Synthesis and characterization of substituted acetophenone-4, 4-dimethyl-3-thiosemicarbazone derivatives with an evaluation of antimicrobial and antioxidant activities

Nagoor Meeran M*, Narayanan Ravisankar

Department of Chemistry, Vel Tech Rangarajan Dr Sagunthala R&D Institute of Science and Technology (Deemed to be University), Avadi, Chennai, Tamilnadu, India

ABSTRACT

Thiosemicarbazones are organic compounds; these are widely used in the medical field. It is primarily in the manufacture of pharmaceutical drugs and bio molecules. These compounds have the azomethine functional group (\(-\text{N} = \text{CH}\)) used to derive organic open chain and heterocyclic compounds. Thiosemicarbazones are similar to semicarbazones, and carbonyl group oxygen is replaced by sulphur. Thiosemicarbazone contains nitrogen and sulfur atoms, so they are used as ligands in coordination chemistry and form metal complexes. Thiosemicarbazone and its metal complexes are used in a variety of ways. It is especially widely used in the preparation of polymers and biochemical products. Thiosemicarbazone and its metal complexes are closely correlated with biological properties and act as antioxidants, antifungals, antimicrobials and anticancer. Subsequently, in the current study, substituted thiosemicarbazones were synthesized and examined for their antimicrobial and antioxidant activity. Newly substituted thiosemicarbazones were obtained from the respective acetophenones and thiosemicarbazides. The structure of the newly prepared compounds was confirmed by spectroscopic studies such as IR, \(^1^H\), \(^1^3^C\) NMR, Mass and elemental analysis. The antimicrobial activity of all the new compounds showed significant activity against the selected bacteria and fungi in the studies. The compound TZ04 exhibited good antioxidant activity compared with standard ascorbic acid and butylated hydroxytoluene.

INTRODUCTION

Thiosemicarbazones is a thiourea derivative, and the general formula is \(R_1 R_2 \text{C} = \text N\text{H} - \text{CS} - \text{NR}' \text{R}''\). \(R_1, R_2, R'\) and \(R''\) may be alkyl/aryl/hydragen or heterocyclic part (Reddy et al., 2016). Thiosemicarbazones are generally prepared from condensation of carbonyl compounds and thiosemicarbazide with alcohol and dehydrating agents. Chemically, Thiosemicarbazones are generally known as Schiff base compounds and contain azomethine (\(-\text{C} = \text{N}\)) group (Mohan et al., 2018). In the presences, azomethine group thiosemicarbazones are intermediate and form heterocyclic compounds (Salman et al., 2017) with suitable agents and also forms, metal complexes (Suvarapu et al., 2012) with metal ions due to in the presence of N and S donor atoms. The derivatives of thiosemicarbazones in organic and metal bonding compounds are of high biological importance.
Due to this, many organic and organo metallic thiosemicarbazone derivatives are the focus of the majority of structural and medical research such as antibacterial (R1 Mosquera et al., 2018), antifungal (de Oliveira Paiva et al., 2014), antimicrobial and antioxidant (Kumar et al., 2018), antiviral (Padmanabhan et al., 2017), antiamoebic (Abid et al., 2008), anticonvulsant (Nevagi et al., 2014), anti-HIV (Rauf et al., 2019), antimalarial (Pingaw et al., 2010), antitumor (Zhang et al., 2017), anticancer (Su et al., 2013), neurotropic (Lukevics et al., 1994), antitrypanosomal Haraguchi et al. (2011), antituberculosis (Velezheva et al., 2011) and anti-inflammatory (Oliveira et al., 2016) activity. The biological activity of metal chelates is based on the formation of metal chelates.

The biological activity of metal complexes varies from metal ions or ligands, and this is indicated to increase or decrease the biological activity of many metal complexes. Based on the above information, we have studied the synthesis, characterization, antimicrobial and antioxidant activities of the substituted acetophenone-4,4-dimethyl-thiosemicarbazones. A thin layer chromatography was used to determine the purity of the compounds. The structures of the synthesized compounds were determined by spectral and elemental analysis. The physical properties of the synthesized compounds are given in the experimental section, and all newly synthesized compounds were screened to antimicrobial and antioxidant activity.

### MATERIALS AND METHODS

The chemicals used in this study are brought from Sigma-Aldrich Chemicals and was used directly in the experimental part without refinement. The compounds were synthesized by the reporting method (Kumar et al., 2018), and the method is described in Scheme 1 and Table 1. Reaction completion was checked and confirmed by thin-layer chromatography. The melting points were determined and are uncorrected using the open capillary tube method. The structures of the products were confirmed by elemental and spectral analysis.

### Synthesis of 2-[1-(4-chlorophenyl) ethylidene]-N,N-dimethylhydrazinecarbothioamide (TZ03)

An equimolar (0.01 mol) mixture of 4-Chloroacetophenone and 4,4-Dimethyl-3-thiosemicarbazide was dissolved in ethanol (20 ml) with five drops of acetic acid and were refluxed for 6–7 hours at 70–80°C. Thin-layer chromatography techniques checked purity. After the reaction was done, the synthesized compound (TZ03) was separated. The extracted product was recrystallized with ethanol. Similarly, other compounds (TZ04, TZ05, TZ03a, ATZ1 and ATZ2) were synthesized by using the same procedure.

### Graph 1: Antimicrobial activity.

### Graph 2: Antioxidant activity.

2-[1-(4-chlorophenyl) ethylidene]-N,N-dimethyl hydrazinecarbothioamide (TZ03)

Yield 68%; m.p 203°C IR (cm$^{-1}$): 3471 (NH), 3062 (C-H), 2985 (C-H), 1589 (C=N), 1504 (C-C); $^1$H NMR (400 MHz) $\delta$: 11.39 (s, 1H), 7.82 (d, $J=10.4$, 2H), 7.44 (d, $J=10.8$, 2H), 3.11 (s, 6H), 2.29 (s, 3H) ppm; $^{13}$C NMR (100 MHz) $\delta$: 177.5 (C$_8$), 174.9 (C$_{14}$), 135.6 (C$_6$), 128.2 (C$_7$ and C$_{11}$), 128.9 (C$_8$ and C$_{10}$), 136.5 (C$_9$), 23.3 (C$_{12}$), 42.6 (C$_{13}$ and C$_{14}$) ppm; EI-MS (m/z): 255 [M$^+$]; C$_{11}$H$_{14}$ClN$_3$S; Elemental analysis-calcd: C, 51.65; H, 5.51; N, 16.42 (%); found: C, 51.66; H, 5.52; N, 16.43 (%).

2-[1-(4-bromophenyl)ethylidene]-N, N-dimethyl hydrazinecarbothioamide (TZ04)

Yield 71%; m.p 209°C IR (cm$^{-1}$): 3425 (NH), 3070 (C-H), 2985 (C-H), 1527 (C=N), 1604 (C-C); $^1$H NMR (400 MHz) $\delta$: 11.37 (s, 1H), 7.90 (d, $J=9.6$, 2H), 7.44 (d, $J=9.2$, 2H), 3.12 (s, 6H), 2.25 (s, 3H) ppm; $^{13}$C NMR (100 MHz) $\delta$: 177.6 (C$_8$), 147.4 (C$_{14}$), 136.5 (C$_9$), 128.6 (C$_7$ and C$_{11}$), 131.7 (C$_8$ and C$_{10}$), 125.4 (C$_9$),...
Scheme 1: Synthesis of substituted acetophenone-4, 4-dimethyl-3-thiosemicarbazones.

Table 1: Compound code of substituted acetophenone-4, 4-dimethyl-3-thiosemicarbazones.

| Compound Code | TZ03 | TZ04 | TZ05 | TZ03a | ATZ1 | ATZ2 |
|---------------|------|------|------|-------|------|------|
| R1            | H    | H    | H    | H     | H    | OH   |
| R2            | H    | H    | H    | OMe   | Me   | H    |
| R3            | Cl   | Br   | CHMe2| OMe   | Me   | OH   |

23.2 (C₁₂), 42.1 (C₁₃ and C₁₄) ppm; EI-MS (m/z): 299 [M⁺]; C₁₁H₁₄BrN₃S; Elemental analysis-calcd: C, 44.01; H, 4.66; N, 14.00 (%); found: C, 44.02; H, 4.69; N, 13.99 (%).

N-dimethyl-2-(1-[4-(propan-2-yl)phenyl] ethyldene) hydrazinecarbothioamide (TZ05)

Yield 67%; m.p 202°C IR (cm⁻¹): 3448 (NH), 3062 (C-H), 2985 (C-H), 1527 (C=N), 1604 (C=C); ¹H NMR (400 MHz) δ: 11.71 (s, 1H), 7.40 (d, J=10.4, 2H), 7.06 (d, J=8.4, 2H), 3.14 (s, 6H), 2.87–2.64 (sep, 1H), 2.33 (s, 3H), 1.16 (d, J=36.4, 6H) ppm; ¹³C NMR (100 MHz): 177.5 (C₂), 147.4 (C₅), 134.7 (C₆), 126.8 (C₇ and C₁₁), 126.2 (C₈ & C₁₀), 150.7 (C₉), 23.3 (C₁₂), 42.5 (C₁₃ and C₁₄), 33.2 (isoprop-C₁₅), 23.3 (isoprop CH-C₁₆ & C₁₇) ppm; EI-MS (m/z): 263 [M⁺]; C₁₄H₂₁N₃S; Elemental analysis-calcd: C, 55.51; H, 6.81; N, 14.93 (%).

Antimicrobial activity

The Kirby-Bauer disc diffusion method (Hussain et al., 2016) of in vitro antibacterial activity was used.
Table 2: Antimicrobial activity of the synthesized compounds.

| Sample Code | Zone of inhibition (mm) of synthesized compounds |
|-------------|-----------------------------------------------|
|             | Staphylococcus aureus | Bacillus subtilis | Salmonella typhi | Escherichia coli | Candida albicans |
|             | Antibacterial activity | Antifungal activity | Antibacterial activity | Antifungal activity |
|             | 100 mcg | 50 mcg | 25 mcg | std | 100 mcg | 50 mcg | 25 mcg | std | 100 mcg | 50 mcg | 25 mcg | std | 100 mcg | 50 mcg | 25 mcg | std |
| TZ03        | 10     | 7     | 4     | 17  | 6     | 4     | 19    | 12  | 8     | 6     | 19    | 8     | -     | -     | 24    |
| TZ04        | 13     | 5     | 2     | 24  | 8     | 6     | 17    | 5   | 3     | 4     | 17    | 7     | 8     | 12    | 6     |
| TZ05        | 8      | 4     | 15    | 9   | 6     | 5     | 16    | 7   | 5     | 3     | 16    | 12    | 8     | 18    | 8     | 7     | 3     | 17    |
| TZ03aθ      | 7      | 6     | 16    | 13  | 9     | 5     | 24    | 11  | 8     | 4     | 21    | 9     | 8     | 5     | 21    | 6     | 4     | 1 -   | 15    |
| ATZ1        | 8      | 5     | 3     | 15  | 8     | 6     | 14    | 7   | 3     | -     | 19    | 10    | 7     | 4     | 21    | 9     | 7     | 4     | 21    |
| ATZ2        | 8      | 6     | 15    | 17  | 5     | 4     | 12    | 9   | 6     | 5     | 17    | 12    | 5     | 8     | 21    | 8     | 6     | 3     | 16    |

Table 3: Antioxidant activity of synthesized compounds.

| Compound | Concentration (µg/ml) | IC50 (µg/ml)* |
|----------|-----------------------|---------------|
|          | 20  | 40  | 60  | 80  | 100 |
| TZ03     | 49.03 | 59.47 | 66.22 | 70.08 | 74.82 | 15.22 |
| TZ04     | 50.27 | 59.2 | 64.68 | 70.96 | 75.58 | 14.83 |
| TZ05     | 49.63 | 59.33 | 65.75 | 70.48 | 76.06 | 15.46 |
| TZ03aθ   | 47.41 | 53.49 | 67.09 | 72.47 | 80.37 | 26.5 |
| ATZ1     | 45.93 | 58.07 | 63.35 | 71.92 | 82.19 | 26.96 |
| ATZ2     | 74.06 | 65.17 | 64.06 | 65.17 | 74.06 | 24.49 |
| BHT      | 54.59 | 64.19 | 72.57 | 81.94 | 94.16 | 11.52 |
| AA       | 58.1 | 64.81 | 75.06 | 87.74 | 98.21 | 8.09 |

*Average of three independent determinations

to evaluate all the synthesized compounds. Bacteria such as *B. subtilis*, *S. aureus*, *S. typhi* and *E. coli* were used to test the activity of the compounds. Ciproflaxacin was used as the reference antibacterial drug. For the anti fungal assay, *Candida albicans* was used to test the activity of the compounds. Fluconazole was kept as the standard drug. The inhibition zone of synthesized compounds was compared with standard drugs. The results of the zone of inhibition for the antimicrobial activity of the synthesized compounds are given in Table 2.

Antioxidant activity

All synthesized compounds for antioxidant activity were screened using the DPPH evaluation method (Meeran and Hussain, 2017). The antioxidant data of all the samples are given in Table 3. DPPH method is a reduction principle of the purple DPPH (free radical) reduced and changes to yellow colored diphenylpicrylhydrazine. The remaining purple colored DPPH exhibited maximum absorption of 517 nm. The 2 ml different concentration of the synthesized compounds or standards were added with 2 ml of DPPH solution (0.1 mM), and these are kept in the dark. After 20 min of incubation at 37°C, the solution absorbance was measured at 517 nm. AA and BHA were used as positive controls. The following formula was used to calculate the percentage of inhibition.

**Inhibition (%) = (blank OD–sample OD/blank OD) × 100.**

RESULTS AND DISCUSSION

The final compounds were purified by recrystallization with ethanol. The structure of the compounds was confirmed based on spectral and elemental analysis. The spectral characterization of 2-[1-(4-chlorophenyl) ethylidene]-N,N-dimethylhydrazinecarbothioamide (TZ03) is described as an example. The IR spectrum revealed 3471, 3062 & 2985 and 1589 cm\(^{-1}\) values respectively. It is obtained due to the compound which contains the characteristics of groups of amide NH, aromatic & aliphatic CH and imine C = N respectively. \(^1\)H NMR spectrum reported a singlet at δ 11.39 ppm assignable to NH proton. Two doublets at δ 7.82 (J=10.4 Hz) ppm and δ 7.44 (J=10.8 Hz) ppm each for two protons are assignable to H-7, H-11 and H-8, H-10 respectively.
A singlet at $\delta$ 3.11 ppm for six protons is due to $\text{N(CH}_3\text{)}_2$ and a singlet at $\delta$ 2.29 ppm is related to $\text{C}_5-\text{CH}_3$ protons. The $^{13}$C NMR spectral results are described below. The signal exhibited at $\delta$ 177.5 ppm due to the thiocarbonyl carbon (C=S) and the carbon of C=SN showed at $\delta$ 147.4 ppm. The aromatic carbons $\text{C}_6$, $\text{C}_7$ and $\text{C}_{11}$, $\text{C}_8$ and $\text{C}_{10}$ and $\text{C}_9$ appeared at $\delta$ 135.6, 128.2, 128.9 and 136.6ppm respectively. The methyl carbon, $\text{C}_{12}$ is observed at 23.3ppm and the peak at 42.6 ppm due to Dimethyl carbons of $\text{C}_{13}$ and $\text{C}_{14}$ (NMe$_2$) respectively. The mass spectrum of the molecular ion peak is reported at 255 [M$^+$]. This value refers to the molecular weight of the compound. Hence, the above spectral data are compatible with the structure of the desired product, 2-{1-[(4-chlorophenyl)ethylidene]-\text{N}, \text{N}-dimethylhydrazinecarbothioamide.}

All synthesized compounds were screened for in vitro antimicrobial activity by the Kirby-Bauer disc diffusion method. The inhibition zone was compared with standards. The results of the antibacterial and anti fungal activity are given in Table 2 and Graph 1. The newly synthesized compounds showed significant activity against selected bacteria. High anti fungal activity was observed in the TZ04 compared to other compounds due to the Bromo substitution of TZ04. The results of the antioxidant activity of synthesized compounds at different concentrations are shown in Table 3. The calculated IC$_{50}$ values are given in Table 3 and Graph 2. The most active compound among the synthesized compounds is TZ04, which gave an IC$_{50}$ value of 14.8 $\mu$g/ml, while AA and BHA gave 8.09 and 11.52 $\mu$g/ml respectively.

**CONCLUSIONS**

Serious substituted acetophenone-4,4-dimethyl-3-thiosemicarbazones derivatives have been synthesized, and the elemental and spectral analysis confirmed the structures of the compounds. Newly synthesized compounds exhibited significant antimicrobial activity against selected bacteria and fungi. The compound TZ04 showed high antioxidant activity. Finally, 2-{1-[(4-bromophenyl)ethylidene]-\text{N}, \text{N}-dimethylhydrazinecarbothioamide is observed to have good anti fungal and high antioxidant activity.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

**REFERENCES**

Abid, M., Agarwal, S. M., Azam, A. 2008. Synthesis and antiamoebic activity of metronidazole thiosemicarbazone analogues. *European Journal of Medicinal Chemistry*, 43(9):2035–2039.

de Oliveira Paiva, R., Kneipp, L. F., Goular, C. M., Albuquerque, M. A., Echevarria, A. 2014. Antifungal activities of thiosemicarbazones and semicarbazones against mycotoxigenic fungi. *Ciência e Agrotecnologia*, 38(6):531–537.

Haraguchi, S. K., Silva, A. A., Vidotti, G. J., Santos, P. D., Garcia, F. P., Pedroso, R. B., Nakamura, C. V., Oliveira, C. M. A., Silva, C. C. 2011. Antiypanosomal Activity of Novel Benzaldehyde-Thiosemicarbazone Derivatives from Kaurenoc Acid. *Molecules*, 16:1166–1180.

Hussain, A. Z., Meeran, M. N., Sankar, A. 2016. Synthesis, characterization and antimicrobial activity of spiro-4-thiazolidione derivatives from 5-substituted indole-2,3-dione. *Der Pharma Chemica*, 8(2):292–296.

Kumar, A., Sarala, V., Siddikha, Y., Vanitha, A., Babu, S., Reddy, S. V., A 2018. Synthesis, characterization antimicrobial and antioxidant activities of 2,4-dihydroxybenzaldehyde-4-phenyl-3-thiosemicarbazone (DHBPTSC) and its Pd(II), Ni(II)dpmm mixed ligand and cu(II) complex having heterocyclic bases. *Journal of Applied Pharmaceutical Sciences*, 8(4):71–78.

Lukevics, E., Erchak, N., Demicheva, L., Germane, S. 1994. Synthesis and Neurotropic Activity of 5-Substituted Furfural Thiosemicarbazones. *Phosphorus. Sulfur and Silicon*, 95:499–500.

Meeran, M. N., Hussain, A. Z. 2017. Synthesis, Characterization and DPPH Scavenging Assay of Isatin Related Spiroheterocyclic Compounds. *Indian Journal of Pharmaceutical Sciences*, 79(4):641–645.

Mohan, C., Kumar, V., Kumari, S. 2018. Synthesis, characterization, and antibacterial activity of Schiff bases derived from thiosemicarbazide, 2-acetyl thiophene and thiophene-2-aldehyde. *International Research Journal of Pharmacy*, 9(7):153–158.

Nevagi, R. J., Dhake, A. S., Narkhede, H. I., Kaur, P. 2014. Design, synthesis and biological evaluation of novel thiosemicarbazide analogues as potent anticonvulsant agents. *Bioorganic Chemistry*, 54:68–72.

ño Mosquera, J.-D. L., ón Muriel, A. A., Cerón, D. P. 2018. Synthesis, antibacterial activity and DNA interactions of lanthanide (III) complexes of N(4)-...
substituted thiosemicarbazones. *Universitas Scientiarum*, 23(2):141–169.

Oliveira, J. F. D., Nonato, F. R., Zafred, R. R. T., Leite, N. M. S., Ruiz, A. L. T. G., Carvalho, J. E. D., Lima, M. D. C. A. D. 2016. Evaluation of anti-inflammatory effect of derivative (E)-N-(4-bromophenyl)-2-(thiophen-2-ylmethylene)-thiosemicarbazone. *Biomedicine & Pharmacotherapy*, 80:388–392.

Padmanabhan, P., Khaleefathullah, S., Kaveri, K., Palani, G., Ramanathan, G., Thennarasu, S., Sivagnanam, U. T. 2017. Antiviral activity of Thiosemicarbazones derived from α-amino acids against Dengue virus. *Journal of Medical Virology*, 89(3):546–552.

Pingaew, R., Prachayasittikul, S., Ruchirawat, S. 2010. Synthesis, Cytotoxic and Antimalarial Activities of Benzoyl Thiosemicarbazone Analogs of Isoquinoline and Related Compounds. *Molecules*, 15(2):988–996.

Rauf, A., Kashif, M. K., Saeed, B. A., Al-Masoudi, N. A., Hameed, S. 2019. Synthesis, anti-HIV activity, molecular modeling study and QSAR of new designed 2-(2-arylidenehydrazinyl)-4-arylthiazoles. *Journal of Molecular Structure*, 1198:126866–126866.

Reddy, M. S., Prathima, B., Saraswathi, M., Babu, S., Sarala, Y., Reddy, V. A. 2016. Synthesis, spectral aspects and biological activities of 5-hydroxy-2-nitrobenzaldehydethiosemicarbazone and their Mn(II), Co(II) and Ni(II) complexes. *Journal of Applied Pharmaceutical Science*, pages 90–96.

Salman, A. S., Mahmoud, N. A., Mohamed, M. A., Azziem, A. A., Elsisi, D. M. 2017. Synthesis, Characterization and in vitro Cytotoxic Evaluation of Some Novel Heterocyclic Compounds Bearing the Indole Ring. *American Journal of Organic Chemistry*, 6(2):39–53.

Srivastava, A., Pandeya, S. N., Khan, A. A. 2011. Synthesis, characterization and analgesic activity of various indole rysemicarbazone & thiosemicarbazone derivatives. *Pharma Research*, 5:231–243.

Su, W., Qian, Q., Li, P., Lei, X., Xiao, Q., Huang, S., Huang, C., Cui, J. 2013. Synthesis, characterization, and anticancer activity of a series of ketone-N4-substituted thiosemicarbazones and their ruthenium(II) arene complexes. *Inorganic Chemistry*, 52(21):12440–12449.

Suvarapu, L. N., Somala, A. R., Koduru, J. R., Baek, S. O., Ammireddy, V. R. 2012. A Critical Review on Analytical and Biological Applications of Thio- and Phenylthiosemi carbazones. *Asian Journal of Chemistry*, 24(5):1889–1898.

Velezheva, V., Brennan, P., Ivanov, P., Kornienko, A., Lyubimov, S., Kazarian, K., Nikonenko, B., Majorov, K., Apt, A. 2016. Synthesis and anti-tuberculosis activity of indole–pyridine derived hydrazides, hydrazide–hydrazones, and thiosemicarbazones. *Bioorganic & Medicinal Chemistry Letters*, 26(3):978–985.

Zhang, B., Luo, H., Xu, Q., Lin, L., Zhang, B. 2017. Antitumor activity of a Trans-thiosemicarbazone Schiff base palladium (II) complex on human gastric adenocarcinoma cells. *Oncotarget*, 8(8):13620–13631.