Synthesis and antibacterial activity of novel Schiff bases of thiosemicarbazone derivatives with adamantane moiety

Jiahui Zhu1,2 · Guosheng Teng1 · Dongfeng Li1 · Ruibin Hou1,2 · Yan Xia1,2

Received: 9 April 2021 / Accepted: 4 June 2021 / Published online: 12 June 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract
Increased bacterial resistance to antibiotics is a major threat to human health, and it is particularly important to develop novel antibiotic drugs. Here, we designed a series of Schiff base thiosemicarbazone derivatives containing an adamantane moiety, and carried out the structural characterization of the compounds and in vitro antibacterial activity tests. Compound 7e was as effective as the commonly used antibiotic ampicillin against the Gram-negative bacterium Escherichia coli, and compound 7g had a good inhibitory effect against Gram-positive Bacillus subtilis. These findings provide data for the development of better thiosemicarbazone antibacterial agents.

Keywords Thiosemicarbazone · Antibacterial · Adamantane

Introduction
In the past 30 years, only limited progress has been made in research into antibacterial drugs with new mechanisms and core structures [1–3]. Amoxicillin, norfloxacin, and ciprofloxacin are the commonest drugs used to treat bacterial infection but are associated with severe side effects. Toxicity and bacterial resistance to the drugs play an important role in the failure of treatment [4, 5]. The development of antibacterial drugs with novel structures is very important to research for clinical application [6].

Recently, considerable attention has been focused on substituted thiosemicarbazone derivatives because of their interesting biological activities. Compounds with a thiosemicarbazone structure are known to possess tranquilizing, muscle relaxing, psychoanaleptic, hypnotic, ulcerogenic, antidepressant, antibacterial, antifungal, analgesic, and anti-inflammatory properties [7–14]. Thiosemicarbazones are a type of Schiff base formed by condensation of thiosemicarbamide and an aldehyde or ketone. Studies have shown that changing the structure of the aldehydes and ketones or introducing different active groups on position N(4) can improve the antibacterial and anticancer activities of those compounds [15]. Therefore, the synthesis of thiosemicarbazone compounds with various structures and the study of their structure–activity relationships have important theoretical significance and potential practical application value.

Adamantane compounds have shown relatively good antivirus, antitumor, and anti-Parkinson’s syndrome activities [16–21]. The introduction of adamantyl groups into the molecular structure of other compounds often enhances the biological activity of the compound. The adamantyl group has relatively good fat solubility, so can greatly increase the membrane permeability of a compound [22–25]. Much research has been carried out on thiosemicarbazone derivatives, but no work has been done screening adamantyl thiosemicarbazone derivatives for their antibacterial activity.

In this paper, novel adamantyl thiosemicarbazone derivatives were synthesized by the condensation of an adamantyl phenyl aldehyde with a thiosemicarbazide. The chemical structure of the compounds was elucidated by infrared (IR), 1H NMR, and 13C NMR spectroscopies, and mass spectrometry. The activities of the compounds were screened in vitro against Bacillus subtilis (a Gram-positive bacterium) and Escherichia coli (Gram-negative). To the best of our knowledge, this is the first report that
thiosemicarbazone analogs having an adamantane moiety inhibits the growth of bacteria.

Results and discussion

Chemistry

Scheme 1 shows the procedure for the synthesis of novel Schiff base derivatives of thiosemicarbazones with an adamantane moiety (compounds 7a–h). The Friedel–Crafts alkylation reaction of bromoadamantane and toluene in the presence of anhydrous potassium carbonate and palladium carbon was used to obtain 1-(p-toluene)adamantane (2). Compound 2 underwent free radical substitution reaction using NBS and BPO to yield benzyl bromide (3), followed by oxidation to give 4 [4-(1-adamantyl)benzaldehyde]. The aromatic amines 5a–h were used to prepare the thiotoluamides 6a–h in basic conditions. Finally, 4 was reacted with 6a–h to produce thiosemicarbazone Schiff base derivatives 7a–h.

The yields of all thiosemicarbazone products were 62–86%. The compounds obtained were stable in both the solid and solution states. Analytical data for these compounds were consistent with their composition. The chemical structure of thiosemicarbazone compounds 7a–h was confirmed on the basis of IR, $^1$H nuclear magnetic resonance (NMR), $^{13}$C NMR, and electrospray ionization mass spectral (ESI/MS) data, which showed their positive ion peaks [M + H]$^+$.

In the IR spectra, N–H, aromatic and aromatic C–Hs, C=N, C=C, C=N, and C=O bands stretching vibrations were observed. N–H bands belonging to the thiosemicarbazone group were recorded around 3250 cm$^{-1}$. Aromatic and aliphatic C–H stretching bands appeared between 3190 and 2910 cm$^{-1}$. C=N and C=C groups gave rise to the bands in the region 1600–1442 cm$^{-1}$. C=N and C=O bands were observed in the region 1287–1124 cm$^{-1}$. In the $^1$H-NMR spectra, adamantyl group protons had peaks between 1.74 and 2.07 ppm. The chemical shift values of aromatic ring protons were between 6.92 and 8.37 ppm, depending on the substituent groups. The characteristic azomethine (–CH=N–) protons appeared between 6.77 and 7.22 ppm. Besides, the characteristic N–H protons were seen at 9.93–11.91 ppm. In the $^{13}$C-NMR spectra, aliphatic and aromatic carbons were observed between 21.05–56.56 and 113.78–163.19 ppm, respectively. The azomethine (–CH=N–) and the thiocarbamoyl carbons were detected at 143.06–143.93 and 175.10–177.60 ppm, respectively. In mass spectrometry (MS) analysis, the mass spectral data were coherent with their molecular formulas.

Antimicrobial activity

The antibacterial activity of the synthesized compounds against B. subtilis (Gram-positive) and E. coli (Gram-negative) was evaluated using the disk inhibition method and the microdilution method to determine MICs.
Table 1 Antibacterial activity of compounds 7a–h at different concentrations

| Comp. | Diameter of inhibition zone of different strains (mm) |
|-------|-----------------------------------------------------|
|       | Escherichia coli (μg/mL) | Bacillus subtilis (μg/mL) |
|       | 1  2  4  8                  | 1  2  4  8                  |
| 7a    | 12 13 15 17                  | 15 16 19 20                  |
| 7b    | 10 11 13 14                  | 6  7  8  9                   |
| 7c    | 11 13 14 15                  | 8 10 11 13                   |
| 7d    | 12 13 14 16                  | 10 11 13 14                  |
| 7e    | 13 14 15 16                  | 9 10 11 12                   |
| 7f    | 8 9 10 11                   | 7 8 9 10                    |
| 7g    | 10 12 13 14                  | 17 18 19 22                  |
| 7h    | 8 10 11 13                  | 10 12 13 15                  |
| Ampicillin | 14.5 15 16 | 18 20 21 22 |
| DMSO  | – – – –                        | – – – –                        |

“–” represents sample no inhibitory effect on the strain

DMSO dimethylsulfoxide

[17, 26, 27]. Ampicillin was used as a positive control drug. The results are shown in Tables 1 and 2.

In the disk inhibition method (Table 1), a diameter of the inhibition zone is 20 mm or more indicates that the drug has a very strong antibacterial effect on the strain; the inhibition zone of 10–20 mm is categorized as strong antibacterial effect; diameter of 5–10 mm indicates a moderate antibacterial effect; and diameter of 5 mm or less indicates that there is little or no antibacterial effect of the compound on the strain [28].

Based on the growth inhibition response, the antibacterial inhibition of compounds 7a and 7g on B. subtilis with a concentration of 8 μg/mL (22 mm) had very strong activity and other concentrations (10–20 mm) including strong activity. The antibacterial inhibition of compounds 7d and 7h on B. subtilis with concentrations of 1–8 μg/mL (10–20 mm) had strong activity, while compounds 7e and 7f with concentrations of 2–8 μg/mL (10–20 mm) indicated strong activity. The antibacterial inhibition of compounds 7b and 7f on B. subtilis with concentrations of 1–8 μg/mL (5–10 mm) had moderate activity. The antibacterial inhibition of compounds 7a and 7e on E. coli with concentrations of 1–8 μg/mL (10–20 mm) had the best inhibitory effect, similar to the effect of positive control ampicillin. Meanwhile compounds 7b–c and 7g–h with concentrations of 1–8 μg/mL (10–20 mm) had strong activity against E. coli, but compound 7f with a concentration of 1–2 μg/mL (5–10 mm) including moderate activity and concentrates of 4–8 μg/mL (10–20 mm) are categorized as strong activity.

The MIC values of the test derivatives indicated that most of the tested candidates exhibited good activity against Gram-negative bacteria as shown in Table 2. Ampicillin inhibits Gram-negative and Gram-positive bacteria with MIC of 0.03–3 μg/mL and 0.02–1.5 mg/mL, respectively. Compounds 7b–d bearing methoxy group on the phenyl had MIC 1–2 μg/mL for E. coli and MIC 4–8 μg/mL for B. subtilis, which was demonstrated that they had potent antibacterial activity compared to ampicillin. While compound 7c bearing no substituent showed less activity against E. coli (MIC = 8 μg/mL) and potent activity against B. subtilis (MIC = 8 μg/mL) compared with positive control. Meanwhile, derivatives 7e–g with electron-withdrawing group displayed potent activity against E. coli (MIC = 1–2 μg/mL) and B. subtilis (MIC = 1–4 μg/mL) which was compared to that of ampicillin. Compound 7h bearing methyl group showed good antibacterial with MIC 1 μg/mL for E. coli compared with that of compound 7a. According to the MIC values as shown in Table 2, it was generally demonstrated that substitution on phenyl moiety of thiosemicarbazone had good potency on Gram-negative bacteria.

Conclusions

In summary, we investigated the antibacterial activity of novel adamantyl thiosemicarbazones prepared by the reaction of benzaldehyde adamantane with different amine-substituted thiosemicarbazides. Of particular note, in vitro antibacterial activity tests showed that compound 7e was as effective against E. coli (a Gram-negative bacterium) as ampicillin, while 7g had good activity against the Gram-positive bacterium B. subtilis. These data lay a foundation for the development of improved thiosemicarbazone antibacterial agents.

Materials and methods

General

The starting materials which include 4-(1-adamantyl) benzaldehyde was prepared according to the procedure
described previously and other required chemicals were purchased from different commercial sources and used without purification unless otherwise stated. The progress of reactions was monitored by using thin-layer chromatography (TLC) with silica gel 60 aluminum-backed plates and the $^1$H NMR and $^{13}$C NMR spectra were recorded via Bruker spectrometer 400 MHz as dilute solutions in a suitable deuterated solvent at 25 °C. The chemical shifts were recorded on the $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.11 (s, 1H), 7.66 (s, 2H), 7.42–7.22 (m, 2H), 7.17–7.05 (m, 1H), 4.81 (s, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 139.79, 128.52, 124.49, 123.78; FT-IR (KBr, cm$^{-1}$): 3300.04, 3156.12, 1636.04, 1493.81, 1284.91, 1208.69, 905.59, 813.48, 502.85.

**N-(2-Methoxyphenyl)hydrazinecarbothioamide (6b)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.94 (s, 1H), 9.18 (s, 1H), 8.74 (s, 1H), 7.04 (s, 2H), 6.90 (s, 1H), 4.83 (s, 2H), 3.84 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 178.87, 149.87, 128.63, 124.26, 121.63, 120.16, 111.28, 56.3; FT-IR (KBr, cm$^{-1}$): v(N–H) 3313.47, 3193.43, v(C–N) 1457.01, v(C = S) 1238.42.

**N-(3-Methoxyphenyl)hydrazinecarbothioamide (6c)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.14 (s, 1H), 7.49 (s, 1H), 7.20 (d, $J = 7.1$ Hz, 2H), 6.67 (d, $J = 6.2$ Hz, 1H), 4.78 (s, 2H), 3.73 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 159.55, 140.90, 129.28, 115.64, 109.96, 109.21, 55.54; FT-IR (KBr, cm$^{-1}$): v(N–H) 3266.26, 3190.51, v(C–N) 1457.42, v(C = S) 1228.66.

**N-(4-Methoxyphenyl)hydrazinecarbothioamide (6d)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.40 (s, 1H), 8.96 (s, 1H), 7.37 (dd, $J = 53.0$, 8.5 Hz, 2H), 6.88 (t, $J = 10.1$ Hz, 2H), 4.70 (s, 2H), 3.73 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 180.45, 156.69, 132.72, 126.03, 113.76, 55.71; FT-IR (KBr, cm$^{-1}$): 3312.45, 3158.14, 1635.19, 1508.62, 1489.55, 1240.97, 829.78, 509.71.

**N-(2-Fluorophenyl)hydrazinecarbothioamide (6e)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.34 (s, 1H), 8.08 (s, 1H), 7.19 (dq, $J = 22.4$, 7.7 Hz, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 170.70, 141.72, 129.94, 119.24, 40.29; FT-IR (KBr, cm$^{-1}$): v(N–H) 3291.84, 3193.52, v(C–N) 1481.12, v(C = S) 1226.07.

**N-(3-Fluorophenyl)hydrazinecarbothioamide (6f)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.29 (s, 1H), 7.86 (s, 1H), 7.45 (s, 1H), 7.32 (s, 1H), 6.91 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 170.70, 141.72, 129.94, 119.24, 40.29; FT-IR (KBr, cm$^{-1}$): v(N–H) 3291.84, 3193.52, v(C–N) 1481.12, v(C = S) 1226.07.

**N-(4-Fluorophenyl)hydrazinecarbothioamide (6g)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.72 (s, 1H), 9.12 (s, 1H), 7.55 (d, $J = 37.9$ Hz, 2H), 7.14 (dt, $J = 17.5$, 8.8 Hz, 2H), 4.78 (s, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 180.34, 136.14, 126.33, 115.13, 114.91; FT-IR (KBr, cm$^{-1}$): 3307.96, 3108.03, 1636.04, 1508.62, 1493.81, 1284.91, 1208.69, 905.59, 813.48, 502.85.

**N-(2-Tolyl)hydrazinecarbothioamide (6h)**

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.54 (s, 1H), 9.04 (s, 1H), 7.49 (d, $J = 6.1$ Hz, 2H), 7.10 (d, $J = 8.1$ Hz, 2H), 4.76 (s, 2H), 2.27 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 180.09, 137.18, 133.68, 128.99, 124.02, 20.96; FT-IR (KBr, cm$^{-1}$): 3291.18, 3191.53, 1615.95, 1537.23, 1060.91, 806.74, 726.66, 441.48.

**Chemistry**

**General procedure for synthesis of derivatives 6a–h**

The anilines (1.0 equiv) were dissolved in 20 mL of DMF, NaOH (1.2 equiv), and CS$_2$ (1.0 equiv) were added and stirred at room temperature for 2 h, hydrazine hydrate (3.0 equiv) was added, and the reaction was heated to 65 °C. TLC monitored the reaction. The reaction was cooled to room temperature and poured into crushed ice, filtered with suction, and recrystallized from ethanol to obtain intermediates 6a–h.
Synthesis of target compound 7a-h

4-(1-adamantyl)benzaldehyde (1.0 equiv) and thiosemicarbazone (6a–h) (1.2 equiv) were dissolved in ethanol (6 mL). Then acetic acid was added and the solution was continued to reflux for 3 h.

2-[4-(Adamantan-1-yl)benzylidene]-N-phenyldihydrazone-1-carbothioamide (7a)

Light yellow solid, yield 49%, m.p. 170–171 °C; 1H NMR (400 MHz, DMSO-d6) δ 11.90 (s, 1H), 10.18 (s, 1H), 8.16 (s, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.66–7.38 (m, 4H), 7.03 (t, J = 8.0 Hz, 1H), 2.06 (s, 3H), 1.87 (s, 6H), 1.74 (s, 6H); 13C NMR (100 MHz, DMSO-d6) δ 176.31, 153.50, 143.43, 139.57, 131.83, 128.48, 127.96, 126.07, 125.66, 125.44, 42.88, 36.62, 36.47, 28.76; FT-IR (KBr, cm⁻¹) 3138.58, 1198.23, 803.73, 744.39, 493.02; HRMS (ESI) calcd for C24H28N3S 420.2110, found 420.2109.

2-[4-(Adamantan-1-yl)benzylidene]-N-(2-methoxyphenyl)dihydrazone-1-carbothioamide (7b)

Light yellow solid, yield 60%, m.p. 187–189 °C; 1H NMR (400 MHz, DMSO-d6) δ 11.89 (s, 1H), 10.00 (s, 1H), 8.36 (d, J = 7.3 Hz, 1H), 8.17 (d, J = 16.8 Hz, 1H), 7.72 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.24–6.89 (m, 3H), 3.91 (s, 3H), 2.07 (s, 3H), 1.90 (d, J = 12.0 Hz, 6H), 1.75 (s, 6H); 13C NMR (100 MHz, DMSO-d6) δ 175.10, 153.73, 151.41, 143.06, 131.67, 127.53, 125.78, 123.50, 120.35, 111.70, 56.56, 42.86, 36.60, 28.74; FT-IR (KBr, cm⁻¹) 3154.70, 2899.16, 1599.45, 1235.88, 1193.39, 1031.00, 743.44; HRMS (ESI) calcd for C25H30N3OS 420.2110, found 420.2109.

2-[4-(Adamantan-1-yl)benzylidene]-N-(3-methoxyphenyl)dihydrazone-1-carbothioamide (7c)

Light yellow solid, yield 53%, m.p. 174–176 °C; 1H NMR (400 MHz, DMSO-d6) δ 11.77 (s, 1H), 9.99 (s, 1H), 8.14 (s, 1H), 7.82 (d, J = 8.0 Hz, 2H), 7.41 (d, J = 8.0 Hz, 2H), 7.34–7.15 (m, 3H), 6.78 (d, J = 7.9 Hz, 1H), 3.77 (s, 3H), 2.07 (s, 3H), 1.88 (s, 6H), 1.74 (s, 6H); 13C NMR (100 MHz, DMSO-d6) δ 176.03, 159.49, 153.55, 143.52, 140.65, 131.77, 129.18, 127.98, 125.46, 118.01, 111.53, 111.17, 55.64, 42.87, 36.60, 28.74; FT-IR (KBr, cm⁻¹) 3125.97, 2899.24, 2845.76, 1597.70, 1546.41, 1463.44, 1288.53, 767.71, 546.23, 448.62; HRMS (ESI) calcd for C25H30N3OS 420.2110, found 420.2108.
Author contributions JZ and YX performed all experiments, purified all compounds, analyzed the data and summarized the results. YX and RH conceived and designed this research and wrote the manuscript. All authors have contributed to the final version and approved the final manuscript.

Funding This work was financially supported by the National Natural Science Foundation of China (21502008) and the National Science Foundation of Jilin Province (00005005031).

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Ventola CL. The antibiotic resistance crisis: Part 1: Causes and threats. Pharm Ther. 2015;40:277–83.
2. Brooks BD. Therapeutic strategies to combat antibiotic resistance. Adv Drug Deliv Rev. 2014;78:14–27. https://doi.org/10.1016/j.addr.2014.10.027
3. Livermore DM. The need for new antibiotics. Clin Microbiol Infect. 2004;10:1–9. https://doi.org/10.1111/j.1465-0691.2004.1004.x
4. Puertoa SA, Fernandeza GJ, Castillo LDJ, Pino SJM, Angulo PG. In vitro activity of beta-lactam and non-beta-lactam antibiotics in extended-spectrum beta-lactamase-producing clinical isolates of Escherichia coli. Diagn Microbiol Infect Dis. 2006;54:135–9. https://doi.org/10.1016/j.diagmicrobio.2005.08.018
5. Krishnamanjeyulu IS, Saravanan G, Vamsi J, Supriya P, Bhavana JU, Sunil Kumar MV. Synthesis, characterization and antimicrobial activity of some novel benzimidazole derivatives. J Adv Pharm Technol Res. 2014;5:21–27. https://doi.org/10.4103/2231-4040.126983
6. Moeller RC Jr. Discovering new antimicrobial agents. Int J Antimicrob Agents. 2011;37:2–9. https://doi.org/10.1016/j.ijantimicag.2010.08.018, 2011
7. De Araújo Neto LN, De Lima M, do CA, De Oliveira JF, et al. Thiophene-thiosemicarbazone derivative (L10) exerts antifungal activity mediated by oxidative stress and apoptosis in C. albicans. Chem Biol Interact. 2020;320:109028 https://doi.org/10.1016/j.chembi.2020.109028
8. Khan SA, Kumar P, Joshi R, Iqbal PF, Saleem K. Synthesis and in vitro antibacterial activity of new steroidal thiosemicarbazone derivatives. Eur J Med Chem. 2008;43:2029–34. https://doi.org/10.1016/j.ejmech.2007.12.004
9. Matsa R, Makam P, Kaushik M, Hoti SL, Kannan T. Thiosemicarbazone derivatives: design, synthesis and in vitro antimalarial activity studies. Eur J Pharm Sci. 2019;137:104986 https://doi.org/10.1016/j.ejps.2019.104986
10. Zhang X, Qi F, Wang S, Song J, Huang J. Synthesis, structure, in silico ADMET evaluation and in vitro antioxidant of (E)-N-(4-ethylphenyl)-2-(isomeric methylbenzylidene) thiosemicarbazone derivatives. J Mol Struct. 2020;1199:126972 https://doi.org/10.1016/j.molstruc.2019.126972
11. Trotsko N, Golus J, Kazimierzczak P, Paneth A, Przekora A, Ginalska G, et al. Design, synthesis and antimycobacterial activity of thiazolidine-2,4-dione-based thiosemicarbazone derivatives. Bioorg Chem. 2020;1016/j.bioorg.2020.103676
12. He Z, Qiao H, Yang F, Zhou W, Gong Y, Zhang X, et al. Novel thiosemicarbazone derivatives containing indole fragment as potent and selective anticancer agent. Eur J Med Chem. 2019;184:111764 https://doi.org/10.1016/j.ejmech.2019.111764
13. Pham VH, Phan TPD, Phan DC, Vu BD. Synthesis and bioactivity of thiosemicarbazones containing adamantane skeletons. Molecules. 2020;25:324 https://doi.org/10.3390/molecules25020324
14. Siddiqui EJ, Azad I, Khan AR, Khan T. Thiosemicarbazone complexes as versatile medicinal chemistry agents: a review. J Drug Deliv Ther. 2019;9:689–703. https://doi.org/10.22270/jddt.v9i3.2888
15. Pervez H, Iqbal MS, Tahir MY, Choudhary MI, Khan KM. Synthesis of some N′-substituted isatin-3-thiosemicarbazones. Nat Prod Res. 2007;21:1178–86. https://doi.org/10.1080/14786410601129770
16. Basarić N, Solhors M, Cindro N, Milinarić-Majerski K, De Clercq E, Balzarini J. Antiproliferative and antiviral activity of three libraries of adamantane derivatives. Arch Pharm. 2014;347:334–40. https://doi.org/10.1002/ardp.2013030435. 2014

2-[(Adamantan-1-yl)benzylidene]-N-(p-tolyl) hydrazine-1-carbothioamide (7h)

Light yellow solid, yield 89%, m.p. 196–197 °C; 1H NMR (400 MHz, DMSO-d6) δ 11.71 (s, 1H), 9.97 (s, 1H), 8.12 (s, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.42 (t, J = 9.5 Hz, 4H), 7.17 (d, J = 8.0 Hz, 2H), 2.31 (s, 3H), 2.06 (s, 3H), 1.88 (s, 6H), 1.74 (s, 6H); 13C NMR (100 MHz, DMSO-d6) δ 176.40, 153.46, 143.30, 137.00, 134.85, 131.86, 128.96, 127.93, 126.09, 125.44, 55.35, 42.87, 36.61, 28.75, 21.05; FT-IR (KBr, cm−1) 3132.79, 2896.53, 2845.57, 1532.84, 1492.85, 1264.08, 1201.14, 1176.71, 835.84, 449.53; HRMS (ESI) calcd for C25H30N3S 404.2160, found 404.2158.

In vitro antibacterial activity evaluation

Minimum inhibitory concentration (MIC) assays using standard microdilution methods were carried out in 96-well microplates based on a modified procedure described previously according to the guidelines of the Clinical and Laboratory Standards Institute [29, 30]. All compounds were prepared as 5 mg/mL solutions in dimethyl sulfoxide (DMSO) and were tested in a final concentration range of 1–8 μg/mL. MICs for the reference antibiotic ampicillin against quality control strains were used to confirm the validity of the screen.

All experiments were performed in duplicate and repeated three times.

Acknowledgements We thank Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Author contributions JZ and YX performed all experiments, purified all compounds, analyzed the data and summarized the results. JZ tested all compounds for their antibacterial activity. DL and GT helped in compiling the data of the manuscript. YX and RH conceived and designed this research and wrote the manuscript. All authors have contributed to the final version and approved the final manuscript.

Funding This work was financially supported by the National Natural Science Foundation of China (21502008) and the Natural Science Foundation of Jilin Province (00005005031).
17. Fesatidou M, Zagaliotis P, Camoutsis C, et al. 5-Adamantan thia-
diazole-based thiazolidinones as antimicrobial agents. Design, synthesis, molecular docking and evaluation. Bioorg Med Chem. 2018;26:4664–76. https://doi.org/10.1016/j.bmc.2018.08.004

18. Anusha S, Mohan CD, Ananda H, et al. Adamantyl-tethered-
biphenylic compounds induce apoptosis in cancer cells by tar-
getting Bcl homologs. Bioorg Med Chem Lett. 2016;26:1056–60. https://doi.org/10.1016/j.bmcl.2015.12.026

19. Göktaş F, Vanderlinden E, Naesens L, Cesur N, Cesur Z. Microwave assisted synthesis and anti-influenza virus activity of 1-adamantyl substituted N-(1-thia-4-azaspiro[4.5]decan-4-yl)car-
boxamide derivatives. Bioorg Med Chem. 2012;20:7155–9. https://doi.org/10.1016/j.bmc.2012.09.064

20. Fytas C, Zoidis G, Tsotinis A, Fytas G, Khan MA, Akhtar S, et al. Novel 1-(2-aryl-2-adamantyl)piperazine derivatives with anti-
proliferative activity. Eur J Med Chem. 2015;93:281–90. https://
doi.org/10.1016/j.ejmech.2015.02.021

21. Aguiar DF, Dutra LLA, Dantas WM, et al. Synthesis, antitumor
and cytotoxic activity of new adamantyl O-acylamidoximes and 3-
aryl-5-adamantane-1, 2, 4-oxadiazole derivatives. Chem Sel. 2019;4:9112–8. https://doi.org/10.1002/slct.201901285

22. Tsuzuki N, Hama T, Kawada M, Hasui A, Konishi R, Shiwa S,
et al. Adamantane as a brain-directed drug carrier for poorly
absorbed drug. 2. AZT derivatives conjugated with the 1-
adamantane moiety. J Pharm Sci. 1994;83:481–4. https://doi.org/
10.1002/jps.2600830407

23. Gerzon K, Krumkals EV, Brindle RL, Marshall FJ, Root MA. The adamantyl group in medicinal agents. I. Hypoglycemic N-
aryl sulfonfonyl-N’-adamantylureas. J Med Chem. 1963;6:760–3. https://doi.org/10.1021/jm00342a029

24. Rapala RT, Kraay RJ, Gerzon K. The adamantyl group in med-
ical agents. II. Anabolic steroid 17-beta-adamantatoes. J Med Chem. 1965;8:580–3. https://doi.org/10.1021/jm00329a007

25. Gerzon K, Kau D. The adamantyl group in medicinal agents. 3. Nucleoside 5’-adamantatoes. The adamantoyl function as a pro-
tecting group. J Med Chem. 1967;10:189–99. https://doi.org/10.
1021/jm00314a014

26. Martin YC, Kofron JL, Traphagen LM. Do structurally similar
molecules have similar biological activity? J Med Chem. 2002;45:4350–8. https://doi.org/10.1021/jm020155c

27. Bender A, Jenkins JL, Scheiber J, Sukuru SCK, Glick M, Davies J. How similar are similarity searching methods? A principal
component analysis of molecular descriptor space. J Chem Inf
Model. 2009;49:108–19. https://doi.org/10.1021/ci800249v

28. Davis WW, Stout TR. Disc plate method of microbiological
antibiotic assay. II. Novel procedure offering improved accuracy. Appl Microbiol. 1971;22:666–70

29. Valentine SC, Contreras D, Tan S, Real LJ, Chu S, Xu HH. Phenotypic and molecular characterization of Acinetobacter ba-
mannii clinical isolates from nosocomial outbreaks in Los Angeles
County, California. J Clin Microbiol. 2008;46:2499–507. https://
doi.org/10.1128/JCM.00367-08

30. CLSI. Methods for dilution antimicrobial susceptibility tests for
bacteria that grow aerobically; Approved Standard-Ninth Edition. M07-A9. Wayne, PA: Clinical and Laboratory Standards Institute; 2012