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Nematicidal Characterization of Newly Synthesized Thiazine Derivatives Using *Caenorhabditis elegans* as the Model Organism

Naqeeb Ullah Khan, Muhammad Sajid,* Ahmad J. Obaidullah, Wajid Rehman,* Hadil Faris Alotaibi, Saira Bibi, and Mohammed M. Alanazi

Cite This: *ACS Omega* 2023, 8, 20767−20778

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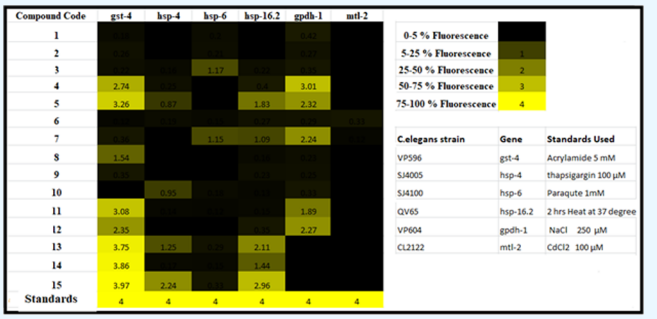
ABSTRACT: In humans, animals, and agriculture, parasitic nematode infection is a very serious issue. Many drugs are being used to control nematode infections. Owing to toxicity and nematodes' resistance to the available drugs, special attention is required to synthesize new drugs that are environmentally friendly with high-level efficacy. In the present study, various substituted thiazine derivatives (1 to 15) were synthesized, and the structures were confirmed by infrared, proton (^{1}H), and ^{13}C NMR spectroscopies. The nematicidal potential of the synthesized derivatives was characterized using *Caenorhabditis elegans* (*C. elegans*) as a model organism. Among all synthesized compounds, 13 (LD_{50} = 38.95 μg/mL) and 15 (LD_{50} = 38.21 μg/mL) were considered the most potent compounds. Most compounds showed excellent anti-egg-hatching activity. Fluorescence microscopy confirmed that compounds 4, 8, 9, 13, and 15 displayed a high apoptotic effect. The expressions of gst-4, hsp-4, hsp16.2, and gpdh-1 genes were high in affected (treated with thiazine derivatives) *C. elegans* in comparison with normal *C. elegans*. The present research revealed that modified compounds are highly effective as they showed the gene level changes in the selected nematode. Due to structural modification in thiazine analogues, the compounds showed various modes of action. The most effective thiazine derivatives could be excellent candidates for novel broad-scale nematicidal drugs.



1. INTRODUCTION

Heterocyclic chemistry is a very rich field based on heterocyclic nuclei from natural and synthetic sources and therefore covers a major portion of organic compounds. Due to heterocyclic nuclei, it withstands the subject of medication and got more consideration in drug development.[1,2] Here in this context, we focused on thiazine and its derivatives owing to its therapeutic profile.[3] Thiazine heterocyclic rings consist of nitrogen and sulfur atoms which exist at various positions in the ring, 1, 2-thiazine, 1, 3- thiazine, and 1,4- thiazine.[4] Thio analogue of morpholine which is called thiomorpholine is the reduced form of thiazine. Some of the thiazine analogues like benzothiazine, phenothiazine, and benz fused analogues of thiazine are of much potential interest owing to their dynamic therapeutic properties.[5,6] The diverse pharmaceutical profile of thiazine is due to N−C−S linkage present in its structure, and owing to this consideration, N−C and C−S linkage relationships are employed for different dyes, tranquilizers, and antitumor, antitubercular, and antimicrobial activities.[7] Moreover, nitrogen affords basic character to thiazine; due to this, it acts as a backbone of cephalosporin, and the thiazine structural unit is also part of various alkaloids like hormones, vitamins, and antibiotics.[8] Benz fused thiazine has displayed better therapeutic potential against multifactorial diseases like diabetic complications, alzheimers, inflammatory conditions, cardiovascular diseases, and tuberculosis.[9] Detailed literature survey about thiazine indicates that it is a versatile motif owing to its potential for antifungal, antitubercular, antibacterial, antiviral, anticonvulsant, antimalarial, antipsychotic, and anti-inflammatory activities.[4,7,10] Until now, no one has published thiazine analogues as antinematodal agents; in the present work, all analogues were evaluated against nematode in order to check out the nematicidal potential and cytotoxic profile of thiazine.

Nematodes are free-living and parasitic roundworms belonging to phylum Nematoda.[11] They are found in host, soil, fresh water, and marine water.[12] Parasitic nematodes have several harmful effects on plant growth, animal productivity, and human body.[13,14] The most effective species of parasitic nematodes that produce infections in animals, livestock, and

Received: March 1, 2023
Accepted: May 25, 2023
Published: June 3, 2023

humans are *Ascaris lumbricoides*, *Trichuris trichiura*, and many species of hookworms.[15] In grazing livestock, the most common infection of parasitic nematodes is a gastrointestinal infection caused by *Trichuris trichiura* (whipworm) and *Ascaris lumbricoides* (roundworm). In developing countries, economic losses in livestock have occurred due to gastrointestinal infection.[16] The per year agriculture loss of parasitic nematode infections is approximately $157 billion U.S. dollars worldwide.[17]

Nematicidal synthetic drugs control many infections caused by parasite nematode. The different drugs used against nematode infections are levamisole, piperazine, oxantel, benzimidazole, avermectins, pyrantel, paraherquamide, morantel, and milbemycins. Due to continuous and heavy reliance on these drugs, the nematodes have developed resistance against several drugs. The drugs which are currently used against worms (anthelmintic) have many side effects. Therefore, we need to develop safer and environment friendly synthetic drugs.[18,19] *Caenorhabditis elegans* (*C. elegans*) is used as a model organism in this research because of its easy culturing and maintenance, short life cycle, simple anatomy, and low testing cost.[20−22] *C. elegans* is nonpathogenic in nature and lives in humid soil (free-living) and used on *E. coli* (OP50) bacteria as foodstuff.[23] The research studies carried out on *C. elegans* anatomy, development, and genetics are applicable to many other nematodes.

2. MATERIAL AND METHODS

2.1. Chemicals. All solvents & chemical reagents were obtained from Sigma Aldrich and Merck. Quantitative real-time polymerase chain reaction (qRT-PCR) products were received from Thermo Fisher Scientific and Biotium. All chemicals were used as received.

2.2. Instruments. ^{1}HNMR and ^{13}CNMR spectra of all thiazine derivatives were performed via applying magnetic field at 500 and 125 MHz, respectively. A fluorescence microscope (Olympus BX-60 (USA)), an inverted microscope (Eclipse TS-100, Nikon, Japan), and quantitative RT-PCR (6321 Eppendorf, USA) were used in the current research.

2.3. Synthesis of Thiazine Derivatives. *2.3.1. Synthesis of Substituted Chalcone.* Substituted chalcone was synthesized via reacting equimolar quantities of various substituted aldehydes and ketones in ethanol, and the reaction mixture was condensed by adding two to three pellets of KOH. The reaction mixture was stirred (at 25 °C, 10 h) and then filtered. The solid precipitate appeared in the mixture followed by recrystallization from methanol, and as a result, pure chalcone was obtained.

2.3.2. Synthesis of Thiazine Derivatives (1 to 15). All derivatives of thiazine were synthesized via reacting equimolar quantities of chalcone and thiourea in ethanol. The reaction mixture was condensed by adding KOH and refluxed for 3−4 h. When the reaction was completed, it was stirred at 25 °C for 24 h. The precipitates formed were filtered, and thiazine derivatives were obtained. 1 to 15 codes were assigned to represent newly synthesized thiazine derivatives.

2.3.2.1. 6-(2-Fluorophenyl)-4-p-tolyl-3,6-di-hydro-2H-1,3-thiazine-2-imine (1). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm**10.76 (s, 1H, NH), 10.21 (s, 1H, NH), 7.71 (t, J = 7.3 Hertz, 1H, Aromatic), 7.55 (d, J = 7.1 Hertz, 1H, Aromatic), 7.36 (d, J = 7.1 Hertz, 1H, Aromatic), 7.29 (d, J = 6.7 Hertz, 2H, Aromatic), 7.16 (d, J = 6.7 Hertz, 2H, Aromatic),7.01 (t, J = 7.2 Hertz, 1H, Aromatic), 6.66 (d, J = 5.4 Hertz, 1H, CH), 5.26 (d, J = 5.1 Hertz, 1H, CH), 2.43 (s, 3H, CH_3) **^{13}CNMR: (125 MHz DMSO-d_6) δ ppm**174.0, 161.6, 150.9, 137.6, 135.0, 131.3, 130.9, 129.0, 128.7, 126.9, 125.7, 124.0, 123.3, 122.4, 105.4, 33.0, 32.3. **Yield:** 85%, **Melting Point:** 158−160 °C, **IR (υ/cm):** NH (3220), C═N (1672), C═C (1561), C−S (1181).

HREI-MS [**H**]$^{+}$: calcd for $C_{17}H_{15}FN_2S$ 298.0940, found 298.0910.

2.3.2.2. 4-(4-Bromophenyl)-6-(2-fluorophenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (2). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.81 (s, 1 H, NH), 10.29 (s, 1H, NH), 7.77 (t, J = 7.4 Hertz, 1H, Aromatic), 7.68 (d, J = 6.8 Hertz, 2H, Aromatic), 7.63 (d, J = 7.1 Hertz, 1H, Aromatic), 7.45 (d, J = 7.4 Hertz, 1H, Aromatic), 7.33 (d, J = 6.6 Hertz, 2H, Aromatic), 7.05 (t, J = 7.2 Hertz, 1H, Aromatic), 6.69 (d, J = 5.4 Hertz, 1H, CH), 5.29 (d, J = 5.1 Hertz, 1H, CH) **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 159.2, 158.3, 151.6, 138.0, 134.5, 132.5, 131.3, 128.7, 127.6, 126.8, 123.0, 119.3, 118.3, 117.8, 106.8, 56.0, **Yield**: 82%, **Melting Point**: 184−186 °C, **IR (υ/cm):** NH(3216), C═N(1675), C═C(1583), C−S(1211), C−F(813).

HREI-MS [**H**]$^{+}$: calcd for $C_{16}H_{12}BrFN_2S$ 361.9889, found 361.9879.

2.3.2.3. 4-(4-Chloro-phenyl)-6- (2-fluorophenyl)-3, 6-di-hydro-2H-1,3-thiazine-2-imine (3). **^{1}HNMR: ^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.83 (s, 1H, NH), 10.28 (s, 1H, NH), 7.82 (t, J = 7.3 Hertz, 1H, Aromatic), 7.61 (d, J = 7.2 Hertz, 1H, Aromatic), 7.44 (d, J = 6.8 Hertz, 2H, Aromatic), 7.42(d, J = 7.3 Hertz, 1H, Aromatic), 7.28 (d, J = 6.5 Hertz, 2H, Aromatic), 7.06 (t, J = 7.0 Hertz, 1H, Aromatic), 6.71 (d, J = 5.4 Hertz, 1H, CH), 5.29 (d, J = 5.2 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 166.2, 165.2, 156.4, 144.1, 139.2, 133.7, 130.6, 126.8, 125.6, 123.0, 122.6, 121.2, 120.8, 119.5, 99.5, 34.2, **Yield**: 79%, **Melting Point:** 168−171 °C, **IR (υ/cm):** NH(3233), C═N(1676), C═C(1569), C−S(1187), C−F(829).

HREI-MS [**H**]$^{+}$**:** calcd for $C_{16}H_{12}ClFN_2S$ 318.0394, found 318.0374.

2.3.2.4. 4-(2,5-Dichlorophenyl)-6-(2-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (4). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.83 (s, 1H. NH), 10.31 (s, 1H. NH), 7.84 (t, J = 7.4 Hertz, 1H, Aromatic), 7.65 (d, J = 7.2 Hertz, 1H, Aromatic), 7.41(d, J = 7.2 Hertz, 1H, Aromatic), 7.33 (d, J = 6.6 Hertz, 1H, Aromatic), 7.31 (d, J = 6.5, Hertz, 1H, Aromatic), 7.23 (s, 1H, Aromatic), 7.09 (t, J = 7.3 Hertz, 1H, Aromatic), 6.75 (d, J = 5.4 Hertz, 1H, CH), 5.34 (d, J = 5.2 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 168.3, 163.3, 153.9, 136.7, 134.4, 135.3, 130.3, 129.9, 128.3, 125.1, 124.8, 123.8, 122.5, 120.2, 91.8, 26.0, **Yield**: 79%, **Melting Point:** 172−174 °C, **IR (υ/cm):** NH (3182), C═N(1667), C═C(1573), C−S(1185), C−F(807).

HREI-MS [**H**]$^{+}$**:** calcd for $C_{16}H_{11}Cl_2FN_2S$ 352.0004, found 351.9974.

2.3.2.5. 6-(2-Fluoro-phenyl)-4-(4-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (5). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.81 (s, 1H. NH), 10.38 (s, 1H. NH), 7.88 (m, 1H, Aromatic), 7.51 (d, J = 7.3 Hertz, 1H, Aromatic), 7.37 (d, J = 6.5 Hertz, 2H, Aromatic), 7.32 (d, J = 6.7 Hertz, 2H, Aromatic), 7.24 (d, J = 6.7 Hertz, 2H, Aromatic), 7.11 (m, 1H, Aromatic), 6.75 (d, J = 5.4 Hertz, 1H, CH), 5.33 (d, J = 5.3 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO-d_6) δ ppm** 169.4, 161.8, 158.3, 156.5, 143.6, 141.3, 138.7, 132.2, 131.0, 129.6, 121.3, 118.5, 117.3, 113.4, 101.3, 44.0, **Yield**: 85%, **Melting Point:** 176−178 °C, **IR (υ/cm):** NH(3179), C═N(1674), C═C(1483), C−S(1178), C−F(832).

https://doi.org/10.1021/acsomega.3c01378
ACS Omega 2023, 8, 20767−20778

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{12}F_2N_2S$ 302.0689, found 302.0649.

2.3.2.6. 4-(4-Chloro-phenyl)-6-(3-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (6). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.72 (s, 1H. NH), 10.16 (s, 1H, NH), 7.37 (m, 1H, Aromatic), 7.53 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.34 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.28 (d, *J* = 6.6 Hertz, 1H, Aromatic), 7.18 (s, 1H, Aromatic), 14 (d, *J* = 6.3 Hertz, 1H, Aromatic), 6.68 (d, *J* = 5.3 Hertz, 1H, CH), 5.30 (d, *J* = 5.2 Hertz, 1H, CH), **^{13}CNMR: (125 MHtz DMSO - d_6) δ ppm** 172.5, 164.3, 159.4, 150.6, 133.4, 131.2, 130.2, 129.1, 128.6, 126.3, 124.3, 119.0, 116.2, 112.4, 97.5, 35.2, **Yield**: 76%, **Melting Point:** 166−168 °C, **IR (υ/cm):** NH(3284), C═N(1683), C═C(1568), C−S(1190), C−F(809).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{12}ClFN_2S$ 318.0394, found 318.0374.

2.3.2.7. 4-(2,5-Dichlorophenyl)-6-(3-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (7). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.76 (s, 1H. NH), 10.19 (s, 1H, NH), 7.41 (d, *J* = 7.2 Hertz, 1H, Aromatic), 7.39 (d, *J* = 7.4 Hertz, 1H, Aromatic), 7.38 (m, 1H, Aromatic), 7.33 (d, *J* = 6.6 Hertz, 1H, Aromatic), 7.27 (s, 1H, Aromatic), 7.22 (s, 1H, Aromatic), 14 (d, *J* = 6.3 Hertz, 1H, Aromatic), 6.69 (d, *J* = 5.3 Hertz, 1H, CH), 5.35 (d, *J* = 5.2 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 166.2, 162.4, 161.3, 147.7, 145.4, 142.3, 137.6, 133.2, 132.7, 128.2, 127.8, 125.7, 124.6, 114.1, 98.3, 27.0, **Yield**: 74%, **Melting Point:** 171−173 °C, **IR (υ/cm):** NH(3192), C═N(1667), C═C(1583), C−S (1186), C−F(806).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{11}Cl_2FN_2S$ 352.0004, found 351.9974.

2.3.2.8. 6-(3-Fluoro-phenyl)-4-(4-fluorophenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (8). **^{1}HNMR: δ ppm** 10.79 (s, 1H, NH.), 10.18 (s, 1H, NH.), 7.39 (m, 1H, Aromatic), 7.37 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.29 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.28 (d, *J* = 6.8 Hertz, 1H, Aromatic), 7.23 (s, 1H, Aromatic), 17 (d, *J* = 6.6 Hertz, 1H, Aromatic), 6.68 (d, *J* = 5.3 Hertz, 1H, CH), 5.31 (d, *J* = 5.2 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 167.9, 165.7, 163.7, 157.6, 148.5, 138.6, 135.4, 132.0, 131.3, 124.5, 118.4, 116.6, 115.3, 108.9, 93.5, 34.8, **Yield**: 76%, **Melting Point:** 178−181 °C, **IR (υ cm^{-1}):** N(3368), C═N(1652), C═C(1550), C−S(1187), C−F (841).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{12}F_2N_2S$ 302.0689, found 302.0649.

2.3.2.9. 4-(4-Bromo-phenyl)-6-(3-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (9). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.71 (s, 1H, NH), 10.17 (s, 1H, NH), 7.73 (d, *J* = 7.4 Hertz, 2H, Aromatic), 7.38 (m, 1H, Aromatic), 7.36 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.26 (d, *J* = 6.7 Hertz, 1H, Aromatic), 7.15 (s, 1H, Aromatic), 7.09 (d, *J* = 6.7 Hertz, 1H, Aromatic), 6.65 (d, *J* = 5.3 Hertz, 1H, CH), 5.25 (d, *J* = 5.2 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 168.9, 159.3, 150.6, 141.5, 138.0, 136.5, 133.5, 130.9, 129.4, 128.6, 127.8, 125.3, 123.2, 111.7, 91.5, 32.8, **Yield**: 69%, **Melting Point:** 181−183 °C, **IR (υ/cm):** NH(3194), C═N(1672), C═C(1562), C−S(1184), C−F(808).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{12}BrFN_2S$ 361.9889, found 361.9879.

2.3.2.10. 6-(3-Fluorophenyl)-4-p-tolyl-3,6-di-hydro-2H-1,3-thiazine-2-imine (10). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.71 (s, 1H, NH), 10.12 (s, 1H, NH), 7.35 (m, 1H, Aromatic), 7.34 (d, *J* = 7.2 Hertz, 2H, Aromatic), 7.23 (d, *J* = 6.6 Hertz, 1H, Aromatic), 7.17 (d, *J* = 7.3 Hertz, 2H, Aromatic), 7.15 (s, 1H, Aromatic), 7.08 (d, *J* = 6.3 Hertz, 1H, Aromatic), 6.64 (d, *J* = 5.3 Hertz, 1H, CH), 5.23 (d, *J* = 5.2 Hertz, 1H, CH), 2.46 (s, 3H, CH_3). **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 171.9, 167.5, 165.6, 153.7, 147.5, 144.0, 142.3, 138.0, 136.0, 134.9, 133.4, 132.3, 126.7, 118.1, 93.7, 45.8, 21.3, **Yield**: 75%, **Melting Point:** 170−171 °C, **IR (υ/cm):** NH(3183), C═N(1672), C═C(1570), C−S(1185), C−F(804).

HREI-MS [**H**]$^+$**:** calcd for $C_{17}H_{15}FN_2S$ 298.0940, found 298.0910.

2.3.2.11. 4-(4-Chlorophenyl)-6-(4-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (11). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.91 (s, 1H, NH), 10.32 (s, 1H, NH), 7.58 (d, *J* = 7.4 Hertz, 2H, Aromatic), 7.47 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.36 (d, *J* = 7.3 Hertz, 2H, Aromatic), 7.21 (d, *J* = 6.9 Hz, 2H, Aromatic), 6.69 (d, *J* = 5.6 Hertz, 1H, CH), 5.31 (d, *J* = 5.3 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 165.3, 156.6, 154.6, 149.1, 145.1, 143.5, 139.3, 137.3, 133.7, 128.7, 120.2, 118.2, 116.5, 114.3, 103.3, 33.1 , **Yield:** 84%, **M.P:** 170−172 °C, **IR (υ/cm):** NH(3194), C═N(1664), C═C(1571), C−S(1185), C−F(809).

HREI-MS [**H**]$^+$: calcd for $C_{16}H_{12}ClFN_2S$ 318.0394, found 318.0374.

2.3.2.12. 4-(2,5-Dichlorophenyl)-6-(4-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (12). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.94 (s, 1H, NH.), 10.32 (s, 1H, NH.), 7.51 (d, *J* = 7.3 Hertz, 2H, Aromatic), 7.41 (d, *J* = 6.8 Hertz, 1H, Aromatic), 7.37 (d, *J* = 6.6 Hertz, 1H, Aromatic), 7.27 (s, 1H, Aromatic), 7.24 (d, *J* = 7.3 Hertz, 2H, Aromatc), 6.72 (d, *J* = 5.6 Hertz, 1H, CH), 5.32 (d, *J* = 5.3 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 160.2, 159.7, 152.3, 137.7, 135.4, 134.3, 132.3, 129.2, 128.1, 126.1, 125.3, 124.8, 121.3, 119.9, 99.7, 26.3, **Yield**: 71%, **Melting Point:** 164−166 °C, **IR (υ/cm):** NH(3131), C═N(1665), C═C(1572), C−S(1186), C−F(808).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{11}Cl_2FN_2S$ 352.0004, found 351.9974.

2.3.2.13. 4(4-Fluoro-phenyl)-6-(4-fluoro-phenyl)-4H-1,3-thiazine-2-amine (13). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.91 (s, 1H, NH), 10.33 (s, 1H, NH), 7.54 (d, *J* = 7.4 Hertz, 2H, Aromatic), 7.47 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.25 (d, *J* = 7.0 Hertz, 2H, Aromatic), 7.21 (d, *J* = 7.3 Hertz, 2H, Aromatic), 6.69 (d, *J* = 5.6 Hertz, 1H, CH), 5.33 (d, *J* = 5.3 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 169.6, 165.1, 150.3, 148.1, 147.2, 142.5, 140.2, 139.9, 134.4, 133.7, 128.7, 126.2, 122.1, 115.4, 96.6, 34.8, **Yield:** 87%, **Melting Point:** 179−182 °C, **IR (υ cm^{-1}):** NH(3187), C═N(1655), C═C(1585), C−S(1194), C−F(806).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{12}F_2N_2S$ 302.0689, found 302.0649.

2.3.2.14. 4-(4-Bromo-phenyl)-6-(4-fluoro-phenyl)-3,6-di-hydro-2H-1,3-thiazine-2-imine (14). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.89 (s, 1H, NH), 10.32 (s, 1H, NH), 7.77 (d, *J* = 7.5 Hertz, 2H, Aromatic), 7.45 (d, *J* = 7.1 Hertz, 2H, Aromatic), 7.41 (d, *J* = 7.3 Hertz, 2H, Aromatic), 7.21 (d, *J* = 6.8 Hertz, 2H, Aromatic), 6.68 (d, *J* = 5.6 Hertz, 1H, CH), 5.27 (d, *J* = 5.3 Hertz, 1H, CH), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 161.3, 159.6, 155.6, 137.0, 134.5, 132.2, 131.5, 130.1, 127.1, 126.6, 123.6, 121.3, 117.2, 116.5, 88.7, 36.2, **Yield:** 82%, **Melting Point:** 170−172 °C, **IR (υ/cm):** NH(3217), C═N(1667), C═C(1570), C−S(1185), C−F (805).

HREI-MS [**H**]$^+$**:** calcd for $C_{16}H_{12}BrFN_2S$ 361.9889, found 361.9879.

https://doi.org/10.1021/acsomega.3c01378
ACS Omega 2023, 8, 20767−20778

2.3.2.15. 6- (4-Fluoro-phenyl)-4-p-tolyl-3,6-di-hhydro-2H-1,3-thiazine-2-imine (15). **^{1}HNMR: (500 MHz DMSO - d_6) δ ppm** 10.87 (s, 1H, NH), 10.28 (s, 1H, NH), 7.41 (d, J = 7.3 Hertz, 2H, Aromatic), 7.38 (d, J = 6.5 Hertz, 2H, Aromatic), 7.19 (d, J = 6.6 Hertz, 2H, Aromatic), 7.15 (d, J = 7.1 Hertz, 2H, Aromatic), 6.66 (d, J = 5.6 Hertz, 1H, CH), 5.29 (d, J = 5.3 Hertz, 1H, CH), 2.51 (s, 3H, CH_3), **^{13}CNMR: (125 MHz DMSO - d_6) δ ppm** 177.3, 164.3, 163.5, 156.1, 148.1, 145.0, 138.3, 135.3, 130.0, 129.0, 128.9, 125.1, 122.7, 120.7, 90.8, 56.8, 33.3, **Yield:** 79%, **Melting Point:** 171−174 °C, **IR (υ/cm):** NH(3365), C═N(1658), C═C(1583), C−S(1189), C−F (809).

HREI-MS $[H]^+$**:** calcd for $C_{17}H_{15}FN_2S$ 298.0940, found 298.0910.

2.4. Biological Activities. *2.4.1. Antinematodal Assay.* For the antinematodal potential of thiazine derivatives, the diluted nematode (30−50 nematode in 100 μL of NGM buffer) was added to each well of the sterile 96-well plate, and then, *C. elegans* were exposed to different concentrations of synthetic compounds and incubated for 24 h at 20 °C. The survival of *C. elegans* was counted under an inverted microscope. Those *C. elegans* which did not show any response to physical stimuli (fine needle) were considered dead.[24,2]

2.4.2. Egg Hatching Analysis. 2.4.2.1. Isolation of Eggs. About 80 to 100 adult (N2 strain) *C. elegans* were treated with 5 mL of hypochlorite solution [250 μL of NaOH (10 M), 650 μL of bleach, and 4.1 mL of H_2O] in a 15 mL tube for 5 min. After 5 min, *C. elegans* were dissolved in hypochlorite solution, and the eggs were isolated. The eggs were washed three times with NGM buffer and centrifuged for 1 min at 1000 rpm. To calculate the effect of thiazine derivatives (1 to 15) on *C. elegans* eggs, the eggs were treated with 20 μg/mL of each thiazine derivative.[25,26]

2.4.2.2. Effect of Thiazine Derivatives. The experiment was carried out in a 96-well plate. About 60−80 eggs were transferred to each well containing 100 μL of S-medium with the concentration of 20 μg/mL thiazine derivatives. In each well were added 60−80 eggs, ≤1% DMSO (negative control), ivermectin 100 μg/mL (positive control), and S-medium. The plate was incubated for 24 h at 20 °C. After 24 h, the plate was checked under an inverted microscope, and the larvae and eggs were counted.[27]

2.4.3. Fluorescence Microscopy. To investigate the apoptotic effect in *C. elegans* cells caused by thiazine derivatives, fluorescence microscopy was carried out. Before the microscopic analysis, the affected worms were stained with a dye (acridine orange, AO).

2.4.3.1. AO Staining Assay. *C. elegans* were treated with thiazine derivatives (at LD_{50} concentration) and incubated for 24 h at 20 °C. After incubation, the dead *C. elegans* were exposed to the AO dye. 2 μL of AO was added to 1000 μL of S-medium; from this, 500 μL of AO was added to dead *C. elegans* and incubated for 1 h in dark at 37 °C. After 1 h, the *C. elegans* were washed with NGM buffer and analyzed using fluorescence microscopy analysis.[28,29]

2.4.4. Determination of Gene Expression through Screening Technique. In the present study, six GFP & RFP fused reporter gene strains (transgenic *C. elegans*) were used to screen out the expression of stress genes in the affected *C. elegans* (treated with thiazine derivatives at a concentration of 20 μg/mL for 24 h). VP596, SJ4005, SJ4100, QV65, VP604, and CL2122 transgenic strains were used to screen out the expression of stress genes (gst-4, hsp-4, hsp-6, hsp-16.2, gpdh-1, and mtl-2).[30−32]

2.4.4.1. Screening Analysis Assay. About 80 to 100 adult *C. elegans* (each transgenic strain) were treated with 5 mL of hypochlorite solution in a 15 mL tube for 5 min. After 5 min, *C. elegans* were dissolved in hypochlorite solution, and the eggs were isolated. The eggs were washed three times with NGM buffer and centrifuged for 1 min at 1000 rpm. The eggs of each transgenic *C. elegans* were added to NA22 (bacteria)-seeded NGM plates and labelled with date and strain name. All plates were incubated for 48 to 55 h at 20 °C. After 48 to 55 h, the plates were filled with L4 or newly adult *C. elegans*. They were washed three times with NGM buffer to remove the bacteria. About 50 to 70 *C. elegans* of each transgenic strain in each well of the 384-well plate were treated with 20 μg/mL of each thiazine derivative and standards for 24 h at 20 °C.[26]

The fluorescence microscope (BX60, USA) was used to score the fluorescence manually in each well of the 384-well plate.

0 = No Fluorescence or less than 5%.
1 = Fluorescence between 5 and 25%.
2 = Fluorescence between 25 and 50%.
3 = Fluorescence between 50 and 75%.
4 = Fluorescence between 75 and 100%.

2.4.5. Expression Analysis of C. elegans Gene Transcripts by RT-PCR. Quantitative RT-PCR was used to measure the gene expression of normal and affected *C. elegans* (treated with 10 μg/mL thiazine derivatives for 3 h at 20 °C).[33,34] These genes were gst-4, hsp-4, hsp-6, hsp-16.2, gpdh-1, mtl-2, and rpl-2.

C. elegans Picking. The lysis solution [98 μL of 2× lysis buffer (KCl (100 mM) 0.745 g, Tris pH 8.2 (20 mM) 0.24 g, $MgCl_2$ (5 mM) 0.047 g, IGEPAL (0.9%) 900 μL, Tween 20 (0.9%) 900 μL, gelatin (0.02%) 20 mg with 2 μL of 20 mg/mL proteinase K] and picking buffer (1 M, KPO_4 buffer) were prepared in separate PCR tubes. 3 μL of lysis buffer was added in the bottom, and 3 μL of picking buffer was added in the lid of each PCR tube. Then, 5−10 *C. elegans* (normal and affected) were added in the lid of each PCR tube, closed, and centrifuged. The tube was then checked, and all worms were in the bottom. Each tube was placed at −80 °C till all were prepared.

2.4.5.2. Isolation of RNA. The PCR was started when the temperature reached 65 °C and then stopped. All tubes were placed in PCR, and the lyses process (65 °C for 10 min to activate lysis and release RNA followed by 85 °C for 2 min to inactivate the proteinase K and denature, or unfold, RNA and hold at 4 °C) was run. After completion of the lyses process, for removal of all dsDNA, 1 μL of ds-DNase master mix (10X buffer, nuclease-free H_2O, ds-DNA enz) was added to each PCR tube, and the ds-DNase program [Destroy dsDNA at 55 °C for 5 min followed by 85 °C for 2 min (to inactivate the ds-DNAenz and hold at 4 °C)] was started. After completion of dsDNase program, only mRNA remained.

2.4.5.3. Synthesis of cDNA. 6 μL of cDNA master mix (5× buffer, 25 mM $MgCl_2$, Oligo(dt)s, dNTPs 10 mM, nuclease-free H_2O, RNasin, GoScript RT) was added to each PCR tube containing the mRNA, and the cDNA program (anneal at 25 °C for 5 min, extend at 42 °C for 1 h, inactivate at 70 °C for 15 min, chill at 4 °C for 5 min, and hold at 15 °C) was started. After completion of cDNA program, the cDNA was diluted by adding 40 μL of nuclease-free water.

2.4.5.4. Quantitative RT-PCR. RT-PCR analysis was carried out with a 10 μL reaction mixture containing 8 μL of RT-PCR master mix (2× buffer, reverse and forward primers, nuclease-free H_2O) and 2 μL of template cDNA. The *rpl-2* was used as a house-keeping gene. The labeled PCR 96-wells plate was seeded and placed in a RT-PCR machine. The RT-PCR program with

https://doi.org/10.1021/acsomega.3c01378
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codes which was mentioned for the 96-well plate was run in the RT-PCR machine. The reaction was started with the initial activation of polymerase at 95 °C for 10 min followed by denaturation of the template at 95 °C for 15 s; annealing and elongation were done at 60 °C for 60 s. After completion of 40 cycles, the melting curve was achieved at 60 to 95 °C to assess the presence of a unique final product. After completion of the RT-PCR program, the assay and CT values were saved, and the PCR 96-well plate was removed from the machine.

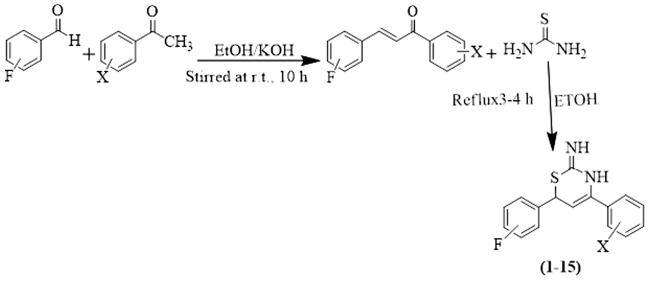
The CT values were put in a normalization calculator, and the expression values for each desired gene were obtained. These expression values of different desired genes were put in graph pad software or in excel sheet to draw the graphs.

2.5. Statistical Analysis. Statistical software (IBM SPSS, version 29) was used to calculate the maximum, minimum, standard deviation, and mean, while Origin software (Version 8.1) was used for graphs. For calculation of gene exposition, Prism Graph-Pad (version 9) was used.

3. RESULTS AND DISCUSSION

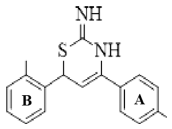
3.1. Chemistry. Chalcones derived from various substituted aldehydes and ketones were synthesized in the presence of KOH in methanol. This reaction mixture was stirred at room temperature for 10 h. The reaction was controlled through TLC, and chalcone was obtained in a good yield. To ensure the purity of the chalcone, recrystallization was done in methanol. The obtained chalcone was then refluxed with thiourea for 3−4 h in the presence of KOH to yield various substituted thiazine derivatives (Scheme 1).

Scheme 1. Synthesis of Thiazine Derivatives (1 to 15)



NMR was used as tool to assign the structures to the synthesized thiazine derivatives. All peaks assigned confirm the proposed structures. Two singlets at 10.76 and 10.21 ppm were assigned to two NH groups present in the thiazine ring. One proton at C.No-4 on the phenyl ring B gives a triplet in the range of 7.71 ppm. One proton on phenyl ring B gives a doublet in the range of 7.71 ppm which is ortho coupling. Proton at C.No-6 on phenyl ring B gives a doublet in the range of 7.36 ppm indicating ortho coupling. Similarly, two protons of 2/6 position on aromatic ring A give a doublet. Furthermore, two protons of the 3/5 position on aromatic ring A also give a doublet. One proton at C.No-5 on phenyl ring B gives a triplet in the range of 7.01 ppm indicating ortho coupling. The proton of the CH group of the thiazine ring gives a doublet in the range of 6.66 ppm. S−CH proton of the thiazine ring also gives a doublet in the range of 5.26 ppm. Three protons of CH_3 groups on ring B at 4-position give a singlet in the range of 2.43 ppm.

Further confirmation of thiazine derivatives was done through FTIR. In all thiazine derivatives, the NH bands appeared in the range from 3100 to 3300 cm^{-1}. Similarly, the characteristic peak

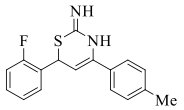
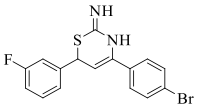
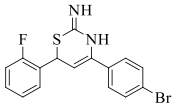
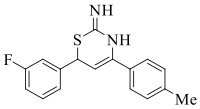
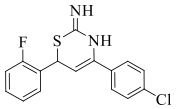
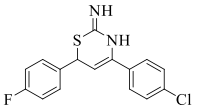
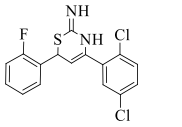
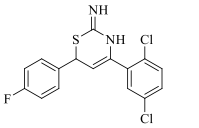
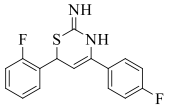
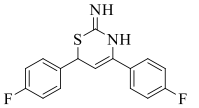
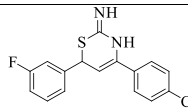
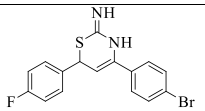
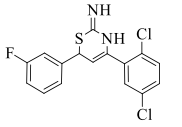
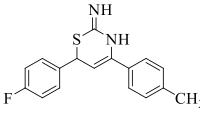
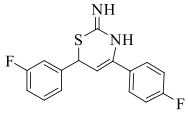


representing C═N was observed around 1672 cm^{-1}. Peaks appeared at 1181 cm^{-1} representing the C−S functional group, while the peak appeared around 832 cm^{-1}.

High-resolution mass spectroscopy was used for further confirmation of synthesized derivatives 1 to 15, and all mass values are very close to the calculated one which further endorsed the formation of our target derivatives (Table 1).

3.2. Biological Activities. *3.2.1. Antinematodal Potential of Thiazine Derivatives.* The LD_{90} and LD_{50} values of all

Table 1. Structures of Thiazine Derivatives (1 to 15)

s.no	structure	s.no	structure
1		9	
2		10	
3		11	
4		12	
5		13	
6		14	
			
7		15	
8			

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synthetic thiazine derivatives were evaluated after 24 h. Compound 15 showed highest activity and killed 100% *C. elegans* at a concentration of 100 μg/mL. The LD_{90} and LD_{50} of compound 15 were 87.49 and 38.21 μg/mL and those of 13 were 98.64 and 38.95 μg/mL, respectively. Compounds 3, 6, and 10 showed good antinematodal activity, and their LD_{90} and LD_{50} were 95.78 and 51.32 μg/mL, 97.51 and 47.89 μg/mL, and 92.57 and 45.63 μg/mL, respectively. While other compounds were relatively less active, the LD_{50} of compounds 1, 9, 7, and 14 were found to be 55.19, 52.11, 57.95, and 53.46 μg/mL respectively. Compounds 2, 4, 5, 8, 11, and 12 were very less active and showed LD_{50} between 70 and 100 μg/mL. All compounds (thiazine derivatives) showed high activity as compared with the control (positive control levamisole 20.35 μg/mL and negative control ≤1% DMSO because all compounds were dissolved in DMSO) as shown in Figure 1 and Table 2.[35]

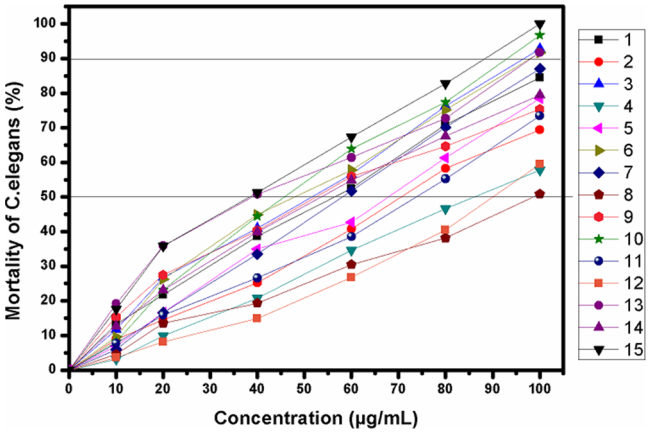

Figure 1. Nematicidal activities of thiazine derivatives (1 to 15).

Li et al.[36] determined the nematicidal potential of newly synthesized compounds. The most potent compounds showed LD_{50} between 2.89 and 8.19 μg/mL compared with fosthiazate (LD_{50} at 72.52 μg/mL).[36] Reddy et al.,[37] synthesized new series of synthetic compounds having compounds from 6a-r and tested against *C. elegans* for nematicidal activity. Among these, the most potent compounds (bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-methylphenyl)-2-benzyl3, 3a,5,6-tetrahydro-2H-pyrazolo-[3,4-d][1,3]thiazol-5-yl]phenyl]methane (6d), bis-[4-methoxy-3-[3-(4-fluorophenyl)-2-methyl-6-(4-methylphenyl) 3, 3a, 5, 6-tetrahydro-2H pyrazolo[3,4-d][1,3] thiazol-5-yl]-phenyl]methane (6f), bis-[4-methoxy-3-[3-(4-fluorophenyl)-6-(4-chlorophenyl)-2-benzyl3,3a,5,6-tetrahydro-2H-pyrazolo-[3,4-d][1,3]thiazol-5-yl]phenyl]methane (6j), etc.) showed LD_{50} between 160 and 210 μg/mL compared with standard oxamyl (LD_{50} at 180 μg/mL).[37] In the reference of above studies, we confirmed that thiazine derivatives 1−15 showed good nematicidal potential having LD_{50} values at lower concentrations than standard fosthiazate and oxamyl (72.52 and 180 μg/mL) and at higher concentrations than levamisole (20.35 μg/mL).

3.3. SAR (Structure−Activity Relationship) Analysis.

For an in-depth analysis of this approach, the SAR was deduced based on the values of antinematicidal activities shown in the table. In comparison to the structural and *C. elegans* mortality rate, a conclusion can be drawn. It has been noticed that among all thiazine derivatives, greater activities were observed in the compounds having fluorine and methyl group on the X position in thiazine. However, when fluorine and methyl group were present on the para position of the benzene ring in thiazine, the mortality rate was observed higher. In addition to this, chlorine-containing compounds (at para position only) also showed higher activities.

3.3.1. Thiazine Derivatives Effect on Egg Hatching. Figure 2 shows the effect of thiazine derivatives (20 μg/mL each) along with positive (ivermectin 100 μg/mL) and negative (≤1% DMSO) controls on *C. elegans* eggs. The unhatched eggs in compounds 1 to 15 were 36.91 ± 02.45, 45.09 ± 03.12, 71.93 ± 01.94, 62.06 ± 02.33, 56.09 ± 02.67, 30.46 ± 03.09, 60.42 ± 02.78, 46.08 ± 02.26, 44.78 ± 03.36, 37.94 ± 02.98, 65.16 ± 01.90, 27.83 ± 03.11, 64.01 ± 02.25, 53.19 ± 02.56, and 70.30 ± 01.76%, respectively, 04.75 ± 01.10% in the negative control, and 100.00 ± 00.00 in the positive control.

At 20 μg/mL, compounds 3, 4, 5, 7, 11, 13, 14, and 15 exhibited more than 50% unhatched eggs. Zhao et al.[38] tested 31

Table 2. Nematicidal Activity of Thiazine Derivatives (1 to 15) in % at Various Concentrations

thiazine derivatives	100 μg/mL	80 μg/mL	60 μg/mL	40 μg/mL	20 μg/mL	10 μg/mL	negative control (≤1% DMSO)
1	84.54 ± 02.17	70.87 ± 03.62	52.45 ± 03.28	38.69 ± 04.08	21.73 ± 03.29	13.36 ± 04.20	03.21 ± 02.33
2	69.41 ± 03.08	58.25 ± 02.37	40.82 ± 02.75	25.16 ± 03.48	14.57 ± 03.83	08.95 ± 04.10	02.95 ± 01.72
3	92.79 ± 02.86	76.25 ± 02.88	56.52 ± 03.15	40.94 ± 03.55	26.84 ± 02.96	11.78 ± 03.55	03.29 ± 01.93
4	57.82 ± 03.21	46.62 ± 03.01	34.67 ± 02.69	20.78 ± 03.93	09.89 ± 03.41	03.21 ± 01.90	02.66 ± 01.13
5	78.51 ± 03.09	61.33 ± 02.15	42.74 ± 03.69	34.93 ± 03.48	16.65 ± 04.26	06.90 ± 03.78	03.88 ± 01.66
6	91.65 ± 03.20	75.19 ± 02.52	57.83 ± 03.25	44.90 ± 02.33	26.23 ± 01.34	09.63 ± 03.57	02.90 ± 01.11
7	87.07 ± 02.91	70.18 ± 02.32	51.75 ± 03.05	33.46 ± 03.22	16.64 ± 02.49	05.95 ± 04.17	03.69 ± 01.33
8	50.87 ± 03.25	38.15 ± 02.66	30.51 ± 02.72	19.35 ± 03.46	13.52 ± 02.81	04.54 ± 02.66	03.77 ± 02.00
9	75.38 ± 02.41	64.66 ± 03.17	55.95 ± 03.81	40.32 ± 02.74	27.49 ± 02.95	15.36 ± 03.13	02.11 ± 01.65
10	96.75 ± 02.84	77.39 ± 03.24	63.95 ± 04.10	44.46 ± 03.61	23.17 ± 04.22	08.21 ± 03.12	03.90 ± 01.33
11	73.48 ± 02.50	55.24 ± 01.33	38.57 ± 03.16	26.62 ± 02.55	15.86 ± 03.29	07.74 ± 03.22	04.80 ± 02.31
12	59.45 ± 04.23	40.53 ± 03.14	26.78 ± 03.55	14.92 ± 02.87	08.13 ± 03.29	03.66 ± 02,10	03.25 ± 01.35
13	91.80 ± 02.94	72.71 ± 02.33	61.39 ± 03.65	50.81 ± 03.21	35.89 ± 02.13	19.22 ± 04.27	03.68 ± 01.10
14	79.45 ± 04.23	67.53 ± 03.14	54.78 ± 03.55	39.92 ± 02.87	23.13 ± 03.29	12.66 ± 02,10	5.50 ± 02.42
15	100.00 ± 0.00	82.77 ± 02.85	67.39 ± 03.51	51.26 ± 03.11	35.90 ± 04.18	22.64 ± 03.31	03.70 ± 01.47
Positive control (Levamisole LD_{50} at 20.35 μg/mL)	50.38 ± 2.42						

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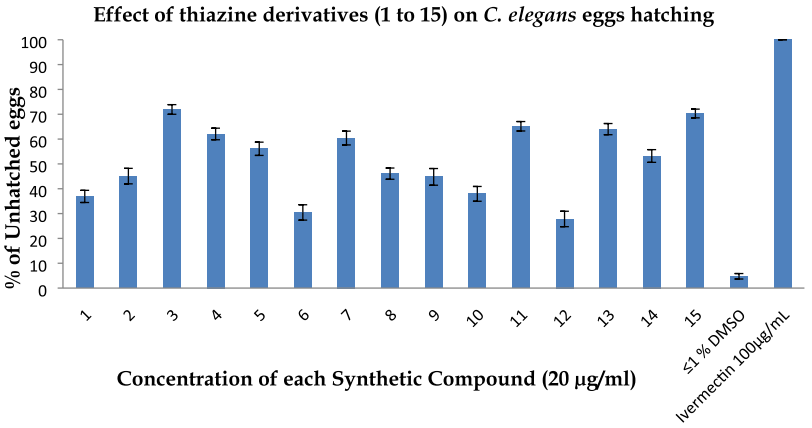

Figure 2. Effect of thiazine derivatives (1 to 15) on egg hatching.

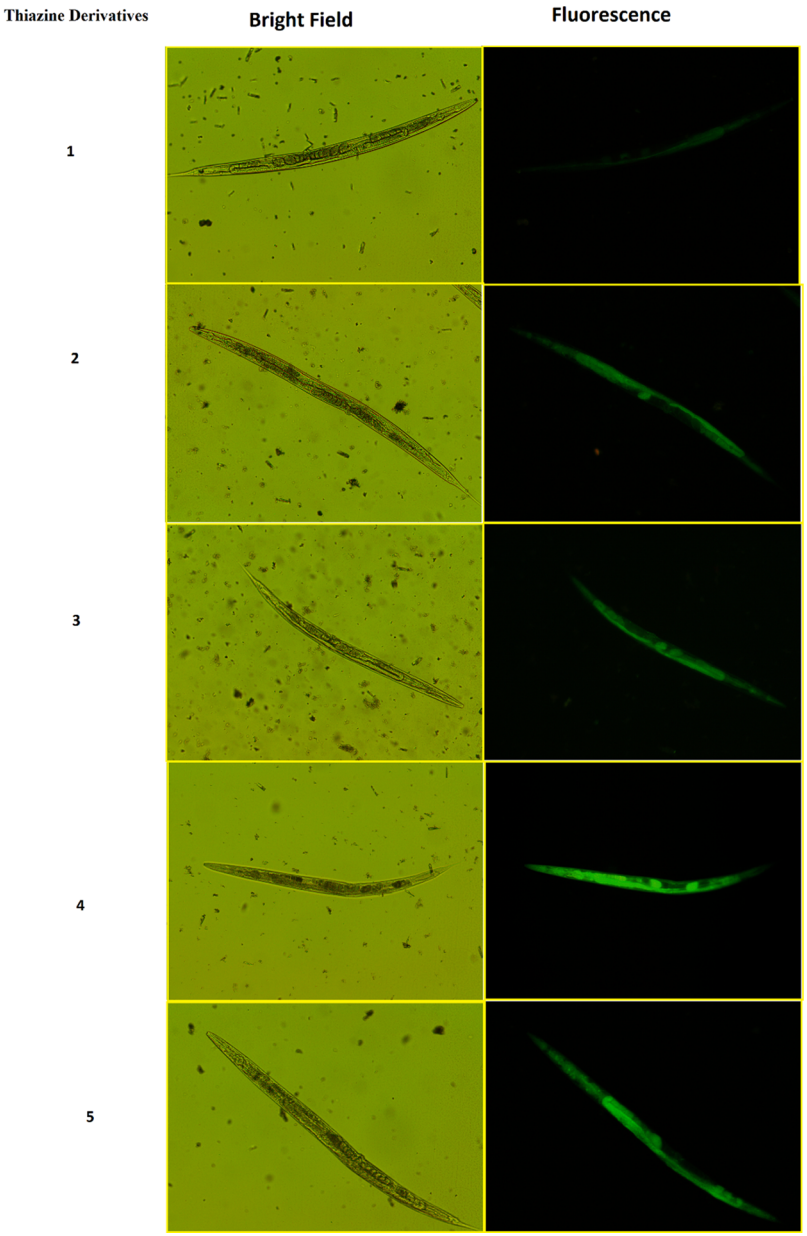

Figure 3. Fluorescence microscopy of affected *C. elegans* [treated with (1 to 5) thiazine derivatives].

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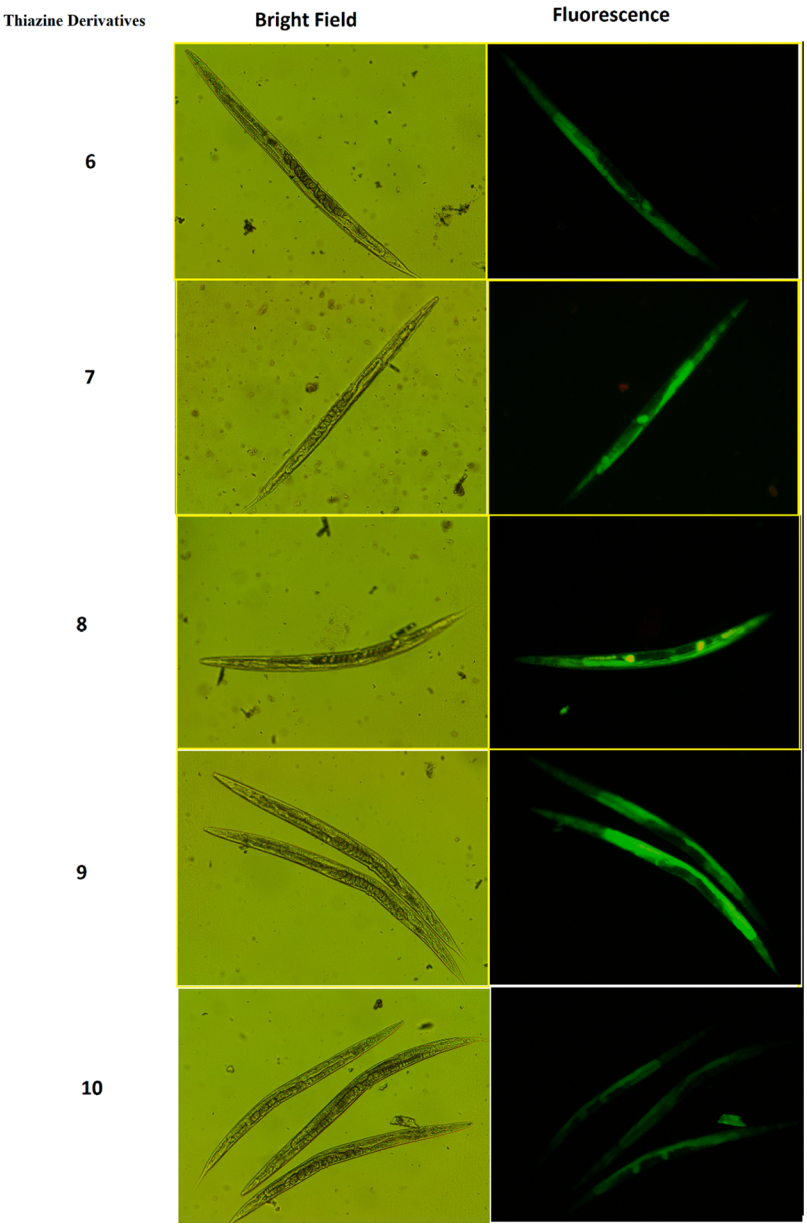

Figure 4. Fluorescence microscopy of affected *C. elegans* [treated with (6 to 10) thiazine derivatives].

quinone derivatives (5 new and 26 already known) against *C. elegans* egg hatching. Among all these tested compounds, only two compounds showed inhibitory effect on egg hatching with the EC_{50} values at 5.60 and 20.35 μg/mL.[38] So compounds 3, 4, 5, 7, 11, 13, 14, and 15 showed excellent results against egg hatching.

3.3.2. Fluorescence Microscopy of C. elegans Affected by Thiazine Derivatives. Fluorescence microscopy of affected *C. elegans* (treated with compounds 1 to 15) is an excellent technique to display apoptosis in *C. elegans* cells. Fluorescence microscopic images of thiazine derivatives are shown in Figure 3 (1 to 5), Figure 4 (6 to 10), and Figure 5 (11 to 15). Fluorescence microscopy of compounds 1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 14, and 15 displayed the fluorescence of green color in gonad cells, indicating the apoptotic effect in gonad cells only. Compounds 4 and 8 displayed the fluorescence of slightly yellowish green color in gonad cells and muscle cells, indicating the apoptotic effect in gonads and muscle cells. The current fluorescence microscopic results provide interesting information regarding the effect of thiazine derivatives on *C. elegans* anatomy that initiated an apoptotic effect in gonadal cells and muscular cells only. Sajid and Azim[24] also observed a similar fluorescence microscopy result in the study of the effect of honey on *C. elegans* gonadal and intestine cells.[24] Figures 3−5 show the fluorescence microscopic analysis that showed apoptosis in gonadal cells and muscular cells only. The thiazine derivatives 4, 8, 9, 13, and 15 displayed highly apoptotic effect confirmed by fluorescence microscopy.

3.3.3. Screening Analysis. In the current research, six GFP & RFP fused transgenic *C. elegans* strains were used to screen out different thiazine derivatives which affected the six stress response genes or stress pathways in *C. elegans*. In Figure 6, each gene showed fluorescence against thiazine derivatives at 20 μg/mL after 24 h of incubation. Fluorescence indicated expression of genes.

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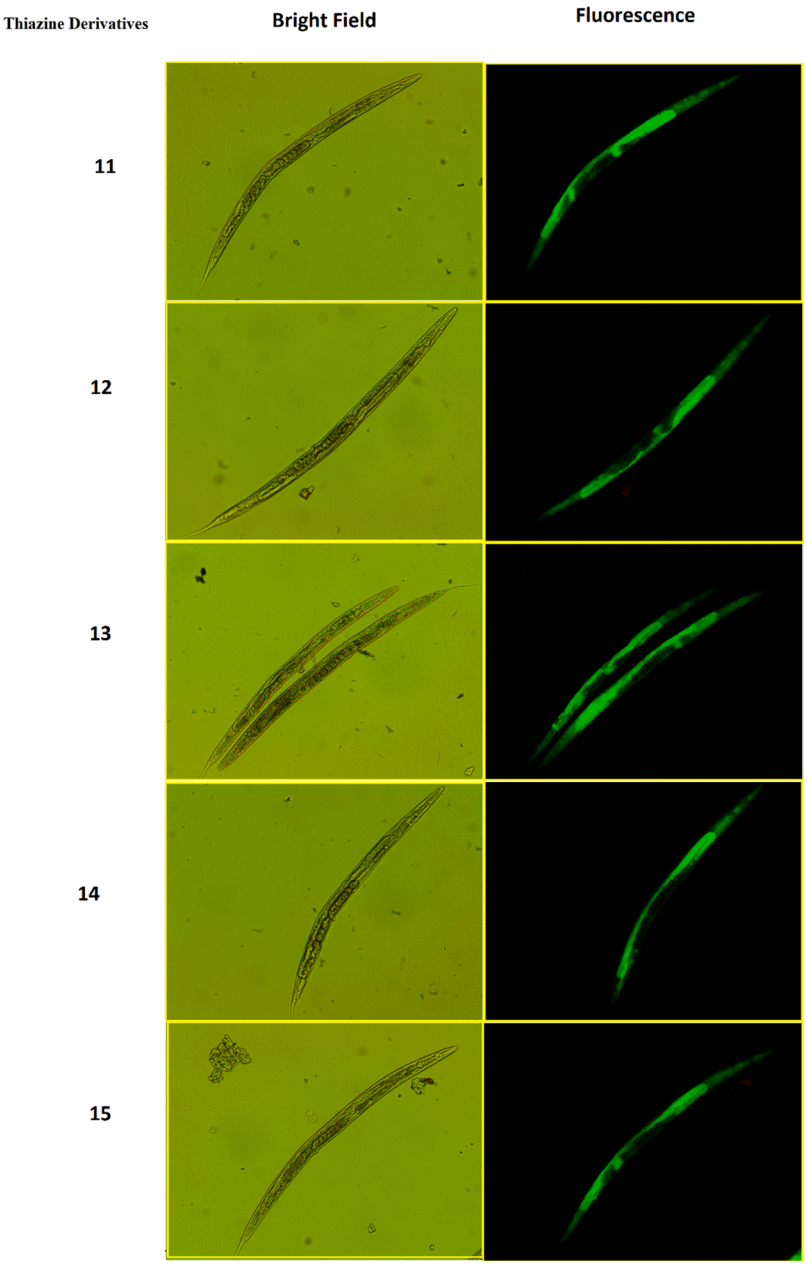

Figure 5. Fluorescence microscopy of affected *C. elegans* [treated with (11 to 15) thiazine derivatives].

3.3.3.1. Screening Analysis of Thiazine Derivatives (1 to 15). The gst-4 gene in transgenic *C. elegans* (VP596) was expressed by compounds 4, 5, 8, 11, 12, 13, 14, and 15. The hsp-4 gene in transgenic *C. elegans* (SJ4005) was expressed by compounds 5, 10, 13, and 15. The hsp-6 gene in transgenic *C. elegans* (SJ4100) was expressed by compounds 3 and 7. The hsp-16.2 gene in transgenic *C. elegans* (QV65) was expressed by compounds 5, 7, 13, 14, and 15. The gpdh-1gene in transgenic *C. elegans* (VP604) was expressed by compounds 4, 5, 7, 11, and 12. The mlt-2 gene in transgenic *C. elegans* (CL2122) was not expressed by any thiazine derivatives. Figure 6 shows that each standard highly expressed their specific stress reporter gene.

Khan et al.[39] also screened out the expression of stress genes in transgenic *C. elegans* affected by plant extracts and then confirmed through quantitative qRT-PCR.[39] In the reference of the above study, it was confirmed that compounds 4, 5, 7, 12, 13, 14, and 15 are the most potent compounds and expressed more genes as shown in Figure 6. This technique shows that thiazine derivatives expressed stress response genes (genes involved in stress). These results were confirmed quantitatively by qRT-PCR because they showed very minute expressions accurately.

3.3.4. Gene Expression Study. Quantitatively, gene expression was done by qRT-PCR to show the gene expression in affected [treated with thiazine derivatives (10 μg/mL) for 3 h at 20 °C] and normal *C. elegans*. The genes gst-4, hsp-4, hsp-6, hsp-16.2, gpdh-1, and mlt-2 are specific for oxidative stress, endoplasmic reticulum stress, mitochondrial stress, heat shock stress or cytosol stress, osmotic stress, and matel RNA stress pathways involved in metabolism, development, neurodegeneration, glycolysis, carbohydrate metabolism, glycolysis, energy metabolism, transcription, translation, and stress responses. The genes that showed a fold change of $\geq$2 (in biological triplicates) are considered as up- or down-regulated.

https://doi.org/10.1021/acsomega.3c01378
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Compound Code	gst-4	hsp-4	hsp-6	hsp-16.2	gpdh-1	mtl-2
1	0.18		0.2		0.42	
2	0.26		0.21		0.27	
3	0.22	0.16	1.17	0.22	0.35	
4	2.74	0.29		0.4	3.01	
5	3.26	0.87		1.83	2.32	
6	0.32	0.19	0.15	0.27	0.29	0.33
7	0.36		1.15	1.09	2.24	0.12
8	1.54			0.18	0.23	
9	0.35			0.23	0.25	
10		0.95	0.16	0.13	0.33	
11	3.08	0.14	0.12	0.12	1.89	
12	2.35			0.35	2.27	
13	3.75	1.25	0.29	2.11		
14	3.86	0.17	0.35	1.44		
15	3.97	2.24	0.33	2.96		
Standards	4	4	4	4	4	4

0-5 % Fluorescence	
5-25 % Fluorescence	1
25-50 % Fluorescence	2
50-75 % Fluorescence	3
75-100 % Fluorescence	4

C.elegans strain	Gene	Standards Used
VP596	gst-4	Acrylamide 5 mM
SJ4005	hsp-4	thapsigargin 100 μM
SJ4100	hsp-6	Paraqute 1mM
QV65	hsp-16.2	2 hrs Heat at 37 degree
VP604	gpdh-1	NaCl 250 μM
CL2122	mtl-2	CdCl2 100 μM

Figure 6. Exepression of stress genes in *C. elegans* (transgenic strains) by thiazine derivatives.

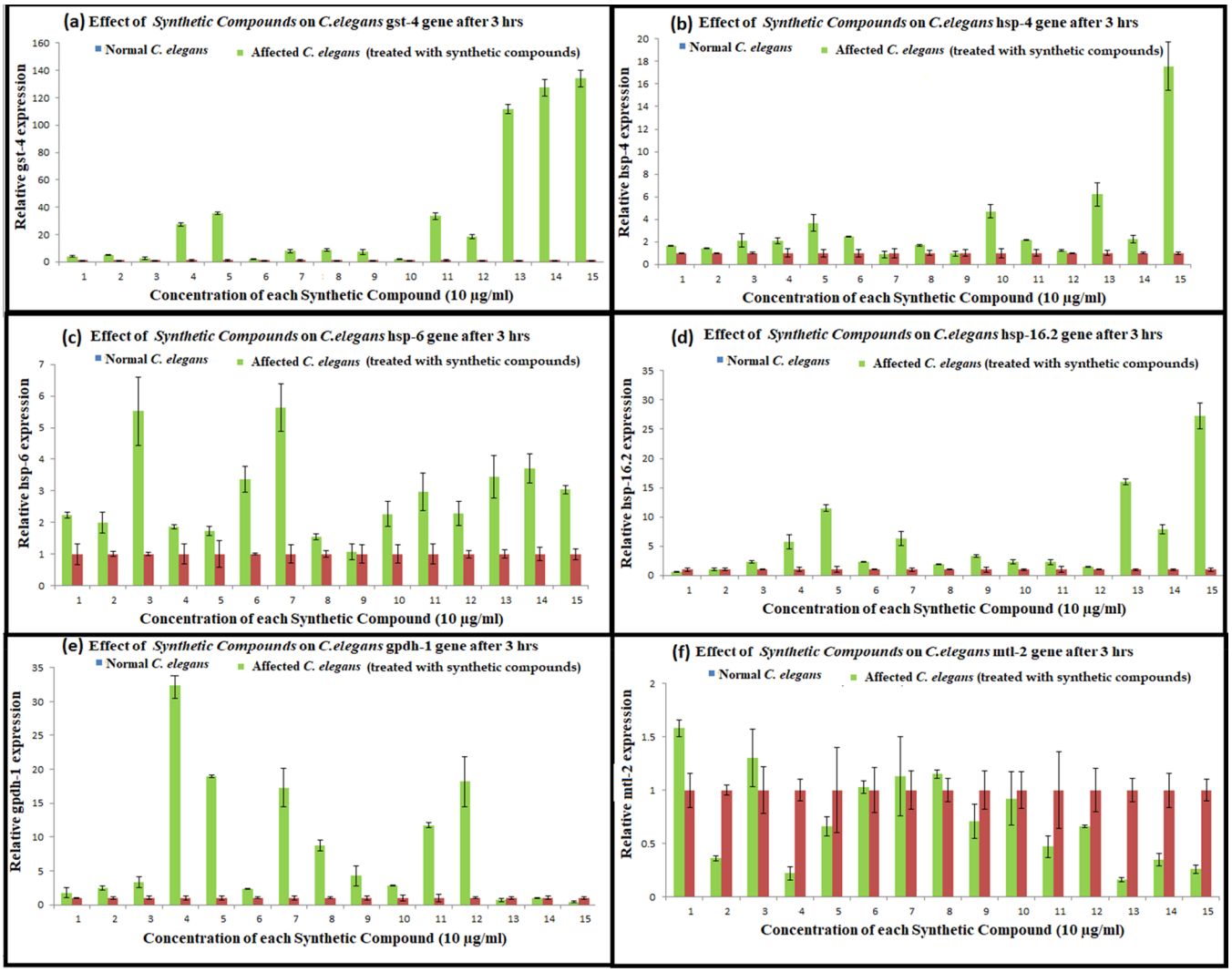

Figure 7. RT-PCR investigation of *C. elegans* stress genes. (a) gst-4, (b) hsp-4, (c) hsp-6, (d) hsp-16.2, (e) gpdh-1, and (f) mtl-2 treated with thiazine derivatives (1 to 15).

The **gst-4** gene expressed in *C. elegans* by all thiazine derivatives except 10 from 1 to 15 was 3.84 ± 0.66, 5.00 ± 0.47, 2.58 ± 0.71, 27.46 ± 1.16, 35.57 ± 0.78, 2.09 ± 0.15, 7.71 ± 1.09, 8.46 ± 0.89, 7.13 ± 1.72, 33.42 ± 2.35, 18.44 ± 1.53, 111.80 ± 3.31, 127.43 ± 6.19, and 134.14 ± 6.06 fold, respectively. The **hsp-4** gene expressed by compounds 3, 4, 5, 6,

https://doi.org/10.1021/acsomega.3c01378
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10, 11,13, 14, and 15 was around 2.11 ± 0.62, 2.10 ± 0.25, 3.66 ± 0.76, 2.42 ± 0.04, 4.70 ± 0.57, 2.18 ± 0.04, 6.21 ± 1.04, 2.25 ± 0.34, and 17.58 ± 2.15 fold. The **hsp-6** gene expressed in *C. elegans* by compounds 1, 3, 6, 7, 10, 11, 12, 13, 14, and 15 was 2.23 ± 0.08, 5.52 ± 1.08, 3.37 ± 0.41, 5.63 ± 0.76, 2.26 ± 0.40, 2.97 ± 0.60, 2.28 ± 0.38, 3.44 ± 0.67, 3.70 ± 0.46, and 3.04 ± 0.13 fold. The **hsp-16.2** gene expressed by compounds 3, 4, 5, 6, 7, 9, 10, 11, 13, 14, and 15 was 2.27 ± 0.20, 5.73 ± 1.20 , 1.51 ± 0.58, 2.36 ± 0.05, 6.34 ± 1.20, 3.35 ± 0.20, 2.35 ± 0.34, 2.30 ± 0.45, 16.04 ± 0.53, 7.88 ± 0.84, and 27.26 ± 2.15 fold. The **gpdh-1** gene expressed by compounds 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 was 2.44 ± 0.29, 3.36 ± 0.81, 32.45 ± 2.03, 18.95 ± 0.17, 2.30 ± 0.06, 17.29 ± 2.88, 8.72 ± 0.77, 4.25 ± 1.44, 2.87 ± 0.02, 11.74 ± 0.33 and 18.17 ± 3.73 fold. The **mtl-2** gene was not expressed in *C. elegans* by any thiazine derivative. When comparing the expression of all these six genes in normal (control no thiazine derivatives), *C. elegans* showed mean value 1.00, as depicted in Figure 7.

In *C. elegans*, genes were expressed by **thiazine derivatives**. This expression might be due to the structural modification in thiazine compounds as different substitutions on the aromatic ring were attached. It has also been observed that the change of position of the substituted group on the aromatic ring, that is, ortho, meta, and para does affect the gene expression.

4. CONCLUSIONS

A new series of thiazine derivatives (1 to 15) were synthesized, and the structures were confirmed by ^{1}H and ^{13}C NMR. Nematicidal potential of the thiazine derivatives was evaluated using *C. elegans* as the model organism. Among the thiazine derivatives, compounds 13 and 15 showed excellent nematicidal potential having LD_{50} at 38 μg/mL. Among all, compounds 4, 8, 9, 13, as well as compound 5 displayed a higher apoptotic effect, revealed by fluorescence microscopy. Our findings confirmed that maximum series of the compounds are very active as they indicated variations in the gene level. This could determine the mechanism of action of these compounds within the phylum Nematoda conserved pathways. All members exhibited altered mechanisms of action owing to structural alteration in thiazine derivatives.

AUTHOR INFORMATION

Corresponding Authors

Muhammad Sajid − *Department of Biochemistry, Hazara University, Mansehra, Khyber Pakhtunkhwa 21300, Pakistan*; Email: sajid931@hotmail.com

Wajid Rehman − *Department of Chemistry, Hazara University, Mansehra, Khyber Pakhtunkhwa 21300, Pakistan*; orcid.org/0000-0003-0128-0377; Email: sono_waj@yahoo.com

Authors

Naqeeb Ullah Khan − *Department of Biochemistry, Hazara University, Mansehra, Khyber Pakhtunkhwa 21300, Pakistan*

Ahmad J. Obaidullah − *Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

Hadil Faris Alotaibi − *Department of Pharmaceutical Sciences, College of Pharmacy, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia*

Saira Bibi − *Department of Chemistry, Hazara University, Mansehra, Khyber Pakhtunkhwa 21300, Pakistan*

Mohammed M. Alanazi − *Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c01378

Funding

The authors extend their appreciation to Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R205), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. Also, it was supported by the Researchers Supporting Project number (RSPD2023R620), King Saud University, Riyadh, Saudi Arabia.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Researchers Supporting Project number (RSPD2023R620), King Saud University, Riyadh, Saudi Arabia. Also, the authors extend their appreciation to Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2023R205), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. We thank Dr. Keith Choe, Department of Biology, University of Florida, USA for technical assistance with gene expression measurements.

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