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DESIGN AND SYNTHESIS OF NEW (*E*)-2-((3-METHYL-5-STYRYL-ISOXAZOL-4-YL) AMINO)-*N*-(4-OXO-2-PHENYLTHIAZOLIDIN-3-YL)- ACETAMIDE DERIVATIVES AS POTENTIAL ANTIMICROBIAL AGENTS

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ABSTRACT

A series of new (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenyl- thiazolidin-3-yl)acetamides **5** have been synthesized from (*E*)-ethyl-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetates synthon **2**. Compound **2** was obtained by reaction of 4-amino-3-methyl 5-styrylisoxazoles **1** with ethyl bromoacetate. Compound **2** on treatment with hydrazine hydrate yields (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl) amino) acetohydrazides **3**. The condensation of compound **3** with different aromatic aldehydes afforded a series of *N*'-benzylidene-2-((3-methyl-5-((*E*)-styryl) isoxazol-4-yl) amino) acetohydrazide Schiff' s bases **4**. Cyclo condensation of compounds **4** with 2-mecrcapto acetic acid in dry benzene affords (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl) amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl) acetamides **5**. Compounds **2-5** were characterized by IR, ^{1}H NMR, ^{13}C NMR, and mass spectral data. The title compounds **5a-i** were evaluated for their antimicrobial activity. The results showed that these compounds showed good antibacterial and antifungal activity compared with that of standard antibiotics.

KEYWORDS: Isoxazolyl thiazolidinone acetamide, antibacterial, antifungal activity.

INTRODUCTION

The increasing incidence of bacterial and fungal resistance to a large number of antimicrobial agents has prompted studies on the development of new potential antimicrobial compounds. The molecular manipulation of promising lead compounds is still a major line of approach to develop new drugs. It involves efforts to combine separate pharmacophoric groups of similar activit into one compound, thus making structural changes in the biological activity. So, the discoveryof novel and potent antimicrobial agents is the best way to overcome microbial resistance anddevelop effective therapies.[1]

Heterocycles are abundant in nature and are of great significance to life, hence they have attracted considerable attention towards the design of biologically active molecules and advanced organic materials. Among a wide variety of heterocycles that have been explored for developing pharmacologically important molecules, isoxazole unit constitutes an important group due to wide variety of biological activity such as antitumor,[2] CNS-active,[3] analgesic,[4] antimicrobial,[5] and chemotherapy.[6] Isoxazoles display a wide range of organic reactivities and could be used as effective means of preparing new molecular scaffolds.[7] Isoxazoles have been repeatedly shown as useful synthons in organic synthesis.[8]

Thiazolidinones display a wide variety of interesting pharmacological properties such as antitubercular,[9] anticonvulsant,[10] antifungal,[11] antitumor,[12] and antimicrobial activity,[13] and are also used in the treatment of arthiritis.[14] They have also been utilized as hypolipidemics and hypocholesteremics.[15] Thiazolidinone herbicides are potent inhibitors of glucose incorporation into cell wall.[16] Besides this, they have been proved to be calcium antagonists with both calcium overload inhibitor activity and antioxidant activity.[17] New hybrid molecules secured by linking isoxazoles with thiazolidinones promise to offer fascinating scaffolds. Design of synthetic methods for the efficient preparation of these heterocycles is necessary. Hence, the synthesis of title compounds was embarked upon to improve specificity and efficacy of these scaffolds against micro organisms. In this context, we have utilized isoxazolyl amino ethyl acetate as useful synthons, to build heterocyclic system. The main objective of the present study is to provide various organic heterocycles which can evolve as better chemotherapeutic agents. As a sequel to our studies on the synthesis and biological activity of heterocycles linked to isoxazole moiety[18-25], we undertook the synthesis of isoxazolyl thiazolidinones in order to explore the pharmacological activity of these compounds. Herein, we report the discovery of thiazolidinones linked to isoxazole from isoxazole amine and their antimicrobial activity.

MATERIALS AND METHODS

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical TLC was performed on Merck precoated 60 F_{254} silica gel plates. Visualization was done by exposing to iodine vapour. IR spectra (KBr pellet) were recorded on a Perkin-Elmer BX series FT-IR spectrometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz spectrometer. ^{13}C NMR spectra were recorded on a Bruker 75 MHz spectrometer. Chemical shift values are given in ppm (δ) with tetramethylsilane as internal standard. Mass Spectral measurements were carried out by EI method on a Jeol JMC-300 spectrometer at 70 eV. Elemental analyses were performed on a Carlo Erba 106 and PerkinElmer model 240 analysers.

ANTIBACTERIAL ACTIVITY

Antibacterial activity of compounds **5a-i** in acetone were performed by the broth dilution method using nutrient agar against Gram-negative bacteria *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Chormobacteium violaceum*, and Gram-positive bacteria *Bacillus subtilis*, *Bacillus sphaericus*, and *Staphylococcus aureus* at 100 µg/ml concentration. The minimum inhibitory concentration (MIC) was done by the broth dilution method.[26] Ciprofloxacin was used as a standard for comparison. The ready-made nutrient broth medium (HiMedia, 24 g) was suspended in distilled water (100 ml) and heated until it dissolved completely. The medium and test tubes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound is dissolved in acetone at a concentration of 100µg/ml and is added to the first test tube, which is serially diluted. A fixed 0.5 ml volume of overnight culture is added to all the test tubes and then incubated at 37 ^{o}C for 24 h. After 24 h, these tubes were measured for turbidity. Results are given in Tables 2.

The results of antibacterial screening (Tables 2) reveal that the compounds **5a–i** displayed a better activity and are more active than the standard drug Ciprofloxacin. The substitution of the electron withdrawing groups such as chloro (**5h** and **5d**) and bromo (**5i** and **5e**) substituents on the benzene ring showed best activity and more active than the standard Ciprofloxacin. But the introduction of electron releasing groups such as methyl (**5b** and **5f**) and methoxy (**5c** and **5g**) substituents on the benzene ring did not exhibit much activity.

Compound **5a** showed least activity because it has no substituent on the benzene ring. However, the degree of inhibition varied both with the test compound and with the bacteria used in the present investigation. In conclusion, compounds **5h** and **5i** showed maximum activity by inhibiting the growth of all the bacteria under investigation compared to standard Ciprofloxacin, hence can be exploited for the formulation of bacteriocides after further studies.

ANTIFUNGAL ACTIVITY

Antifungal activity of compounds **5a-i** were determined by using the agar cup bioassay method.[27] with Clotrimazole as the standard. The compounds were tested for their antifungal activity against five test organisms, *Aspergillus niger*, *Chrysosporium tropicum*, *Rhizopus oryzae*, *Fusarium moniliforma* and *Curvularia lunata* using the agar cup bioassay method at 100 µg/ml concentrations. The ready-made potato dextrose agar medium (HiMedia, 39 g) was suspended in distilled water (1000 ml) and heated until it dissolved completely. The medium and Petri dishes were autoclaved at a pressure of 15 lb/inc^2 for 20 min. The medium was poured into sterile Petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 ml of (week-old) culture of the test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving plant extract in acetone (100 µg/ml). Agar inoculation cups were scooped out with a 6 mm sterile cork borer and the lids of the dishes were replaced. To each cup, 100 (µg/ml) of the test solution was added. Controls were maintained with acetone and Clotrimazole (100 µg/ml). The treated and the controls were kept at room temperature for 72–96 h. Inhibition zones were determined and their diameter was calculated in millimeter. Three to four replicates were maintained for each treatment. Results are given in Tables 3.

The antifungal activity data (Table 3) reveal that compounds 5a-i are highly toxic toward all the fungi under investigation. Compounds 5i, 5h, 5e and 5d exhibited high antifungal activity by inhibiting the growth of fungi to a remarkable extent, when compared to standard drug Clotrimazole which may be due to the presence of chloro and bromo substituents on the benzene ring. Compound 5a showed good activity. Compounds 5g, 5f, 5c and 5b are moderately active. However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under investigation. It is noteworthy that 5i and 5h showed better activity, when compared with the standard drug

EXPERIMENTAL SECTION

General procedure for the synthesis of (*E*)-ethyl-2-((3-methyl-5-styrylisoxazol-4-yl)-amino)acetates (2a-e)

A mixture of 4-amino-3-methyl-5-styrylisoxazole **1**[28] (0.01 mol), anhydrous K_2CO_3 (0.01 mole) and ethyl bromoacetate (0.01 mole) in dry THF (20 mL) was refluxed with continuous stirring for 5 h. After completion of the reaction (monitored by TLC), the reaction mixture was poured onto crushed ice and the solid filtered off and recrystallized from ethylacetate to get pure (*E*)-ethyl-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetates **2a-e**.

(*E*)-Ethyl-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetate 2a

Yield 75%, mp 170-172 °C. IR (KBr) cm^{-1}: 3400 (NH), 1740 (CO_2Et). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.28 (t, 3H, CH_3), 2.29 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.22 (q, 2H, CH_2), 5.10 (bs, 1H, NH, D_2O exchangeable), 6.74 (d, 1H, CH=CH, *J* = 12 Hz), 6.91 (d, 1H, CH=CH, *J* = 12 Hz), 7.20-7.54 (m, 5H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.10, 14.24, 46.62, 61.08, 100.43, 119.40, 120.34, 125.00, 126.23, 128.02, 128.64, 128.93, 130.02, 156.55, 159.65, 168.45. EI-MS (70 eV) m/z 287 $[M+H]^+$. Anal.Calcd. for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 67.16; H, 6.30; N, 9.73%.

(*E*)-Ethyl-2-((3-methyl-5-(4-methylstyryl)isoxazol-4-yl)amino)acetate 2b

Yield 70%, mp 167-169 °C. IR (KBr) cm^{-1}: 3410 (NH), 1746 (CO_2Et). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.20 (t, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.10 (s, 2H, CH_2), 4.25 (q, 2H, CH_2), 5.20 (bs, 1H, NH, D_2O exchangeable), 6.70 (d, 1H, CH=CH, *J* = 12 Hz), 6.89 (d, 1H, CH=CH, *J* = 12 Hz), 7.18-7.50 (m, 4H, Ar-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.04, 24.30, 14.19, 46.60, 61.24, 100.28, 119.38, 120.56, 125.16, 126.20, 128.17, 128.60, 128.84, 130.16, 156.50, 159.57, 169.38. EI-MS (70 eV) m/z 301 $[M+H]^+$. Anal.Calcd. for $C_{17}H_{20}N_2O_3$: C, 67.98; H, 6.71; N, 9.33. Found: C, 67.94; H, 6.74; N, 9.30%.

(*E*)-Ethyl-2-((5-(4-methoxystyryl)-3-methylisoxazol-4-yl)amino)acetate 2c

Yield 72%, mp 174-176 °C. IR (KBr) cm^{-1}: 3406 (NH), 1751 (CO_2Et). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.26 (t, 3H, CH_3), 2.21 (s, 3H, CH_3), 3.40 (s, 3H, OCH_3), 4.13 (s, 2H, CH_2), 4.27 (q, 2H, CH_2), 5.23 (bs, 1H, NH, D_2O exchangeable), 6.75 (d, 1H, CH=CH, *J* = 12 Hz), 6.93 (d, 1H, CH=CH, *J* = 12 Hz), 7.24-7.54 (m, 4H, Ar-H). EI-MS (70 eV) m/z 317 $[M+H]^+$. Anal.Calcd. for $C_{17}H_{20}N_2O_4$: C, 64.54; H, 6.37; N, 8.86. Found: C, 64.57; H, 6.35; N, 8.90%.

(*E*)-Ethyl-2-((5-(4-chlorostyryl)-3-methylisoxazol-4-yl)amino)acetate 2d

Yield 69%, mp 177-179 °C. IR (KBr) cm^{-1}: 3430 (NH), 1753 (CO_2Et). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.27 (t, 3H, CH_3), 2.25 (s, 3H, CH_3), 4.16 (s, 2H, CH_2), 4.24 (q, 2H, CH_2), 5.09 (bs, 1H, NH, D_2O exchangeable), 6.73 (d, 1H, CH=CH, J = 12 Hz), 6.87 (d, 1H, CH=CH, J = 12 Hz), 7.24-7.55 (m, 4H, Ar-H). EI-MS (70 eV) m/z 321 $[M]^+$, 323 $[M+2]^+$. Anal.Calcd. for $C_{16}H_{17}ClN_2O_3$: C, 59.91; H, 5.34; N, 8.73. Found: C, 59.93; H, 5.30; N, 8.70%.

(*E*)-Ethyl 2-((5-(4-bromostyryl)-3-methylisoxazol-4-yl)amino)acetate 2e

Yield 72%, mp 164-166 °C. IR (KBr) cm^{-1}: 3410 (NH), 1742 (CO_2Et). ^1H NMR (300 MHz, $CDCl_3$) δ: 1.21 (t, 3H, CH_3), 2.22 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 4.23 (q, 2H, CH_2), 5.15 (bs, 1H, NH, D_2O exchangeable), 6.75 (d, 1H, CH=CH, J = 12 Hz), 6.86 (d, 1H, CH=CH, J = 12 Hz), 7.21-7.49 (m, 4H, Ar-H). EI-MS (70 eV) m/z 365 $[M]^+$, 367 $[M+2]^+$. Anal.Calcd. for $C_{16}H_{17}BrN_2O_3$: C, 52.62; H, 4.69; N, 7.67. Found: C, 52.65; H, 4.64; N, 7.70%.

General procedure for the synthesis of (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino) acetohydrazides (3a-e)

To a solution of (*E*)-ethyl-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetate **2** (0.01 mole), in methanol, hydrazine hydrate (0.01 mole) (99%) was added. The resulting reaction mixture was refluxed on steam bath for 6-8 h. The excess methanol was distilled off. The resulting residue was washed with cold water, dried and recrystallized from ethanol to afforded (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino) acetohydrazides **3a-e**.

(*E*)-2-((3-Methyl-5-styrylisoxazol-4-yl)amino)acetohydrazide 3a

Yield 80%, mp 182-184 °C. IR (KBr) cm^{-1}: 3452 (NH), 3340 (NH_2), 1634 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.23 (s, 3H, CH_3), 3.62 (s, 2H, CH_2), 4.24 (s, 2H, NH_2, D_2O exchangeable), 5.06 (bs, 1H, NH, D_2O exchangeable), 6.72 (d, 1H, CH=CH, J = 12 Hz), 6.93 (d, 1H, CH=CH, J = 12 Hz), 7.18-7.60 (m, 5H, Ar-H), 9.18 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.04, 45.22, 100.21, 119.74, 120.23, 125.31, 126.56, 128.34, 128.46, 128.73, 130.32, 156.50, 159.48, 169.37. EI-MS (70 eV) m/z 273 $[M+H]^+$. Anal.Calcd. for $C_{14}H_{16}N_4O_2$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.72, H, 5.90; N, 20.53%.

(*E*)-2-((3-Methyl-5-(4-methylstyryl)isoxazol-4-yl)amino)acetohydrazide 3b

Yield 76%, mp 181-183 °C. IR (KBr) cm^{-1}: 3420 (NH), 3318 (NH_2), 1630 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.21 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.67 (s, 2H, CH_2), 4.21

(s, 2H, NH_2, D_2O exchangeable), 5.12 (bs, 1H, NH, D_2O exchangeable), 6.70 (d, 1H, CH=CH, J = 12 Hz), 6.87 (d, 1H, CH=CH, J = 12 Hz), 7.21-7.63 (m, 4H, Ar-H), 9.14 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.13, 25.04, 45.20, 100.35, 119.71, 120.20, 125.27, 126.53, 128.42, 128.38, 128.67, 130.44, 156.48, 159.42, 168.89. EI-MS (70 eV) m/z 287 $[M+H]^+$. Anal.Calcd. for $C_{15}H_{18}N_4O_2$: C, 62.92; H, 6.34; N, 19.57. Found: C, 62.90, H, 6.30; N, 19.60%.

(*E*)-2-((5-(4-Methoxystyryl)-3-methylisoxazol-4-yl)amino)acetohydrazide 3c

Yield 71%, mp 187-189 °C. IR (KBr) cm^{-1}: 3415 (NH), 3329 (NH_2), 1638 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.18 (s, 3H, CH_3), 3.46 (s, 3H, OCH_3), 3.82 (s, 2H, CH_2), 4.20 (s, 2H, NH_2, D_2O exchangeable), 5.18 (bs, 1H, NH, D_2O exchangeable), 6.79 (d, 1H, CH=CH, J = 12 Hz), 6.88 (d, 1H, CH=CH, J = 12 Hz), 7.20-7.63 (m, 4H, Ar-H), 9.10 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 303 $[M+H]^+$. Anal.Calcd. for $C_{15}H_{18}N_4O_3$: C, 59.59; H, 6.00; N, 18.53. Found: C, 59.62, H, 6.03; N, 18.50%.

(*E*)-2-((5-(4-Chlorostyryl)-3-methylisoxazol-4-yl)amino)acetohydrazide 3d

Yield 67%, mp 189-191 °C. IR (KBr) cm^{-1}: 3410 (NH), 3331 (NH_2), 1634 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.09 (s, 3H, CH_3), 3.79 (s, 2H, CH_2), 4.18 (s, 2H, NH_2, D_2O exchangeable), 5.15 (bs, 1H, NH, D_2O exchangeable), 6.77 (d, 1H, CH=CH, J = 12 Hz), 6.80 (d, 1H, CH=CH, J = 12 Hz), 7.23-7.60 (m, 4H, Ar-H), 9.18 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 307 $[M]^+$, 309 $[M+2]^+$. Anal.Calcd. for $C_{14}H_{15}ClN_4O_2$: C, 54.82; H, 4.93; N, 18.26. Found: C, 54.80, H, 4.90; N, 18.23%.

(*E*)-2-((5-(4-Bromostyryl)-3-methylisoxazol-4-yl)amino)acetohydrazide 3e

Yield 72%, mp 190-192 °C. IR (KBr) cm^{-1}: 3432 (NH), 3326 (NH_2), 1643 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.15 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 4.26 (s, 2H, NH_2, D_2O exchangeable), 5.11 (bs, 1H, NH, D_2O exchangeable), 6.75 (d, 1H, CH=CH, J = 12 Hz), 6.86 (d, 1H, CH=CH, J = 12 Hz), 7.20-7.66 (m, 4H, Ar-H), 9.20 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 351 $[M]^+$, 353 $[M+2]^+$. Anal.Calcd. for $C_{14}H_{15}BrN_4O_2$: C, 47.88; H, 4.31; N, 15.95. Found: C, 47.91, H, 4.34; N, 15.91%.

General procedure for the synthesis of *N*'-Benzylidene-2-((3-methyl-5-((*E*)-styryl)isoxazol-4-yl)amino)acetohydrazides (4a-i)

A mixture of (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino) acetohydrazide **3** (0.01 mole), and appropriate aromatic aldehydes (0.01 mole) was refluxed in absolute ethanol (25 mL) for

7 h. The reaction mixture was cooled; the solid separated was filtered and recrystallized from methanol to give *N*'-benzylidene-2-((3-methyl-5-((*E*)-styryl)isoxazol-4-yl)amino)aceto-hydrazides **4a-i**.

N'-Benzylidene-2-((3-methyl-5-((E)-styryl)isoxazol-4-yl)amino)acetohydrazide 4a

Yield 78%, mp 210-212 °C. IR (KBr) cm^{-1}: 3410 (NH), 1645 (C=O, amide), 1620 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.10 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 5.20 (bs, 1H, NH, D_2O exchangeable), 6.65 (d, 1H, CH=CH, J = 12 Hz), 6.81 (d, 1H, CH=CH, J = 12 Hz), 7.20-7.72 (m, 10H, Ar-H), 8.24 (bs, 1H, NH, D_2O exchangeable), 8.48 (s, 1H, CH=N-). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 11.36, 46.42, 100.32, 119.69, 121.52, 124.30, 125.52, 126.22, 126.30, 126.50, 128.00, 128.62, 128.85, 130.05, 131.08, 133.06, 135.23, 145.20, 156.45, 159.37, 170.16. EI-MS (70 eV) m/z 361 $[M+H]^+$. Anal. Calcd. for $C_{21}H_{20}N_4O_2$: C, 69.98; H, 5.59; N, 15.55. Found: C, 69.95, H, 5.62; N, 15.53%.

N'-Benzylidene-2-((3-methyl-5-((E)-4-methylstyryl)isoxazol-4-yl)amino)acetohydrazide 4b

Yield 75%, mp 204-206 °C. IR (KBr) cm^{-1}: 3418 (NH), 1640 (C=O, amide), 1626 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.14 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.95 (s, 2H, CH_2), 5.18 (bs, 1H, NH, D_2O exchangeable), 6.63 (d, 1H, CH=CH, J = 12 Hz), 6.79 (d, 1H, CH=CH, J = 12 Hz), 7.18-7.70 (m, 9H, Ar-H), 8.20 (bs, 1H, NH, D_2O exchangeable), 8.42 (s, 1H, CH=N-). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.04, 25.12, 46.30, 100.41, 119.57, 121.46, 124.26, 125.50, 126.23, 126.45, 126.38, 128.06, 128.60, 128.78, 130.11, 131.15, 133.21, 135.43, 145.24, 156.40, 159.35, 169.95. EI-MS (70 eV) m/z 375 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.54, H, 5.90; N, 14.98%.

N'-Benzylidene-2-((5-((E)-4-methoxystyryl)-3-methylisoxazol-4yl)amino)acetohydrazide 4c

Yield 70%, mp 201-203 °C. IR (KBr) cm^{-1}: 3435 (NH), 1650 (C=O, amide), 1632 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.10 (s, 3H, CH_3), 3.42 (s, 3H, OCH_3), 3.87 (s, 2H, CH_2), 5.13 (bs, 1H, NH, D_2O exchangeable), 6.61 (d, 1H, CH=CH, J = 12 Hz), 6.82 (d, 1H, CH=CH, J = 12 Hz), 7.23-7.71 (m, 9H, Ar-H), 8.32 (bs, 1H, NH, D_2O exchangeable), 8.40 (s, 1H, CH=N-). EI-MS (70 eV) m/z 391 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35. Found: C, 67.71, H, 5.65; N, 14.30%.

N'-Benzylidene-2-((5-((*E*)-4-chlorostyryl)-3-methylisoxazol-4-yl)amino)acetohydrazide 4d

Yield 68%, mp 211-213 °C. IR (KBr) cm^{-1}: 3424 (NH), 1653 (C=O, amide), 1628 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.08 (s, 3H, CH_3), 3.83 (s, 2H, CH_2), 5.21 (bs, 1H, NH, D_2O exchangeable), 6.65 (d, 1H, CH=CH, *J* = 12 Hz), 6.81 (d, 1H, CH=CH, *J* = 12 Hz), 7.20-7.80 (m, 9H, Ar-H), 8.29 (bs, 1H, NH, D_2O exchangeable), 8.44 (s, 1H, CH=N-). EI-MS (70 eV) m/z 395 $[M]^+$, 397 $[M+2]^+$.Anal. Calcd for $C_{21}H_{19}ClN_4O_2$: C, 63.88; H, 4.85, N, 14.19. Found: C, 63.90, H, 4.81; N, 14.21%.

N'-Benzylidene-2-((5-((*E*)-4-bromostyryl)-3-methylisoxazol-4-yl)amino)acetohydrazide 4e

Yield 65%, mp 217-219 °C. IR (KBr) cm^{-1}: 3421 (NH), 1646 (C=O, amide), 1630 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.23 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 5.24 (bs, 1H, NH, D_2O exchangeable), 6.68 (d, 1H, CH=CH, *J* = 12 Hz), 6.87 (d, 1H, CH=CH, *J* = 12 Hz), 7.23-7.81 (m, 9H, Ar-H), 8.34 (bs, 1H, NH, D_2O exchangeable), 8.49 (s, 1H, CH=N-). EI-MS (70 eV) m/z 439 $[M]^+$, 441 $[M+2]^+$.Anal. Calcd.for $C_{21}H_{19}BrN_4O_2$: C, 57.41; H, 4.36, N, 12.75. Found: C, 57.39, H, 4.39; N, 12.72%.

2-((3-Methyl-5-((*E*)-styryl)isoxazol-4-yl)amino)-*N*'-(4methylbenzylidene)acetohydrazide 4f

Yield 70%, mp 207-209 °C. IR (KBr) cm^{-1}: 3434 (NH), 1648 (C=O, amide), 1632 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.10 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.90 (s, 2H, CH_2), 5.21 (bs, 1H, NH, D_2O exchangeable), 6.67 (d, 1H, CH=CH, *J* = 12 Hz), 6.82 (d, 1H, CH=CH, *J* = 12 Hz), 7.20-7.71 (m, 9H, Ar-H), 8.24 (bs, 1H, NH, D_2O exchangeable), 8.48 (s, 1H, CH=N-). EI-MS (70 eV) m/z 375 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{22}N_4O_2$: C, 70.57; H, 5.92; N, 14.96. Found: C, 70.60, H, 5.94; N, 14.95%.

N'-(4-Methoxybenzylidene)-2-((3-methyl-5-((*E*)-styryl)isoxazol-4-yl)amino)acetohydrazide 4g

Yield 67%, mp 214-215 °C. IR (KBr) cm^{-1}: 3430 (NH), 1642 (C=O, amide), 1629 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.08 (s, 3H, CH_3), 3.41 (s, 3H, OCH_3), 3.84 (s, 2H, CH_2), 5.11 (bs, 1H, NH, D_2O exchangeable), 6.66 (d, 1H, CH=CH, *J* = 12 Hz), 6.85 (d, 1H, CH=CH, *J* = 12 Hz), 7.20-7.70 (m, 9H, Ar-H), 8.45 (bs, 1H, NH, D_2O exchangeable), 8.53

(s, 1H, CH=N-). EI-MS (70 eV) m/z 391 $[M+H]^+$. Anal. Calcd. for $C_{22}H_{22}N_4O_3$: C, 67.68; H, 5.68; N, 14.35. Found: C, 67.65, H, 5.70; N, 14.38%.

_N_'-(4-Chlorobenzylidene)-2-((3-methyl-5-((_E_)-styryl)isoxazol-4-yl)amino)acetohydrazide 4h

Yield 73%, mp 216-218 °C. IR (KBr) cm^{-1}: 3432 (NH), 1650 (C=O, amide), 1632 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.14 (s, 3H, CH_3), 3.80 (s, 2H, CH_2), 5.29 (bs, 1H, NH, D_2O exchangeable), 6.63 (d, 1H, CH=CH, J = 12 Hz), 6.80 (d, 1H, CH=CH, J = 12 Hz), 7.23-7.84 (m, 9H, Ar-H), 8.31 (bs, 1H, NH, D_2O exchangeable), 8.46 (s, 1H, CH=N-). EI-MS (70 eV) m/z 395 $[M]^+$, 397 $[M+2]^+$. Anal. Calcd. for $C_{21}H_{19}ClN_4O_2$: C, 63.88; H, 4.85, N, 14.19. Found: C, 63.92, H, 4.80; N, 14.22%.

_N_'-(4-Bromobenzylidene)-2-((3-methyl-5-((_E_)-styryl)isoxazol-4-yl)amino)acetohydrazide 4i

Yield 67%, mp 195-197 °C. IR (KBr) cm^{-1}: 3431 (NH), 1644 (C=O, amide), 1629 (C=N). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.18 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 5.27 (bs, 1H, NH, D_2O exchangeable), 6.64 (d, 1H, CH=CH, J = 12 Hz), 6.83 (d, 1H, CH=CH, J = 12 Hz), 7.20-7.85 (m, 9H, Ar-H), 8.30 (bs, 1H, NH, D_2O exchangeable), 8.45 (s, 1H, CH=N-). EI-MS (70 eV) m/z 439 $[M]^+$, 441 $[M+2]^+$. Anal. Calcd. for $C_{21}H_{19}BrN_4O_2$: C, 57.41; H, 4.36, N, 12.75. Found: C, 57.44, H, 4.33; N, 12.71%.

General procedure for the synthesis of (_E_)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-_N_-(4-oxo-2-phenylthiazolidin-3-yl)acetamides (5a-i)

A mixture of (*N*'-benzylidene-2-((3-methyl-5-((*E*)-styryl)isoxazol-4-yl)amino)acetohydrazides **4** (0.01 mole) and mercaptoacetic acid (0.02 mole) in dry benzene (20 mL) was refluxed for 8 h. Progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled and poured onto crushed ice. The solid thus obtained was filtered, washed with water and recrystallized from benzene yielding (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamides **5a-i**.

(_E_)-2-((3-Methyl-5-styrylisoxazol-4-yl)amino)-_N_-(4-oxo-2-phenylthiazolidin-3-yl)acetamides 5a

Yield 80%, mp 220-222 °C. IR (KBr) cm^{-1}: 3408 (NH), 1650 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.22 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 4.10 (s, 2H, thiazole-CH_2), 5.31 (bs, 1H, NH, D_2O exchangeable), 5.83 (s, 1H, -SCHN-), 6.71 (d, 1H, CH=CH, *J* = 12 Hz), 6.90 (d, 1H, CH=CH, *J* = 12 Hz), 7.18-7.70 (m, 10H, Ar-H), 8.20 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.06, 34.87, 46.64, 59.85, 100.43, 119.62, 121.53, 124.45, 125.26, 126.44, 126.50, 126.76, 128.06, 128.46, 128.81, 130.20, 131.18, 133.15, 135.38, 156.54, 159.46, 168.35, 170.08. EI-MS (70 eV) m/z 435 $[M+H]^+$. Anal. Calcd. for $C_{23}H_{22}N_4O_3S$: C, 63.58; H, 5.10; N, 12.89; S, 7.38. Found: C, 63.62; H, 5.14; N, 12.85; S, 7.34%.

(*E*)-2-((3-Methyl-5-(4-methylstyryl)isoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamide 5b

Yield 76%, mp 223-225 °C. IR (KBr) cm^{-1}: 3421 (NH), 1646 (C=O, amide). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.17 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 4.19 (s, 2H, thiazole-CH_2), 5.27 (bs, 1H, NH, D_2O exchangeable), 5.80 (s, 1H, -SCHN-), 6.70 (d, 1H, CH=CH, *J* = 12 Hz), 6.86 (d, 1H, CH=CH, *J* = 12 Hz), 7.20-7.76 (m, 9H, Ar-H), 8.23 (bs, 1H, NH, D_2O exchangeable). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 12.12, 25.19, 34.75, 46.60, 59.78, 100.41, 119.60, 121.51, 124.34, 125.33, 126.40, 126.48, 126.66, 128.13, 128.53, 128.66, 130.19, 131.22, 133.18, 135.30, 156.50, 159.44, 168.32, 169.87. EI-MS (70 eV) m/z 449 $[M+H]^+$. Anal. Calcd. for $C_{24}H_{24}N_4O_3S$: C, 64.27; H, 5.39; N, 12.49; S, 7.15. Found: C, 64.30; H, 5.36; N, 12.51; S, 7.18%.

(*E*)-2-((5-(4-Methoxystyryl)-3-methylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamide 5c

Yield 70%, mp 217-219 °C. IR (KBr) cm^{-1}: 3444 (NH), 1651 (C=O, amide). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.10 (s, 3H, CH_3), 3.48 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2), 4.21 (s, 2H, thiazole-CH_2), 5.20 (bs, 1H, NH, D_2O exchangeable), 5.84 (s, 1H, -SCHN-), 6.73 (d, 1H, CH=CH, *J* = 12 Hz), 6.80 (d, 1H, CH=CH, *J* = 12 Hz), 7.18-7.73 (m, 9H, Ar-H), 8.26 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 465 $[M+H]^+$. Anal. Calcd. for $C_{24}H_{24}N_4O_4S$: C, 62.05; H, 5.21; N, 12.06; S, 6.90. Found: C, 62.09; H, 5.19; N, 12.08; S, 6.93%.

(*E*)-2-((5-(4-Chlorostyryl)-3-methylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamide 5d

Yield 77%, mp 208-210 °C. IR (KBr) cm^{-1}: 3438 (NH), 1647 (C=O, amide). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.08 (s, 3H, CH_3), 3.73 (s, 2H, CH_2), 4.24 (s, 2H, thiazole-CH_2), 5.25 (bs, 1H, NH, D_2O exchangeable), 5.80 (s, 1H, -SCHN-), 6.70 (d, 1H, CH=CH, *J* = 12 Hz), 6.82 (d, 1H, CH=CH, *J* = 12 Hz), 7.21-7.74 (m, 9H, Ar-H), 8.20 (bs, 1H, NH, D_2O exchangeable).

EI-MS (70 eV) m/z 469 $[M]^+$, 471 $[M+2]^+$. Anal. Calcd. for $C_{23}H_{21}ClN_4O_3S$: C, 58.91; H, 4.51; N, 11.95; S, 6.84. Found: C, 58.89; H, 4.53; N, 11.92; S, 6.80%.

(*E*)-2-((5-(4-Bromostyryl)-3-methylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamide 5e

Yield 82%, mp 201-203 °C. IR (KBr) cm^{-1}: 3430 (NH), 1643 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.15 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 4.20 (s, 2H, thiazole-CH_2), 5.24 (bs, 1H, NH, D_2O exchangeable), 5.81 (s, 1H, -SCHN-), 6.77 (d, 1H, CH=CH, *J* = 12 Hz), 6.88 (d, 1H, CH=CH, *J* = 12 Hz), 7.17-7.70 (m, 9H, Ar-H), 8.25 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 513 $[M]^+$, 515 $[M+2]^+$. Anal. Calcd. for $C_{23}H_{21}BrN_4O_3S$: C, 53.81; H, 4.12; N, 10.91; S, 6.25. Found: C, 53.85; H, 4.09; N, 10.87; S, 6.21%.

(*E*)-2-((3-Methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-(*p*-tolyl)thiazolidin-3-yl)acetamide 5f

Yield 83%, mp 228-230 °C. IR (KBr) cm^{-1}: 3442 (NH), 1640 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.11 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 3.84 (s, 2H, CH_2), 4.21 (s, 2H, thiazole-CH_2), 5.23 (bs, 1H, NH, D_2O exchangeable), 5.84 (s, 1H, -SCHN-), 6.72 (d, 1H, CH=CH, *J* = 12 Hz), 6.83 (d, 1H, CH=CH, *J* = 12 Hz), 7.18-7.74 (m, 9H, Ar-H), 8.27 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 449 $[M+H]^+$. Anal. Calcd. for $C_{24}H_{24}N_4O_3S$: C, 64.27; H, 5.39; N, 12.49; S, 7.15. Found: C, 64.25; H, 5.42; N, 12.45; S, 7.13%.

(*E*)-*N*-(2-(4-Methoxyphenyl)-4-oxothiazolidin-3-yl)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetamide 5g

Yield 75%, mp 211-213 °C. IR (KBr) cm^{-1}: 3440 (NH), 1648 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.06 (s, 3H, CH_3), 3.41 (s, 3H, OCH_3), 3.72 (s, 2H, CH_2), 4.26 (s, 2H, thiazole-CH_2), 5.26 (bs, 1H, NH, D_2O exchangeable), 5.80 (s, 1H, -SCHN-), 6.71 (d, 1H, CH=CH, *J* = 12 Hz), 6.81 (d, 1H, CH=CH, *J* = 12 Hz), 7.19-7.72 (m, 9H, Ar-H), 8.28 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 465 $[M+H]^+$. Anal. Calcd. for $C_{24}H_{24}N_4O_4S$: C, 62.05; H, 5.21; N, 12.06; S, 6.90. Found: C, 62.07; H, 5.23; N, 12.10; S, 6.87%.

(*E*)-*N*-(2-(4-Chlorophenyl)-4-oxothiazolidin-3-yl)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetamide 5h

Yield 71%, mp 231-233 °C. IR (KBr) cm^{-1}: 3451 (NH), 1642 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.17 (s, 3H, CH_3), 3.71 (s, 2H, CH_2), 4.20 (s, 2H, thiazole-CH_2), 5.23 (bs, 1H, NH, D_2O exchangeable), 5.84 (s, 1H, -SCHN-), 6.75 (d, 1H, CH=CH, *J* = 12 Hz), 6.87

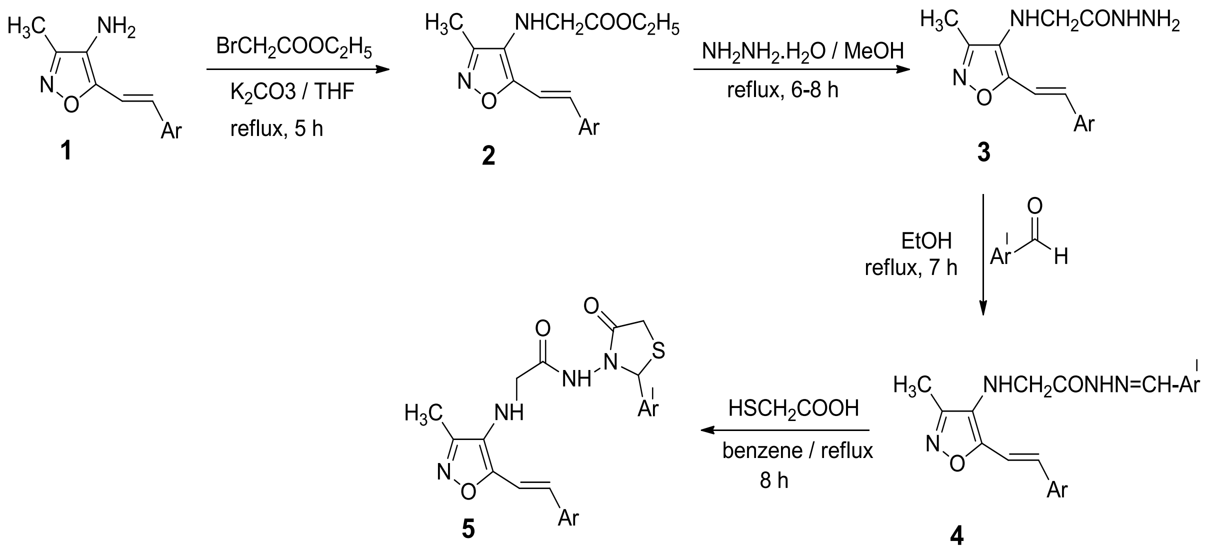
(d, 1H, CH=CH, *J* = 12 Hz), 7.15-7.63 (m, 9H, Ar-H), 8.25 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 469 $[M]^+$, 471 $[M+2]^+$. Anal. Calcd. for $C_{23}H_{21}ClN_4O_3S$: C, 58.91; H, 4.51; N, 11.95; S, 6.84. Found: C, 58.87; H, 4.49; N, 11.94; S, 6.79%.

(*E*)-*N*-(2-(4-Bromophenyl)-4-oxothiazolidin-3-yl)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetamide 5i

Yield 79%, mp 224-226 °C. IR (KBr) cm^{-1}: 3428 (NH), 1647 (C=O, amide). ^1H NMR (300 MHz, $CDCl_3$) δ: 2.14 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 4.28 (s, 2H, thiazole-CH_2), 5.20 (bs, 1H, NH, D_2O exchangeable), 5.80 (s, 1H, -SCHN-), 6.75 (d, 1H, CH=CH, *J* = 12 Hz), 6.91 (d, 1H, CH=CH, *J* = 12 Hz), 7.19-7.75 (m, 9H, Ar-H), 8.22 (bs, 1H, NH, D_2O exchangeable). EI-MS (70 eV) m/z 513 $[M]^+$, 515 $[M+2]^+$. Anal. Calcd. for $C_{23}H_{21}BrN_4O_3S$: C, 53.81; H, 4.12; N, 10.91; S, 6.25. Found: C, 53.79; H, 4.10; N, 10.94; S, 6.20%.

RESULTS AND DISCUSSION

The synthesis of compounds **2**, **3**, **4** and **5** was accomplished by the synthetic sequence shown in Scheme 1. The reaction of 4-amino-3-methyl-5-styrylisoxazole **1.**[28] with ethyl bromoacetate in the presence of K_2CO_3 in dry THF furnished (*E*)-ethyl-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetate **2**. Hydrazinolysis of **2** with hydrazine hydrate in refluxing methanol to give (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)acetohydrazide **3** in good yield. The IR spectra of carbohydrazide **3** showed absorption bands in the 3452 cm^{-1} (NH), 3340 cm^{-1} (NH_2) and 1634 (-C=O, amide groups) regions, respectively. The ^1H NMR spectrum of compound **3** exhibited a singlet due to the –CO-NH-NH_2NH proton at δ 9.18 ppm. Methylene protons –CH_2 and –NH_2 resonated as singlets at 3.62 and 4.24 ppm, respectively. Compound **3** with different aromatic aldehydes in absolute ethanol under reflux condition afforded *N*'-benzylidene-2-((3-methyl-5-((*E*)-styryl)- isoxazol-4-yl)amino)acetohydrazide **4** in excellent yields. The IR spectra of compound **4** showed characteristic bands at 3410 cm^{-1} (NH), 1645 cm^{-1} (C=O, amide) and 1620 cm-1 (-HC=N-azomethine). The ^1H NMR spectra did not only show the absence of NH_2 protons at 4.24, but also the presence of the N=CH proton at 8.48 ppm. Data from the elemental analyses and ESI mass spectra further confirmed the assigned structures of **4a-i**. Cyclocondensation of **4** with mercaptoacetic acid in boiling dry benzene led to the formation of new (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamides **5** in good yields (Scheme 1). The ^1H NMR spectrum of compound **5** displayed a distinct signal at δ 4.10 due to the methylene protons of the thiazolidinone ring confirming the formation of the thiazolidinone ring. The mass spectrum of compound **5a** confirmed the structure by exhibiting the molecular ion peak $[M+H]^+$ at *m/z* 435.



5a,	Ar = C_6H_5	Ar' = C_6H_5
5b,	Ar = 4-$CH_3C_6H_4$	Ar' = C_6H_5
5c,	Ar = 4-$OCH_3C_6H_4$	Ar' = C_6H_5
5d,	Ar = 4-ClC_6H_4	Ar' = C_6H_5
5e,	Ar = 4-BrC_6H_4	Ar' = C_6H_5
5f,	Ar = C_6H_5	Ar' = 4-$CH_3C_6H_4$
5g,	Ar = C_6H_5	Ar' = 4-$OCH_3C_6H_4$
5h,	Ar = C_6H_5	Ar' = 4-ClC_6H_4
5i,	Ar = C_6H_5	Ar' = 4-BrC_6H_4

Scheme 1. Synthesis of (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)acetamides (5a-i)

Table 1: Synthesis of (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenyl-thiazolidin-3-yl)acetamides (5a-i)

Compd.	**Ar**	**Ar'**	**Yield (%)**
5a	C_6H_5	C_6H_5	80
5b	4-$CH_3C_6H_4$	C_6H_5	76
5c	4-$OCH_3C_6H_4$	C_6H_5	70
5d	4-ClC_6H_4	C_6H_5	77
5e	4-BrC_6H_4	C_6H_5	82
5f	C_6H_5	4-$CH_3C_6H_4$	83
5g	C_6H_5	4-$OCH_3C_6H_4$	75
5h	C_6H_5	4-ClC_6H_4	71
5i	C_6H_5	4-BrC_6H_4	79

Table 2: Antibacterial activity of (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamides (5a-i)

Compound	**Minimum inhibitory Concentration in µg/mL (MIC)**					
	Gram + ve bacteria			**Gram –ve bacteria**		
	B.subtilis	***B.sphaericus***	***S.aureus***	***P.aeruginosa***	***K.aerogenes***	***C.violaceum***
5a	20	22	20	24	20	20
5b	19	23	25	23	18	15
5c	18	22	25	22	20	16
5d	12	10	13	14	11	14
5e	12	11	10	10	14	12
5f	18	12	22	24	17	18
5g	18	12	16	14	18	18
5h	8	16	9	10	7	5
5i	9	7	8	6	5	8
Ciprofloxacin	20	24	28	25	22	20

- Negative control (acetone) – No activity
- Values are indicated in µg/mL

Table 3: Antifungal activity of (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamides (5a-i) in µg/mL

Compound	**Minimum inhibitory Concentration in µg/mL (MIC)**				
	A. niger	***C. tropicum***	***R. oryzae***	***F.moniliformae***	***C. lunata***
5a	16	14	10	12	15
5b	16	18	14	15	16
5c	20	22	20	24	18
5d	10	14	16	14	16
5e	10	11	13	12	14
5f	18	14	12	14	12
5g	12	20	18	12	17
5h	8	9	10	11	11
5i	9	8	7	6	10
Clotrimazole	28	26	22	25	20

- Negative control (acetone) – No activity

CONCLUSION

In conclusion, we report the synthesis of new (*E*)-2-((3-methyl-5-styrylisoxazol-4-yl)amino)-*N*-(4-oxo-2-phenylthiazolidin-3-yl)acetamides using inexpensive and commercially available materials with potential medicinal properties. This synthesis benefits from a simple method of purification, which does not require chromatography. This ease of purification compliments this synthetic technology practical, easy to perform and facile. The title compounds **5a–i** exhibited promising antimicrobial activity.

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