Synthesis, Antimicrobial Activity and Molecular Docking Studies of 1, 3-Thiazole Derivatives Incorporating Adamantanyl Moiety

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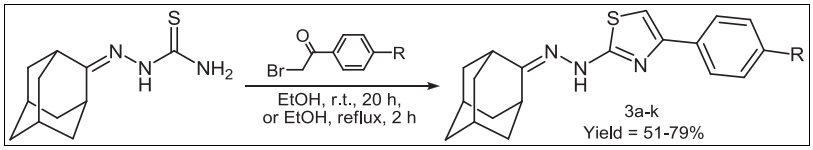

Synthesis, characterization and investigation of antimicrobial activity of 11 novel adamantanyl-thiazoles are presented. Their structures were determined using ^{1}H and ^{13}C NMR, EI(+)-MS, HRMS, and elemental analyses. Among the derivatives, compound **3c** showed very strong activity, especially against *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019 with minimal inhibitory concentration (MIC) values ranging from 1.95 to 7.81 μg/ml. Compounds **3a** and **3b** showed good antifungal activity. Among the examined compounds, the widest spectrum of antibacterial activity possessed **3f** that showed good activity, especially against *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 10240, *Bacillus subtilis* ATCC 6633 with MIC values ranging from 31.25 to 62.5 μg/ml. Molecular docking studies of all compounds on the active sites of microbial enzymes indicated possible targets sterol 14α-demethylase, secreted aspartic proteinase (SAP), *N*-myristoyltransferase (NMT), and topoisomerase II. Thiazoles **3a–k** showed more favorable affinity to SAP and NMT than the native ligand.

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INTRODUCTION

In the past two decades, the incidence of fungal infections in hospitalized, immunosuppressed or HIV-infected patients increased significantly [1]. One of the main reasons for this phenomenon is the widespread use of broad-spectrum antibiotics, immunosuppressive agents, anticancer, and anti-AIDS drugs, which leads to the multidrug resistant microorganisms [2]. The best known examples of such organisms are methicillin-resistant *Staphylococcus aureus* (MRSA) [3] and vancomycin-resistant enterococci [4]. However, majority of these infections are caused by *Candida* spp., with over 50% as a result of *Candida albicans*, which occurs naturally in the human body [5]. A possible solution to the observed drug resistance of microorganisms is the responsible use of already existing drugs and the search for innovative drugs possessing a different mechanism of action. In the present project, we have turned out attention to adamantane derivatives.

The cage-like structure of adamantane is generally used to increase the lipophilicity of the biologically active compounds. In the recent years, adamantanyl derivatives have been reported to have wide and interesting biological properties such as antibacterial and antifungal activities [6–10]. 1-Aminoadamantane (amantadine) and its derivatives were shown to have high antiviral activity against influenza A [11,12] and HIV viruses, [13] as well as Parkinson's and Alzheimer's diseases [14,15].

Another group of compounds important in medicinal chemistry are azoles and their derivatives that for several years have been widely studied because of their varied biological activities, such as antibacterial [16–18], antifungal [19–21] and antitumor activities [22].

Continuing our previous investigation on the effect of systematic structural modifications of thiazole ring on the antimicrobial activity [23–26], we decided to incorporate adamantanyl moiety into the thiazole ring to obtain compounds with relatively high lipophilicity and biological availability. We also inserted halogens, alkoxy-, nitro-, cyano-, pyrene-, and other groups in the *para*-position in the phenyl ring. These substituents are useful to modulate electronic effects in the phenyl ring of drugs. Next, their reference strains of 20 microorganisms was evaluated. The microorganisms came from American Type Culture Collection (ATCC), routinely used for the evaluation of antimicrobials. We have also performed molecular modeling and docking studies of all compounds on the active sites of sterol 14α-demethylase (CYP51), secreted aspartic proteinase (SAP), *N*-myristoyltransferase (NMT), and topoisomerase II (Topo II), in order to find their possible target.

RESULTS AND DISCUSSION

Chemistry. Synthesis of desired thiazole compounds consists of two steps. In the first step, 2-adamantanone

thiosemicarbazone (**2**) was obtained through reaction of 2-adamantanone (**1**) with thiosemicarbazide in absolute ethanol in the presence of catalytic amount of glacial acetic acid and under reflux. The product was obtained with 42% yield. In the next step, thiazoles **3a–k** with adamantanyl moiety were prepared through Hantzsch cyclization reaction of thiosemicarbazone **2** prepared in the first step with *para*-substituted bromoacetophenones **4a–h**, 3-(2-bromoacetyl)-2*H*-chromen-2-one (**4i**), 2-bromo-1-(pyren-1-yl)ethanone (**4j**) and 1-adamantyl bromomethyl ketone (**4k**) in refluxing ethanol with good yield (51–79%) and with high chemical purity. Obtained thiazoles **3a–k** do not require additional purification through precipitation from reaction mixtures. However, for microbiological analysis, they were purified further by column chromatography. The reaction pathway has been summarized in Scheme 1.

The structure of all compounds was confirmed by spectroscopic methods (^{1}H NMR, 700 and 400 MHz, ^{13}C NMR, 100 MHz, EI(+)-MS and HRMS), and elemental analysis. The ^{1}H NMR spectrum of **2** presents typical three proton signals of NH_2 and NH groups at 7.45, 7.89 and 10.12 ppm, respectively. These tree signals are because of the exchange of H between the terminal NH_2 and S. The ^{1}H NMR spectra of thiazoles **3a–k** showed singlet at δ (6.40–7.71) as a result of thiazole 5H proton and singlet at δ (10.79–11.20) indicating the presence of hydrazide NH proton, which confirms the conversion of substrates in to the expected products. The mass spectra of all compounds are fully consistent with the assigned structures. In all cases [M^+], peaks were observed. All reactions were repeated at least two times and are fully reproducible.

Biological evaluation. According to the data presented in Table 1, on the basis of minimal inhibitory concentration (MIC) values obtained by the broth microdilution method, it was shown that compounds **3a–c** showed the highest bioactivity with fungicidal or fungistatic effect. Minimum concentrations that inhibited the growth of *Candida* spp. ATCC strains ranged from 1.95 to 31.25 μg/ml and minimal fungicidal concentration (MFC) = 15.62–62.5 μg/ml. Among them, **3c** exhibited very strong activity, especially against *C. albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019. In turn, compound **3f** had activity with MIC between 31.25 and 250 μg/ml and MFC = 250–>1000 μg/ml. Compounds **3d**, **3e**, **3j** and **3k** showed moderate or mild activity (MIC = 250–1000 μg/ml and MFC > 1000 μg/ml) or had no inhibitory effect on the growth of reference yeasts species (Table 1). It was also shown that examined compounds **3g–i** had no influence on the growth of reference strains of fungi belonging to *Candida* spp.

The widest spectrum of antibacterial activity among the examined compounds possessed **3f**. This compound was found to be active against gram-positive bacteria, both some pathogenic staphylococci (*Staphylococcus epidermidis* ATCC 12228) with MIC = 31.25 μg/ml, (*S. aureus* ATCC 25923) with MIC = 250 μg/ml and streptococci (*Staphylococcus pyogenes* ATCC 19615) with MIC = 500 μg/ml and opportunistic bacteria, such as *Micrococcus luteus* ATCC 10240 (MIC = 62.5 μg/ml), *Bacillus subtilis* ATCC 6633 (MIC = 62.5 μg/ml) or *Bacillus cereus* ATCC 10876 (MIC = 1000 μg/ml). The minimal bactericidal concentration (MBC) of compound **3f** for these bacteria was equal or greater than 1000 μg/ml. The remaining compounds exhibited a lower bacteriostatic activity (MIC = 125–1000 μg/ml, MBC ≥ 1000 μg/ml) or no activity to reference strains of gram-positive bacteria. Moreover, the compounds **3a**, **3g** and **3j** had no influence also on the growth of gram-positive bacteria. The results

Scheme 1. Synthesis of thiazoles **3a–k** with adamantanyl moiety.

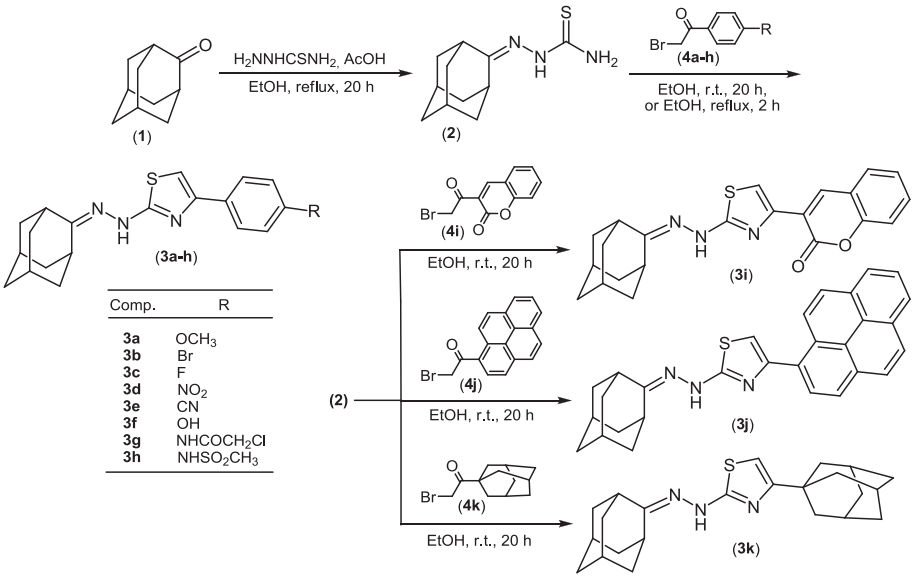

Table 1

Antimicrobial activity data in MIC (MBC/MFC) [μg/ml] for thiazoles **3a–k**.

Species	MIC (MBC/MFC) [μg/ml] of the tested compounds											
	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	CIP/VA*/ FLU**
S. aureus ATCC 6538	—	1000 (>1000)	—	1000 (>1000)	—	—	—	—	1000 (>1000)	—	—	0.244
S. aureus ATCC 25923	—	1000 (>1000)	—	—	—	250 (>1000)	—	1000 (>1000)	—	—	—	0.488
S. epidermidis ATCC 12228	—	—	—	—	—	31.25 (>1000)	—	—	—	—	—	0.122
M. luteus ATCC 10240	—	1000 (>1000)	1000 (>1000)	1000 (>1000)	1000 (>1000)	62.5 (>1000)	—	500 (>1000)	1000 (>1000)	—	125 (>1000)	0.976
B. subtilis ATCC 6633	—	1000 (>1000)	—	1000 (>1000)	—	62.5 (>1000)	—	—	1000 (>1000)	—	—	0.031
B. cereus ATCC 10876	—	—	—	—	—	1000 (>1000)	—	—	—	—	—	0.061
S. pyogenes ATCC 19615	—	—	—	—	—	500 (>1000)	—	—	—	—	—	0.244*
C. albicans ATCC 2091	15.62 (62.5)	31.25 (62.5)	15.62 (31.25)	—	—	250 (>1000)	—	—	—	1000 (>1000)	1000 (>1000)	0.245**
C. albicans ATCC 10231	15.62 (62.5)	15.62 (62.5)	1.95 (31.25)	1000 (>1000)	500 >1000	62.5 (250)	—	—	—	1000 (>1000)	1000 (>1000	0.976**
C. parapsilosis ATCC 22019	15.62 (62.5)	31.25 (31.25)	7.81 (15.62)	—	250 (>1000)	31.25 (250)	—	—	—	1000 (>1000)	1000 (>1000)	1.953**
logP	5.78	6.05	5.71	5.41	5.37	5.62	5.51	5.03	5.52	7.54	5.84	—

The standard antibiotics used as positive controls: ciprofloxacin (CIP) or vancomycin (VA
*) for bacteria and fluconazole (FLU)
**for fungi. Lipophilic parameter, logP, was calculated for each molecule by using ALOGPS 2.1 program, http://www.vcclab.org.

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of our study indicated that all examined compounds **3a–k** had no inhibitory effect on the growth of reference strains of gram-negative bacteria.

From the obtained results, it is evident that the major role in antifungal activity of compounds **3a–c**, **3f** is played by OCH_3, Br, F, and OH substituents. Additionally, antibacterial activity is determined by the presence of OH group. Also, it is clear that $NHCOCH_2Cl$ group makes the compound **3g** totally inactive. This is an interesting result because in earlier studies, a derivative containing such substituent showed very strong antifungal activity [25]. The calculations showed that the most active thiazoles with adamantanyl moiety are those possessing the lipophilicity in the range 5.62–6.05 (Table 1). Compounds with the logP values above and below this range do not exhibit significant activity. This observation indicates that lipophilicity of compounds probably plays an important role in their antimicrobial activity.

Molecular modeling studies. With the hope of identifying the fungal cellular targets of title compounds, docking simulations were performed. In our studies, the following enzymes were included: CYP51, SAP, NMT, and Topo II, which were considered in literature as attractive targets for discovering selective inhibitors to combat fungal infections [27–30]. Although no structure-activity relationships trends were observed when the docking conformations, the Gibbs free energy, and the interactions between title compounds and residues of the binding sites were analyzed in detail, all compounds were recognized as potential inhibitors of CYP51, SAP, NMT, and Topo II. Factors that may limit antifungal potency of compounds **3d**, **3e** and **3g–k** are lack of penetration of the cell wall or membrane, and removal of compound by active efflux mechanisms. Evidently, enzymatic studies are necessary to develop our knowledge of the molecular basis of antifungal activity of title compounds. The docking results are presented in Table 2 and are shown in details in Figure 1.

Table 2

Binding free energy ΔG_b (kcal/mol) corresponding to the best docking poses of compounds from series **3a–k**.

Compounds	CYP51	SAP	NMT	Topo II
Native ligand	−11.5	−8.0	−9.1	−11.8
3a	−8.8	−8.5	−9.7	−8.9
3b	−9.7	−9.0	−9.4	−9.4
3c	−9.8	−9.4	−10.5	−9.6
3d	−9.2	−9.3	−9.8	−10.0
3e	−8.3	−9.5	−9.5	−9.7
3f	−8.5	−9.0	−9.0	−9.3
3g	−7.0	−8.9	−9.8	−7.9
3h	−7.4	−9.2	−9.5	−9.4
3i	−7.5	−9.8	−11.7	−6.9
3j	−7.9	−10.3	−12.5	−7.0
3k	−9.5	−9.1	−10.0	−8.7

CONCLUSION

To conclude, we have developed an efficient and economic method for the synthesis of disubstituted thiazoles containing adamantanyl moiety. Microbiological studies in the synthesized compounds have shown that type of substituent in the *para*-position of benzene ring is very important for their activity. The results of antifungal and antibacterial screenings reveal that 4 of the 11 thiazoles **3a–c** and **3f** possessing substituents OCH_3, Br, F and OH respectively, show attractive activity. The results of antimicrobial screenings reveal that compound **3c** showed very strong activity, especially against *C. albicans* ATCC 10231 and *C. parapsilosis* ATCC 22019 with MIC values ranging from 1.95 to 7.81 µg/ml. Among the examined compounds, the widest spectrum of antibacterial activity possessed **3f** that showed good activity, especially against *S. epidermidis* ATCC 12228, *M. luteus* ATCC 10240, *B. subtilis* ATCC 6633 with MIC values ranging from 31.25 to 62.5 µg/ml. All compounds were recognized as potential inhibitors of CYP51, SAP, NMT, and Topo II and showed more favorable affinity to SAP and NMT than the native ligand. The bioactivity results provide good starting templates for further structural optimization of this kind of derivatives.

EXPERIMENTAL

Materials and methods. All experiments were carried out under air atmosphere. Reagents were generally the best quality commercial-grade products and were used without further purification. ^{1}H NMR (700 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance III multinuclear instrument. FAB(+)-MS and HRMS analyses were performed by the Team Mass Spectrometry of the Institute of Organic Chemistry of the Polish Academy of Sciences in Warsaw. MS spectra were recorded on AutoSpec Premier (Waters) spectrometer. Melting points were determined in open glass capillaries and are uncorrected. Analytical TLC was performed using Macherey-Nagel Polygram Sil G/UV_{254} 0.2 mm plates. 2-Adamantanone, thiosemicarbazide and appropriate bromoketones were commercial materials.

Biological assay. The examined compounds were screened *in vitro* for antibacterial and antifungal activities using the broth microdilution method according to European Committee on Antimicrobial Susceptibility Testing [31] and Clinical and Laboratory Standards Institute guidelines [32] against a panel of reference strains of 20 microorganisms, including gram-positive bacteria (*S. aureus* ATCC 6538, *S. aureus* ATCC 43300, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *S. pyogenes* ATCC 19615, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus mutans* ATCC 25175, *B. subtilis* ATCC 6633, *B. cereus* ATCC 10876, *M. luteus* ATCC 10240), gram-negative bacteria (*Escherichia coli* ATCC 3521, *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453, *Bordetella bronchiseptica* ATCC 4617, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC

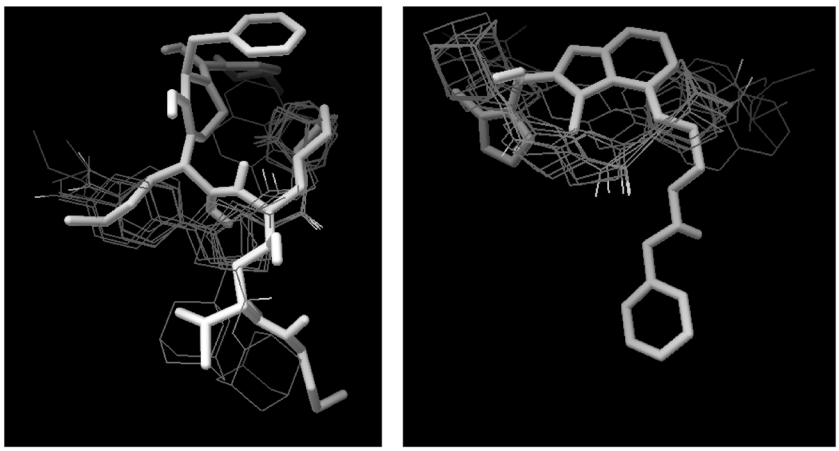

Figure 1. Superimposition of the native ligand (rendered as tubes) and the best conformations of compounds **3a–k** docked to the binding site of secreted aspartic proteinase SAP (*left*) and *N*-mirystoyltransferase NMT (*right*).

9027) and fungi belonging to yeasts (*C. albicans* ATCC 2091, *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019). These microorganisms came from ATCC, routinely used for the evaluation of antimicrobials.

All the used microbial cultures were first subcultured on nutrient agar or Sabouraud agar at 35 °C for 18–24 h or 30 °C for 24–48 h for bacteria and fungi, respectively. The surface of Mueller-Hinton agar or Mueller-Hinton agar with 5% sheep blood (for bacteria) and RPMI 1640 with MOPS (for fungi) were inoculated with the suspensions of bacterial or fungal species. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of McFarland standard scale 0.5 – approximately 1.5×10^8 colony forming units (CFU)/ml for bacteria and 0.5 McFarland standard scale – approximately 5×10^5 CFU/ml) for fungi.

Samples containing 5, 1 and 0.5 mg of tested compounds **3a–k** were dissolved in 1 ml dimethylsulphoxide (DMSO). Next, 50 µl of the tested compound was dropped into the wells ($d = 6$ mm) on the mentioned previously agar media. The agar plates were preincubated at room temperature for 1 h; next, they were incubated at 37 °C for 24 h and 30 °C for 48 h for bacteria and fungi, respectively. After the incubation period, the zones of growth inhibition were measured and average values were calculated. The wells containing DMSO without the tested compound was used as controls.

Furthermore, bacterial and fungal suspensions were put onto petri dishes with solid media containing 1 mg/ml of examined compounds **3a–k**, and they were incubated in similar conditions. The inhibition of microorganisms' growth was judged by comparison with a control culture prepared without any sample tested. Ciprofloxacin, vancomycin or fluconazole (Sigma) were used as reference antibacterial or antifungal compounds, respectively.

Subsequently, MIC of the compounds was examined by the microdilution broth method, using their twofold dilutions in Mueller-Hinton broth or Mueller-Hinton broth with 5% sheep blood (for bacteria) and RPMI 1640 broth with MOPS (for fungi) prepared in 96-well polystyrene plates. Final concentrations of the compounds ranged from 1000 to 0.122 µg/ml. Microbial suspensions were prepared in sterile saline (0.85% NaCl) with an optical density of 0.5 McFarland standard. Next, 2 µl of each bacterial or fungal suspension was added per each well containing 200 µl broth and various concentrations of the examined compounds. After incubation (37 °C, 24 h), the MIC was assessed spectrophotometrically (OD 600) as the lowest concentration of the samples showing complete bacterial or fungal growth inhibition. Appropriate DMSO, growth and sterile controls were carried out. The medium with no tested substances was used as control.

The MBC or MFC are defined as the lowest concentration of the compounds that is required to kill a particular bacterial or fungal species. MBC/MFC was determined by removing 20 µl of the culture using for MIC determinations from each well and spotting onto appropriate agar medium. The plates were incubated for 37°C for 24 h and 30 °C for 48 h for bacteria and fungi, respectively. The lowest compound concentrations with no visible growth observed was assessed as a bactericidal/fungicidal concentration. All the experiments were repeated three times, and representative data are presented.

In this study, no bioactivity was defined as a MIC >1000 µg/ml, mild bioactivity as a MIC in the range 501–1000 µg/ml, moderate bioactivity with MIC from 126 to 500 µg/ml, good bioactivity as a MIC in the range 26–125 µg/ml, strong bioactivity with MIC between 10 and 25 µg/ml and very strong bioactivity as a MIC <10 µg/ml [33].

The MBC/MIC or MFC/MIC ratios were calculated in order to determine bactericidal/fungicidal (MBC/MIC ≤ 4, MFC/MIC ≤ 4) or bacteriostatic/fungistatic (MBC/MIC >4, MFC/MIC >4) effect of the tested compounds.

Automated docking setup. Flexible ligand–receptor docking was performed using the AutodockVina program using the default settings [34]. Models of the CYP51, SAP, NMT, and Topo II binding sites based on the structure deposited in the Protein Data Bank [35] under the PDB ID 2CIB [36], 1EAG [37], 1IYL [38], 1Q1D [39] were employed. Default docking parameters and flexible space of $24 \times 24 \times 24$ Å^3 were validated by re-docking native ligand that docked exactly in the position present in the crystal structure. Subsequently, all the compounds were docked using the same docking parameters.

2-Adamantanone thiosemicarbazone (2). To a stirred solution of 2-adamantanone (**1**) (1.50 g, 0.01 mmoles) in absolute ethyl alcohol (25 ml), thiosemicarbazide (0.91 g, 0.01 mmoles) and glacial acetic acid (0.5 ml) were added. The reaction mixture was stirred under reflux for 20 h. After the mixture had cooled to room temperature, a white solid began to separate. The product was filtered off and

subsequently washed with ethyl alcohol to yield 0.95 g (42%); mp 190–192 °C; ^{1}H NMR (DMSO-d_6, 700 MHz), δ 3.33 (m, 1H, CH), 3.39 (m, 1H, CH), 1.67 (m, 2H, 2CH), 1.73 (m, 2H, 2CH), 1.77 (m, 2H, 2CH), 1.83 (m, 2H, 2CH), 1.90 (m, 4H, 4CH), 7.45 (s, 1H, NH_2), 7.89 (s, 1H, NH_2), 10.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.61 (2C), 31.43, 36.28, 37.79 (2C), 39.15 (2C), 39.27, 163.78, 178.97. *Anal*. Calcd. for $C_{11}H_{17}N_3S$: C, 59.16; H, 7.67; N, 18.81. Found: C, 59.14; H, 7.69; N, 18.84.

General procedure for the reaction of 2-adamantanone thiosemicarbazone (2) *with appropriate bromoketones* ***4a–k***. All the experiments were carried out under atmospheric air. To a stirred solution of 2-adamantanone thiosemicarbazone (**2**) (1.12 g, 1.00 mmoles) in absolute ethyl alcohol (20 ml), a solution (5 ml) of 2-bromo-1-(4-methoxyphenyl)ethanone (0.244 g, 1.00 mmoles) in absolute ethyl alcohol was added. The mixture was stirred at room temperature for 20 h or under reflux for 2 h. The separated precipitate was collected by filtration, suspended in water and neutralized with $NaHCO_3$ solution. The crude product was purified by silica gel column chromatography (230–400 mesh) using (*n*-hexane/AcOEt 90:10) to afford the desired products **3a–k**.

2-(2-Adamantanylidenehydrazinyl)-4-(4-methoxyphenyl)thiazole (3a). Yield 0.18 g (51%), mp 227–229 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.74–1.80 (m, 2H), 1.81–1.87 (m, 4H), 1.90–1.95 (m, 2H), 1.96–2.01 (m, 4H), 2.50–2.52 (m, 1H), 2.55–2.58 (m, 1H), 3.80 (s, 3H, OCH_3), 6.87 (d, J = 9 Hz, 2H, 2CH), 7.07 (s, 1H, CH); 7.78 (d, J = 9 Hz, 2H, 2CH), 10.80 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.62 (2C), 31.80, 36.27, 37.63 (2C), 39.14 (2C), 39.16, 55.64, 101.68, 114.47 (2C), 127.44, 127.49 (2C), 137.18, 164.30, 153.16, 170.43. EI(+)-MS (*m/z*, %): 353.1 [(M^+), 100], 320.1 (25), 206.0 (84), 204.9 (38), 190.9 (24), 164.0 (42), 149.0 (25), 80.1 (16). HRMS (*m/z*) calculated for $C_{20}H_{23}N_3OS$: 353.1562. Found: 353.1566. *Anal*. Calcd. for $C_{20}H_{23}N_3OS$: C, 67.96; H, 6.56; N, 11.89. Found: C, 67.99; H, 6.54; N, 11.91.

2-(2-Adamantanylidenehydrazinyl)-4-(4-bromophenyl)thiazole (3b). Yield 0.26 g (65%), mp 236–237 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.75–1.79 (m, 2H), 1.81–1.88 (m, 4H), 1.91–1.95 (m, 2H), 1.96–2.00 (m, 4H), 2.54–2.56 (m, 2H), 7.33 (s, 1H, CH); 7.60 (d, J = 9 Hz, 2H, 2CH), 7.81 (d, J = 9 Hz, 2H, 2CH), 10.83 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.49 (2C), 31.99, 36.22, 37.66 (2C), 39.13 (2C), 39.29, 104.87, 121.35, 128.31 (2C), 132.04 (2C), 132.83, 146.70, 165.25, 170.52. EI(+)-MS (*m/z*, %): 401.0 [(M^+), 97], 402.9 (100), 255.9 (90), 253.9 (90), 174.0 (70), 148.1 (32), 79.0 (36), 41.5 (30) HRMS (*m/z*) calculated for $C_{19}H_{20}BrN_3S$: 401.0561. Found: 401.0564. *Anal*. Calcd. for $C_{19}H_{20}BrN_3S$: C, 56.72; H, 5.01; N, 10.44. Found: C, 56.75; H, 4.98; N, 10.47.

2-(2-Adamantanylidenehydrazinyl)-4-(4-fluorophenyl)thiazole (3c). Yield 0.27 g (79%), mp 251–253 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.75–1.80 (m, 2H), 1.81–1.88 (m, 4H), 1.90–1.95 (m, 2H), 1.96–2.01 (m, 4H), 2.47–2.51 (m, 1H), 2.55–2.57 (m, 1H), 7.23 (m, 1H, CH), 7.24 (m, 2H, 2CH), 7.89 (m, 2H, 2CH), 10.82 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.59 (2C), 31.68, 36.23, 37.61 (2C), 39.13 (2C), 39.19, 108.55, 124.55 (2C), 126.77 (2C), 141.15, 146.59, 148.55, 163.25, 171.00. EI(+)-MS (*m/z*, %): 341.1 [(M^+), 96], 308.1 (22), 193.9 (100), 154.0 (51), 149.1 (14), 79.3 (18). HRMS (*m/z*) calculated for $C_{19}H_{20}FN_3S$: 341.1362. Found: 341.1376. *Anal*. Calcd. for $C_{19}H_{20}$ FN_3S: C, 66.83; H, 5.90; N, 12.31; Found: C, 66.81; H, 5.90; N, 12.33.

2-(2-Adamantanylidenehydrazinyl)-4-(4-nitrophenyl)thiazole (3d). Yield 0.29 g (79%), mp 238–239 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.74–1.86 (m, 6H), 1.88–2.00 (m, 6H), 2.52–2.55 (m, 1H), 3.35–3.40 (m, 1H), 7.63 (s, 1H, CH), 8.09 (d, J = 9 Hz, 2H, 2CH), 8.27 (d, J = 9 Hz, 2H, 2CH), 10.96 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.62 (2C), 31.68, 36.23, 37.61 (2C), 39.13 (2C), 39.19, 108.55, 124.55 (2C), 126.77 (2C), 141.15, 146.59, 148.55, 163.25, 171.00. EI(+)-MS (*m/z*, %): 368.1 [(M^+), 96], 220.9 (100), 148.1 (24), 79.3 (27), 41.5 (22). HRMS (*m/z*) calculated for $C_{19}H_{20}N_4O_2S$: 368.1307. Found: 368.1309. *Anal*. Calcd. for $C_{19}H_{20}N_4O_2S$: C, 61.94; H, 5.47; N, 15.21. Found: C, 61.92; H, 5.46; N, 15.24.

4-((2-Adamantanylidenehydrazinyl)thiazol-4-yl)benzonitrile (3e). Yield 0.25 g (72%), mp 256–257 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.70–1.85 (m, 6H), 1.97–2.00 (m, 6H), 2.52–2.55 (m, 1H), 3.35–3.40 (m, 1H), 7.54 (s, 1H, CH); 7.85 (d, J = 8 Hz, 2H, 2CH), 8.01 (d, J = 8 Hz, 2H, 2CH), 10.95 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.61 (2C), 31.78, 36.26, 37.61 (2C), 38.12 (2C), 39.17, 107.59, 110.96, 119.41, 126.64 (2C), 133.13 (2C), 138.81, 147.93, 163.82, 170.82. EI(+)-MS (*m/z*, %): 348.1 [(M^+), 90], 315.1 (19), 200.9 (100), 199.0 (37), 148.1 (25), 79.3 (23), 41.5 (17). HRMS (*m/z*) calculated for $C_{20}H_{20}N_4S$: 348.1422. Found: 348.1420. *Anal*. Calcd. for $C_{20}H_{20}N_4S$: C, 68.93; H, 5.79; N, 16.08. Found: C, 68.96; H, 5.82; N, 16.11.

4-((2-Adamantanylidenehydrazinyl)thiazol-4-yl)phenol (3f). Yield 0.20 g (59%), mp 213–214 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.75–1.80 (m, 2H), 1.81–1.86 (m, 4H), 1.90–1.94 (m, 2H), 1.96–2.00 (m, 4H), 2.54–2.56 (m, 1H), 3.37–3.40 (m, 1H), 6.79 (d, J = 9 Hz, 2H, 2CH), 6.97 (s, 1H, CH); 7.66 (d, J = 9 Hz, 2H, 2CH), 9.53 (bs, 1H, OH, D_2O exchangeable), 10.79 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.52 (2C), 32.43, 36.11, 37.70 (2C), 39.11 (2C), 39.14, 101.48, 115.99 (2C), 127.64, 127.93 (2C), 144.84, 158.50, 167.80, 170.08. EI(+)-MS (*m/z*, %): 339.1 [(M^+), 100], 306.1 (25), 191.9 (98), 150.0 (63), 149.1 (17), 79.3 (17), 41.5 (14). HRMS (*m/z*) calculated for $C_{19}H_{21}N_3OS$: 339.1405. Found: 339.1418. *Anal*. Calcd. for $C_{19}H_{21}N_3OS$: C, 67.23; H, 6.24; N, 12.38. Found: C, 67.20; H, 6.21; N, 12.40.

2-Chloro-n-(4-((2-adamantanylidenehydrazinyl)thiazol-4-yl) phenyl)acetamide (3g). Yield 0.17 g (41%), mp 198–200 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.75–1.80 (m, 2H), 1.81–1.88 (m, 4H), 1.92–1.96 (m, 2H), 1.97–2.01 (m, 4H), 2.50–2.58 (m, 1H), 3.38–3.40 (m, 1H), 4.29 (s, 2H, $COCH_2$), 7.17 (s, 1H, CH), 7.65 (d, J = 9 Hz, 2H, 2CH), 7.81 (d, J = 9 Hz, 2H, 2CH), 10.45 (s, 1H, NH), 10.90 (bs, 1H, NH, D_2O exchangeable). *Anal*. Calcd. for $C_{21}H_{23}ClN_4OS$: C, 60.78; H, 5.59; N, 13.50. Found: C, 60.80; H, 5.60; N, 13.53.

N-(4-((2-adamantanylidenehydrazinyl)thiazol-4-yl)phenyl) methanesulfonamide (3h). Yield 0.27 g (65%), mp 165–168 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.70–1.85 (m, 6H), 1.86–2.01 (m, 6H), 2.52–2.56 (m, 2H), 3.00 (s, 3H, CH_3), 7.13 (s, 1H, CH); 7.22 (d, J = 9 Hz, 2H, 2CH), 7.79 (d, J = 9 Hz, 2H, 2CH), 9.80 (s, 1H, NH), 10.80 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.67 (2C), 31.54, 36.34, 37.58 (2C), 39.14 (2C), 39.19, 39.74, 102.62, 120.17 (2C), 126.87 (2C), 131.19, 137.98, 150.18, 162.26, 170.68. EI(+)-MS (*m/z*, %): 416.1 [(M^+), 100], 337.1 (35), 268.9 (41), 189.9 (72), 148.1 (17), 79.3 (21), 41.5 (17). HRMS (*m/z*) calculated for $C_{20}H_{24}N_4O_2S_2$: 416.1341. Found: 416.1349. *Anal*. Calcd. for $C_{20}H_{24}N_4O_2S_2$: C, 57.67; H, 5.81; N, 13.45. Found: C, 57.70; H, 5.80; N, 13.44.

3-((2-Adamantanylidenehydrazinyl)thiazol-4-yl)-2h-chromen-2-one (3i). Yield 0.30 g (77%), mp 260–268 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.76–1.80 (m, 2H), 1.82–1.88 (m, 4H), 1.92–1.96 (m, 2H), 1.97–2.02 (m, 4H), 2.55–2.58 (m, 1H), 3.38–3.42 (m, 1H), 7.41 (t, J = 8 Hz, 1H, CH), 7.48 (d, J = 8 Hz, 1H, CH), 7.65 (m, 1H, CH), 7.71 (s, 1H, CH); 8.55 (s, 1H, CH), 10.95 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.60 (2C), 31.83, 36.25, 37.61 (2C), 39.12 (2C), 39.19, 110.49, 116.38, 119.53, 120.42, 125.27, 129.24, 132.28, 138.82, 142,68, 152.78, 159,17, 164.13, 169.79. EI(+)-MS (*m/z*, %): 391.1 [(M^+), 198], 358.1 (30), 243.9 (100), 211.0 (20), 172.0 (29), 149.1 (9), 79.3 (16), 41.5 (13). HRMS (*m/z*) calculated for $C_{22}H_{21}N_3O_2S$: 391.1354. Found: 391.1363. *Anal*. Calcd. for $C_{22}H_{21}N_3O_2S$: C, 67.50; H, 5.41; N, 10.73. Found: C, 67.53; H, 5.43; N, 10.76.

1-((2-Adamantanylidenehydrazinyl)thiazol-4-yl)pyrene (3j). Yield 0.24 g (46%), mp 258–259 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.80–1.84 (m, 2H), 1.85–1.90 (m, 4H), 1.96–2.00 (m, 2H), 2.01–2.04 (m, 4H), 2.60–2.70 (m, 1H), 3.40–3.45 (m, 1H), 7.24 (s, 1H, CH), 8.10–8.14 (m, 1H, CH), 8.22–8.28 (m, 4H, 4CH), 8.32–8.38 (m, 3H, 3CH), 8.72–8.80 (m, 1H, CH), 11.19 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.55 (2C), 32.37, 36.15, 37.75 (2C), 39.16 (2C), 39.31, 108.45, 124.21, 124.45, 125.19, 125.24, 125.94 (2C), 126.22, 127.06 (2C), 127.75, 128.23, 128.51, 128.56, 128,59, 130.78, 131.32, 131.61, 167.48, 169.74. EI(+)-MS (*m/z*, %): 447.1 [(M^+), 100], 300.0 (73), 257.9 (52), 226.0 (29), 149.1 (10), 79.3 (10), 41.5 (7). HRMS (*m/z*) calculated for $C_{29}H_{25}N_3S$: 447.1769. Found: 447.1783. *Anal*. Calcd. for $C_{29}H_{25}N_3S$: C, 77.82; H, 5.63; N, 9.39. Found: C, 77.85; H, 5.66; N, 9.42.

1-((2-Adamantanylidenehydrazinyl)thiazol-4-yl)adamantane (3k). Yield 0.21 g (46%), mp 263–265 °C; ^{1}H NMR (DMSO-d_6, 700 MHz): δ 1.69–2.06 (m, 27H), 2.58–2.60 (m, 1H), 3.30–3.35 (m, 1H), 6.40 (s, 1H, CH); 11.20 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (DMSO-d_6, 100 MHz), δ (ppm): 27.48 (2C), 28.08 (6C), 32.53, 35.73, 36.06, 36.46 (3C), 37.70 (2C), 38.94 (2C), 40.75, 101.26, 153.68, 169.03, 169.03. EI(+)-MS (*m/z*, %): 381.2 [(M^+), 100], 234.0 (58), 177.0 (15), 148.1 (11), 79.3 (21), 41.5 (14). HRMS (*m/z*) calculated for $C_{23}H_{31}N_3S$: 381.2239. Found: 381.2251. *Anal*. Calcd. for $C_{23}H_{31}N_3S$: C, 72.40; H, 8.19; N, 11.01. Found: C, 72.44; H, 8.18; N, 11.03.

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