Synthesis, Antimicrobial and Antiinflammatory Activities of Novel 5-(1-Adamantyl)-1,3,4-thiadiazole Derivatives

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Original article

Synthesis, antimicrobial and anti-inflammatory activities of novel 5-(1-adamantyl)-1,3,4-thiadiazole derivatives

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ABSTRACT

New 1-adamanyl-1,3,4-thiadiazole derivatives namely, 5-(1-adamantyl)-1,3,4-thiadiazoline-2-thione **3**, 5-(1-adamantyl)-3-(benzyl- or 4-substituted benzyl)-1,3,4-thiadiazoline-2-thione **4a**–**d**, 5-(1-adamantyl)-3-(4-substituted-1-piperazinylmethyl)-1,3,4-thiadiazoline-2-thiones **5a**–**c**, 2-[5-(1-adamantyl)-2-thioxo-1,3,4-thiadiazolin-3-yl]acetic acid **7**, (±)-2-[5-(1-adamantyl)-2-thioxo-1,3,4-thiadiazolin-3-yl] propionic acid **9**, 3-[5-(1-adamantyl)-2-thioxo-1,3,4-thiadiazolin-3-yl]propionic acid **11**, *N*-[5-(1-adamantyl)-1,3,4-thiadiazol-2-yl]-*N*′-arylthioureas **15a**–**c** and 5-(1-adamantyl)-1,3,4-thiadiazoline-2-one **16**, were synthesized and tested for in vitro activities against a panel of Gram-positive and Gram-negative bacteria and the yeast-like pathogenic fungus *Candida albicans*. Compounds **7**, **9**, **15b** and **15c** displayed marked activity against the tested Gram-positive bacteria, while compound **3** was highly active against the tested Gram-negative bacteria. Compounds **4b**, **7** and **15c** were weakly or moderately active against *C. albicans*. In addition, the in vivo anti-inflammatory activity of the synthesized compounds was determined using the carrageenan-induced paw oedema method in rats. The propionic acid derivative **9** produced good dose-dependent anti-inflammatory activity.

1. Introduction

Covalent conjugation of biologically active compounds acting by different mechanisms could, in a favorable case, lead to synergism to obtain compounds with improved activity and reduced toxicity. Adamantane derivatives has long been known for their antiviral activity against Influenza A [1–4] and HIV viruses [5–8]. Several adamantane derivatives were also associated with central nervous [9–11], antimicrobial [6,12–15], and anti-inflammatory activities [12,13,15–18]. In addition, 1,3,4-thiadiazole nucleus constitutes the active part of several biologically active compounds, including antibacterial [19–21], antimycotic [22,23], and anti-inflammatory agents [24–26]. Acetic acid and propionic acid derivatives constitute the most important class of nonsteroidal anti-inflammatory agents [27].

Recently, we reported the anti-inflammatory and antimicrobial activities of novel series of 3-(1-adamantyl)-substituted-1,2,4-triazolin-5-thiones and 2-(1-adamantyl)-1,3,4-oxadiazolin-5-thiones, carrying an acetic or propionic acid moiety [12,13,15]. In continuation to our interest in the chemical and pharmacological properties of adamantane derivatives, we report herein the synthesis, antimicrobial, and anti-inflammatory activities of new series of 5-(1-adamantyl)-1,3,4-thiadiazoles and related derivatives.

2. Results and discussion

2.1. Chemistry

5-(1-Adamantyl)-1,3,4-thiadiazoline-2-thione **3** was prepared starting from adamantane-1-carboxylic acid hydrazide **1** *via* treatment with potassium hydroxide and carbon disulphide to afford potassium *N*′-(1-adamantylcarbonyl) dithiocarbazate **2** in almost quantitative yield [28]. Dehydrative cyclization of compound **2** using sulphuric acid at room temperature yielded the target compound **3**, which was reacted with benzyl- or 4-substituted benzyl chlorides to yield the corresponding 5-(1-adamantyl)-3-(benzyl- or 4-substituted benzyl)-1,3,4-thiadiazoline-2-thiones **4a**–**d**. On the other hand, the reaction of compound **3** with 1-methyl-, ethyl- or phenylpiperazine and formaldehyde solution in ethanol at room temperature yielded the corresponding

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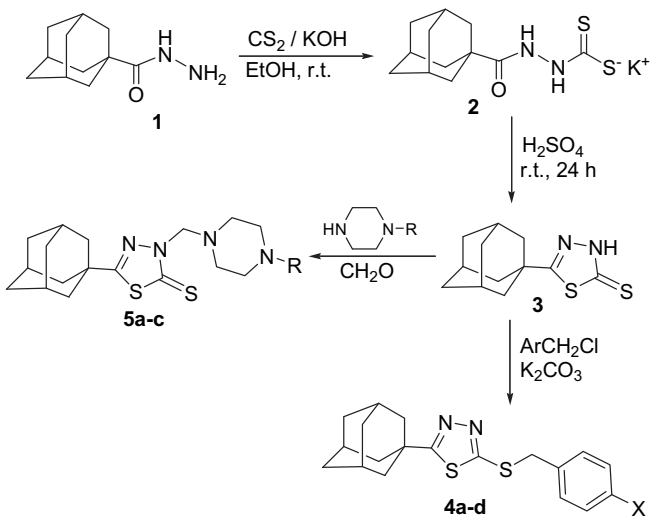


Scheme 1. Synthesis of compounds **4a**–**d** and **5a**–**c**.

5-(1-adamantyl)-3-(4-substituted-1-piperazinylmethyl)-1,3,4-thiadiazoline-2-thiones **5a**–**c** (Scheme 1, Table 1).

The reaction of compound **3** with ethyl bromoacetate, ±-ethyl 2-bromopropionate or ethyl 3-bromopropionate, in ethanol, in the presence of anhydrous potassium carbonate yielded the corresponding ethyl esters **6**, **8** and **10**, which were consequently hydrolyzed by heating in 10% aqueous sodium hydroxide solution to afford the corresponding carboxylic acids **7**, **9** and **11** (Scheme 2, Table 1).

5-(1-Adamantyl)-2-amino-1,3,4-thiadiazole **14** was previously prepared in 48% yield from adamantane-1-carboxylic acid hydrazide **1** *via* reaction with potassium thiocyanate and hydrochloric acid to yield 1-(1-adamantylcarbonyl)-3-thiosemicarbazide **12**, followed by dehydrative cyclization with sulphuric acid at room temperature (Method A) [29]. Compound **14** was also prepared in 59% yield *via* one-step three-component reaction of adamantane-1-carboxylic acid **13**, thiosemicarbazide and phosphorus oxychloride (Method B). Compound **14** was reacted with phenyl-, 4-fluorphenyl- or 4-chlorophenylisothiocyanate to yield the corresponding *N*-[5-(1-adamantyl)-1,3,4-thiadiazol-2-yl]-*N*′-arylthioureas **15a**–**c** in poor yields. 5-(1-Adamantyl)-1,3,4-thiadiazoline-2-one **16** was prepared through deamination of compound **14** *via* treatment with sodium nitrite in cold aqueous hydrochloric acid solution followed by boiling for 10 min (Scheme 3, Table 1). The structures of all the newly synthesized compounds were confirmed by elemental analyses in addition to the IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data which were in full agreement with their structures.

2.2. In vitro antimicrobial activity

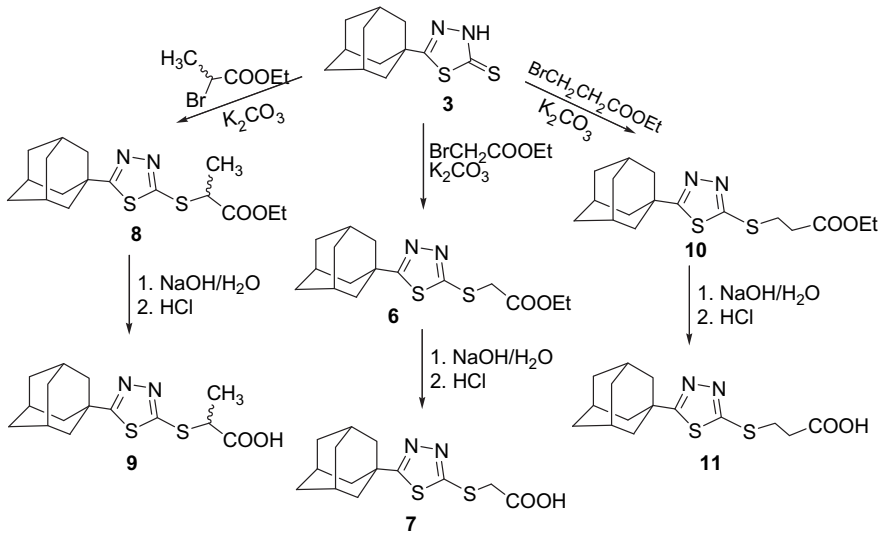
The newly synthesized compounds **3**, **4a**–**d**, **5a**–**c**, 7, **9**, **11**, **15a**–**c** and **16** were tested for their in vitro growth inhibitory activity against the standard strains of the Institute of fermentation of Osaka (IFO) namely; *Staphylococcus aureus* IFO 3060, *Bacillus subtilis* IFO 3007, *Micrococcus luteus* IFO 3232 (Gram-positive bacteria), *Escherichia coli* IFO 3301, *Pseudomonas aeruginosa* IFO 3448 (Gram-negative bacteria), and the yeast-like pathogenic fungus *Candida albicans* IFO 0583. The primary screening was carried out using the agar disc-diffusion method using Müller–Hinton agar medium [30].

The results of the preliminary antimicrobial testing of compound **3**, **5**, **4a**–**d**, **5a**–**c**, **7**, **9**, **11**, **15a**–**c** and **16** (200 μg/disc), the antibacterial antibiotics Ampicillin trihydrate, Gentamicin (100 μg/disc) and the antifungal drug Clotrimazole (100 μg/disc) are shown in Table 2. The results revealed that the compounds showed varying degrees of inhibition against the tested microorganisms. In general, strong activity was displayed by the compounds **3**, **7**, **9**, **15b** and **15c**, which produced growth inhibition zones ≥19 mm against one or more of the tested microorganisms. Meanwhile, compounds **5a**, **5c** and **15a** showed moderate activity (growth inhibition zones 14–18 mm), compounds **4b**, **5b** and **11** exhibited weak activity (growth inhibition zones 10–13 mm) and compounds **4a**, **4c**, **4d** and **16** were practically inactive against the tested microorganisms. The Gram-positive bacteria *B. subtilis* and to a lesser extent *S. aureus* and *M. luteus* are considered the most sensitive among the tested microorganisms. The tested compounds were generally inactive against the Gram-negative bacteria, only compound **3** showed strong activity against *E. coli* and *P. aeruginosa*. The inhibitory activity of the compounds against *C. albicans* was rather lower than their antibacterial activity, only compounds **4b**, **7** and **11** displayed moderate or weak activity. The minimal inhibitory concentrations (MIC) [31] for the most active compounds **3**, **7**, **9**, **15b**, **15c** which are shown in Table 3, were in accordance with the results obtained in the primary screening.

According to the results of the antimicrobial activity, it seems difficult to abstract definite structure–activity relationship. However, we can conclude that the antibacterial activity greatly diminished on introduction of the benzyl- or 4-substituted benzyl moieties (compounds **4a**–**d**). Meanwhile, the introduction of the 4-substituted-1-piperazinylmethyl moieties (compounds **5a**–**c**) led

Table 1
Melting points, yield percentages, molecular formulae, molecular weights and analytical data of compounds **4a**–**d**, **5a**–**c**, **7**, **9**, **11**, **15a**–**c** and **16**.

Comp. No.	R/X	M.p. (°C)	Yield (%)	Mol. Formula (Mol. Wt.)	Analysis: % Calcd. (Found)			
					C	H	N	S
3	–	155–6	62	$C_{12}H_{16}N_2S_2$ (252.4)	57.10 (57.23)	6.39 (6.51)	11.10 (10.89)	25.41 (25.12)
4a	H	139–41	75	$C_{19}H_{22}N_2S_2$ (342.52)	66.62 (66.40)	6.47 (6.52)	8.18 (8.09)	18.72 (18.65)
4b	F	133–5	77	$C_{19}H_{21}FN_2S_2$ (360.51)	63.30 (63.20)	5.87 (5.90)	7.77 (7.68)	17.79 (17.58)
4c	Cl	148–50	86	$C_{19}H_{21}ClN_2S_2$ (376.97)	60.54 (60.56)	5.62 (5.64)	7.43 (7.42)	17.01 (16.96)
4d	NO_2	196–8	90	$C_{19}H_{21}N_3O_2S_2$ (387.52)	58.89 (58.90)	5.46 (5.47)	10.84 (10.84)	16.55 (16.53)
5a	CH_3	93–5	36	$C_{18}H_{28}N_4S_2$ (364.57)	59.30 (59.25)	7.74 (7.81)	15.37 (15.40)	17.59 (17.55)
5b	C_2H_5	112–4	45	$C_{19}H_{30}N_4S_2$ (378.60)	60.28 (60.02)	7.99 (8.12)	14.80 (14.68)	16.94 (16.90)
5c	C_6H_5	149–51	52	$C_{23}H_{30}N_4S_2$ (426.64)	64.75 (64.52)	7.09 (7.11)	13.13 (13.10)	15.03 (15.0)
7	–	208–10	59	$C_{14}H_{18}N_2O_2S_2$ (310.43)	54.17 (54.15)	5.84 (5.85)	9.02 (9.0)	20.66 (20.51)
9	–	150–2	62	$C_{15}H_{20}N_2O_2S_2$ (324.46)	55.53 (55.43)	6.21 (6.20)	8.63 (8.61)	19.77 (19.58)
11	–	149–51	55	$C_{15}H_{20}N_2O_2S_2$ (324.46)	55.53 (55.38)	6.21 (6.27)	8.63 (8.60)	19.77 (19.76)
15a	H	295–7	33	$C_{19}H_{22}N_4S_2$ (370.53)	61.59 (61.60)	5.98 (6.11)	15.12 (15.10)	17.31 (17.28)
15b	4-F	>300	27	$C_{19}H_{21}FN_4S_2$ (388.53)	58.74 (58.71)	5.45 (5.55)	14.42 (14.39)	16.51 (16.50)
15c	4-Cl	291–3	34	$C_{19}H_{21}ClN_4S_2$ (404.98)	56.35 (56.40)	5.23 (5.24)	13.83 (13.62)	15.84 (15.68)
16	–	165–7	61	$C_{12}H_{16}N_2OS$ (236.33)	60.99 (61.03)	6.82 (6.84)	11.85 (12.02)	13.57 (13.48)

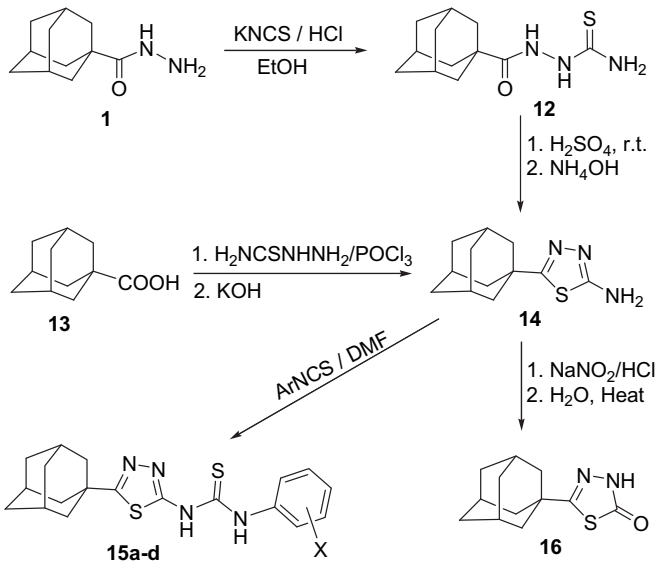


Scheme 2. Synthesis of compounds 7, 9 and **11**.

to the lack of the Gram-negative activity, while the moderate Gram-positive activity was retained. The acetic and propionic acid derivatives **7**, **9** and **11** were characterized by marked activity against the Gram-positive bacteria but totally inactive against the Gram-negative bacteria. In addition, compound **7** showed moderate activity against *C. albicans*. The thiourea derivatives **15a**–**c** were generally active against the Gram-positive bacteria and totally inactive against the tested Gram-negative bacteria and *C. albicans*. The thiadiazoline-2-one 16 was completely inactive against the tested microorganisms.

2.3. Acute anti-inflammatory activity

The acute in vivo anti-inflammatory activity of the newly synthesized compounds **4a**, **5b**, **7**, **9** and **11** was determined following the carrageenan-induced paw oedema method in rats [32]. The selection of the representative compounds and dose levels was made after carrying out pilot experiments which showed the absence of significant anti-inflammatory activity in compounds **3**, **15a**–**c** and **16**.



Scheme 3. Synthesis of compounds **15a**–**c** and **16**.

The compounds were tested at 20 and 40 mg/kg dose levels which showed no signs of acute toxicity. The results of the anti-inflammatory activity of the tested compounds (20 & 40 mg/kg) and the potent anti-inflammatory drug Indomethacin (5 mg/kg) are listed in Table 4. The tested compounds showed varying degrees of activity. The highest activity was shown by the propionic acid derivative **9**, which produced strong dose-dependent inhibition of carrageenan-induced paw oedema (>50%). The activity of the 2-propionic acid derivative **9** was superior to the acetic and 3-propionic acid derivatives **7** and **11** which were weakly or moderately active. The ethylpiperazine derivative **5b** showed weak activity at 20 mg/kg dose level; increasing the dose to 40 mg/kg did not result in improving the anti-inflammatory activity. There are in fact a high number of enzyme/receptors involved in the inflammatory process. Without specific tests it is quite difficult to hypothesize the mechanism of action of active compounds, the active compounds may exert their action *via*

Table 2
Antimicrobial activity of compounds **3**, **4a**–**d**, **5a**–**c**, **7**, **9**, **11**, **15a**–**c** and **16** (200 μg/8 mm disc), the broad spectrum antibacterial drugs Gentamicin (100 μg/8 mm disc), Ampicillin (100 μg/8 mm disc) and the antifungal drug Clotrimazole (100 μg/8 mm disc) against *Staphylococcus aureus* IFO 3060 (*SA*), *Bacillus subtilis* IFO 3007 (*BS*), *Micrococcus luteus* IFO 3232 (ML), *Escherichia coli* IFO 3301 (*EC*), *Pseudomonas aeuroginosa* IFO 3448 (*PA*), and *Candida albicans* IFO 0583 (*CA*).

Comp. No.	Diameter of Growth Inhibition Zone (mm)[a]					
	SA	*BS*	*ML*	*EC*	*PA*	*CA*
3	13	13	–	27	20	–
4a	–	–	–	–	–	–
4b	–	–	–	–	–	14
4c	–	–	–	–	–	–
4d	–	–	–	–	–	–
5a	13	14	14	–	–	–
5b	12	13	–	–	–	–
5c	–	14	–	–	–	–
7	18	22	16	–	–	17
9	18	20	13	–	–	–
11	11	13	–	–	–	11
15a	13	14	15	–	–	–
15b	14	19	11	–	–	–
15c	15	20	12	–	–	–
16	–	–	–	–	–	–
Gentamicin	26	25	18	20	19	NT
Ampicillin	23	21	19	17	17	NT
Clotrimazole	NT	NT	NT	NT	NT	21

[a] (–): Inactive (inhibition zone < 10 mm). (NT): Not tested.

Table 3
The minimal inhibitory concentrations (MIC, μg/ml) of compounds **3**, **7**, **9**, **15b**, **15c**, the broad spectrum antibacterial drugs Gentamicin, Ampicillin and the antifungal drug Clotrimazole against *Staphylococcus aureus* IFO 3060 (*SA*), *Bacillus subtilis* IFO 3007 (*BS*), *Micrococcus luteus* IFO 3232 (*ML*), *Escherichia coli* IFO 3301 (*EC*), *Pseudomonas aeruginosa* IFO 3448 (*PA*), and *Candida albicans* IFO 0583 (*CA*).

Comp. No.	Minimal Inhibitory Concentration (MIC, μg/ml)[a]					
	SA	*BS*	*ML*	*EC*	*PA*	*CA*
3	ND	ND	ND	0.5	2	ND
7	ND	2	ND	ND	ND	ND
9	ND	4	ND	ND	ND	ND
15b	ND	4	ND	ND	ND	ND
15c	ND	2	ND	ND	ND	ND
Gentamicin	2	2	2	0.5	1	ND
Ampicillin	2	0.5	2	2	2	ND
Clotrimazole	ND	ND	ND	ND	ND	2

[a] ND: Not determined.

Table 4
Anti-inflammatory effect of intraperitoneal injection of (20 & 40 mg/kg) of compounds **4a**, **5b**, **7**, **9**, **11** and Indomethacin (5 mg/kg) against carrageenan-induced paw oedema in rats.

Comp. No.	Mean % Reduction of paw oedema from control[a]	
	20 mg/kg	40 mg/kg
Control[b]	0 (±0.036)	
4a	1.12 (±0.099)[c]	0.92 (±0.082)[c]
5b	11.32 (±0.131)[d]	10.62 (±0.140)[d]
7	29.54 (±0.097)[d]	32.70 (±0.129)[d]
9	50.60 (±0.132)[d]	65.19 (±0.144)[e]
11	7.47 (±0.060)[d]	36.83 (±0.171)[d]
Indomethacin (5 mg/kg)	52.79 (±0.044)	

[a] Results are expressed as mean % inhibition ± S.E.M. ($n = 5$) and compared with student "*t*" test.
[b] The group was injected with 1 ml of 0.5% aqueous carboxymethyl cellulose solution.
[c] Inactive: Significantly different from Indomethacin at $p < 0.05$.
[d] Activity comparable to Indomethacin (significantly different from Indomethacin at $p < 0.05$.
[e] Significantly different (higher than) Indomethacin at $p < 0.05$.

inhibition of the cyclooxygenase enzymes like other nonsteroidal anti-inflammatory agents. In addition, the recently reported activity of some adamantane derivatives as selective inhibitors of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) [33,34] should be taken into consideration. The 11β-hydroxysteroid dehydrogenase type 1 converts cortisone to the active glucocorticoid cortisol, which is responsible for various metabolic disorders including water retention, thus the inhibition of 11β-HSD1 would result in increasing the intracellular cortisone level.

3. Conclusion

In this study, new series of 5(1-adamanyl)-1,3,4-thiadiazole were synthesized and their antimicrobial and anti-inflammatory activity were determined. Several newly synthesized derivatives displayed promising antimicrobial and anti-inflammatory activities compared to known antibacterial, antifungal and anti-inflammatory drugs. Though, the mechanism of the biological activity needs further investigations, which are in progress.

4. Experimental protocols

Melting points (°C) were measured in open glass capillaries using a Branstead 9001 Electrothermal melting point apparatus and are uncorrected. NMR spectra were obtained on a Bruker AC 500 Ultra Shield NMR spectrometer (Fällanden, Switzerland) operating at 500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C, the chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane (TMS) as internal standard; coupling constants (J) are expressed in Hz. Electrospray ionization mass spectra (ESI-MS) were recorded on a Waters QuatroMicro triple quadrupole tandem mass spectrometer at 4.0 and 3.5 kV for positive and negative ions, respectively. Monitoring the reactions and checking the purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminium sheets (60 F_{254}, Merck) and visualization with ultraviolet light (UV) at 365 and 254 nm. The bacterial strains and *C. albicans* fungus were obtained from the Institute of Fermentation of Osaka (IFO), Osaka, Japan. The reference drugs Ampicillin trihydrate (CAS 7177-48-2), Clotrimazole (CAS 23593-75-1) and Indomethacin (CAS 53-86-1) were obtained from Sigma–Aldrich Chemie GmbH, Taufkirchen, Germany. The Sprauge-Dawley rats were purchased from local animal house (Abu-Rawash, Giza, Egypt). The animal experiments for the determination of the anti-inflammatory activity were carried out in agreement with the pertinent legal and ethical standards of the international guidelines.

4.1. 5-(1-Adamantyl)-1,3,4-Thiadiazoline-2-Thione (3)

Potassium *N*′-(1-adamantylcarbonyl)dithiocarbazate **2** (15.43 g, 0.05 mol) was added portion wise to 98% sulphuric acid (15 mL) and the resulted clear solution was stirred at room temperature for 24 h. The mixture was cautiously added to crushed ice (200 gm), stirred for 1 h, refrigerated for 2 h, and the separated white precipitate was filtered, washed with water, dried and crystallized from ethanol to yield 7.8 gm (62%) of the title compound **3** (M.p. 155–6 °C). ^{1}H NMR ($CDCl_3$): δ 1.77–1.84 (m, 6H, Adamantane-H), 2.10 (s, 6H, Adamantane-H), 2.14 (s, 3H, Adamantane-H), 6.14 (s, 1H, NH). ^{13}C NMR: 28.36, 36.24, 38.86, 43.39 (Adamantane-C), 156.09 (C-5), 184.35 (C=S). ESI-MS, *m/z* (Rel. Int.): 252 (M^+, 100), 209 (12), 195 (17), 135 (87), 119 (18), 59 (79).

4.2. 5-(1-Adamantyl)-3-(benzyl- or 4-substituted benzyl)-1,3,4-thiadiazoline-2-thiones (4a-d)

A mixture of 5-(1-adamantyl)-1,3,4-thiadiazoline-2-thione **3** (0.5 g, 2.0 mmol), the appropriate benzyl- or 4-substituted benzyl chloride (2.0 mmol) and anhydrous potassium carbonate (0.28 g, 2.0 mmol), in ethanol (15 mL) was heated under reflux for 2 h, and the solvent was distilled off under reduced pressure. Water (15 mL) was added to the residue and the separated crude product was filtered, washed with water, dried and crystallized from ethanol (**4c**, **4d**) or aqueous ethanol (**4a**, **4b**). **4a**: ^{1}H NMR ($CDCl_3$): δ 1.77–1.83 (m, 6H, Adamantane-H), 2.07 (s, 6H, Adamantane-H), 2.13 (s, 3H, Adamantane-H), 4.55 (s, 2H, Ar**C**$\mathbf{H}_2$), 7.29–7.34 (m, 3H, Ar-H), 7.44 (s, 2H, Ar-H). ^{13}C NMR: 28.42, 36.35, 38.10, 43.40 (Adamantane-C), 38.34 (Ar***C***H_2), 127.78, 128.69, 129.27, 136.21 (Ar-C), 163.68 (C-5), 180.80 (C=S). ESI-MS, *m/z* (Rel. Int.): 343 (M^+ +1, 14), 342 (M^+, 66), 309 (12), 148 (85), 135 (19), 105 (14), 91 (100), 77 (18). **4b**: ^{1}H NMR ($CDCl_3$): δ 1.77–1.81 (m, 6H, Adamantane-H), 2.07 (s, 6H, Adamantane-H), 2.12 (s, 3H, Adamantane-H), 4.52 (s, 2H, ArC***H***$_2$), 7.0 (t, 2H, Ar-H, $J = 8.5$ Hz), 7.41–7.43 (m, 2H, Ar-H). ^{13}C NMR: 28.41, 36.27, 38.36, 43.27 (Adamantane-C), 37.17 (Ar***C***H_2), 115.48, 130.94, 132.16, 161.30 (Ar-C), 163.33 (C-5), 180.95 (C=S). ESI-MS, *m/z* (Rel. Int.): 361 (M^+ +1, 19), 360 (M^+, 79), 345 (35), 327 (11), 166 (94), 135 (25), 123 (13), 109 (100). **4c**: ^{1}H NMR ($CDCl_3$): δ 1.73 (s, 6H, Adamantane-H), 1.96 (s, 6H, Adamantane-H), 2.04 (s, 3H, Adamantane-H), 4.52 (s, 2H, ArC***H***$_2$), 7.38 (d, 2H, Ar-H, $J = 8.5$ Hz), 7.45 (d, 2H, Ar-H, $J = 8.5$ Hz), ^{13}C NMR: 28.35, 36.25, 38.43, 43.31 (Adamantane-C), 38.0 (Ar***C***H_2), 129.06, 131.53, 132.74, 136.39 (Ar-C), 163.99 (C-5), 180.84 (C=S). EI-MS, *m/z* (Rel. Int.): 378 (M^+ +2, 14), 376 (M^+, 33), 343 (5), 184 (29), 182 (80), 141 (4), 139 (11), 135 (18), 127 (39),

125 (100). **4d**: ^{1}H NMR ($CDCl_3$): δ 1.76–1.83 (m, 6H, Adamantane-H), 2.06 (s, 6H, Adamantane-H), 2.12 (s, 3H, Adamantane-H), 4.60 (s, 2H, ArCH_2), 7.64 (d, 2H, Ar-H, $J = 8.5$ Hz), 8.17 (d, 2H, Ar-H, $J = 8.5$ Hz), ^{13}C NMR: 28.37, 36.29, 38.42, 43.40 (Adamantane-C), 36.59 (ArCH_2), 123.83, 130.47, 144.44, 148.33 (Ar-C), 162.32 (C-5), 181.88 (C=S). ESI-MS, *m/z* (Rel. Int.): 388 ($M^+ +1$, 23), 387 (M^+, 100), 354 (6), 265 (4), 193 (34), 182 (80), 150 (7), 136 (38), 135 (47), 121 (15).

4.3. 5-(1-Adamantyl)-3-(4-substituted-1-piperazinylmethyl)-1,3,4-thiadiazoline-2-thiones (**5a–c**)

A mixture of 5-(1-adamantyl)-1,3,4-thiadiazoline-2-thione **3** (0.5 gm, 2.0 mmol), the *N*-substituted piperazine (2.0 mmol) and 37% formaldehyde solution (1 mL), in ethanol (8 mL), was heated under reflux for 2 h and stirred at room temperature for 24 h. The crude product was separated in case of compound **5c**, while in case of compounds **5a** and **5b** it was necessary to add water (5 ml) to precipitate the products. The crude products were filtered, washed with water, dried and crystallized from ethanol (**5c**) or aqueous ethanol (**5a**, **5b**). **5a**: ^{1}H NMR ($CDCl_3$): δ 1.73–1.80 (m, 6H, Adamantane-H), 1.94 (s, 3H, Adamantane-H), 2.05 (s, 6H, Adamantane-H), 2.34 (s, 3H, CH_3), 2.61–2.78 (m, 4H, Piperazine-H), 2.99 (s, 4H, Piperazine-H), 5.25 (s, 2H, CH_2). ^{13}C NMR: 27.98, 36.05, 38.43, 42.11 (Adamantane-C), 46.52 (CH_3), 50.12, 52.68 (Piperazine-C), 70.13 (CH_2), 168.33 (C-5), 186.75 (C=S). ESI-MS, *m/z* (Rel. Int.): 364 (M^+, 1), 252 (9), 135 (46), 113 (100), 98 (7). **5b**: ^{1}H NMR ($CDCl_3$): δ 1.08 (t, 3H, CH_3CH$_2$, $J = 7.0$ Hz), 1.71–1.80 (m, 6H, Adamantane-H), 1.88 (s, 3H, Adamantane-H), 1.92 (s, 6H, Adamantane-H), 2.48 (q, 2H, CH_3CH_2, $J = 7.0$ Hz), 2.58 (br. s, 4H, Piperazine-H), 2.93 (s, 4H, Piperazine-H), 5.24 (s, 2H, CH_2). ^{13}C NMR: 11.22 (CH_3CH$_2$), 28.12, 36.15, 38.99, 42.08 (Adamantane-C), 49.71 (CH_3CH_2), 51.98, 52.23 (Piperazine-C), 70.01 (CH_2), 169.26 (C-5), 188.01 (C=S). ESI-MS, *m/z* (Rel. Int.): 378 (M^+, 1), 266 (3), 252 (30), 135 (100), 127 (56). **5c**: ^{1}H NMR ($CDCl_3$): δ 1.73–1.80 (m, 6H, Adamantane-H), 2.10 (s, 6H, Adamantane-H), 2.14 (s, 3H, Adamantane-H), 3.03 (s, 4H, Piperazine-H), 3.20 (s, 4H, Piperazine-H), 5.31 (s, 2H, CH_2), 6.93–7.28 (m, 5H, Ar-H). ^{13}C NMR: 28.36, 36.25, 38.86, 43.39 (Adamantane-C), 49.47, 50.42 (Piperazine-C), 70.33 (CH_2), 116.35, 120.04, 129.14, 151.27 (Ar-C), 165.85 (C-5), 184.34 (C=S). ESI-MS, *m/z* (Rel. Int.): 426 (M^+, 1), 263 (2), 265 (1), 252 (100), 195 (7), 175 (66), 135 (65).

4.4. 2-[5-(1-Adamantyl)-2-thioxo-1,3,4-thiadiazolin-3-yl]acetic acid (7), (±)-2-[5-(1-Adamantyl)-2-thioxo-1,3,4-thiadiazolin-3-yl]-propionic acid (9), and 3-[5-(1-Adamantyl)-2-thioxo-1,3,4-thiadiazolin-3-yl]propionic acid (**11**)

A mixture of 5-(1-adamantyl)-1,3,4-thiadiazoline-2-thione **3** (0.5 gm, 2.0 mmol), the appropriate ethyl bromoester (2.0 mmol) and anhydrous potassium carbonate (0.28 gm, 2.0 mmol), in ethanol (15 mL), was heated under reflux for 2 h, and the solvent was distilled off under reduced pressure. 10% Aqueous sodium hydroxide (15 mL) was added to the residue and the mixture was heated under reflux for 1 h and filtered hot. The cold filtrate was acidified with hydrochloric acid to *p*H 2–3 and allowed to stand for 3 h. The separated crude product was filtered, washed with water, dried and crystallized from aqueous ethanol. **7**: ^{1}H NMR ($CDCl_3$): δ 1.71–1.82 (m, 6H, Adamantane-H), 1.94–1.96 (m, 6H, Adamantane-H), 2.05 (s, 3H, Adamantane-H), 2.12 (s, 2H, NCH_2), 11.80 (br. s, 1H, COOH). ^{13}C NMR: 28.14, 36.15, 38.64, 42.24 (Adamantane-C), 40.49 (NCH_2), 174.33 (C-5), 183.13 (C=S), 188.85 (C=O). ESI-MS, *m/z* (Rel. Int.): 311 ($M^+ +1$, 11), 310 (M^+, 18), 319 (100). **9**: ^{1}H NMR ($CDCl_3$): δ 1.69–1.85 (m, 9H, Adamantane-H, CH_3), 1.93 (s, 6H, Adamantane-H), 2.04 (s, 3H, Adamantane-H), 2.14–2.16 (m, 1H, NCH), 11.78 (br. s, 1H, COOH). ^{13}C NMR: 17.11 (CH_3), 27.85, 36.43, 38.59, 43.38 (Adamantane-C), 40.50 (NCH), 174.27 (C-5), 181.71 (C=S), 184.12 (C=O). ESI-MS, *m/z* (Rel. Int.): 325 ($M^+ +1$, 13), 324 (M^+, 18), 323 (100). **11**: ^{1}H NMR ($CDCl_3$): δ 1.73–1.83 (m, 8H, Adamantane-H, NCH_2), 1.94–1.96 (m, 8H, Adamantane-H, CH_2CO), 2.05 (s, 3H, Adamantane-H), 11.85 (br. s, 1H, COOH). ^{13}C NMR: 27.85, 36.15, 38.60, 42.23 (Adamantane-C), 28.14 (NCH_2), 40.49 (CH_2CO), 174.29 (C-5), 183.91 (C=S), 188.92 (C=O). ESI-MS, *m/z* (Rel. Int.): 325 ($M^+ +1$, 20), 324 (M^+, 23), 323 (100).

4.5. 5-(1-Adamantyl)-2-amino-1,3,4-thiadiazole (**14**)

Method A [29]: 98% Sulphuric acid (20 mL) was added to 1-(1-adamantylcarbonyl)-3-thiosemicarbazide **12** (12.67 gm, 0.05 mol), and the mixture was stirred for 24 h at room temperature. The mixture was then poured onto crushed ice (200 gm), neutralized with concentrated ammonium hydroxide solution and stirred for 20. The separated crude product was filtered, washed with water, dried and crystallized from aqueous ethanol to yield 5.65 g (48%) of the title compound **14** (M.p. 201–3 °C) [29]. **Method B:** Phosphorus oxychloride (20 ml) was added to adamantane-1-carboxylic acid **13** (9 gm, 0.05 mol) and the mixture was stirred for 20 min at room temperature. Thiosemicarbazide (4.56 gm, 0.05 mol) was added and the mixture was heated under reflux for 1 h. On cooling, water (50 mL) was added dropwise and cautiously with continuous stirring to decompose the excess phosphorus oxychloride. The mixture was then heated under reflux for 4 h. On cooling, the mixture was neutralized by addition of potassium hydroxide pellets and refrigerated overnight. The precipitated crude product was filtered, washed with water, dried and crystallized from aqueous ethanol to yield 6.94 g (59%) of the title compound **14** (M.p. 201–3 °C). ^{1}H NMR ($CDCl_3$): δ 1.79 (s, 6H, Adamantane-H), 2.04 (s, 6H, Adamantane-H), 2.11 (s, 3H, Adamantane-H), 5.25 (s, 2H, NH_2). ^{13}C NMR: 28.46, 36.45, 38.11, 43.24 (Adamantane-C), 166.82 (C-5), 171.34 (C-2). ESI-MS, *m/z* (Rel. Int.): 235 (M^+, 92), 202 (9), 178 (32), 135 (100).

4.6. N-[5-(1-Adamantyl)-1,3,4-thiadiazol-2-yl]-N′-arylthioureas (**15a–c**)

The appropriate arylisothiocyanate (2.0 mmol) was added to a solution of 5-(1-adamantyl)-2-amino-1,3,4-thiadiazole **14** (0.47 gm, 2.0 mmol) in dry DMF (10 mL) and the mixture was heated under reflux for 6 h. On cooling, the mixture was poured onto cold water (15 mL) and the separated precipitate was filtered, washed with water and crystallized from ethanol (**15b**, **15c**) or aqueous ethanol (**15a**) to yield compounds **15a–c**. **15a**: ^{1}H NMR (DMSO-d_6): δ 1.72 (s, 6H, Adamantane-H), 1.91 (s, 6H, Adamantane-H), 2.02 (s, 3H, Adamantane-H), 6.97 (s, 3H, Ar-H), 7.34–7.45 (m, 2H, Ar-H), 11.05 (br. s, 2H, NH). ^{13}C NMR: 28.38, 36.45, 37.72, 43.27 (Adamantane-C), 122.19, 125.15, 129.07, 136.58 (Ar-C), 168.0 (C-5), 172.44 (C-2), 186.42 (C=S). ESI–MS, *m/z* (Rel. Int.): 370 (M^+, 3), 235 (94), 220 (4), 135 (59), 91 (100). **15b**: ^{1}H NMR (DMSO-d_6): δ 1.75 (s, 6H, Adamantane-H), 1.99 (s, 6H, Adamantane-H), 2.06 (s, 3H, Adamantane-H), 6.82–6.86 (m, 2H, Ar-H), 7.22–7.36 (m, 2H, Ar-H), 11.0 (br. s, 2H, NH). **15c**: ^{1}H NMR (DMSO-d_6): δ 1.74–1.82 (m, 6H, Adamantane-H), 2.03 (s, 6H, Adamantane-H), 2.10 (s, 3H, Adamantane-H), 7.02 (d, 2H, Ar-H, $J = 7.5$ Hz), 7.31 (d, 2H, Ar-H, $J = 7.5$ Hz), 10.87 (br. s, 2H, NH). ^{13}C NMR: 28.03, 36.44, 38.10, 43.22 (Adamantane-C), 122.96, 128.85, 129.05, 138.55 (Ar-C), 167.48 (C-5), 171.99 (C-2), 188.59 (C=S).

4.7. 5-(1-Adamantyl)-1,3,4-thiadiazoline-2-one (**16**)

10% Aqueous sodium nitrite solution (10 mL) was added dropwise to an ice-cooled suspension of 5-(1-adamantyl)-2-amino-

1,3,4-thiadiazole **14** (2.35 gm, 0.01 mol) and hydrochloric acid (5 mL) in cold water (20 mL), with continuous stirring over a period of 20 min. The temperature was then allowed to rise to room temperature and the mixture was heated to boiling for 10 min, cooled and allowed to stand overnight. The separated crude product was filtered, washed with water, dried and crystallized from aqueous ethanol to yield 1.44 gm (61%) of compound **16**. ^{1}H NMR ($CDCl_3$): δ 1.74–1.82 (m, 6H, Adamantane-H), 2.01 (s, 3H, Adamantane-H), 2.14 (s, 6H, Adamantane-H), ^{13}C NMR: 28.48, 36.38, 38.82, 43.69 (Adamantane-C), 150.75 (C-5), 180.26 (C=O). ESI-MS, *m/z* (Rel. Int.): 236 (M^+, 17), 235 ($M^{++}-1$, 43), 220 (100), 163 (25), 135 (83), 100 (8).

4.8. Determination of antimicrobial activity (agar disc-diffusion method)

Sterile filter paper discs (8 mm diameter) were moistened with the compound solution in dimethylsulphoxide of specific concentration (200 μg/disc), the antibacterial antibiotics Gentamicin and Ampicillin trihydrate (100 μg/disc) and the antifungal drug Clotrimazole (100 μg/disc) were carefully placed on the agar culture plates that had been previously inoculated separately with the microorganisms. The plates were incubated at 37 °C, and the diameter of the growth inhibition zones were measured after 24 h in case of bacteria and 48 h in case of *C. albicans*.

4.9. Determination of the minimal inhibitory concentration (MIC)

Compounds **3**, **7**, **9**, **15b**, **15c**, Gentamicin, Ampicillin trihydrate and Clotrimazole were dissolved in dimethylsulphoxide at concentration of 128 μg/mL. The two-fold dilutions of the solution were prepared (128, 64, 32,…, 0.5 μg/mL). The microorganism suspensions at 106 CFU/mL (colony forming unit/ml) concentrations were inoculated to the corresponding wells. The plates were incubated at 36 °C for 24 and 48 h for the bacteria and *C. albicans*, respectively. The MIC values were determined as the lowest concentration that completely inhibited visible growth of the microorganism as detected by unaided eye.

4.10. Determination of in vivo anti-inflammatory activity

Male Sprague-Dawley rats weighing 140–190 gm were maintained at room temperature (20–23 °C). The animals were randomly divided into 12 groups each of 5 animals. The animals were housed with food and water *ad libitum* and allowed to be accustomed to their environment for two days before testing. Each group was injected with the specific dose of the test compound (20 and 40 mg/kg), or Indomethacin (5 mg/kg) intraperitoneally as a uniform suspension in 1 ml of 0.5% (w/v) aqueous carboxymethyl cellulose solution, 1 h before injection of 0.1 mL of carrageenan (1% solution in normal saline) into the plantar tissue of the right hind paw. The left hind paw was injected with 0.1 mL of normal saline solution. Four hours after carrageenan injection, the volume of paw oedema (mL) was determined using water plethysmometer. The percentage protection against inflammation was calculated as follows:

$$(V_c - V_d/V_d)\times 100$$

where V_c is the mean percentage increase in paw volume in the absence of the test compound (control) and V_d is the mean percentage increase in paw volume after injection of the test compound. The values are expressed as the mean ± S.E.M. Statistical significance between the control and treated groups was performed using the Student "*t*" test.

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