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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

5-Nitro-heteroarylidene analogs of 2-thiazolylimino-4-thiazolidinones as a novel series of antibacterial agents

Nouraddin Hosseinzadeh · Mohammad Hasani · Alireza Foroumadi · Hamid Nadri · Saeed Emami · Nasrin Samadi · Mohammad Ali Faramarzi · Parastoo Saniee · Farideh Siavoshi · Neda Abadian · Yasaman Mahmoudjanlou · Amirhossein Sakhteman · Alireza Moradi · Abbas Shafiee

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Abstract In the pursuit of novel antibacterial agents with the 2-thiazolylimino-4-thiazolidinone as a core structure, a series of 5-nitro-heteroarylidene and 5-(2-oxoindolin-3-ylidene) analogs of 2-thiazolylimino-5-arylidene-4-thiazolidinone were synthesized and their antibacterial activities were evaluated against some strains of Gram-positive and Gram-negative bacteria, as well as *Helicobacter pylori* strains. Biological data indicated that 5-nitrofuran analog **5a** and 5-nitroimidazole analog **7a** containing no substitutions on the thiazole ring were the most potent compounds.

Keywords Antibacterial activity · *Helicobacter pylori* · 4-Thiazolidinones · Thiazole · 5-Nitroheterocycles

Introduction

The treatment of some infectious diseases still remains a challenging problem due to the emergence of multi-drug resistant pathogens including both Gram-positive and Gram-negative bacteria (Muroi *et al.*, 2004; Pfeltz and Wilkinson, 2004; Tenover and McDonald, 2005). The synthesis and pharmacological evaluation of thiazole derivatives have been frequently reported over the years because of their various therapeutic effects (Hutchinson *et al.*, 2002). Among the synthesized structures, 2-arylimino-4-thiazolidinone derivatives exhibited various pharmacological effects such as antimicrobial, antifungal, antituberculosis, anticonvulsant, anti-inflammatory, and antineoplastic activity (Bell *et al.*, 1995; Hargrave *et al.*, 1983; Patt *et al.*, 1992; Tsubouchi *et al.*, 1994). Moreover, a new class of antimicrobial agents bearing 2-thiazolylimino-5-arylidene-4-thiazolidinone scaffold have been reported (Vicini *et al.*, 2006; Vicini *et al.*, 2008). It was found that the 5-arylidene moiety plays an important role in the antimicrobial activity of this class of compounds (Vicini *et al.*, 2008).

N. Hosseinzadeh · A. Foroumadi · A. Shafiee (✉)
Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran 14176, Iran
e-mail: ashafiee@ams.ac.ir

M. Hasani
School of Chemistry, College of Science, University of Tehran, Tehran, Iran

H. Nadri · A. Sakhteman · A. Moradi
Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

S. Emami
Department of Medicinal Chemistry and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran

N. Samadi · N. Abadian
Department of Drug and Food Control, Faculty of Pharmacy and Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran

M. A. Faramarzi · Y. Mahmoudjanlou
Department of Pharmaceutical Biotechnology, Faculty of Pharmacy and Drug Design and Development Research Center, Tehran University of Medical Sciences, Tehran, Iran

P. Saniee · F. Siavoshi
Microbiology Department, Faculty of Sciences, University of Tehran, Tehran, Iran

On the other hand, it should be noted that the antibacterial activity of nitroimidazoles, nitrofurans, and nitrothiophenes have been established (Apt, 2010; Graham and Fischbach, 2010; Lienhardt *et al.*, 2010). Also, antimicrobial agents containing nitroimidazole or nitrofuran scaffolds have been used to treat *Helicobacter pylori* and the other microbial infections with varying degrees of success (Foroumadi *et al.*, 2009).

Based on these findings and in pursuit of the previous studies to develop new antimicrobial agents (Foroumadi *et al.*, 2009; Jazayeri *et al.*, 2009; Khalaj *et al.*, 2011; Mirzaei *et al.*, 2008), we have designed novel structures with the 2-thiazolylimino-4-thiazolidinone as a core structure, in which various 5-nitro-heteroarylidene scaffolds have been attached at the 5-position instead of bezylidene moiety (Fig. 1). Thus, we describe here, the synthesis of 5-nitrofuran, 5-nitrothiophene, and 5-nitroimidazole analogs of 2-thiazolylimino-5-arylidene-4-thiazolidinone (compounds **5**–**7**, respectively) and their antibacterial activity against some strains of Gram-positive and Gram-negative bacteria as well as *H. pylori*. Beside of the 5-nitro-heteroarylidene analogs **5**–**7**, 5-(2-oxo-indolin-3-ylidene) derivatives **8** were considered as new type of 5-arylidene-4-thiazolidinones containing fused 5-bezylidene moiety (Fig. 1).

Materials and methods

Chemistry

All chemicals and solvents used in this study were purchased from Merck AG and Aldrich. Melting points were determined on a Kofler hot stage apparatus (C. Reihert, Vienna, Austria). ^{1}H-NMR spectra were recorded using a Bruker 500 spectrometer (Bruker, Rheinstetten, Germany) and chemical shifts are expressed as δ with tetramethylsilane (TMS) as internal standard. The IR spectra were taken using Nicolet FT-IR Magna 550 spectrometer (Nicolet, Madison, WI, USA). The mass spectra were run on a Finnigan MAT TSQ-70 spectrometer (Finnigan Mat, Bremen, Germany) at 70 eV. Elemental analyses were carried out on a CHN–O rapid elemental analyzer (GmbH-Germany) for C, H, and N, and the results are within ±0.4 % of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC. Yields are based on the purified products and were not optimized. Column chromatography was performed on Merck silica gel (70-230 mesh).

Experimental

*General procedure for the synthesis of 2-thiazolylimino-5-arylidene-4-thiazolidinones (**5**–**8**)*

To a well-stirred solution of thiazolidin-4-one **4a–d** (4 mmol) in acetic acid (35 mL), buffered with sodium acetate (8 mmol) was added appropriate aldehyde or isatin (6 mmol). The mixture was irradiated in the microwave oven at 450 W for 15 min (5 × 3 min). The precipitated product was filtered off and washed with cold water. The crude product was recrystallized from dioxane to give pure compounds **5**–**8**.

*(5Z)-5-((5-Nitrofuran-2-yl)methylene)-2-(thiazol-2-ylimino) thiazolidin-4-one (**5a**)* Yield: 85 %; mp: 293–295 °C; IR (KBr, cm^{-1}): 3419 (N–H), 1716 (C=O), 1615 (N=C), 1541, and 1347 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 7.32 (d, 1H, J = 3.4 Hz), 7.54 (d, 1H, J = 4.0 Hz), 7.61 (s, 1H), 7.72 (d, 1H, J = 4.0 Hz), 7.83 (d, 1H, J = 3.4 Hz), 12.83

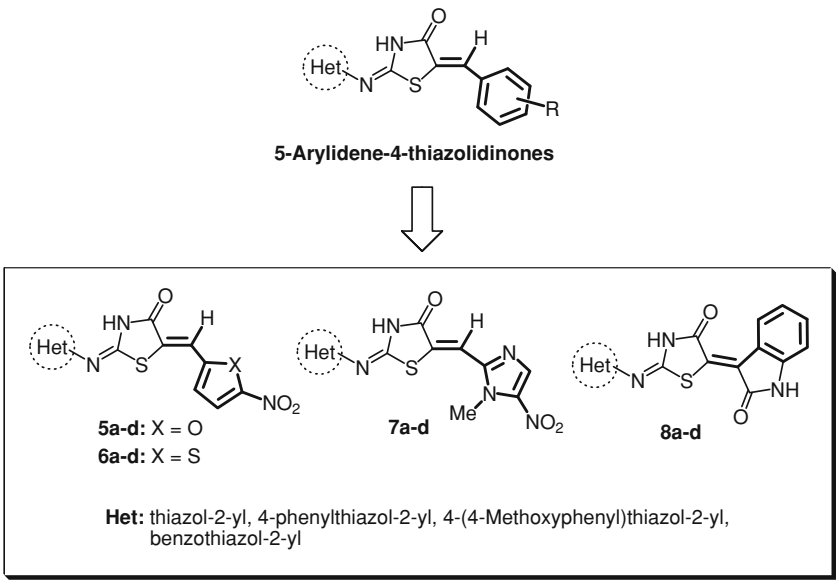


Fig. 1 Designed 5-heteroarylidene-4-thiazolidinone derivatives **5**–**8** as antibacterial agents

(s, 1H, NH). MS (*m/z*, %): 322 (M^+, 42), 276 (87), 184 (29), 126 (41). Anal. Calcd. For $C_{11}H_6N_4O_4S_2$: C, 40.99; H, 1.88; N, 17.38. Found: C, 40.58; H, 1.81; N, 17.43.

*(5Z)-5-((5-Nitrofuran-2-yl)methylene)-2-(4-phenylthiazol-2-ylimino)thiazolidin-4-one (**5b**)* Yield: 80 %; mp: >300 °C; IR (KBr, cm^{-1}): 3430 (N–H), 1696 (C=O), 1588 (N=C), 1555, and 1348 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 7.37–7.42 (m, 2H), 7.56 (t, 2H, $J = 7.10$ Hz), 7.67 (s, 1H), 7.86 (d, 1H, $J = 3.4$ Hz), 7.97 (s, 1H), 8.14 (d, 2H, $J = 7.25$ Hz), 12.87 (s, 1H, NH). MS (*m/z*, %): 398 (M^+, 31), 352 (64), 261 (45), 201 (29). Anal. Calcd. For $C_{17}H_{10}N_4O_4S_2$: C, 51.25; H, 2.53; N, 14.06. Found: C, 51.28; H, 2.59; N, 14.01.

*(5Z)-2-(4-(4-Methoxyphenyl)thiazol-2-ylimino)-5-((5-nitrofuran-2-yl)methylene)thiazolidin-4-one (**5c**)* Yield: 91 %; mp: >300 °C; IR (KBr, cm^{-1}): 3138 (N–H), 1712 (C=O), 1584 (N=C), 1553, and 1359 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 3.81 (s, 3H), 7.09 (d, 2H, $J = 8.25$ Hz), 7.35 (d, 1H, $J = 3.4$ Hz), 7.65 (s, 1H), 7.79 (s, 1H), 7.85 (d, 1H, $J = 3.4$ Hz), 8.06 (d, 2H, $J = 8.25$ Hz), 12.85 (s, 1H). MS (*m/z*, %): 428 (M^+,43), 382 (24), 291 (33), 231 (58). Anal. Calcd. For $C_{18}H_{12}N_4O_5S_2$: C, 50.46; H, 2.82; N, 13.08. Found: C, 50.61; H, 2.90; N, 13.11.

*(5Z)-2-(Benzodthiazol-2-ylimino)-5-((5-nitrofuran-2-yl)methylene)thiazolidin-4-one (**5d**)* Yield: 73 %; mp: 299–303 °C; IR (KBr, cm^{-1}): 3436 (N–H), 1723 (C=O), 1596 (C=N), 1544, and 1350 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 7.35-7.41 (m, 2H), 7.55 (t, 1H, $J = 7.2$ Hz), 7.67 (s, 1H), 7.83–7.85 (m, 2H), 8.03 (d, 1H, $J = 7.6$ Hz), 13.05 (s, 1H). MS (*m/z*, %): 372 (M^+, 78), 326 (71), 176 (100), 95 (60). Anal. Calcd. For $C_{15}H_8N_4O_4S_2$: C, 48.38; H, 2.17; N, 15.05. Found: C, 48.29; H, 2.21; N, 15.11.

*(5Z)-5-((5-Nitrothiophen-2-yl)methylene)-2-(thiazol-2-ylimino)thiazolidin-4-one (**6a**)* Yield: 72 %; mp: 269–272 °C; IR (KBr, cm^{-1}): 3425 (N–H), 1716 (C=O), 1600 (N=C), 1559, and 1363 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 7.55 (d, 1H, $J = 4.0$ Hz), 7.70 (d, 1H, $J = 3.8$ Hz), 7.81 (d, 1H, $J = 4.0$ Hz), 8.01 (s, 1H), 8.24 (d, 1H, $J = 3.8$ Hz), 12.95 (s, 1H, NH). MS (*m/z*, %): 338 (M^+, 46), 292 (24), 185 (52), 126 (61), 95 (61), 73 (91), 43 (100). Anal. Calcd. For $C_{11}H_6N_4O_3S_3$: C, 39.04; H, 1.79; N, 16.56. Found: C, 39.21; H, 1.82; N, 16.66.

*(5Z)-5-((5-Nitrothiophen-2-yl)methylene)-2-(4-phenylthiazol-2-ylimino)thiazolidin-4-one (**6b**)* Yield: 68 %; mp: >300 °C; IR (KBr, cm^{-1}): 3417 (N–H), 1704 (C=O), 1572 (N=C), 1544, and 1332 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 7.43 (t, 1H, $J = 7.25$ Hz), 7.54 (t, 2H, $J = 7.10$ Hz), 7.75 (d, 1H, $J = 3.8$ Hz), 7.95 (s, 1H), 8.03 (s, 1H), 8.1 (d, 2H, $J = 7.25$ Hz), 8.25 (d, 1H, $J = 3.8$ Hz), 12.81 (s, 1H, NH). MS (*m/z*, %): 414 (M^+, 75), 368 (58), 261 (27), 202 (49). Anal. Calcd. For $C_{17}H_{10}N_4O_3S_3$: C, 49.26; H, 2.43; N, 13.52. Found: C, 49.41; H, 2.40; N, 13.42.

*(5Z)-2-(4-(4-Methoxyphenyl)thiazol-2-ylimino)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (**6c**)* Yield: 74 %; mp: >300 °C; IR (KBr, cm^{-1}): 3429 (N–H), 1714 (C=O), 1595 (N=C), 1560, and 1384 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 3.83 (s, 3H), 7.08 (d, 2H, $J = 8.25$ Hz), 7.73 (d, 1H, $J = 3.8$ Hz), 7.76 (s, 1H), 7.98 (s, 1H), 8.01 (d, 2H, $J = 8.25$ Hz), 8.23 (d, 1H, $J = 3.8$ Hz), 12.91 (s, 1H, NH). MS (*m/z*, %): 444 (M^+, 56), 398 (35), 291 (28), 231 (42). Anal. Calcd. For $C_{18}H_{12}N_4O_4S_3$: C, 48.64; H, 2.72; N, 12.60. Found: C, 48.71; H, 2.75; N, 12.69.

*(5Z)-2-(Benzodthiazol-2-ylimino)-5-((5-nitrothiophen-2-yl)methylene)thiazolidin-4-one (**6d**)* Yield: 82 %; mp: 283–287 °C; IR (KBr, cm^{-1}): 3444 (N–H), 1720 (C=O), 1596, (N=C), 1550, and 1328 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 7.40 (t, 1H, $J = 7.2$ Hz), 7.52 (t, 1H, $J = 7.1$ Hz), 7.72 (d, 1H, $J = 3.8$ Hz), 7.91 (d, 1H, $J = 8.0$ Hz), 8.1–8.2 (m, 2H), 7.91–8.05 (m, 3H), 8.23 (d, 1H, $J = 3.8$ Hz), 13.05 (s, 1H, NH). MS (*m/z*, %): 388 (M^+, 53), 341 (65), 236 (41), 175 (59). Anal. Calcd. For $C_{15}H_8N_4O_3S_3$: C, 46.38; H, 2.08; N, 14.42. Found: C, 46.28; H, 2.03; N, 14.49.

*(5Z)-5-((1-Methyl-5-nitro-1H-imidazol-2-yl)methylene)-2-(thiazol-2-ylimino)thiazolidin-4-one (**7a**)* Yield: 63 %; mp: >300 °C; IR (KBr, cm^{-1}): 3440 (N–H), 1724 (C=O), 1615, and 1689 (N=C), 1548, and 1351 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 4.06 (s, 3H), 7.50 (d, 1H, $J = 4.0$ Hz), 7.59 (s, 1H), 7.74 (d, 1H, $J = 4.0$ Hz), 8.43 (s, 1H), 12.76 (s, 1H, NH). MS (*m/z*, %): 336 (M^+, 86), 211 (57), 183 (100), 96 (53). Anal. Calcd. For $C_{11}H_8N_6O_3S_2$: C, 39.28; H, 2.40; N, 24.99. Found: C, 39.39; H, 2.43; N, 25.11.

*(5Z)-5-((1-Methyl-5-nitro-1H-imidazol-2-yl)methylene)-2-(4-phenylthiazol-2-ylimino)thiazolidin-4-one (**7b**)* Yield: 73 %; mp: >300 °C; IR (KBr, cm^{-1}): 3443 (N–H), 1712 (C=O), 1596 (N=C), 1558, and 1342 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 4.06 (s, 3H), 7.38 (t, 1H, $J = 7.25$ Hz), 7.56 (t, 2H, $J = 7.10$ Hz), 7.63 (s, 1H), 7.93 (s, 1H), 8.03 (d, 2H, $J = 7.25$ Hz), 8.47 (s, 1H), 12.79 (s, 1H). MS (*m/z*, %): 412 (M^+, 39), 366 (32), 261 (42), 201 (65). Anal. Calcd. For $C_{17}H_{12}N_6O_3S_2$: C, 49.51; H, 2.93; N, 20.38. Found: C, 49.07; H, 2.85; N, 20.21.

*(5Z)-2-(4-(4-Methoxyphenyl)thiazol-2-ylimino)-5-((1-methyl-5-nitro-1H-imidazol-2-yl) methylene)thiazolidin-4-one (**7c**)* Yield: 78 %; mp: >300 °C; IR (KBr, cm^{-1}): 3428 (N–H), 1712 (C=O), 1596 (N=C), 1561, and 1369 (NO_2); ^{1}H NMR

(DMSO-d_6) δ: 3.85 (s, 3H), 4.07 (s, 3H), 7.13 (d, 2H, $J = 8.25$ Hz), 7.63 (s, 1H), 7.76 (s, 1H), 7.97 (d, 2H, $J = 8.25$ Hz), 8.53 (s, 1H), 12.77 (s, 1H, NH). MS (*m/z*, %): 442 (M^+, 69), 396 (71), 291 (25), 260 (38), 231 (49). Anal. Calcd. For $C_{18}H_{14}N_6O_4S_2$: C, 48.86; H, 3.19; N, 18.99. Found: C, 48.65; H, 3.27; N, 19.09.

(5Z)-2-(Benzodthiazol-2-ylimino)-5-((1-methyl-5-nitro-1H-imidazol-2-yl)methylene)thiazolidin-4-one ***(7d)*** Yield: 81 %; mp: >300 °C; IR (KBr, cm^{-1}): 3405 (N–H), 1720 (C=O), 1562, and 1370 (NO_2); ^{1}H NMR (DMSO-d_6) δ: 4.08 (s, 3H), 7.39 (t, 1H, $J = 7.2$ Hz), 7.52 (t, 1H, $J = 7.2$ Hz), 7.67 (s, 1H), 7.90 (d, 1H, $J = 8.3$ Hz), 8.02 (d, 1H, $J = 7.6$ Hz), 8.42 (s, 1H), 12.98 (s, 1H, NH). MS (*m/z*, %): 386 (M^+, 34), 340 (81), 235 (32), 175 (28). Anal. Calcd. For $C_{15}H_{10}N_6O_3S_2$: C, 46.62; H, 2.61; N, 21.75. Found: C, 46.78; H, 2.65; N, 21.85.

(5Z)-5-(2-Oxoindolin-3-ylidene)-2-(thiazol-2-ylimino)thiazolidin-4-one ***(8a)*** Yield: 79 %; mp: >300 °C; IR (KBr, cm^{-1}): 3374 (N–H), 1708 (C=O), 1693 (C=O), 1580 (N=C); ^{1}H NMR (DMSO-d_6) δ: 6.91 (d, 1H, $J = 7.65$ Hz), 7.05 (t, 1H, $J = 7.5$ Hz), 7.35 (t, 1H, $J = 7.3$ Hz), 7.51 (d, 1H, $J = 4.0$ Hz), 7.73 (d, 1H, $J = 4.0$ Hz), 8.83 (d, 1H, $J = 7.65$ Hz), 11.18 (s, 1H, NH), 12.86 (s, 1H, NH). MS (*m/z*, %): 328 (M^+, 27), 197 (45), 185 (62), 132 (24), 125 (52). Anal. Calcd. For $C_{14}H_8N_4O_2S_2$: C, 51.21; H, 2.46; N, 17.06. Found: C, 51.45; H, 2.38; N, 17.19.

(5Z)-5-(2-Oxoindolin-3-ylidene)-2-(4-phenylthiazol-2-ylimino) thiazolidin-4-one ***(8b)*** Yield: 82 %; mp: >300 °C; IR (KBr, cm^{-1}): 3207 (N–H), 1715 (C=O), 1697 (C=O), 1583 (N=C); ^{1}H NMR (DMSO-d_6) δ: 6.95 (d, 2H, $J = 7.5$ Hz), 7.07 (t, 1H, $J = 7.5$ Hz), 7.38 (m, 2H), 7.49 (t, 2H, $J = 7.10$ Hz), 7.94 (s, 1H), 8.04 (d, 2H, $J = 7.3$ Hz), 8.85 (d, 1H, $J = 7.6$ Hz), 11.12 (s, 1H, NH), 12.93 (s, 1H, NH). MS (*m/z*, %): 404 (M^+, 83), 273 (34), 201 (41), 144 (65), 88 (29). Anal. Calcd. For $C_{20}H_{12}N_4O_2S_2$: C, 59.39; H, 2.99; N, 13.85. Found: C, 59.54; H, 3.11; N, 13.64.

(5Z)-2-(4-(4-Methoxyphenyl)thiazol-2-ylimino)-5-(2-oxoindolin-3-ylidene)thiazolidin-4-one ***(8c)*** Yield: 84 %; mp: >300 °C; IR (KBr, cm^{-1}): 3426 (N–H), 1721, 1693 (C=O), 1581 (N=C); ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H), 6.95 (d, 1H, $J = 7.5$ Hz), 7.02–7.06 (m, 3H), 7.37 (t, 1H, $J = 7.4$ Hz), 7.76 (s, 1H), 7.97 (d, 2H, $J = 8.25$ Hz), 8.85 (d, 1H, $J = 7.65$ Hz), 11.09 (s, 1H, NH), 12.89 (s, 1H, NH). MS (*m/z*, %): 434 (M^+, 73), 302 (37), 291 (54), 263 (28), 89 (22). Anal. Calcd. For $C_{21}H_{14}N_4O_3S_2$: C, 58.05; H, 3.25; N, 12.89. Found: C, 58.42; H, 3.08; N, 12.62.

(5Z)-2-(Benzothiazol-2-ylimino)-5-(2-oxoindolin-3-ylidene) thiazolidin-4-one ***(8d)*** Yield: 85 %; mp: >300 °C; IR (KBr, cm^{-1}): 3184 (N–H), 1717 (C=O), 1687 (C = O), 1562 (N=C); ^{1}H NMR (DMSO-d_6) δ: 6.93 (d, 1H, $J = 7.65$), 7.06 (t, 1H, $J = 7.5$ Hz),7.35–7.39 (m, 2H), 7.50 (t, 1H, $J = 7.2$ Hz), 7.90 (d, 1H, $J = 8.4$ Hz), 8.00 (d, 1H, $J = 7.6$ Hz), 8.84 (d, 1H, $J = 7.65$ Hz), 11.21 (s, 1H, NH), 13.10 (s, 1H, NH). MS (*m/z*, %): 378 (M^+, 84), 275 (38), 247 (21), 235 (22). Anal. Calcd. For $C_{18}H_{10}N_4O_2S_2$: C, 57.13; H, 2.66; N, 14.81. Found: C, 57.35; H, 2.51; N, 14.72.

Biological activities

Microdilution method (minimum inhibitory concentration determination)

The minimum inhibitory concentrations (MICs) of synthetic compounds against microorganisms including Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, and *Bacillus subtilis* ATCC 6633) and Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, and *Pseudomonas aeruginosa* ATCC 9027) were determined by microdilution method by using 96 U-shaped wells plates (Jazayeri *et al.*, 2009). A stock solution of compounds (200 μg/ml) was prepared in Mueller–Hinton broth (MHB) by 10 % v/v DMSO. Then twofold serial dilution of the stock solution of each compound (100 μl) was prepared by using MHB (100 μl) in 12 wells. The stock microbial suspension with twofold test inoculum (1×10^6 CFU/ml) was prepared from a 24 h-old culture. Then aliquot of 100 μl of twofold test strain inoculum was added to each well to reach the final inoculum size of 5×10^5 CFU/ml. After 24 h incubation at 35–37 °C, the microdilution plates were tested for the absence or presence of visible growth in comparison with that of the growth in drug-free control well. The endpoint MIC is the lowest concentration of the compound at which the test strain does not demonstrate visible growth.

H. pylori growth inhibition assay (disk diffusion method)

Growth inhibition assay was done using the filter paper disk diffusion method on Mueller–Hinton agar with 7 % of defibrinated horse blood under microaerophilic conditions at 37 °C (Foroumadi *et al.*, 2009; Mirzaei *et al.*, 2008). Different doses of compounds were tested. The compounds were dissolved in methanol and then dropped on the paper disks (6 mm diameter) using a microsyringe. After drying the paper disks in the fume hood, they were placed on the agar surface that was inoculated with *H. pylori*. Following incubation for 3–5 days at 37 °C, the inhibition zone around each disk (average diameter) was recorded. All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by title compounds.

Results and discussion

Chemistry

The target compounds were synthesized according to the multi-step reaction protocol reported earlier (Vicini *et al.*, 2006). The general synthetic pathway was depicted in Scheme 1. 2-Bromo-1-phenylethanone (**1a**, R = H) or 2-bromo-1-(4-methoxyphenyl)ethanone (**1b**, R = MeO) was reacted with thiourea in refluxing ethanol to yield 4-phenylthiazol-2-amine (**2b**) or 4-(4-methoxyphenyl)thiazol-2-amine (**2c**), respectively. 2-Aminothiazole (**2a**) and 2-aminobenzothiazole (**2d**) were commercially available. Reaction of appropriate amine **2a–d** with chloroacetyl chloride in DMF at room temperature for 2 h, furnished compound **3a–d** in good yield (Geronikaki *et al.*, 2003; Vicini *et al.*, 1990). 2-Chloroacetamides **3a–d** efficiently reacted with ammonium thiocyanate in refluxing ethanol to give 2-(heteroarylimino) thiazolidin-4-ones **4a–d**. To obtain the final compounds **5–8**, intermediate **4** and appropriate 5-nitroheteroaryl-2-carboxaldehyde or isatin were mixed in acetic acid buffered with sodium acetate and irradiated in a microwave oven at 450 W for 15 min (5 × 3 min). By using microwave-assisted synthesis method, it was possible to decrease the reaction time up to 15 min respect to 2–4 h in conventional heating method. The yields of the reactions were also improved in several cases comparing to the previously described method (Vicini *et al.*, 2006, 2008).

The proposed mechanism for the formation of the 2-(heteroarylimino) thiazolidin-4-ones has been previously described (Vicini *et al.*, 2006, 2008). The new synthesized compounds **5–8** were characterized by ^{1}H NMR, IR, and mass spectroscopy. Theoretically, all final compounds **5–8** exist as potential *E* and *Z* geometrical isomers. Configuration of the exocyclic C=C double bond in compounds **5–7** was assigned as (*Z*)-geometry according to ^{1}H NMR spectroscopy data (Fig. 2). The vinylic hydrogen in compounds **5–7** was detected at 7.59–8.01 ppm as a sharp singlet peak which was deshielded by an adjacent carbonyl group. In 5-(2-oxoindolin-3-ylidene) derivatives **8**, the H-4 proton of indoline ring was appeared at downfield (δ 8.83–8.85 ppm) due to the proximity of carbonyl group (Fig. 2). These findings were in agreement with spectroscopic data of 4-thiazolidinones and 2,4-thiazolidinediones analogs (Bruno *et al.*, 2002; Ottana *et al.*, 2005). The proton emerging at 12.76–13.10 ppm in the ^{1}H NMR spectra was attributed to

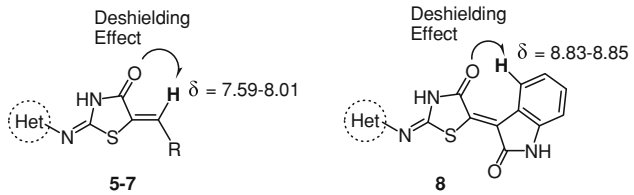
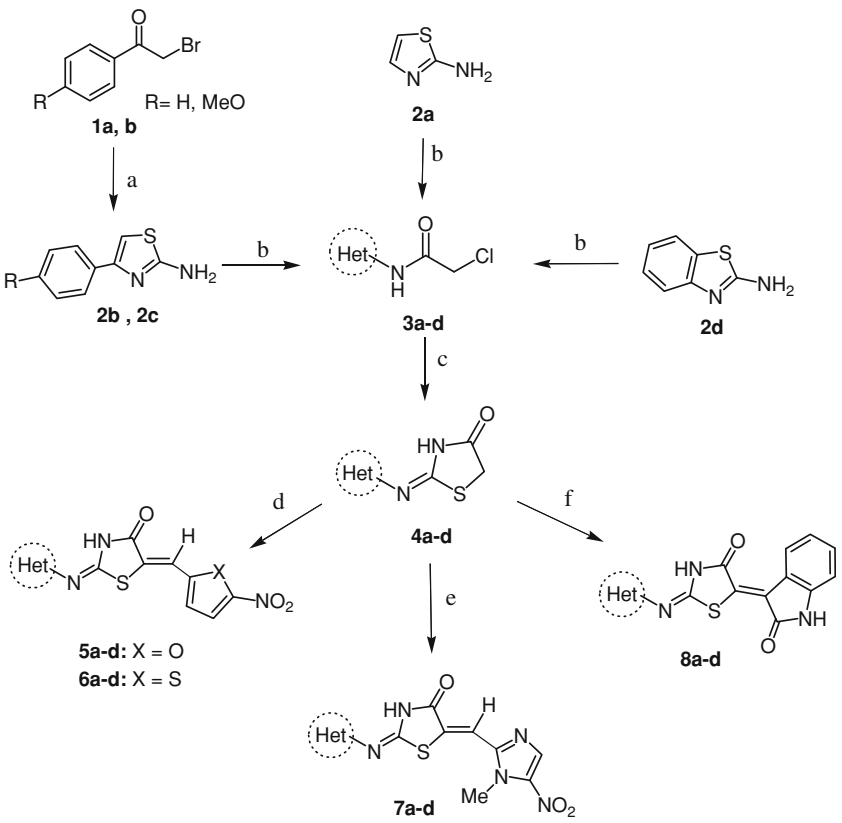


Fig. 2 The geometry assignment of the exocyclic C=C double bond in compounds **5–8** according to ^{1}H NMR spectroscopy data



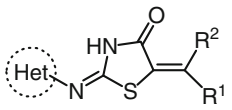
Scheme 1 Synthesis of the compounds **5–8**. Reagents and conditions: (*a*) EtOH, reflux, 1.5 h; (*b*) $ClCOCH_2Cl$, DMF, rt, 2 h; (*c*) NH_4SCN, EtOH, reflux, 2 h; (*d*) 5-nitrofurfural or 5-nitrothiophene-2-carboxaldehyde, CH_3COOH, CH_3COONa, MW; (*e*) 1-methyl-5-nitro-1*H*-imidazole-2-carbaldehyde, CH_3COOH, CH_3COONa, MW; (*f*) isatin, CH_3COOH, CH_3COONa, MW

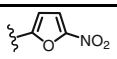
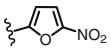
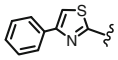
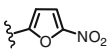
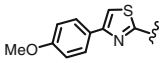
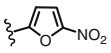
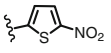
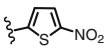
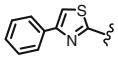
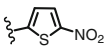
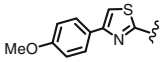
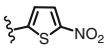
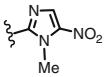
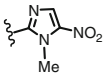
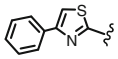
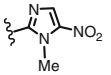
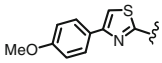
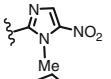
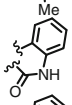
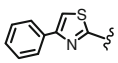
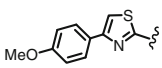
the NH of thiazolidinone ring. The feature of a thiazolidinone ring in the solid state is also supported by the IR spectral data (NH group band at ~3,400 cm^{-1} and a strong C=O band at 1,690–1,714 cm^{-1}) for the majority of the compounds.

Antibacterial activity

The antibacterial activity of compounds **5–8** in comparison with the reference drug ciprofloxacin was reported in Table 1. The MIC values of test compounds revealed that

Table 1 Minimum inhibitory concentrations (MICs, μg/ml) of compounds **5–8**



Compounds	R^1	R^2	Het	*S. a.*[a]	*S. e.*	*B. s.*	*E. c.*	*K. p.*	*P. a.*
5a		H		0.97	0.006	0.19	1.56	1.56	0.39
5b		H		50	25	50	>100	>100	>100
5c		H		25	12.5	12.5	>100	>100	100
5d		H		0.97	0.39	0.39	3.125	6.25	>100
6a		H		1.56	0.39	0.78	>100	>100	>100
6b		H		>100	>100	>100	>100	>100	>100
6c		H		>100	>100	>100	100	>100	>100
6d		H		0.39	0.19	0.78	>100	>100	>100
7a		H		0.78	0.19	0.19	12.5	0.39	>100
7b		H		>100	50	50	>100	>100	>100
7c		H		>100	25	50	>100	100	>100
7d		H		25	25	12.5	>100	25	>100
8a				25	12.5	6.25	>100	>100	>100
8b				>100	>100	>100	>100	>100	50
8c				>100	100	>100	100	>100	>100
8d				>100	>100	>100	>100	>100	>100
Ciprofloxacin				0.19	0.097	0.19	0.012	0.006	0.39

[a] *Staphylococcus aureus* ATCC 6538p, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* ATCC 10031, *Pseudomonas aeruginosa* ATCC 9027

unsubstituted thiazole analogs **5a**, **6a**, **7a**, and benzothiazole derivatives **5d** and **6d** exhibited good antibacterial activity against Gram-positive bacteria including *S. aureus*, *S. epidermidis*, and *B. subtilis* (MICs = 0.006–1.56 μg/ml). Also, compounds **5b**, **5c**, **7d**, and **8a** showed moderate growth inhibitory activity at concentrations less than 50 μg/ml against all tested Gram-positive strains.

Generally, most of the tested compounds exhibited higher activity against Gram-positive bacteria rather than Gram-negatives. Among them, compounds **5a**, **5d**, and **7a** displayed moderate to good activity against *E. coli* and *K. pneumoniae* (MICs = 0.39–12.5 μg/ml). The rest of remaining compounds showed no significant activity against Gram-negative organisms. Notably, compound **5a** showed high activity against *P. aeruginosa* with MIC value of 0.39 μg/ml.

The antibacterial data demonstrated that compounds bearing 5-nitroheterocycles including furan, thiophene and *N*-methylimidazole had anti-Gram positive potential. In contrast, compounds **8b–d** with an attached 2-oxoindoline group did not show any antibacterial activity. It could be concluded that the introduction of a 2-oxoindoline moiety on position 5 of thiazolidinone significantly diminishes the antibacterial activity.

By comparing the MIC values of thiazolylimino-derivatives in each 5-nitroheterocycle series, it obviously revealed that the introduction of phenyl or 4-methoxyphenyl on thiazole ring have largely decreased the antibacterial activity, and compounds with no substitution on thiazole ring were the most active compounds in each series. Moreover, compounds with benzothiazole moiety instead of thiazole ring showed to be slightly less active.

Anti-H. pylori activity

The anti-*H. pylori* activity of the target compounds along with commercially available antibiotic metronidazole was evaluated using paper disc diffusion bioassay method. The synthesized compounds were evaluated against three strains of *H. pylori* at four doses (8, 16, 32, and 64 μg/disk). The antibacterial activity of compounds was considered as follow: strong response, zone diameter >20 mm; moderate response, zone diameter 16–20 mm; weak response, zone diameter 11–15 mm; and little or no response, zone diameter <10 mm. The anti-*H. pylori* activity of tested compounds was depicted in Table 2.

The inhibitory activity of test compounds against *H. pylori* strains revealed that compounds **5a** and **7a** had complete growth inhibition at the doses of 8, 16, 32, and 64 μg/disk (inhibition zone diameter >50 mm), while compounds **7b**, **7c**, and **8c** have shown no inhibitory activity even at the high dose of 64 μg/disk. Also, compounds **5d**, **6a**, **6c**, and **7d** showed a strong activity at all tested concentrations (inhibition zone diameter >20 mm).

From the bioassay data, it is evident that nitroheterocycle analogs which have no substitution on thiazole ring exhibited higher anti-*H. pylori* activity (compounds **5a**, **6a**, and **7a**). This pattern was also observed in 2-oxoindoline series. Compound **8a** was the most effective compound in this series. Thus, it is worthwhile to note that the introduction of a phenyl or 4-methoxyphenyl as a substituent on the 4-position of thiazole ring decreases the anti-*H. pylori* activity. However, the potency is relatively dependent on the type of attached group at the 5 position of

Table 2 Inhibition zone diameters (mm) of test compounds at different doses against *H. pylori* strains

Compounds[a]	Strain 1				Strain 2				Strain 3			
	8 μg	16 μg	32 μg	64 μg	8 μg	16 μg	32 μg	64 μg	8 μg	16 μg	32 μg	64 μg
5a	>50[b]	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
5b	10	13	15	20	10	12	18	23	13	15	18	20
5c	≤6	10	12	14	15	17	19	20	12	16	19	26
5d	20	24	28	33	22	24	26	30	25	28	30	34
6a	36	40	42	45	25	28	30	34	33	35	40	45
6c	22	25	27	30	30	32	35	39	22	25	30	35
7a	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
7b	6	6	6	6	6	6	6	6	6	6	6	6
7c	6	6	6	6	6	6	6	6	6	6	6	6
7d	23	26	29	32	27	29	33	35	22	24	26	30
8a	10	12	13	15	12	14	18	20	10	10	14	15
8b	6	6	6	6	6	6	6	6	6	6	6	14
8c	6	6	6	6	6	6	6	6	6	6	6	6
8d	6	6	10	13	11	13	16	18	6	6	6	10

[a] Metronidazole was used as standard drug. The inhibition zone diameters of metronidazole at the dose of 16 μg/disk were 14–27 mm

[b] The values represent the mean. $P < 0.05$ was considered statistically significant

Table 3 The two top models of MLR analysis in QSAR study

MLR models	R	R_{cv}	PRESS
$Y = [93.99\ (X_{5v}) \pm 13.51] - [1.57 \times 10^3\ (R8P^+) \pm 497.16] - [120.69 \pm 28.27]$	0.88	0.81	9.57×10^3
$Y = [125.92\ (X_{5v}) \pm 17.87] - [2.23 \times 10^3\ (R8P^+) \pm 512.73] - [1.08 \times 10^4\ (GATS7m) \pm 4.58 \times 10^3] - [74.02 \pm 31.4]$	0.92	0.85	8.17×10^3

thiazolidinone nucleus. Generally, the pattern of anti-*H. pylori* activity for test compounds was in accordance with the pattern of antibacterial activity against Gram-positive bacteria.

QSAR study

In an attempt to investigate the role of structural properties on the activity of the synthesized compounds, two common methods of QSAR analysis including multiple linear regression (MLR) and Kohonen maps were conducted (Ballabio *et al.*, 2009). Based on antibacterial data, among the tested organisms, Gram-positive bacteria *S. epidermidis* was more sensitive to designed compounds. Thus, the most discriminative index of antibacterial activity for the compounds (*S. epidermidis*) was used as the activity vector (Y) to obtain its correlation with the structural features ($Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + \cdots + b_nX_n$). For this purpose, the structures were drawn in ChemOffice10 and saved as cdx format. Primary 3D generation and optimization of the structures was done using Openbabel 2.5 in Linux operating system. Afterwards, final geometry optimization of the compounds was performed by AM1 semiempirical method in HyperChem 8 (Hypercube) (Miri *et al.*, 2004; Khalafi-Nezhad *et al.*, 2005). For doing the optimization procedure in an automatic mode, our application implemented in vb.Net was used in order to perform dynamic data exchange (DDE) with HyperChem. Dragon software was subsequently taken to calculate 1171 descriptors of the optimized structures (Todeschini *et al.*, 2002). The resulted matrix of descriptors together with their corresponding activity vector was entered into Matlab for further analysis. QSAR analysis was preceded by a pretreatment process during which correlation of each descriptor vector with the others in the data matrix has been calculated and the ones with higher autocorrelations ($r \geq 0.85$) were eliminated. The resulted matrix contained 127 descriptors. The stepwise feature selection method in combination with MLR was used for primary model generation. Afterwards, the two top models according to the rule of five were selected for the leave one out cross validation (LOO-CV) test (Table 3). Among the two models the second one with three descriptors had a more R_{cv} (0.85) than the model with two descriptors ($R_{cv} = 0.81$). The predicted residual error sum of squares (PRESS) in the second model was also less than the first one. According to data in Table 3, the first selected descriptor in both models was X_{5v} (valence connectivity index chi-5) which is among the topological descriptors. The second descriptor was R_8p^+ (R maximal of lag 8/weighted by atomic polarizability) which is among the Getaway descriptors. The next descriptor in the second model was GATAS7M (Geary autocorrelation −lag 7/weighted by atomic mass) and is among the Getaway descriptors. The value for this descriptor is highly related to the number of atoms in a molecule. In the final equation, the activity of compounds is also related to this descriptor with a minus coefficient. It could be concluded that the compounds with less number of atoms or less number of substituents are more likely to be active than their larger counterparts.

To verify that the selected descriptors are predictive enough and able to make an acceptable classification in the synthesized compounds, a supervised self organizing Kohonen map was used (Ballabio *et al.*, 2009). This method is similar to the action of human neural system in such a way that each neuron accepts different signals from neighboring neuron and is capable of processing them. The Kohonen map is characterized by a squared toroidal space, which include a grid of N^2 neurons, where N is the number of neurons for each side of the squared space. Each neuron contains as many elements (weights) as the number of input variables. The matrix of the three selected descriptors

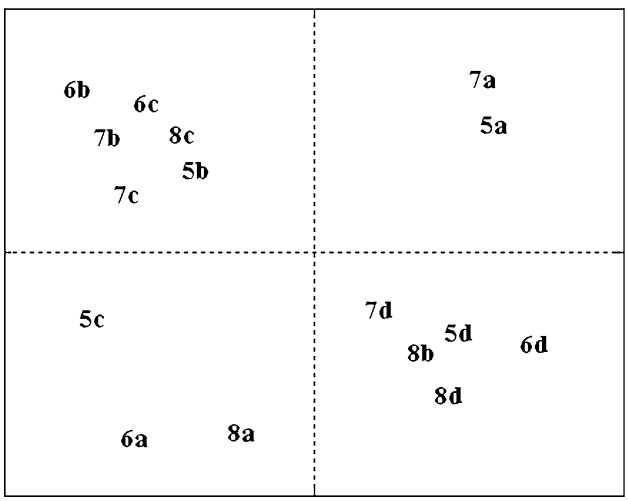


Fig. 3 Visualization of the Kohonen map for the input data. The compounds were clustered based on the similarities of the descriptors in the second MLR model

was entered into a 2 × 2 neural network with 1,000 iterations. When finished with training step, the network was visualized for clustering of the compounds. The result of neural network clustering is depicted in Fig. 3. It reveals that the three descriptor model is able to cluster the compounds space in a reasonable way. According to this classification, two active compounds **5a**, **7a** were placed in one cluster and the compounds with the moderate to good activity such as **5c**, **6a**, and **8a** were clustered in the neighboring neuron.

Conclusion

In summary, we have described the synthesis of some novel antibacterial agents with the 2-thiazolylimino-4-thiazolidinone as a core structure. Structurally, two distinct series of compounds including 5-nitro-heteroarylidene and 5-(2-oxo-indolin-3-ylidene) analogs of 2-thiazolylimino-5-arylidene-4-thiazolidinone were synthesized and characterized as (*Z*)-isomers. The antibacterial activity of title compounds was evaluated against some strains of Gram-positive and Gram-negative bacteria as well as *H. pylori* strains. Biological data indicated that 5-nitrofuran analog **5a** and 5-nitroimidazole analog **7a** containing no substitutions on the thiazole ring were the most potent compounds. The result of QSAR study revealed that the compounds with less number of atoms or less number of substituents are more likely to be more active than their counterparts with higher molecular weight.

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