Synthesis, Antimicrobial, and Anti-Proliferative Activities of Novel 4-(Adamantan-1-yl)-1-arylidene-3-thiosemicarbazides, 4-Arylmethyl N-(Adamantan-1-yl)piperidine-1-carbothioimidates, and Related Derivatives

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Abstract: The reaction of 4-(adamantan-1-yl)-3-thiosemicarbazide 3 with various aromatic aldehydes yielded the corresponding thiosemicarbazones 4a–g. 1-Adamantyl isothiocyanate 2 was reacted with 1-methylpiperazine or piperidine to yield the corresponding N-(adamantan-1-yl)carbothioamides 5 and 6, respectively. The latter was reacted with benzyl or substituted benzyl bromides to yield the S-arylmethyl derivatives 7a–c. Attempted cyclization of 1,3-bis(adamantan-1-yl)thiourea 8 with chloroacetic acid via prolonged heating to the corresponding thiazolidin-4-one 9 resulted in desulfurization of 8 to yield its urea analogue 10. The thiazolidin-4-one 9 and its 5-arylidene derivatives 11a,b were obtained via microwave-assisted synthesis. The in vitro antimicrobial activity of the synthesized compounds was evaluated against a panel of Gram-positive and Gram-negative bacteria and yeast-like pathogenic fungus Candida albicans. Compounds 7a–c displayed marked broad spectrum antibacterial activities (minimal inhibitory concentration (MIC), 0.5–32 µg/mL) and compounds 4a and 4g showed good activity against Candida albicans. Nine representative compounds were evaluated for anti-proliferative activity towards three human tumor cell lines. Compounds 7a–c displayed significant generalized anti-proliferative activity against all the tested cell lines with IC_{50} < 10 µM.

Keywords: adamantane; carbothioimidates; thiazolidin-4-ones; antimicrobial activity; anti-proliferative activity
1. Introduction

As a result of the evolution of new resistant bacterial, fungal, and viral strains, the development of new chemotherapeutic agents is becoming the major priority in pharmaceutical research with the aim to discover newer, more potent molecules with higher specificity and reduced toxicity than the existing ones. Adamantane-based derivatives are currently used as efficient therapies for the treatment of various pathological disorders [1–3]. As a result of the high lipophilicity of adamantane, the incorporation of an adamantyl moiety into structure of bioactive compounds positively modulates the biological activity. Amantadine [4,5] and rimantadine [6] were early approved as potent therapy against Influenza A viral infections, and tromantadine is currently used against herpes simplex skin viral infections [7]. In addition, several adamantane-based analogues were proven to possess significant inhibitory activity against human immunodeficiency viruses (HIV) [8–10]. Antitumor activity was reported for some adamantane derivatives, the synthetic retinoid CD437 was discovered as a potent inducer of apoptosis in human head and neck squamous cell carcinoma [11,12]. ABC294640 is a recently approved anticancer drug for the treatment of patients with advanced solid tumors [13,14]. Moreover, several adamantane-based derivatives were recognized as potent bactericidal and fungicidal agents [15–17]. SQ109 is a newly developed drug for the treatment of tuberculosis (TB); it was approved for use against drug-susceptible and drug-resistant TB strains [18]. The related dipiperidine derivative SQ609 was further discovered as a lead compound with potent long acting activity against Mycobacterium tuberculosis [19] (Figure 1).

![Figure 1. Adamantane-based chemotherapeutic drugs.](image)

On the other hand, thiosemicarbazide and thiosemicarbazone derivatives [20–22], isothiourea [16,23,24], and 4-thiazolidinone [25–27] derivatives were reported to possess marked chemotherapeutic properties.

In view of the above mentioned observations and in continuation to an ongoing study on the chemical and pharmacological properties of adamantane-based derivatives [10,16,17], herein we report the synthesis and characterization of novel adamantane derivatives containing thiosemicarbazide, isothiourea, or 4-thiazolidinone moieties as potential antibacterial, antifungal, and/or anti-proliferative agents.
2. Results and Discussion

2.1. Chemical Synthesis

Adamantan-1-yl isothiocyanate 2 was prepared in good yield starting from 1-adamantylamine 1 following our previously described procedure [28] via modification of the general methods of Munch et al. [29] and Spilovska et al. [30]. The intermediate 4-(adamantan-1-yl)-3-thiosemicarbazide 3 was previously reported as a minor byproduct during the reaction of N-(adamantan-1-yl)-4-ethoxycarbonylpiperidine-1-carbothioamide with excess hydrazine hydrate, in ethanol, at reflux temperature [29]. In the present investigation, compound 3 was prepared in high yield (94%) via treatment of adamantan-1-yl isothiocyanate 2 with hydrazine hydrate in ethanol at reflux temperature for one hour. 4-(Adamantan-1-yl)-3-thiosemicarbazide 3 was then condensed with the aromatic aldehydes; 2-hydroxybenzaldehyde, 4-nitrobenzaldehyde, 2,4-difluorobenzaldehyde, 2,6-difluorobenzaldehyde, 3,4-dichlorobenzaldehyde, 2,6-dichlorobenzaldehyde, or benzod[1,3]dioxole-4-carboxaldehyde via heating in ethanol to yield the corresponding azomethine derivatives 4a–g in 68%–85% yield (Scheme 1). Compound 4a was previously reported as a potential treatment for neurodegenerative diseases such as Alzheimer’s disease [31]. The structures of compounds 4a–g were confirmed on the basis of 1H nuclear magnetic resonance (NMR), 13C NMR, and electrospray ionization mass spectral (ESI/MS) data, which showed their negative ion peaks [M − H]−.

![Scheme 1. Synthetic approach for the target compounds 4a–g.](image)

Adamantan-1-yl isothiocyanate 2 was reacted with 1-methylpiperazine or piperidine in boiling ethanol to yield the corresponding N-(adamantan-1-yl)carbothioamides 5 and 6, respectively. Compound 6 was previously reported [28]. The reaction of the carbothioamide derivative 6 with benzyl or 4-substituted benzyl bromides in N,N-dimethylformamide (DMF) in the presence of anhydrous potassium carbonate at room temperature yielded the corresponding S-arylmethyl (isothiourea) derivatives 7a–c in good yields (Scheme 2). The structures of compounds 5 and 7a–c were confirmed on the basis of 1H and 13C NMR spectra, in addition to ESI/MS data, which showed their positive ion peaks [M + H]⁺.
The reaction of adamantan-1-yl isothiocyanate 2 with 1-adamantylamine 1 by heating in ethanol under reflux for four hours yielded the symmetric thiourea derivative 8 in 75% yield. The reaction of monosubstituted or 1,3-disubstituted symmetric thiourea derivatives with chloroacetic acid or ethyl chloro- or bromoacetate in the presence of sodium acetate was reported to yield the corresponding iminothiazolidin-4-one derivatives [25–27,32]. Attempted reaction of the symmetric thiourea derivative 8 with chloroacetic acid via heating in ethanol in the presence of sodium acetate for up to four hours to get the intermediate 3-(adamantan-1-yl)-2-(adamantan-1-ylimino)thiazolidin-4-one 9 was unsuccessful and the reactants were recovered unchanged. Increasing the reaction time to 10–12 h resulted in desulphurization of the thiourea derivative 9 to yield the corresponding urea analogue 10. Although this type of desulfurization is uncommon, similar reactions were previously reported [33,34]. The assignment of the structures of compound 10 was based on its physical and spectral data, which were identical to the reported data [35,36]. In addition, the structure was further supported by single crystal X-ray diffraction (Figure 2).

Microwave irradiation was introduced as a useful alternative to traditional heating for the synthesis of several heterocyclic derivatives [37–39]. Thus, it was of interest to try this environmentally friendly tool to get thiazolidin-4-one derivative 9. After several pilot experiments to optimize the irradiation time and intensity, it was found that microwave irradiation for 10 min, a maximum power of 700 W is the optimum condition for this reaction, and the product was attained in 72% yield. The successful microwave assisted synthesis of compound 9 prompted us to try a three-component one step reaction of compound 8 with chloroacetic acid and the appropriate aromatic aldehyde, in ethanol,
in the presence of anhydrous sodium acetate. The trial was successful and the products 11a and 11b were obtained in fair yields after being irradiated for 10 min at a maximum power of 700 W (Scheme 3).

Scheme 3. Synthetic approach for the target compounds 8, 9, and 11a,b.

2.2. In Vitro Antimicrobial Activity

The in vitro growth inhibitory activity of the newly synthesized compounds 4a–g, 5, 7a–c, 8, 9, 11a, and 11b was assessed against the standard bacterial strains of the American type culture collection (ATCC), Staphylococcus aureus ATCC 6571, Bacillus subtilis ATCC 5256, Micrococcus luteus ATCC 27141 (Gram-positive bacteria), Escherichia coli ATCC 8726, Pseudomonas aeruginosa ATCC 27853 (Gram-negative bacteria), and the yeast-like pathogenic fungus Candida albicans MTCC 227. The primary antimicrobial screening was carried out using the semi-quantitative agar-disc diffusion method with Müller–Hinton agar medium [40]. The results of the preliminary antimicrobial testing of compounds 4a–g, 5, 7a–c, 8, 9, 11a, and 11b (200 μg/disc); the antibacterial antibiotics Gentamicin sulfate, Ampicillin trihydrate, and the antifungal drug Clotrimazole (100 μg/disc); and the calculated log p-values (Clog P) of the compounds (calculated using the CS ChemOffice Ultra version 8.0, CambridgeSoft, Cambridge, MA, USA) are listed in Table 1.

The results indicated that the tested compounds showed various levels of activity against the tested microorganisms. Potent antibacterial activity was displayed by the compounds 4a, 4c, 4d, 4e, 4f, 7a, 7b, and 7c, which displayed growth inhibition zones ≥18 mm against one or more of the tested microorganisms. Meanwhile, the compounds 4b and 5 showed moderate activity (growth inhibition zones 14–17 mm); the compounds 8, 11a, and 11b were poorly active (growth inhibition zones 10–13 mm); and compound 9 was practically inactive (growth inhibition zones ≤10 mm) against the tested microorganisms. In general, the tested Gram-positive bacteria are considered the most sensitive among the tested bacterial strains and the activity against the tested Gram-negative bacteria was generally lower than that of the Gram-positive bacteria. Compounds 4a, 4c, 4d, 4e, 4f, 4g, 7a, 7b, and 7c displayed potent activity particularly against the Gram-negative bacteria Escherichia coli and the optimum antibacterial activity was attained by compounds 4a, 4d, 4f, 7b, and 7c, which exhibited potent broad spectrum activity against all the tested bacterial strains. The antifungal activity of the compounds against Candida albicans was generally lower than their antibacterial activity, compounds 4a and 4g showed potent activity; compound 4f displayed moderate activity; and compounds 4b, 4c, 4f, 7a, 7b, and 7c displayed marginal activity compared with Clotrimazole.
In addition, the antibacterial activity of compounds 4c, 4f, and 4g was found to be.

The minimal inhibitory concentrations (MICs) of the most active compounds 4a, 4c, 4d, 4e, 4f, 4g, 7a, 7b, and 7c, as well as the antibacterial antibiotics Gentamicin sulfate, Ampicillin trihydrate, and the antifungal drug Clotrimazole, were determined using the microdilution susceptibility method in Müller–Hinton broth and Sabouraud liquid medium [41]. The MIC values were almost consistent with the results obtained in the primary screening.

According to the results of the antimicrobial activity, it could be concluded that the 4-(adamantan-1-yl)-1-arylidene-3-thiosemicarbazides 4a–g and the 4-arylmethyl N′-(adamantan-1-yl) piperidine-1-carbothioimides 7a–c are the most active derivatives. Among the 4-(adamantan-1-yl)-1-arylidene-3-thiosemicarbazide series 4a–g, it was observed that the hydroxy- and benzodioxole substituents (4a and 4g) are optimal for antifungal activity. The 2,6-dihalophenyl analogues 4d and 4f showed higher potency against the tested Gram-negative bacteria compared with their 2,4- or 3,4-dihalophenyl analogues 4c and 4e, which showed potent activity against the tested Gram-positive bacteria. In addition, the antibacterial activity of compounds 4a–g was found to be.
correlated to their lipophilicity, in contrast to their antifungal activity. The thiourea derivatives 5 and 8 showed moderate and weak activity against the tested Gram-positive bacteria. The insertion of the isothiourea moiety (compounds 7a–c) greatly enhanced the antibacterial potency and spectrum as the piperidine-4-carbothioimidates 7a–c displayed marked broad spectrum antibacterial activity with marginal antifungal activity. On the other hand, the conversion of the thiourea derivative 8 to its corresponding thiazolidine analogue 9 resulted in a total loss of antibacterial activity. The insertion of a 4-arylidene moiety on the thiazolidine derivative 9, which resulted in the more lipophilic derivatives 11a and 11b, resulted in limited improvement in the activity against the tested Gram-positive bacteria.

2.3. In Vitro Anti-Proliferative Activity

The in vitro anti-proliferative activity of nine representative compounds (4a, 4d, 4f, 4g, 7a, 7b, 7c, 9, and 11a) was assessed against three human tumor cell lines; namely, HL-60 (human promyelocytic leukemia cell line), HT-29 (human colorectal cancer cell line), and MCF7 (human breast cancer cell line) using the 3-[4,5-dimethylthiazoyl-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay \[42–44\]. Compounds 4a, 4d, 4f, 4g, 7a, 7b, 7c, 9, and 11a were selected after preliminary pilot experiments, which proved that the other derivatives were almost inactive (IC\(_{50}\) > 100 \(\mu\)M). The results of the anti-proliferative activity of compounds 4a, 4d, 4f, 4g, 7a, 7b, 7c, 9, 11a, and the potent anticancer drug Doxorubicin [45] are shown in Table 2.

**Table 2.** In vitro anti-proliferative activity of the tested compounds 4a, 4d, 4f, 4g, 7a, 7b, 7c, 9, 11a, and Doxorubicin expressed as IC\(_{50}\) values against HL-60 (human promyelocytic leukemia cell line), HT-29 (human colorectal cancer cell line), and MCF7 (human breast cancer cell line).

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>IC(_{50}) ((\mu)M) (\pm) SD</th>
<th>HL-60</th>
<th>HT-29</th>
<th>MCF7</th>
</tr>
</thead>
<tbody>
<tr>
<td>4a</td>
<td>&gt;100</td>
<td>75.60 ± 0.77</td>
<td>92.67 ± 2.70</td>
<td></td>
</tr>
<tr>
<td>4d</td>
<td>32.65 ± 1.52</td>
<td>25.46 ± 1.22</td>
<td>22.40 ± 1.20</td>
<td></td>
</tr>
<tr>
<td>4f</td>
<td>24.98 ± 2.02</td>
<td>47.50 ± 1.27</td>
<td>32.75 ± 2.90</td>
<td></td>
</tr>
<tr>
<td>4g</td>
<td>24.24 ± 1.33</td>
<td>18.99 ± 1.02</td>
<td>24.02 ± 0.88</td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>6.62 ± 0.52</td>
<td>4.25 ± 0.08</td>
<td>1.55 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>7b</td>
<td>5.30 ± 0.99</td>
<td>3.67 ± 1.02</td>
<td>0.95 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>7c</td>
<td>8.43 ± 1.01</td>
<td>2.68 ± 0.32</td>
<td>0.46 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>11a</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>1.05 ± 0.12</td>
<td>0.32 ± 0.02</td>
<td>0.11 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

\(\text{IC}_{50}\) values presented as the mean \(\pm\) SD of three separate determinations.

The results indicated that the tested compounds displayed variable degrees of anti-proliferative activity against the tested cancer cell lines. The optimal activity was attained by the isothiourea derivatives 7a, 7b, and 7c with IC\(_{50}\) <10 \(\mu\)M against the tested cell lines. Meanwhile, compounds 4d, 4f, and 4g showed moderate activity with IC\(_{50}\) values>10–50 \(\mu\)M; compound 4a exhibited marginal activity against HT-29 and MCF7 cell lines. In addition, the thiazolidinone derivatives 9 and 11a did not show any activity on the tested cell lines (IC\(_{50}\) > 100 \(\mu\)M).

According to the results of the anti-proliferative activity, it could be concluded that the arylmethyl N'-{(adamantan-1-yl)piperidine-1-carbothioimidates 7a–c and, to a lesser extent, the 4-(adamantan-1-yl)-1-arylidene-3-thiosemicarbazides 4a,d,f,g, are the most active derivatives. In the adamantyl piperidine-4-thiocarboxamide series 7a–c, it seems that the conjugation of the adamantyl moiety with an isothiourea fragment is optimal for anti-proliferative activity, regardless of the nature of the arylmethyl substituents (X). In addition, the benzodioxole substituent enhanced the anti-proliferative activity of the 4-(adamantan-1-yl)-1-arylidene-3-thiosemicarbazide.
3. Materials and Methods

3.1. General Information

Melting points (°C) were measured in open glass capillaries using IA9100 electrothermal melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AC 500 Ultra Shield NMR spectrometer at 500.13 MHz for $^1$H and 125.76 MHz for $^{13}$C and Bruker Ascend 700 NMR spectrometer at 700.17 MHz for $^1$H and 176.08 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. Deuteriochloroform (CDCl$_3$) and deuteriodimethyl sulfoxide (DMSO-d$_6$) were used as solvents. Electrospray ionization mass spectra (ESI-MS) were recorded on an Agilent 6410 Triple Quad tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) at 4.0 kV for positive ions. Microwave irradiation was carried on using a domestic microwave oven (Haas HMW 20WN) at 700 W. Elemental analyses (C, H, N, and S) were in agreement with the proposed structures within ±0.4% of the theoretical values (Table S1). Monitoring of the reactions and checking of the purity of the final products were carried out by thin layer chromatography (TLC) using silica gel precoated aluminum sheets (60 F$_{254}$; Merck Schuchardt, Darmstadt, Germany), as well as visualization with ultraviolet light (UV) at 365 and 254 nm. The reference drugs Gentamicin sulfate (CAS 1405-41-0), Ampicillin trihydrate (CAS 7177-48-2), Clotrimazole (CAS 23593-75-1), and Doxorubicin (CAS 23214-92-8) were purchased from Sigma-Aldrich Chemie GmbH, Germany. The microanalytical data (C, H, N and S) and the experimental details of the determination of in vitro antimicrobial activity, in vitro anti-proliferative activity are given in supplementary materials.

3.2. 4-(Adamantan-1-yl)-3-thiosemicarbazide 3

Hydrazine hydrate (98%, 5 mL) was added to a hot solution of 1-adamantyl isothiocyanate 2 (1.93 g, 0.01 mol) in ethanol (10 mL) and the mixture was heated under reflux with stirring for one hour. On cooling, the precipitated crude product was filtered, washed with cold ethanol, dried, and crystallized from ethanol to yield 2.12 g (94%) of the target compound 3 as fine colorless needle crystals; m.p. 195–197 °C [29].

3.3. 4-(Adamantan-1-yl)-1-arylidene-3-thiosemicarbazides 4a–g

A mixture of 4-(adamantan-1-yl)-3-thiosemicarbazide 3 (0.45 g, 2.0 mmol) and the appropriate aromatic aldehyde (2.0 mmol), in ethanol (10 mL), was heated under reflux with stirring for four hours. On cooling, the precipitated crude products were filtered, washed with cold ethanol, dried, and crystallized from ethanol (4a, 4c, 4d, and 4g) or ethanol/chloroform (4b, 4e, and 4f) as transparent needle or block crystals.

4-(Adamantan-1-yl)-1-(2-hydroxybenzylidene)-3-thiosemicarbazide 4a: Yield 70%; m.p. 194–196 °C (EtOH); Mol. Formula (Mol. Wt.): C$_{18}$H$_{23}$N$_{3}$OS (329.46). $^1$H NMR (DMSO-d$_6$, 700.17 MHz): δ 1.66 (s, 6H, Adamantane-H), 2.08 (s, 3H, Adamantane-H), 2.27 (s, 6H, Adamantane-H), 6.84–6.89 (m, 2H, Ar-H), 7.23 (t, 1H, Ar-H, J = 7.0 Hz), 7.48 (s, 1H, NH), 7.68-7.71 (m, 1H, Ar-H), 8.38 (s, 1H, CH=N), 10.0 (br. s, 1H, OH), 11.30 (s, 1H, NH). $^{13}$C NMR (DMSO-d$_6$, 176.08 MHz): δ 29.47, 36.37, 40.21, 53.80 (Adamantane-C), 124.52, 128.37, 140.82, 148.09 (Ar-C), 139.24 (CH=N), 175.27 (C=S). ESI-MS, m/z: 328.3 [M – H]$^-$.

4-(Adamantan-1-yl)-1-(4-nitrobenzylidene)-3-thiosemicarbazide 4b: Yield 85%; m.p. 153–155 °C (EtOH/CHCl$_3$); Mol. Formula (Mol. Wt.): C$_{18}$H$_{22}$N$_{4}$O$_2$S (358.46). $^1$H NMR (DMSO-d$_6$, 700.17 MHz): δ 1.66 (s, 6H, Adamantane-H), 2.09 (s, 3H, Adamantane-H), 2.30 (s, 6H, Adamantane-H), 7.58 (s, 1H, NH), 7.94 (d, 2H, Ar-H, J = 7.0 Hz), 8.24 (d, 2H, Ar-H, J = 7.0 Hz), 8.15 (s, 1H, CH=N), 11.64 (s, 1H, NH). $^{13}$C NMR (DMSO-d$_6$, 176.08 MHz): δ 29.47, 36.37, 40.21, 53.80 (Adamantane-C), 124.45, 128.37, 140.82, 148.09 (Ar-C), 139.24 (CH=N), 175.27 (C=S). ESI-MS, m/z: 357.3 [M – H]$^-$.
4-(Adamantan-1-yl)-1-(2,4-difluorobenzylidene)-3-thiosemicarbazide 4d: Yield 68%; m.p. 193–195 °C (EtOH); Mol. Formula (Mol. Wt.): C₁₉H₂₁F₂N₃S (349.44). ¹H NMR (DMSO-d₆, 700.17 MHz): δ 1.66 (s, 6H, Adamantane-H), 2.08 (s, 3H, Adamantane-H), 2.23 (s, 6H, Adamantane-H), 7.53 (s, 1H, NH), 7.67–7.86 (m, 2H, Ar-H), 7.97 (d, 1H, Ar-H, J = 7.0 Hz), 8.03 (s, 1H, CH=N), 11.49 (s, 1H, NH). ¹³C NMR (DMSO-d₆, 176.08 MHz): δ 29.47, 36.38, 40.32, 53.73 Adamantane-C), 127.35, 129.03, 131.44, 132.20, 135.28, 132.49 (Ar-C), 139.24 (CH=N), 175.15 (C=S). ESI-MS, m/z: 380.4 [M − H]⁻, 382.4 [M + 2 − H]⁻.

4-(Adamantan-1-yl)-1-(2,6-dichlorobenzylidene)-3-thiosemicarbazide 4e: Yield 78%; m.p. 226–228 °C (EtOH/CHCl₃); Mol. Formula (Mol. Wt.): C₁₉H₂₁Cl₂N₃S (382.35). ¹H NMR (DMSO-d₆, 700.17 MHz): δ 1.61–1.65 (m, 6H, Adamantane-H), 2.05 (m, 2H, Adamantane-H), 2.21 (s, 6H, Adamantane-H), 7.38–7.41 (m, 1H, Ar-H), 7.54–7.57 (m, 2H, Ar-H & NH), 8.37 (s, 1H, CH=N), 11.69 (s, 1H, NH). ¹³C NMR (DMSO-d₆, 176.08 MHz): δ 29.43, 36.28, 41.32, 53.41 (Adamantane-C), 129.5, 130.19, 131.26, 134.16 (Ar-C), 136.07 (CH=N), 175.35 (C=S). ESI-MS, m/z: 380.4 [M − H]⁻, 382.4 [M + 2 − H]⁻.

4-(Adamantan-1-yl)-1-(2,6-difluorobenzylidene)-3-thiosemicarbazide 4f: Yield 75%; m.p. 238–240 °C (EtOH/CHCl₃); Mol. Formula (Mol. Wt.): C₁₉H₂₁F₂N₃S (382.35). ¹H NMR (DMSO-d₆, 700.17 MHz): δ 1.61–1.65 (m, 6H, Adamantane-H), 2.05 (m, 2H, Adamantane-H), 2.21 (s, 6H, Adamantane-H), 7.38–7.41 (m, 1H, Ar-H), 7.54–7.57 (m, 2H, Ar-H & NH), 8.37 (s, 1H, CH=N), 11.69 (s, 1H, NH). ¹³C NMR (DMSO-d₆, 176.08 MHz): δ 29.43, 36.28, 41.32, 53.41 (Adamantane-C), 129.5, 130.19, 131.26, 134.16 (Ar-C), 136.07 (CH=N), 175.35 (C=S). ESI-MS, m/z: 380.4 [M − H]⁻, 382.4 [M + 2 − H]⁻.

4-(Adamantan-1-yl)-1-(3,4-dichlorobenzylidene)-3-thiosemicarbazide 4g: Yield 82%; m.p. 184–186 °C (EtOH); Mol. Formula (Mol. Wt.): C₁₉H₂₁Cl₂O₂S (357.47). ¹H NMR (DMSO-d₆, 700.17 MHz): δ 1.63–1.66 (s, 6H, Adamantane-H), 2.04–2.06 (m, 3H, Adamantane-H), 2.18 (s, 3H, Adamantane-H), 2.26 (s, 3H, Adamantane-H), 6.12 (s, 2H, OCH₂O), 6.88–6.95 (m, 2H, Ar-H), 7.19 (d, 1H, Ar-H, J = 7.0 Hz), 7.57 (s, 1H, NH), 8.40 (s, 1H, CH=N), 11.46 (s, 1H, NH). ¹³C NMR (DMSO-d₆, 176.08 MHz): δ 29.46, 36.43, 41.32, 53.42 (Adamantane-C), 102.03 (OCH₂O), 109.72, 116.93, 119.33, 122.38, 146.32, 148.24 (Ar-C), 136.08 (CH=N), 174.98 (C=S). ESI-MS, m/z: 356.3 [M + H]⁺.

3.4. N-(Adamantan-1-yl)-4-methylpiperazin-1-carbothioamide 5

A mixture of 1-adamantyl isothiocyanate 2 (387 mg, 2 mmol) and 1-methylpiperazines (200 mg, 2.0 mmol), in ethanol (15 mL), was heated under reflux for two hours. On cooling, the precipitated crude product was filtered, washed with cold ethanol, dried, and crystallized from n-hexane to yield 470 mg (80%) as transparent needle crystals; m.p. 184–186 °C; Mol. Formula (Mol. Wt.): C₁₉H₂₁N₂S (293.47). ¹H NMR (DMSO-d₆, 700.17 MHz): δ 1.62 (s, 6H, Adamantane-H), 2.03 (s, 3H, Adamantane-H), 2.18 (s, 3H, CH₃), 2.24 (s, 6H, Adamantane-H), 2.27 (t, 4H, Piperazine-H, J = 4.9 Hz), 3.67 (t, 4H, Piperazine-H, J = 4.9 Hz), 6.54 (s, 1H, NH). ¹³C NMR (DMSO-d₆, 176.08 MHz): δ 29.56, 36.57, 41.39, 54.20 (Adamantane-C), 45.92 (CH₃), 47.61, 54.85 (Piperazine-C), 180.82 (C=S). ESI-MS, m/z: 294.0 [M + H]⁺.

3.5. 4-Arylmethyl N'-adamantan-1-yl)piperidine-1-carbothioimidates 7a–c

The appropriate arylmethyl bromide (2 mmol) and anhydrous potassium carbonate (276 mg, 2 mmol) were added to a stirred solution of N-(adamantan-1-yl)piperidine-1-carbothioamide 6 (557 mg, 2 mmol), in N,N-dimethylformamide (10 mL), and the mixture was stirred for 24 h at room temperature.
Cold water (20 mL) was then added and the precipitated crude products were filtered, washed with water, dried, and crystallized from ethanol to yield the target compounds 7a–c as transparent block crystals.

4-Benzyl N'-(adamantan-1-yl)piperidine-1-carbothioimidate 7a: Yield 78%; m.p. 79–80 °C; Mol. Formula (Mol. Wt.): C_{23}H_{32}N_{2}S (368.23). $^1$H NMR (DMSO-$d_6$, 700.17 MHz): $\delta$ 1.55–1.75 (m, 18H, Adamantane-H & Piperidine-H), 1.92 (s, 3H, Adamantane-H), 3.14–3.36 (m, 4H, Piperidine-H), 3.97 (s, 2H, Benzylic CH$_2$), 7.29–7.30 (m, 5H, Ar-H). $^{13}$C NMR (DMSO-$d_6$, 176.08 MHz): $\delta$ 24.0, 25.81, 50.16 (Piperidine-C), 29.74, 36.58, 43.17, 54.20 (Adamantane-C), 127.36, 128.78, 129.17, 138.86 (Ar-C), 151.0 (C=O). ESI-MS, $m/z$: 392.1 [M + H]$^+$.

4-Bromobenzyl N'-(adamantan-1-yl)piperidine-1-carbothioimidate 7b: Yield 86%; m.p. 96–98 °C; Mol. Formula (Mol. Wt.): C$_{25}$H$_{33}$BrN$_2$S (447.47). $^1$H NMR (CDCl$_3$, 700.17 MHz): $\delta$ 1.63–2.19 (m, 21H, Adamantane-H & Piperidine-H), 3.91–3.93 (m, 4H, Piperidine-H), 4.13 (s, 2H, Benzylic CH$_2$), 7.19 (d, 2H, Ar-H, $J = 7.0$ Hz), 7.52 (d, 2H, Ar-H, $J = 7$ Hz). $^{13}$C NMR (CDCl$_3$, 176.08 MHz): $\delta$ 23.54, 26.04, 53.77 (Piperidine-C), 29.63, 35.61, 42.42, 59.14 (Adamantane-C), 39.70 (Benzylic CH$_2$), 122.93, 130.52, 132.51, 133.23 (Ar-C), 167.73 (C=O). ESI-MS, $m/z$: 449.1 [M + 2 + H]$^+$, 447.1 [M + H]$^+$.

4-Nitrobenzyl N'-(adamantan-1-yl)piperidine-1-carbothioimidate 7c: Yield 94%; m.p. 116–118 °C; Mol. Formula (Mol. Wt.): C$_{25}$H$_{33}$N$_3$O$_2$S (413.58). $^1$H NMR (DMSO-$d_6$, 700.17 MHz): $\delta$ 1.53–1.57 (m, 12H, Adamantane-H & Piperidine-H), 1.71 (s, 6H, Adamantane-H), 1.91 (s, 3H, Adamantane-H), 3.11–3.13 (m, 4H, Piperidine-H), 4.10 (s, 2H, Benzylic CH$_2$), 7.54 (d, 2H, Ar-H, $J = 7.0$ Hz), 8.19 (d, 2H, Ar-H, $J = 7.0$ Hz). $^{13}$C NMR (DMSO-$d_6$, 176.08 MHz): $\delta$ 25.04, 25.70, 50.25 (Piperidine-C), 29.70, 36.50, 43.16, 54.29 (Adamantane-C), 37.02 (Benzylic CH$_2$), 123.94, 130.47, 146.74, 147.31 (Ar-C), 150.0 (C=O). ESI-MS, $m/z$: 414.1 [M + H]$^+$.

3.6. 1,3-Bis(adamantan-1-yl)thiourea 8

A mixture of 1-adamantylamine 2 (1.51 g, 0.01 mol) and 1-adamantyl isothiocyanate 3 (1.93 g, 0.01 mol), in ethanol (10 mL), was heated under reflux for four hours. On cooling, the precipitated crude product was filtered, washed with cold ethanol, dried, and crystallized from ethanol to yield 2.58 g (75%) of the title compound 8 as white amorphous powder; m.p.; 161–163 °C; Mol. Formula (Mol. Wt.): C$_{21}$H$_{32}$N$_2$S (344.56). $^1$H NMR (DMSO-$d_6$, 700.17 MHz): $\delta$ 1.53–1.57 (m, 12H, Adamantane-H), 2.01 (s, 6H, Adamantane-H), 2.15 (s, 12H, Adamantane-H), 6.84 (s, 2H, NH). $^{13}$C NMR (DMSO-$d_6$, 176.08 MHz): $\delta$ 29.48, 36.53, 41.70, 53.12 (Adamantane-C), 179.85 (C=S). ESI-MS, $m/z$: 345.0 [M + H]$^+$.

3.7. 3-(Adamantan-1-yl)-2-(adamantan-1-ylimino)thiazolidin-4-one 9

A mixture of 1,3-bis(adamantan-1-yl)thiourea 8 (690 mg, 2.0 mmol), chloroacetic acid (190 mg, 2.0 mmol), and anhydrous sodium acetate (165 mg, 2.0 mmol), in ethanol (1.0 mL), was irradiated in a microwave oven for 10 min at a maximum power of 700 W. Cold water (20 mL) was then added to the mixture and the precipitated crude products were filtered, washed with water, dried, and crystallized from ethanol to yield 554 mg (72%) of the title compound 9 as white amorphous powder; m.p.; 256–258 °C; Mol. Formula (Mol. Wt.): C$_{23}$H$_{32}$N$_2$OS (384.58). $^1$H NMR (CDCl$_3$, 700.17 MHz): $\delta$ 1.60–1.70 (m, 12H, Adamantane-H), 1.82 (s, 3H, Adamantane-H), 1.97–2.11 (m, 15H, Adamantane-H), 3.87 (s, 2H, SCH$_2$). $^{13}$C NMR (CDCl$_3$, 176.08 MHz): $\delta$ 29.58, 29.86, 36.06, 36.48, 42.57, 44.80, 50.90, 55.0 (Adamantane-C), 139.50 (C=N), 158.0 (C=O). ESI-MS, $m/z$: 385.1 [M + H]$^+$.

3.8. 3-(Adamantan-1-yl)-2-(adamantan-1-ylimino)-5-aryldenethiazolidin-4-ones 11a,b

A mixture of 1,3-bis(adamantan-1-yl)thiourea 8 (345 mg, 1.0 mmol), chloroacetic acid (95 mg, 1.0 mmol), the appropriate aromatic aldehyde (1.0 mmol), and anhydrous sodium acetate (82 mg, 1.0 mmol), in ethanol (1.0 mL), was irradiated in a microwave oven for 10 min at a maximum power of 700 W. Cold water (20 mL) was then added to the mixture and the precipitated crude products were
filtered, washed with water, dried, and crystallized from ethanol to yield the target compound 11a,b as white amorphous powder.

3-(Adamant-1-yl)-2-(adamantan-1-ylimino)-5-(4-chlorobenzylidene)-thiazolidin-4-one 11a: Yield 45%; m.p. 247–249 °C; Mol. Formula (Mol. Wt.): C_{30}H_{30}ClN_{2}O_{5} (507.13). ¹H NMR (CDCl₃, 700.17 MHz): δ 1.68–1.7 (m, 3H, Adamantane-H), 1.76–1.77 (m, 9H, Adamantane-H), 2.04 (s, 6H, Adamantane-H), 2.16–2.18 (m, 6H, Adamantane-H), 2.70 (s, 6H, Adamantane-H), 7.43 (s, 1H, CH=C), 7.44–7.46 (m, 4H, Ar-H). ¹³C NMR (CDCl₃, 176.08 MHz): δ 29.69, 30.49, 36.42, 36.45, 39.52, 42.22, 56.43, 65.69 (Adamantane-C), 124.63 (C-5), 124.98, 130.81, 135.0 (Ar-C), 136 (Ethylene-C), 137.59 (C-ethylene), 166.54 (C=O). ESI-MS, m/z: 509.1 [M + 2 + H]^+, 507.1 [M + H]^+.

3-(Adamant-1-yl)-2-(adamantan-1-ylimino)-5-(4-nitrobenzylidene)-thiazolidin-4-one 11b: Yield 28%; m.p. 288–290 °C; Mol. Formula (Mol. Wt.): C_{30}H_{35}N_{2}O_{5}S (517.68). ¹H NMR (DMSO-d₆, 500.15 MHz): δ 1.68–1.70 (m, 15H, Adamantane-H), 2.11–2.17 (m, 15H, Adamantane-H), 7.70 (s, 1H, CH=C), 7.81 (d, 2H, Ar-H, J = 7.0 Hz), 8.36 (d, 2H, Ar-H, J = 7.0 Hz). ¹³C NMR (DMSO-d₆, 176.08 MHz): δ 29.33, 36.04, 40.64, 41.03, 52.45, 56.47 (Adamantane-C), 124.77 (C-5), 124.98, 129.1, 130.81, 135.0 (Ar-C), 136 (Ethylene-C), 137.59 (C=O), 166.54 (C=O). ESI-MS, m/z: 517.0 [M + H]^+.

4. Conclusions

A series of adamantane-linked thiosemicarbazones (4a–g), isothioureas (7a–c), and thiazolidin-4-ones (9, 11a, 11b) was prepared and characterized, and their in vitro antimicrobial and anti-proliferative activities were evaluated. The adamantyl isothiourea derivatives 7a–c displayed strong broad-spectrum antibacterial activity (MIC, 0.5–32 µg/mL) and the thiosemicarbazone derivatives 4a and 4g showed marked antifungal activity against Candida albicans. The anti-proliferative activity assessment of 4a, 4d, 4f, 4g, 7a, 7b, 7c, 9, and 11a against the human tumor cell lines HL-60, HT-29, and MCF7 revealed that the isothiourea derivatives 7a–c are highly active, with IC₅₀ < 10 µM against the tested cell lines, and the thiosemicarbazone derivatives showed moderate activity, with IC₅₀ values >10–50 µM. It could be concluded that adamantane-linked isothioureas (7a–c) and, to a lesser extent, the thiosemicarbazones (4a–g), are considered to be good candidates as newer antibacterial, antifungal, and anticancer agents. The biological screening results are considered as preliminary and further investigations including experimental and molecular docking studies for the exploration of the mechanism of their biological activity are required for optimization of their chemotherapeutic activities.

Supplementary Materials: The microanalytical data (C, H, N and S) and the experimental details of the determination of in vitro antimicrobial activity, in vitro anti-proliferative activity can be found online.

Author Contributions: M.A.A.-A. and A.A.E.-E. designed and managed the project and supervised the research progress. A.A.A.-M. synthesized the target compounds. F.A.M.A.-O. analyzed the results. H.M.H. performed the in vitro anti-proliferative activity testing. A.M.E.-M. conceived the in vitro antimicrobial testing. All authors discussed the contents of the manuscript and approved the submission.

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**Sample Availability:** Sample of the compounds are available from the correspondent author.