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RESEARCH ARTICLE

Synthesis and biological evaluation of 5-benzylidenerhodanine-3-acetic acid derivatives as AChE and 15-LOX inhibitors

Negah Shafii[1], Mehdi Khoobi[2], Mohsen Amini[2], Amirhossein Sakhteman[3], Hamid Nadri[3], Alireza Moradi[3], Saeed Emami[4], Ebrahim Saeedian Moghadam[2], Alireza Foroumadi[2], and Abbas Shafiee[1,2]

[1]Tehran University of Medical Sciences, International Campus (TUMS-IC), Tehran, Iran, [2]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran, [3]Department of Medicinal Chemistry, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran, and [4]Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazanderan University of Medical Sciences, Sari, Iran

Abstract

A series of 5-benzylidenerhodanine-3-acetamides bearing morpholino-, 4-arylpiperazinyl-, or 4-benzylpiperidinyl- moieties were synthesized and their inhibitory activities against acetylcholinesterase (AChE) were evaluated. Alteration of amide part and substitution on the benzylidene moiety resulted in change of anti-AChE activity. The most active compound was the 1-benzylpiperidinyl derivative containing 4-(dimethylamino)benzylidene scaffold. Notably, the intermediate compounds, namely 5-arylidene-rhodanine-3-acetic acids (**3**), showed mild inhibitory activity against 15-lipoxygenase (15-LOX), while the final compound **4** showed no activity against 15-LOX.

Keywords

2-Thioxothiazolidin-4-one, 15-lipoxygenase, acetylcholinesterase, docking study, inhibitors, rhodanines

History

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Introduction

Rhodanines (2-thioxothiazolidin-4-ones) and their analogues have been known as privileged structures in drug discovery. Compounds containing the rhodanine ring have demonstrated a broad range of biological activities[1]. Particularly, 5-arylidene rhodanines have been reported as small molecule inhibitors of diverse enzyme targets such as β-lactamase[2], hepatitis C virus (HCV) NS3 protease[3], aldose reductase[4], UDP-*N*-acetylmuramate/L-alanine ligase[5], protein mannosyl transferase, JNK-stimulating phosphatase, cyclooxygenase, 5-lipoxygenase[6] and tyrosinase[7].

Historically, rhodanines have become a therapeutic class of compounds since the introduction of epalrestat (*Z,E*-[5-(2-methyl-3-phenyl-allylidene)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid) into clinical use for the treatment of diabetic complications[8]. Epalrestat is a rhodanine-3-acetic acid analogue and acts as an aldose reductase inhibitor[9]. Although, the rhodanine-based compounds are suspected to undergo conjugate addition *in vivo*, they are frequently employed in drug design, as there is no adverse effect (e.g. mutagenicity) correlated to this scaffold[10]. According to a long-term clinical study with epalrestat, the structure can be bioavailable and well-tolerated[11]. Bulic et al. have been described a series of 5-arylidene-rhodanine-3-acetic acids as tau aggregation inhibitors potentially useful in the management of Alzheimer's disease and related dementias[12]. Also, amide derivatives of 5-arylidene-rhodanine-3-acetic acid have been reported as anti-inflammatory agents[13,14].

In view of these facts, although extensive studies on the diverse bioactivity of 5-arylidene-rhodanines have been reported, the anti-acetylcholinesterase (AChE) activities of these prototypes have never appeared in the literature. Accordingly as part of our ongoing studies in developing new anti-AChE agents[15–18], we have synthesized 5-benzylidenerhodanine-3-acetamides and evaluated their inhibitory activity against AChE. Also, the intermediate compounds 5-arylidene-rhodanine-3-acetic acids were screened for 15-lipoxygenase (15-LOX) inhibitory activity.

Experimental

Carbonyldiimidazole (CDI), dicyclohexylcarbodiimide (DCC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), hydroxyl benzotriazole (HOBt) and other commercially available reagents were used without further purification. TLC was conducted on silica gel 250 micron, F254 plates. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrograph (KBr disks). ^{1}H NMR spectra were recorded on a NMR instrument Bruker 500 MHz. The chemical shifts (δ) and coupling constants (*J*) are expressed in parts per million and Hertz, respectively. Mass spectra of the products were obtained with an HP (Agilent Technologies, Santa Clara, CA) 5937 Mass Selective Detector. Elemental analyses were carried out by a CHN-Rapid Heraeus elemental analyzer. The results of elemental analyses (C, H, N) were within ±0.4% of the calculated values.

Address for correspondence: Abbas Shafiee, Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran. Tel: +98 21 66406757. Fax: +98 21 66461178. E-mail: ashafiee@ams.ac.ir

General procedure for the synthesis of compounds 3a-l

Compounds **3a–l** were prepared according to the literature[6].

General procedure for the synthesis of compounds 4a–n

A solution of compound **3** (1.0 mmol), EDC (1.1 mmol) and HOBt (1.0 mmol) in acetonitrile (10 ml) was stirred for 1 h at room temperature. Then, the corresponding amine (1.0 mmol) was added and stirred for 24 h at ambient temperature. After completion of the reaction (monitored by TLC), the solvent was evaporated and the residue was crystallized from chloroform-petroleum ether to give compound **4**.

(*Z*)-5-(4-Cholorobenzylidene)-3-(2-morpholino-2-oxoethyl)-2-thioxothiazolidin-4one (4a)

Yield 69%, yellow solid, m.p. 225–227 °C, IR (cm^{-1}, KBr): ν_{max} 3325 (C–H aromatic), 2926 and 2850 (C–H aliphatic), 1716 and 1668 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.73 (s, 1H, vinylic H), 7.48 and 7.45 (2 d, 4H, $H_{2,3,5,6}$, $J = 8.7$ Hz), 4.96 (s, 2H, CH_2CO), 3.8 and 3.73 (2t, 4H, CH_2O, $J = 4.45$ Hz), 3.64 and 3.59 (2t, 4H, CH_2N, $J = 4.45$ Hz). EI-MS (*m/z*, %) 382 (M^+, 19.6), 349 (26.7), 297 (34.6), 268 (37.7), 224 (13.3), 168 (100), 143 (14), 133 (23.6), 114 (14), 99 (31.4), 86 (98.4), 72 (53.5), 56 (77.9). Anal. ($C_{16}H_{15}ClN_2O_3S_2$): C, H, N, Calcd 50.19, 3.95, 7.32. Found. 50.32, 3.71, 7.12.

(*Z*)-5-(4-(Dimethylamino)benzylidene)-3-(2-morpholino-2-oxoethyl)-2-thioxothiazolidin-4-one (4b)

Yield 52%, orange solid, m.p. <300 °C, IR (cm^{-1}, KBr): ν_{max} 3327 (C–H aromatic), 2926 and 2851 (C–H aliphatic), 1707 and 1644 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.72 (s, 1H, vinylic H), 7.42 (d, 2H, $H_{2,4}$, $J = 8.4$ Hz), 6.75 (d, 2H, $H_{3,5}$, $J = 8.4$ Hz), 4.95 (s, 2H, CH_2CO), 3.80-3.59 (m, 8H, Morpholine), 3.10 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 193.2, 167.1, 163.4, 152.5, 133.7, 132.0, 120.2, 114.0, 112.7, 66.4, 47.9, 45.4, 45.2, 42.4. EI-MS (*m/z*, %) 391 (M^+, 88), 358 (15.7), 278 (13.3), 224 (14), 177 (100), 161 (13.3), 56 (14). Anal. ($C_{18}H_{21}N_3O_3S_2$): C, H, N, Calcd 55.22, 5.41, 10.73. Found. 55.48, 5.19, 10.53.

(*Z*)-5-(4-Methoxybenzylidene)-3-(2-morpholino-2-oxoethyl)-2-thioxothiazolidin-4-one (4c)

Yield 41%, yellow solid, m.p. 201–203 °C, IR (cm^{-1}, KBr): ν_{max} 2927 and 2850 (C–H aliphatic), 1702 and 1667 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.76 (s, 1H, vinylic H), 7.49 (d, 2H, $H_{2,4}$, $J = 8.75$ Hz), 7.01 (d, 2H, $H_{3,5}$, $J = 8.85$ Hz), 4.95 (s, 2H, CH_2CO), 3.89 (s, 3H, OMe), 3.80 and 3.72 (2t, 4H,CH_2O, $J = 4.6$ Hz), 3.65 and 3.59 (2t, 4H, CH_2N, $J = 4.6$ Hz). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 193.7, 167.1, 163.2, 162.1, 134.2, 133.5, 125.8, 119.2, 115.6, 66.4, 56.0, 47.9, 45.5, 45.2. EI-MS (*m/z*, %) 378 (M^+, 78.7), 345 (59), 293 (63), 264 (46.4), 164 (100), 149 (99.2), 121 (45.6), 99 (20.4), 86 (72.4), 77 (27.5), 70 (41.7), 56 (55). Anal. ($C_{17}H_{18}N_2O_4S_2$): C, H, N, Calcd 53.95, 4.79, 7.40. Found. 53.68, 4.54, 7.73.

(*E* and *Z*)-3-(2-Morpholino-2-oxoethyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (4d)

Yield 70%, yellow solid, m.p. 231–233 °C, IR (cm^{-1}, KBr): ν_{max} 3000 (C–H aromatic), 2935–2853 (C–H aliphatic), 1720 and 1671 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz, *E/Z* = 27/73): δ 7.86 (*E*) and 7.72 (*Z*) (2 s, 1H, vinylic H), 6.76 (*E*) and 6.74 (*Z*) (2 s, 2H, $H_{2,6}$ phenyl), 4.96 (*E*) and 4.55 (*Z*) (2 s, 2H, CH_2CO), 3.94 (*E*) and 3.93 (*Z*) (2 s, 3H, OMe), 3.93 (*E*) and 3.92 (*Z*) (2 s, 6H, OMe), 3.80 (*E*), 3.73 (*Z*) (2 m, 4H, Morpholine), 3.66 (*E*) and 3,59 (*Z*) (2 m, 4H, Morpholine). EI-MS (*m/z*, %) 438 (M^+, 82.7), 405 (22), 353 (20), 325 (40), 265 (11), 224 (100), 209 (89), 181 (19.7), 166 (12.6), 86 (21.2), 70 (14.1), 56 (13.3). Anal. ($C_{19}H_{22}N_2O_6S_2$): C, H, N, Calcd 52.04, 5.06, 6.39. Found. 51.86, 5.25, 6.14.

(*Z*)-3-(2-Oxo-2-(4-phenylpiperazine-1-yl)ethyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene) thiazolidin-4-one (4e)

Yield 67%, yellow solid, m.p. 192–194 °C, IR (cm^{-1}, KBr): ν_{max} 2932 and 2833 (C–H aliphatic), 1651 and 1599 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.71 (s, 1H, vinylic H), 7.34 (m, 2H, phenyl), 6.97 (m, 3H, phenyl), 6.75 (s, 2H, $H_{2,6}$), 5.02 (s, 2H,CH_2CO), 3.93 (s, 9H, OMe), 3.81 and 3.26 (m, 8H, piperazine). EI-MS (*m/z*, %) 513 (M^+, 42.5), 480 (45.6), 375 (18), 352 (15.7), 324 (14), 265 (11.8), 224 (28.3), 209 (41.7), 181 (16.5), 160 (62.7), 132 (100), 120 (15), 104 (35.4), 91 (11.8), 77 (19.7), 56 (56). Anal. ($C_{25}H_{27}N_3O_5S_2$): C, H, N, Calcd 58.46, 5.30, 8.18. Found. 58.22, 5.51, 8.01.

(*Z*)-3-(2-(4-(2-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)-5-(4-methoxybenzylidene)-2-thioxothiazolidin-4-one (4f)

Yield 49%, yellow solid, m.p. 259–260 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.76 (s, 1H, vinylic H), 7.49 (d, 2H, $H_{2,6}$, $J = 8.85$ Hz), 7.14–6.90 (m, 6H, fluorophenyl and $H_{3,5}$ methoxyphenyl), 5.01 (s, 2H, benzylic H), 3.89 (s, 3H, OMe), 3.82-3.10 (m, 8H, piperazine). EI-MS (*m/z*, %) 471 (M^+, 34.6), 438 (56.7), 315 (28.3), 292 (18.9), 264 (30.7), 220 (12.6), 178 (100), 164 (69.3), 150 (80.3), 138 (15), 122 (48), 109 (11.8), 95 (11.8), 77 (17.3), 56 (67). Anal. ($C_{23}H_{22}FN_3O_3S_2$): C, H, N, Calcd 58.58, 4.70, 4.03. Found 58.39, 4.93, 4.36.

(*Z*)-3-(2-(4-(3-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (4g)

Yield 52%, yellow solid, m.p. 202–204 °C, IR (cm^{-1}, KBr): ν_{max} 3414 (C–H aromatic), 2928 and 2837 (C–H aliphatic), 1718–1648 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.70 (s, 1H, vinylic H), 7.32 (d, 2H, $H_{2,6}$ fluorophenyl, $J = 8.95$ Hz), 6.99 (d, 1H, H_4 fluorophenyl), 6.77 (dd, 1H, H_5 fluorophenyl, $J = 2.6$ Hz), 6.74 (s, 2H, $H_{2,6}$), 4.99 (s, 2H, CH_2CO), 3.93 (s, 9H, OMe), 3.79 and 3.19 (m, 8H, piperazine). EI-MS (*m/z*, %) 531 (M^+, 8.6), 498 (11), 279 (10.2), 241 (16.5), 224 (25.2), 209 (30.7), 167 (26.7), 149 (68.5), 129 (96), 111 (44), 97 (40), 83 (52.7), 71 (58.3), 57 (100). Anal. ($C_{25}H_{26}FN_3O_5S_2$): C, H, N, Calcd 56.48, 4.93, 7.90. Found. 56.19, 4.67, 7.70.

(*Z*)-3-(2-(4-(4-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)-2-thioxo-5-(3,4,5-trimethoxybenzylidene)thiazolidin-4-one (4h)

Yield 64%, yellow solid, m.p. 200–202 °C, IR (cm^{-1}, KBr): ν_{max} 3432 (C–H aromatic), 2922 and 2853 (C–H aliphatic), 1706 and 1664 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.71 (s, 1H, vinylic H), 7.00 (t, 2H, $H_{2,6}$ fluorophenyl, $J = 8$ Hz), 6.92 (m, 2H, $H_{3,5}$ fluorophenyl), 6.74 (s, 2H, $H_{2,6}$), 5.01 (s, 2H, CH_2CO), 3.93 (s, 9H, OMe), 3.76 and 3.17 (m, 8H, piperazine). EI-MS (*m/z*, %) 531 (M^+, 48), 498 (56), 375 (16.5), 352 (19.7), 324 (18), 265 (11.8), 224 (35.4), 209 (50.3), 178 (79.5), 150 (100), 138 (15), 122 (36.2), 109 (10.2), 95 (17.3), 56 (50.4). Anal. ($C_{25}H_{26}FN_3O_5S_2$): C, H, N, Calcd 56.48, 4.93, 7.90. Found. 56.75, 4.65, 7.61.

(*Z*)-3-(2-(4-(3,4-Dichlorophenyl)piperazin-1-yl)-2-oxoethyl)-5-(2,3-dimethoxybenzylidene)-2-thioxothiazolidin-4-one (4i)

Yield 25%, yellow solid, m.p. 144–146 °C, IR (cm^{-1}, KBr): ν_{max} 3431(C–H aromatic), 2929–2841 (C–H aliphatic), 1710 and 1670

(C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.13 (s, 1H, vinylic H),7.34 (d, 1H, H_2 phenyl, $J = 8.8$ Hz), 7.17 (t, 1H, H_3 phenyl, $J = 8$ Hz), 7.08 and 7.04 (m, 3H, dicholorophenyl), 6.83 (m, 1H, H_4, $J = 2.5$ Hz), 5.01 (s, 2H, CH_2CO), 3.90 (s, 6H, OMe), 3.82 and 3.12 (d, 8H, piperazine). EI-MS (*m/z*, %) 551 (M^+, 30.7), 518 (52.7), 345 (30), 322 (66), 294 (81.8), 270 (25.2), 228 (100), 213 (17.3), 200 (93.7), 179 (42.5), 161 (21.2), 149 (26), 136 (21.2), 121 (18), 108 (12.6), 91 (11), 72 (26), 56 (86.6). Anal. ($C_{24}H_{23}Cl_2N_3O_4S_2$): C, H, N, Calcd 52.18, 4.20, 7.61. Found. 52.35, 4.01, 7.34.

(*Z*)-3-(2-(4-Benzylpiperidin-1-yl)-2-oxoethyl)-5-(4-(dimethylamino)benzylidene)-2-thioxothiazolidin-4-one (4j)

Yield 63%, yellow solid, m.p. 229–230 °C, IR (cm^{-1}, KBr): ν_{max} 2910 and 2853 (C–H aliphatic), 1710 and 1647 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.71 (s, 1H, vinylic H), 7.45 (d, 2H, $H_{2,6}$, $J = 8.5$ Hz), 7.32 (t, 2H, $H_{3,5}$ benzyl, $J = 8.2$ Hz), 7.23 (t, 1H, H_4 benzyl, $J = 8.2$ Hz), 7.17 (d, 2H, $H_{2,6}$ benzyl, $J = 8.2$ Hz), 6.89 (d, 2H, $H_{3,5}$, $J = 8.5$ Hz), 4.97 and 4.93 (2 d, 2H, CH_2CO), 4.54 and 3.85 (2 d, 2H, CH_2 benzylic, $J = 13.8$ Hz), 2.60 (m, 4H, CH_2N), 1.80–1.20 (m, 5H, CH_2 and CH). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 193.2, 167.1, 162.6, 152.5, 140.4, 135.3, 133.7, 129.4, 128.6, 126.3, 120.2, 114.1, 112.7, 45.6, 44.8, 42.4, 42.3, 37.6, 31.7. EI-MS (*m/z*, %) 479 (M^+, 47.2), 446 (22), 378 (31.4), 345 (22.8), 293 (21.2), 278 (11), 264 (22), 231 (11), 177 (100), 164 (81), 149 (33.8), 121 (14), 91 (19.6), 72 (10.2), 56 (11). Anal. ($C_{26}H_{29}N_3O_2S_2$): C, H, N, Calcd 65.11, 6.09, 8.76. Found. 65.34, 6.32, 8.53.

(*E* and *Z*)-3-(2-(4-Benzylpiperidin-1-yl)-2-oxoethyl)-5-(3-hydroxy-4-methoxybenzylidene)-2-thioxothiazolidin-4-one (4k)

Yield 34%, yellow solid, m.p. 278–280 °C, IR (cm^{-1}, KBr): ν_{max} 2923 and 2853 (C–H aliphatic), 1706 and 1646 (C=O); ^{1}H NMR ($CDCl_3$, 500 MHz, *E/Z* = 22/78): δ 7.83 (*E*) and 7.68 (*Z*) (2 s, 1H, vinylic H), 7.37 (*E*) and 7.32 (*Z*) (t, 2H, $H_{3,5}$ benzyl, $J = 7.3$ Hz), 7.26 (*E*) and 7.22 (*Z*) (t, 1H, H_4 benzyl, $J = 7.3$ Hz), 7.17 (*E*) and 7.08 (*Z*) (m, 2H, phenyl), 6.95 (*E*) and 6.58 (*Z*) (dd, 1H, H_6 phenyl, $J = 8.2$ and 1.9 Hz), 4.95 (*E*) and 4.53 (*Z*) (m, 2H, CH_2CO), 4.53 (*E*) and 4.16 (*Z*) (m, 2H, CH_2N), 3.98 (*Z*) and 3.97 (*E*) (2 s, 3H, OMe), 2.60 (*E*) and 2.43 (*Z*) (m, 2H, CH_2 benzylic), 1.85-1.30 (*E* and *Z*) (m, 5H, CH_2 and CH Piperidine). EI-MS (*m/z*, %) 480 (M^+, 7), 466 (26.7), 449 (14.1), 438 (39.3), 413 (15), 384 (45.6), 368 (16.5), 356 (26), 325 (26), 313 (10.2), 298 (15), 280 (15), 265 (19), 251 (14.1), 237 (13.4), 224 (90), 209 (100), 180 (79.5), 165 (48.8), 150 (60.6), 137 (39.3), 122 (33.8), 109 (21.2), 91 (60), 69 (26.7), 56 (43.3). Anal. ($C_{25}H_{26}N_2O_4S_2$): C, H, N, Calcd 62.22, 5.43, 5.80. Found. 62.54, 5.27, 5.67.

2-((*Z*)-5-(4-Chlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-N-(1-benzylpiperidin-4-yl)acetamide (4l)

Yield 43%, yellow solid, m.p. > 250 °C. IR (cm^{-1}, KBr): ν_{max} 3300 (NH), 3088 and 3023 (C–H aromatic), 2930 (C–H aliphatic), 1714 and 1656 (C=O); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.50-8.60 (br, s, 1H, NH), 7.70 (s, 1H, vinylic H), 8.00–8.30 (m, 4H, Ar-H), 7.40–7.80 (m, 5H, Ar-H of benzyl), 4.80 (s, 2H, $CO\underline{CH_2}N$), 3.35–3.65 (br, s, 3H, $\underline{CH}NH$ & benzylic $\underline{CH_2}$), 2.40–2.85 (m, 4H, $2CH_2N$ piperidine), 1.40–2.20 (m, 4H, 2 $\underline{CH_2}CH_2N$ piperidine). EI-MS (*m/z*, %) 485 (M^+, 27), 385 (5), 314 (7.7), 297 (6.9), 268 (7.5), 230 (3.8), 203 (12.3), 172 (93.8), 165 (31.5), 146 (6), 133 (16.9), 125 (23), 99 (3), 91 (98), 82 (100), 65 (7.5), 42 (10). Anal. ($C_{24}H_{24}ClN_3O_2S_2$): C, H, N, Calcd 59.31, 4.98, 8.65. Found. 59.65, 4.76, 8.91.

2-((*Z*)-5-(4-(Dimethylamino)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-N-(1-benzylpiperidin-4-yl) acetamide (4m)

Yield 40%, orange solid, m.p. >250 °C. IR (cm^{-1}, KBr): ν_{max} 3292 (NH), 3082 and 3021 (C–H aromatic), 2930 (C–H aliphatic), 1698 and 1655 (C=O); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.20 (br, s, 1H, NH), 7.70 (s, 1H, vinylic H), 6.85–7.50 (m, 9H, Ar-H), 4.60 (s, 2H, $CO\underline{CH_2}N$), 3.35–3.50 (br, s, 3H, $\underline{CH}NH$ and benzylic $\underline{CH_2}$), 3.05 (s, 6H, 2 CH_3), 2.50–2.95 (m, 4H, $\underline{CH_2}N$ piperidine), 1.40–2.30 (m, 4H, 2 $\underline{CH_2}CH_2N$ piperidine). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 193.3, 167.1, 164.1, 152.4, 135.2, 133.6, 129.2, 128.6, 127.4, 120.2, 114.3, 112.7, 62.4, 52.1, 46.7, 46.5, 31.8. EI-MS (*m/z*, %) 494 (M^+,4.5), 465 (8.3), 439 (25), 421 (5.3), 368 (9), 334 (7.5), 292 (11.3), 248 (12), 215 (13.6), 187 (6.8), 172 (96), 148 (43), 105 (41), 91 (100), 82 (53), 77 (18.9), 65 (13.6), 43 (25.5). Anal. ($C_{26}H_{30}N_4O_2S_2$): C, H, N, Calcd 63.13, 6.11, 11.33. Found. 63.51, 6.28, 11.03.

2-((*Z*)-5-(4-Methylbenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-N-(1-benzylpiperidin-4-yl)acetamide (4n)

Yield 38%, yellow solid, m.p. >250 °C. IR (cm^{-1}, KBr): ν_{max} 3299 (NH), 3082 and 3020 (C–H aromatic), 2924 (C–H aliphatic), 1716 and 1663 (C=O); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.05–8.27 (br, s, 1H, NH), 7.80 (s, 1H, vinylic H), 7.20–7.70 (m, 9H, Ar-H), 4.62 (s, 2H, $CO\underline{CH_2}N$), 3.30–3.60 (br, s, 3H, $\underline{CH}NH$ and benzylic $\underline{CH_2}$), 2.45–2.95 (m, 4H, $2CH_2N$ piperidine), 2.38 (s, 3H, CH_3), 1.35–2.30 (m, 4H, 2 $\underline{CH_2}CH_2N$ piperidine). ^{13}C NMR (DMSO-d_6, 125 MHz): δ 193.9, 167.1, 164.4, 142.1, 134.0, 131.7, 131.2, 130.6, 130.1, 130.0, 129.3, 121.4, 59.5, 51.0, 46.5, 44.7, 29.0, 21.5. EI-MS (*m/z*, %) 465 (M^+, 14.3), 276 (14), 248 (18.9), 172 (100), 148 (35), 132 (11.3), 118 (7.5), 91 (90), 82 (53), 72 (4.5), 56 (2.2), 42 (1.5). Anal. ($C_{25}H_{27}N_3O_2S_2$): C, H, N, Calcd 64.49, 5.84, 9.02. Found. 64.21, 5.76, 9.28.

Molecular modelling studies

All docking studies were performed using Autodock Vina (ver. 1.1.1, Florida, CA)[19]. The crystal structures of AChE complexed with E2020 (code ID: 1EVE) and soybean lypoxygenase complexed with 13(*S*)-hydroproxy-9(*Z*)-2,11(*E*)-octadecadienoic acid (code ID: 1IK3) were retrieved from protein data bank. In both cases, the co-crystallized ligand and water molecules were removed. Then the Fe in the structure of soybean lipoxygenase was modified to Fe(III)–OH and both proteins were converted to pdbqt format using Autodock Tools (1.5.4, Florida, CA)[20].

The 2D structures of ligands were sketched using MarvinSketch 5.8.3, 2012, ChemAxon (http://www.chemaxon.com) and then converted to 3D and pdbqt format by Openbabel (ver. 2.3.1, Pittsburgh, PA)[21].

The docking parameters were as follows:

LOX: size_x = 20; size_y = 20; size_z = 20; center_x = 19.693; center_y = 0.054; center_z = 17.628; exhaustiveness = 100; num_modes = 15.

AChE: center_x = 2.023; center_y = 63.295; center_z = 67.062; size_x = 25; size_y = 25; size_z = 25; exhaustiveness = 80; num_modes = 15. The other parameters were left as default for both of target enzymes. Finally, the conformations with the most favorable free energy of binding were selected for analyzing the interactions between the target enzyme and inhibitor. All the 3D models are generated using the Chimera 1.6 software (San Francisco, CA)[22].

AChE inhibition assay

The inhibitory potency of target compounds on AChE was determined using Ellman's method[23]. The test compounds were

dissolved in DMSO (1 ml) and phosphate buffer (9 ml, pH = 8) and then diluted using phosphate buffer to achieve five concentrations in the range from 10^{-3} to 10^{-7} M. The reaction mixture included 100 µl of DTNB 0.1 M, 50 µl of enzyme (2 U/ml) and 50 µl of inhibitor solution. The changing of the absorbance was measured at 412 nm for 2 min after addition of 10 µl of substrate (acetylthiocholine iodide) 0.15 M to the reaction mixture. The IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration versus percent of inhibition). All experiments were performed on a UV-2100 Rayleigh Double Beam Spectrophotometer in triplicate.

15-LOX inhibition assay

LOX inhibitory activity of target compounds was studied by a -spectrophotometric assay[24]. The tested compounds were dissolved in DMSO (1 ml) and phosphate buffer (9 ml, 0.1 M, pH = 8). This stock solution was added to test solution containing enzyme (final concentration: 167 U/ml), phosphate buffer (pH = 8) to achieve concentrations in range of 10^{-3} to 10^{-6}. After incubation of test solution for 4 min, substrate (linoleic acid, final concentration: 134 µM) was added and change in the absorbance was measured for 60 s at 234 nm. The enzyme solution was kept in ice and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant. All experiments were performed and then analyzed as mentioned above for AChE assay.

Results and discussion

Chemistry

In this work, we describe synthesis of a novel series of 2-((*Z*)-5-benzylidene-4-oxo-2-thioxothiazolidin-3-yl)acetamide **4a–n**. As outlined in Scheme 1, initially, an aldol condensation of rhodanine-3-acetic acid **1** with different aldehydes **2** in the presence of catalytic amount of *S*-proline yielded intermediate **3**. Under these conditions, (*Z*)-configuration was obtained as major product. In the ^{1}H NMR spectroscopy, the vinylic proton located on the exocyclic double bond was assigned as a singlet ranging from 7.71 to 8.13 ppm. According to the literature reports, vinilic proton in the (*Z*)-isomers appeared at 7.7–8.5 ppm downfield compared to the (*E*)-isomers[25,26]. It would be due to the deshielding effect of the carbonyl group. Finally, the target compounds **4a–n** were obtained via condensation of the carboxylic acids **3** with appropriate amines. Several coupling agents including CDI, DCC, EDC and HOBt in different solvents were investigated, but the best result was obtained by using EDC/HOBt in acetonitrile.

In vitro anti-AChE activity

The potential of the target compounds **4a–n** to inhibit AChE activity was assessed by the Ellman method. The inhibitory activity (IC_{50} values, µM) of the test compounds in comparison to tacrine as reference drug was listed in Table 1. The most active compound was 1-benzylpiperidinyl derivative **4m** with IC_{50} value of 21.0 µM. The *N*-(3-fluorophenyl)piperazinyl derivative **4g** with IC_{50} value of 22.4 µM was as potent as compound **4m**. Furthermore, compounds **4b**, **4f**, **4h–l**, and **4n** showed remarkable inhibitory activity against AChE in concentrations less than 64 µM.

Among the morpholine compounds **4a–d**, 4-(*N,N*-dimethylamino)-benzylidene derivative **4b** exhibited higher activity. As evidenced from IC_{50} values of **4a**, **4c** and **4d**, the introduction of 4-chloro-, 4-methoxy- and 3,4,5-trimethoxy- substituents dramatically diminished the activity. In the *N*-phenylpiperazine series **4e–i**, the unsubstituted analogue **4e** showed weak activity (IC_{50} value = 285 µM), while remaining congeners **4f–i** showed good activities in the range of 22.4–61.9 µM. The comparison of IC_{50} values of unsubstituted compound **4e** with 3-fluoro- and 4-fluoro- derivatives (**4g** and **4h**, respectively) revealed that fluoro- group increased the anti-AChE activity. Moreover, by comparing compound **4e** with **4d** or **4f** with **4c** demonstrated that *N*-phenylpiperazine moiety is more favorable than morpholine attached to the acetamide linker. In the case of compounds containing 4-(*N,N*-dimethylamino)- substituent, morpholine derivative **4b** and 4-benzylpiperidine analogue **4j** showed nearly same activity, and 1-benzylpiperidin-4-yl derivative **4m** was more potent than the latter compounds.

In vitro anti-15-LOX activity

Since the 5-LOX inhibitory activity of 5-benzylidenerhodanine-3-acetic acid has been reported,[6] thus we investigated the 15-LOX inhibitory potential of the intermediate compounds **3a–e** against soybean 15-LOX enzyme (Table 2). For structure–activity relationships study, the 5-benzylidenerhodanine-3-acetic acid series was completed by synthesizing compounds **3f–l** and evaluating their 15-LOX inhibitory activity. The results in comparison with quercetin were summarized in Table 2. All compounds with the exception of compounds **3b** and **3g** showed mild inhibitory activity against 15-LOX (IC_{50} values = 147–421 µM). The 2-hydroxy-4-bromo and 2,4-dihydroxy derivatives (compounds **3f** and **3h**, respectively) with IC_{50} values $\leq$149 µM, were the most active compounds. The comparison of unsubstituted compound **3l** with substituted benzylidene compounds **3c**, **3g**, and **3i** revealed that the introduction of 4-(*N,N*-dimethylamino), 4-methoxy, 3-bromo-4,5-dimethoxy and 2-hydroxy substituents decreased the anti-LOX activity. In contrast, the insertion of 3,4,5-trimethoxy, 2,3-dimethoxy, 2-hydroxy-4-methoxy, 2,4-dihydroxy, 2-methoxy, and 2-bromo substituents on the benzylidene moiety of compound **3l** resulted in more active compounds. Interestingly, the inhibitory activity of 3,4,5-trimethoxy analogue (compounds **3d**) was superior than that of 4-methoxy derivative **3c**. Also, compound **3h** having 2,4-dihydroxy group showed higher inhibition than 2-hydroxy congener **3i**. These findings demonstrated that the second hydroxyl substitution on the 4-position of 2-hydroxybenzylidene compound **3i** improved the inhibitory activity against 15-LOX. By comparing the IC_{50} values of compounds **3j** and **3i**, it is revealed that the *O*-methylation of the 2-OH is favorable. Notably, formation of amides with various amines that yield final compounds **4a–n**, led to inactive compounds toward lipoxygenase enzyme.

Docking studies

Molecular docking study was performed using the most active compound **4m** to investigate the possible interactions with amino

Scheme 1. Synthesis of 5-benzylidenerhodanine-3-acetamides (**4**).

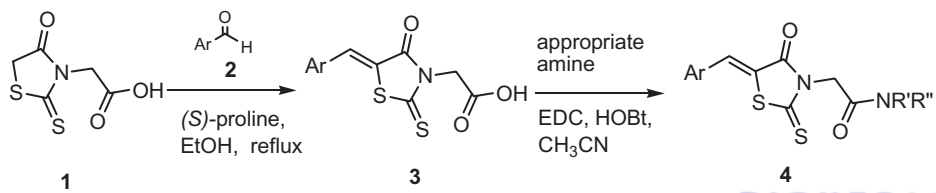
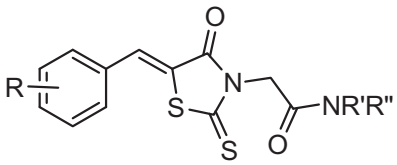
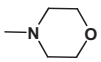
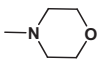
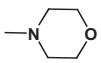
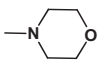
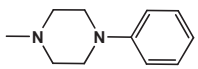
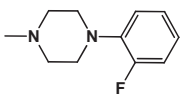
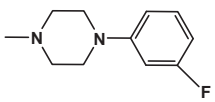
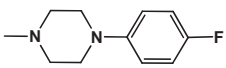
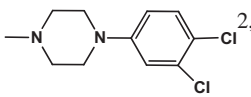
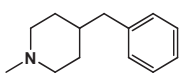
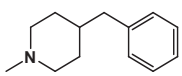
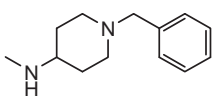
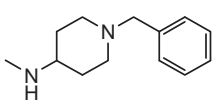
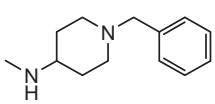


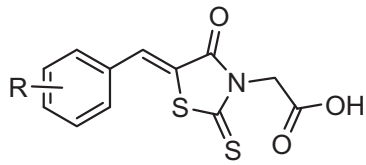
Table 1. Chemical structure and *eel*AChE inhibition data of target compounds **4a**–**n**.



Compounds	NR′R″	R	IC_{50} (μM)*
4a		4-Chloro-	285.0 ± 9.1
4b		4-(*N*,*N*-dimethylamino)-	52.3 ± 1.7
4c		4-Methoxy-	664.0 ± 21.0
4d		3,4,5-Trimethoxy-	675.0 ± 22.5
4e		3,4,5-Trimethoxy-	285.0 ± 9.2
4f		4-Methoxy-	44.1 ± 1.4
4g		3,4,5-Trimethoxy-	22.4 ± 0.7
4h		3,4,5-Trimethoxy-	52.3 ± 1.7
4i		2,3-Dimethoxy-	61.9 ± 2.2
4j		4-(*N*,*N*-dimethylamino)-	57.9 ± 1.8
4k		3-Hydroxy-4-methoxy-	63.0 ± 2.3
4l		4-Chloro-	47 ± 2.6
4m		4-(*N*,*N*-dimethylamino)-	21 ± 1.1
4n		4-Methyl-	63 ± 3.8
Tacrine	–	–	0.41

*Data are expressed as mean ± S.E. of at least three different experiments.

Table 2. Chemical structure and soybean 15-LOX inhibition activity data of 5-arylidene-rhodanine-3-acetic acids **3a**–**l**.



Compounds	R	IC_{50} (μM)* soybean 15-LOX
3a	4-Chloro-	156 ± 3.6
3b	4-(*N*,*N*-dimethylamino)-	NA†
3c	4-Methoxy-	390 ± 9.1
3d	3,4,5-Trimethoxy-	164 ± 3.8
3e	2,3-Dimethoxy-	175 ± 3.9
3f	2-Hydroxy-4-bromo-	149 ± 3.4
3g	3-Bromo-4,5-dimethoxy	NA
3h	2,4-Dihydroxy	147 ± 3.6
3i	2-Hydroxy	421 ± 9.7
3j	2-Methoxy	251 ± 5.9
3k	2-Bromo	203 ± 4.5
3l	H	366 ± 8.5
Quercetin	–	18 ± 0.5

*Data are expressed as mean ± S.E. of at least three different experiments.
†No activity.

acid residues on the active site of the AChE. The interaction of **4m** and the residues in the active site of *eel*AChE was illustrated in Figure 1. The compound was spanning along the narrow gorge of the enzyme from peripheral anionic binding site (PAS) to catalytic anionic site (CAS). The active site of the enzyme is lined with aromatic residues, which comprise 40% of the residues in the binding pocket. The most active compound **4m** takes advantage of these residues by making a variety of interactions. The positively charged piperidine nitrogen makes a cation–π interaction with Phe330 that is essential for ligand recognition. The benzyl ring of the compound shows π–π stacking with the aromatic ring of the Trp84. In such orientation, the pendant fragment including the rhodanine is stabilized by making π–π stacking with Trp279 through the 4-(dimethylamino)phenyl part of the molecule that is positioned at the rim of the gorge.

In the case of 15-LOX enzyme, the best LOX inhibitor, **3h**, was docked into the LOX active site pocket while the Fe core had been modified to Fe(III)-OH. Analysis of docking results revealed that free carboxylic group of compound **3 h**, oriented toward Fe–OH and interfered with it through a hydrogen bonding. It is further stabilized through additional hydrogen bonding formed between 2-hydroxy group of phenyl moiety and Ser510 and His513 (Figure 2). As mentioned above, all final compounds **4a**–**k** were inactive toward lipoxygenase enzyme. This reconfirmed the importance of free carboxylic group for binding of these compounds to target enzyme and their LOX inhibitory activity.

Conclusion

We have synthesized a series of 5-benzylidenerhodanine-3-acetamides bearing morpholino-, 4-arylpiperazinyl-, or 4-benzylpiperidinyl- moieties and evaluated their inhibitory activity against AChE. Alteration of amide part and substitution on the benzylidene moiety resulted in changing of anti-AChE activity. The better result was obtained with 4-(3-fluorophenyl)piperazin-1-yl derivative **4g** containing 3,4,5-trimethoxybenzylidene scaffold and 1-benzylpiperidinyl derivative **4m** bearing 4-(*N*,*N*-dimethylamino)benzylidene moiety.

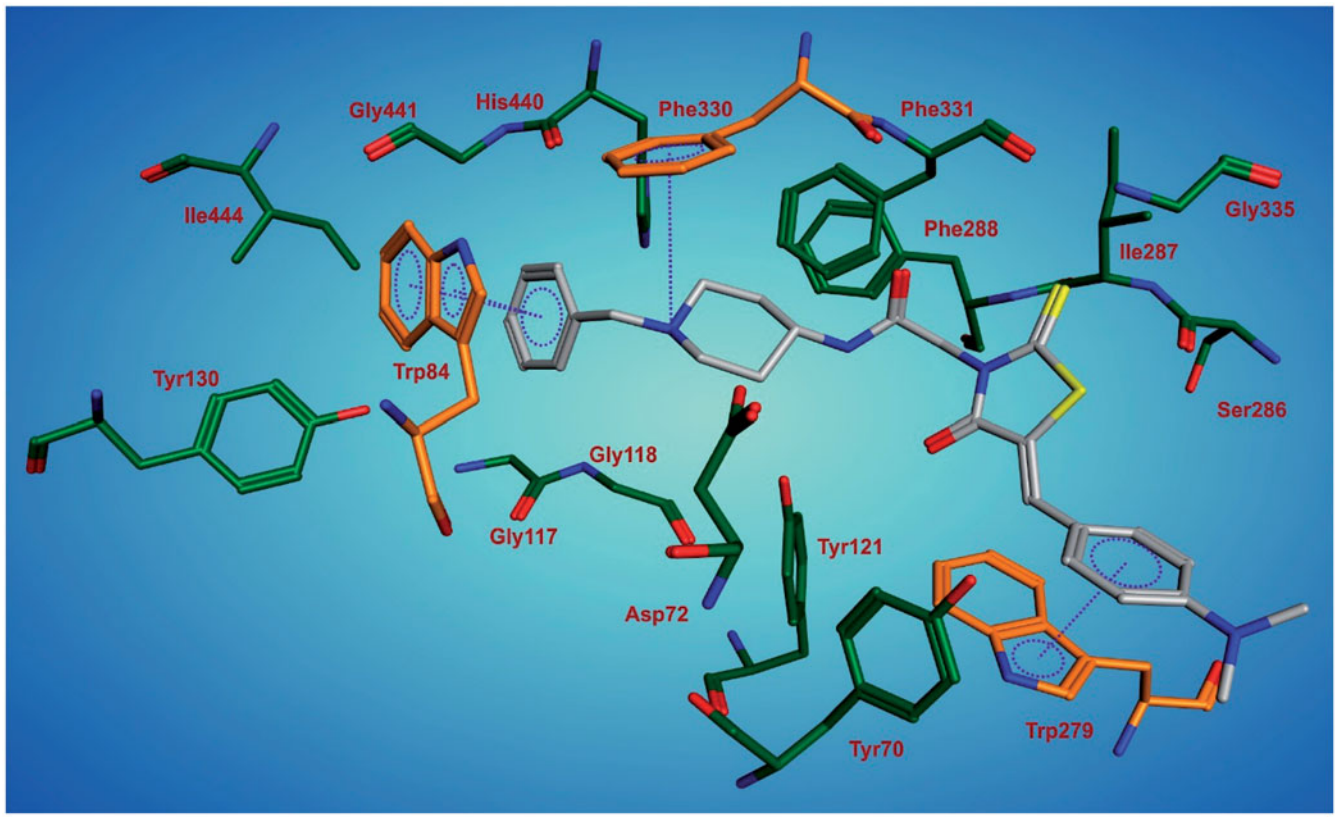


Figure 1. A representative model for interaction of compound **4m** and the AChE. The residues involved in the interaction are shown.

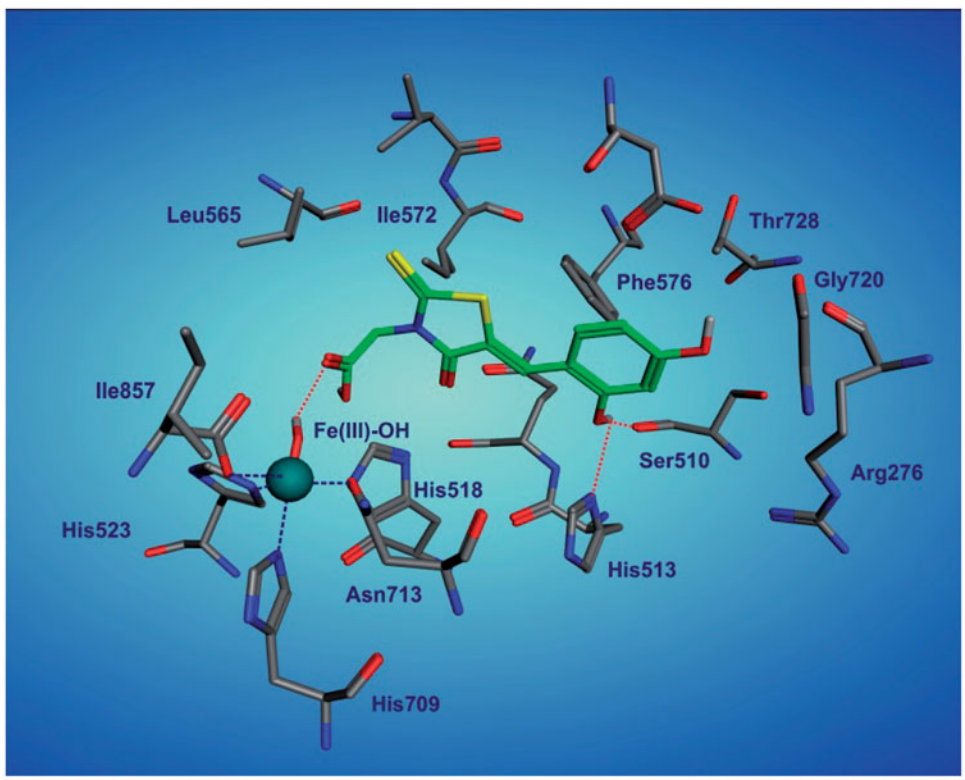


Figure 2. Representation of the best docked pose of **3h** in the active site of 15-LOX enzyme. Hydrogen bonds are shown as red-dotted lines and Fe(III) also is shown as ball model. (For interpretation of the references to colour in this caption, the reader is referred to the online version of this article.)

Besides AChE, most intermediate compounds **3** showed mild inhibitory activity against 15-LOX. The 2-hydroxy-4-bromo and 2,4-dihydroxy derivatives (compounds **3f** and **3h**, respectively) were the most active compounds.

Acknowledgements

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Declaration of interest

The authors have declared no conflict of interest.

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Supplementary material available online

Supplementary Figures.