₃).

The formation of the final compounds 4a-h (Figure 1) was monitored by TLC. In addition, the UV spectra showed absorption bands at λ_max 258–394 nm and 236–258 nm due to an increase of the electron density of the ring system and π→π* transitions, respectively.

The formation of compounds 4a-h was confirmed by spectroscopic techniques. In the FT-IR spectra, the presence of amide NH and C=O absorption peaks confirmed the proposed structures. In the ¹H NMR spectra, the absence of hydroxy groups belonging to the benzoic acid derivatives and the presence of 8.01 ppm signals due to the NH-N=C=O groups gives clear evidence for the formation of the target compounds. The signals corresponding to indane protons were observed between (4.5–3.5) ppm as singlets, which were in agreement with the expected structures.

**Conclusion**

The present study reports the synthesis and characterization of novel spiroheterocyclic compounds based on 1,3,4-thiadiazole derivatives derived from thiocarbohydrazide. The final step was performed through traditional heating in the presence of phosphorus oxychloride using a free-solvent and represents as an easy procedure to obtain spirothiadiazoles 4a-h with high purities and yields. The structures of the products were confirmed by spectral methods.

**Experimental**

The melting points (mp) were measured with an SMP30 Stuart electric melting point device. ¹H NMR spectra were recorded at the Turkish EGE University using a 400 MHz spectrometer, and DMSO-d₆ or CDCl₃ as the solvent. FT-IR spectra were recorded on Shimadzu Prestige-21, spectrometer as KBr disks. Ultraviolet (UV) spectra were obtained on a T92+UV spectrophotometer (PG instruments) using methanol as the solvent. Thin-layer chromatography was performed using aluminum plates (20 x 20 cm) coated with silica gel 60 F254 (Merck), and column chromatography was performed using silica gel (Sigma-Aldrich) and petroleum ether (40–60) methanol as the eluent (20:80).

**Thiocarbohydrazide (1)**

Carbon disulfide (13 mL, 22 mmol) was gradually added to a vigorously stirred solution of 85% hydrazine hydrate (24 mL, 44 mmol) in distilled water (15 mL) over 30 min. The reaction temperature was raised to 100–110 °C, and the mixture was refluxed for 2 h before being cooled in an ice bath.
The precipitated thiocarbohydrazide was washed with ethanol and recrystallized from a minimum amount of hot water to give compound 1. Pale yellow solid; yield: 14 g (76%); mp 169–170 °C; R_f = 0.720 (hexane/EtOAc = 5:1). 1H NMR (400 MHz, CDCl_3): δ 7.81 (s, 2H, NH), 2.72 (s, 4H, NH_2). FT-IR (KBr): 3306 (NH_2), 3274, 3203 (NH), 1489 (N-N), 1384, (C-N), 1282 (C=S) cm⁻¹; UV (MeOH): λ_max = 355, 272 nm.

### Indan-2-thiocarbohydrazone (2)

In a round-bottomed flask (100 mL), 2-indanone (1.32 g, 10 mmol) was dissolved in methanol (25 mL) and a solution of thiocarbohydrazide 1 (1.06 g, 10 mmol) was added dropwise followed by glacial acetic acid (2 drops) to the stirred mixture. After heating at reflux temperature for 8 h, the mixture was cooled, filtered, and evaporated. The residue was washed with cold water to remove the acid. Recrystallization from ethanol gave compound 2. Light gray solid; yield: 1.1 g (50%); mp 182–183 °C; R_f = 0.600 (hexane/EtOAc = 5:1). 1H NMR (400 MHz, CDCl_3): δ 9.30 (s, 1H, NH-N=C), 7.13–7.22 (m, 4H, aromatic), 2.98 (s, 1H, S=C-NH), 2.26 (s, 4H, CH_2 indane), 1.90 (s, 2H, NH_2). FT-IR (KBr): 3390 (NH_2), 3204 (NH), 3036 (C=H), 2922, 2744 (CH_3), 1597 (N=C), 1280 (C=S) cm⁻¹; UV (MeOH): λ_max = 354, 246 nm.

### 2-(1-Acetylhydrazino)-4H-acetyl-5'-spiro(indano-2-yl)-1,3,4-thiadiazoline (3)

To a round-bottomed flask, acetic anhydride (1.5 mL) was slowly added to the solution of compound 2 (0.4 g, 1.8 mmol) in pyridine (5 mL). The mixture was stirred until the acetic anhydride was consumed (TLC). The reaction was then refluxed for 3 h and cooled by adding ice-water to quench the excess of acid which was formed during the reaction. The resulting precipitate was recrystallized from H_2O-EtOH to give compound 3. Gray solid; yield 0.45 g (82%); mp 200–201 °C; R_f = 0.686 (hexane/EtOAc = 5:1). 1H NMR (400 MHz, CDCl_3): δ 8.58 (s, 1H, NH-C=O), 7.75–7.35 (m, 4H, ArH), 2.5 (s, 3H, H_3C-C=O), 2.26 (s, 4H, CH_2 indane), 2.03 (s, 1H, NH-NH-C=O). FT-IR (KBr): 3244 (NH), 3058 (C=H), 2922, 2744 (CH_3), 1707, (C=O), 1597 (N=C cycl.), 1015 (N-N), 748 (C=S-C) cm⁻¹; UV (MeOH): λ_max = 346, 258 nm.

### Compounds 4a-h

A mixture of compound 3 (0.16 g, 5 mmol) and the corresponding benzoic acid derivative (5 mmol) in a beaker (50 mL) was grinded to a smooth powder. Next, phosphorus oxychloride (1 mL, 11 mmol) was gradually added while stirring, and then, the reaction mixture was reflux for 4 h. The mixture was poured into ice-water after cooling and neutralized with sodium bicarbonate. The resulting solid was dried, and recrystallized from methanol to give the product 4.

N’-Acetyl-N-(3’-acetyl-1,3-dihydro-3’H-spiro[indene-2,2’-[1,3,4]thiadiazol]-5’-yl)benzohydrazide (4a). Bright yellow solid; yield 0.17 g (80%); mp 118–119 °C; R_f = 0.760 (hexane/EtOAc = 5:1). UV (MeOH): λ_max = 376, 234 nm. 1H NMR (400 MHz, CDCl_3): δ 8.1–7.6 (m, 9H, ArH), 8.1–7.6 (m, 5H, NH), 3.6 (m, 4H, S=C-NH).
CH₃ indane), 2.5 (s, 3H, H,C-C=O), 2.3 (s, 3H, H,C-C=O). FT-IR (KBr): 2924 (C-H), 2866 (CH₃ indane), 1704 (C=O amide), 1597 (C=N cycl.), 1172 (C-O-C cycl.), 1017 (N-N), 709 (C-S-C) cm⁻¹.

N'-Acetyl-N-(3'-acetyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4] thiadiazol]-5'-yl)-2-chlorobenzohydrazide (4b). Bright yellow solid; yield 0.19 g (94%); mp 153–154 °C; Rf 0.754 (hexane/EtOAc = 5:1); UV (MeOH): λmax = 374, 236 nm. ¹H NMR (400 MHz, CDCl₃): δ 8.0–7.4 (m, 8H, ArH), 3.9 (m, 4H, CH₃ indane), 2.5 (s, 3H, H,C-C=O), 2.3 (s, 3H, H,C-C=O). FT-IR (KBr): 3034 (C–H), 2888 (CH₂ indane), 1692 (C=O amide), 1597 (C=N cycl.), 1194 (C-O-C cycl.), 1019 (N-N), 708 (C-S-C) cm⁻¹.

N'-Acetyl-N-(3'-acetyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4] thiadiazol]-5'-yl)-4-methylbenzohydrazide (4c). Yellow solid; yield 0.12 g (57%); mp 193–194 °C; Rf 0.740 (hexane/EtOAc = 5:1); UV (MeOH): λmax = 394, 258 nm. ¹H NMR (400 MHz, CDCl₃): δ 8.0–7.5 (m, 8H, ArH), 3.5 (m, 4H, CH₃ indane), 2.5 (s, 3H, H,C-C=O), 2.0 (s, 3H, H,C-C=O). FT-IR (KBr): 3054 (C–H), 2928 (CH₂ indane), 1680 (C=O amide), 1589 (C=N cycl.), 1283 (C-O-C cycl.), 1122 (N-N), 698 (C-S-C) cm⁻¹.

N'-Acetyl-N-(3'-acetyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4] thiadiazol]-5'-yl)-4-chlorobenzohydrazide (4d). Bright yellow solid; yield 0.1 g (51%); mp 126–128 °C; Rf 0.784 (hexane/EtOAc = 5:1); UV (MeOH): λmax = 394, 258 nm. ¹H NMR (400 MHz, CDCl₃): δ 8.0–7.5 (m, 8H, ArH), 3.5 (m, 4H, CH₃ indane), 2.5 (s, 3H, H,C-C=O), 2.0 (s, 3H, H,C-C=O). FT-IR (KBr): 3045 (C–H), 2928 (CH₃ indane), 1680 (C=O amide), 1588 (C=N cycl.), 1274 (C-O-C cycl.), 1122 (N-N), 754 (C-Cl), 714 (C-S-C) cm⁻¹.

N'-Acetyl-N-(3'-acetyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4] thiadiazol]-5'-yl)-4-methylbenzohydrazide (4e). Bright yellow solid; yield 0.13 g (51%); mp 100–103 °C; Rf 0.673 (hexane/EtOAc = 5:1); UV (MeOH): λmax = 320, 236 nm. ¹H NMR (400 MHz, CDCl₃): δ 8.0–7.5 (m, 8H, ArH), 3.5 (m, 4H, CH₃ indane), 2.5 (s, 6H, H,C-C=O), 2.0 (s, 3H, H,C-C=O). FT-IR (KBr): 2956 (C-H), 2822 (CH₃ indane), 1678 (C=O amide), 1587 (C=N cycl.), 1281 (C-O-C cycl.), 1030 (N-N), 697 (C-S-C) cm⁻¹.

N'-Acetyl-N-(3'-acetyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4] thiadiazol]-5'-yl)-2-nitrobenzohydrazide (4f). Bright yellow solid; yield 0.12 g (56%); mp 259–260 °C; Rf 0.660 (hexane/EtOAc = 5:1); UV (MeOH): λmax = 376, 242 nm. ¹H NMR (400 MHz, CDCl₃): δ 8.2–7.6 (m, 8H, ArH), 3.5 (m, 4H, CH₃ indane), 2.5 (s, 3H, H,C-C=O), 2.0 (s, 3H, H,C-C=O). FT-IR (KBr): 3037 (C–H), 2927 (CH₃ indane), 1716 (C=O amide), 1597 (C=N cycl.), 1274 (C-O-C cycl.), 1014 (N-N), 708 (C-S-C) cm⁻¹.

N'-Acetyl-N-(3'-acetyl-1,3-dihydro-3'H-spiro[indene-2,2'-[1,3,4] thiadiazol]-5'-yl)-3-chlorobenzohydrazide (4c). Bright yellow solid; yield 0.1 g (51%); mp 126–128 °C; Rf 0.784 (hexane/EtOAc = 5:1); UV (MeOH): λmax = 258, 236 nm. ¹H NMR (400 MHz, CDCl₃): δ 8.0–7.5 (m, 8H, ArH), 3.5 (m, 4H, CH₃ indane), 2.7 (s, 3H, OCH₃), 2.1 (s, 6H, H,C-C=O). FT-IR (KBr): 3036 (C–H), 2922 (CH₃ indane), 1716 (C=O amide), 1596 (C=N cycl.), 1188 (C-O-C cycl.), 1107 (N-N), 747 (C-Cl), 707 (C-S-C) cm⁻¹.

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S.H.K. and M.H.A. contributed to the conceptualization and writing of the original draft. The authors have read and agreed to the published version of the manuscript.

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Our study did not require an ethical board approval because it did not contain human or animal trials.

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Supplemental material
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