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Synthesis, Anti-inflammatory, Analgesic, and Antibacterial Activities of Some Triazole, Triazolothiadiazole, and Triazolothiadiazine Derivatives

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This study is concerned with the synthesis of new 1,2,4-triazoles, 1,3,4-thiadiazoles, and 1,3,4-thiadiazines derivatives. Derivatives **3a-i** were obtained by condensation of 4-amino-3-(4-pyridine)-5-mercapto-1,2,4-triazole **1** with the appropriate aldehyde. Compounds **4a-i** were synthesized in a one pot reaction involving compounds **3a-i**, formaldehyde, and morpholine. Condensation of compound **1** with the appropriate acids or 4-substituted phenacyl bromide gave compounds **6a-d** and **8a-f** respectively. The chemical structures of the newly synthesized derivatives were elucidated using different spectral and elemental methods of analysis. All compounds were evaluated for their anti-inflammatory activity and the most potent derivatives were tested for their analgesic activity using indomethacin as a reference drug. In addition, ulcerogenicity and LD_{50} for the most active compounds were evaluated. Moreover, the antibacterial activities of the newly synthesized derivatives were investigated.

Key words: 1,2,4-Triazoles, Triazolothiadiazoles, Triazolothiadiazines, Anti-inflammatory, Analgesic, Antibacterial

INTRODUCTION

Currently available non-steroidal anti-inflammatory drugs (NSAIDs) alleviate pain by inhibiting the cyclooxygenase (COX) enzyme which catalyzes the synthesis of prostaglandins. The NSAIDs prevent the prostaglandins from being synthesized, thereby reducing or eliminating the pain (Karthikeyan et al., 2006). Additionally, there are analgesics which are commonly associated with anti-inflammatory drugs but have no anti-inflammatory effects; an example is paracetamol (Kritsanida et al., 2002). There are at least two COX isoforms - COX-1 and COX-2 (Kritsanida et al., 2002; Karthikeyan et al., 2006). Constitutive COX-1 is responsible for providing cytoprotection in the gastrointestinal (GI) tract, whereas inducible COX-2 mediates inflammation (Holla et al., 2005). Some investigators are concerned about the long term usage of NSAIDs because they cause gastric erosions which can cause stomach ulcers, and in extreme cases result in life threatening severe hemorrhage. Thus, the discovery of COX-2 provided the rationale for the development of drugs devoid of GI effects while retaining clinical efficacy as anti-inflammatory agents. Therefore, the development of novel compounds having anti-inflammatory and analgesic activity with improved safety and ideally, antimicrobial activities, is still a necessity (Shivarama Holla et al., 2006).

Substituted 4-amino-1,2,4-triazol-5-thione derivatives and the *N*-bridged heterocycles derived from them have been reported to possess antibacterial (Karthikeyan et al., 2006), antiviral (Karthikeyan et al., 2006), anticancer (Shivarama Holla et al., 2006), antitubercular (Walczak et al., 2004), analgesic (Amir and Shikha, 2004), anti-inflammatory (Berk et al., 2001; Amir and Shikha, 2004), anticonvulsant (Stillings et al., 1986), and anti-depressant properties (Kane et al., 1988). Moreover, Mannich bases have been reported as potential biological agents. They find applications as antitubercular (Joshi et al., 2004), antimalarial (Francisca et al., 2004), vasorelaxing (Ferlin et al., 2002), anticancer

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(Shivarama Holla et al., 2003), and analgesic agents (Malinka et al., 2005). Prompted by the aforementioned biological and pharmaceutical activities, and in continuation of our previous work (Hamdy and Mostafa, 2006; Mohamed et al., 2006; Raafat, 2006; Raafat and Ali, 2006; Raafat and Mohammed, 2008), we have attempted to develop new approaches for the synthesis of polyfunctionally substituted heterocyclic compounds with expected biological activity. This study presents the synthesis of a new series of Schiff and Mannich bases, containing 1,2,4-triazole, triazolothiadiazoles, and triazolothiadiazines, and the screening of their anti-inflammatory, antibacterial, and analgesic activities. In addition, ulcerogenic and LD_{50} activities were also investigated.

MATERIALS AND METHODS

Chemistry

Melting points were determined in open capillary tubes on an electrothermal melting point apparatus (Stuart Scientific, model SMPI) and all were uncorrected. Precoated silica gel plates (Kieselgel 0.25 mm, 60 f-254, Merck) were used for TLC for monitoring of reactions, and the spots were visualized under a UV lamb (Spectroline, model CM-10). Infrared spectra were recorded as KBr disks on a Shimadzu spectrophotometer (IR-470) at the Faculty of Pharmacy, Assiut University. ^{1}H-NMR spectra were run on an EM-360 Varian 60 MHz NMR spectrometer at the Faculty of Pharmacy, Assiut University, and DMSO-d_6 (unless otherwise stated) was used as a solvent and TMS as an internal standard (chemical shifts are taken in δ ppm). Mass spectra were produced using a JEOL JMS 600 mass spectrometer at the Microanalytical Center, Assiut University, Egypt. Elemental analyses were done on a Perkin-Elmer 240 elemental analyzer at the unit of microanalysis, Assiut University or Cairo University, Egypt.

4-Amino-1,2,4-triazole-3-thiones were prepared according a reported procedures (Raafat and Ali, 2006). Moreover, during this work, other groups reported the synthesis and characterization of Schiff base hydraones bearing a 3-(4-pyridyl)-5-mercapto-1,2,4-triazoles moiety (Khanmohammadi et al., 2008).

General method for synthesis of 4-[1-substituted methylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione compounds (3a-i)

To a suspension of aryl aldehyde, **2** (0.005 mol) in dioxane (10 mL), was added an equimolar amount of 3-(4-pyridyl)-4-amino-5-mercapto-1,2,4-triazole, **1** in the presence of a few drops of conc. sulfuric acid as a catalyst. The reaction mixture was refluxed for 9 h, cooled, and the precipitated solid was filtered and recrystallized from ethanol.

4-[Phenylmethylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3a)
M.p. 203-204ºC, yield 65%; IR (KBr) ν_{max} (cm^{-1}): 1344 (C=S), 1535 (C-N), 1470 (C=N), 3490 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.20-9.50 (9H, m, aromatic protons), 10.75 (IH, s, CH), 13.85 (1H, s, triazole, NH). EI-MS *m/z*: 280.92 (M^+-1/20.5%), 281.15 (M^+/0.6%), 192.93 (72.6%), 177.94 (100%), 105.01 (38.8%). Anal. Calcd. (%) for $C_{14}H_{11}N_5S$: C, 59.77; H, 3.94; N, 24.89; S, 11.40. Found: C, 59.87; H, 3.85; N, 24.90; S, 11.60.

4-[4-Bromophenylmethylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3b)
M.p. 295-300ºC, yield 80%; IR (KBr) ν_{max} (cm^{-1}): 1345 (C=S), 1467 (C=N), 3425 (NH), 1557 (C-N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.20-9.80 (8H, m, aromatic protons), 10.30 (1H, s, N=CH), 13.85 (1H, NH, triazole). EI-MS *m/z*: 360.60 (M^+/6.7%), 362.63 (M^+ +2/8.0%), 177.81 (100%), 118.91 (42.8%). Anal. Calcd. (%) for $C_{14}H_{10}N_5SBr$: C, 46.68; H, 2.80; N, 19.44; S, 8.90. Found: C, 46.98; H, 2.50; N, 19.84; S, 8.90.

4-(4-Chlorophenylmethylidene) amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3c)
M.p. 220-224ºC, yield 75%; IR (KBr) ν_{max} (cm^{-1}): 1344 (C=S), 1450 (C=N), 3455 (NH), 1534 (C-N). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.20-10.20 (8H, m, aromatic protons), 10.30 (1H, s, N=CH), 13.85 (1H, s, NH triazole). EI-MS *m/z*: 314.63 (M^+-1/6.4), 316.66 (M^++1/2.7), 177.81 (46.60%), 136.83 (100%). Anal. Calcd. (%) for $C_{14}H_{10}N_5SCl$: C, 53.25; H, 3.19; N, 22.18; S, 10.15. Found: C, 53.35; H, 3.33; N, 22.25; S, 10.19.

4-[4-Hydroxyphenylmethylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3d)
M.p. 200-205ºC, yield 70%; IR (KBr) ν_{max} (cm^{-1}): 1350 (C=S), 1458 (C=N), 3450 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.50-9.60 (8H, m, aromatic protons), 10.20 (1H, s, N=CH), 15.50 (1H, s, NH triazole). EI-MS *m/z*: 297.47 (M^+/1.5%), 281.17 (18.0%). Anal. Calcd. (%) for $C_{14}H_{11}ON_5S$: C, 56.55; H, 3.73; N, 23.55; S, 10.78. Found: C, 53.55; H, 3.74; N, 22.59; S, 10.80.

4-[4-Nitrophenylmethylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3e)
M.p. 270-272ºC, yield 88%; IR (KBr) ν_{max} (cm^{-1}): 1337 (C=S), 1469 (C=N), 3410 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.60-9.60 (8H, m, aromatic protons), 11.60 (1H, s, N=CH), 16.60 (1H, s, NH triazole). EI-MS *m/z*: 325.49

(M^+-/13.9%), 280.63 (12.1%), 177.76 (100%). Anal. Calcd. (%) for $C_{14}H_{10}O_2N_6S$: C, 51.53; H, 3.09; N, 25.75; S, 9.83. Found: C, 51.63; H, 3.00; N, 25.95; S, 9.95

4-(4-Dimethylamino)phenylmethylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3f)

M.p. 215-220°C, yield 80%; IR (KBr) ν_{max} (cm^{-1}): –1352 (C=S), 1571 (C-N), 1460 (C=N), 3490 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.40 (6H, S, CH_3), 7.30-9.50 (8H, m, aromatic protons), 10.02 (IH, s, CH), 15.90 (1H, s, triazole, NH). EI-MS *m/e*: 324.32 (M^+/2.1%), 326.11 (4.6%), 177.55 (100.0%). Anal. Calcd. (%) for $C_{16}H_{16}N_6S$: C, 59.24; H, 4.97; N, 25.91; S, 9.88. Found: C, 59.34; H, 5.10; N, 25.90; S, 10.00.

4-[4-Methoxyphenylmethylidene]amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3g)

M.p. 210-212°C, yield 55%; IR (KBr) ν_{max} (cm^{-1}): –1560 (C=N), 3495 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.50 (3H, s, OCH_3), 8.70 (dd, NCH), 8.20-9.40 (8H, m, aromatic protons), 14.20 (1H, s, triazole, NH). EI-MS *m/z*: 310.86 (M^+-1/1.0%), 278.91 (100.0%), 120.94 (77.5 %), 101.06 (74.70%). Anal. Calcd. (%) for $C_{15}H_{13}N_5S$: C, 57.86; H, 4.21; N, 22.49; S, 10.30. Found: C, 57.96; H, 4.42; N, 22.60; S, 10.20.

3-(4-Pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione 3-(4-pyridyl)-4-[1-(2-thienyl)methylidene]amino-4,5-dihydro-1*H*-1,2,4-triazole-5-thione (3h)

M.p. 255-257°C, yield 85%; IR (KBr) ν_{max} (cm^{-1}): 1348 (C=S), 1461 (C=N), 3500 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.90-9.37 (7H, m, aromatic protons), 10.20 (1H, s, N=CH), 15.40 (1H, s, NH triazole). EI-MS *m/z*: 287.40 (M^+/1.2%), 289.03 (9.5%), 177.06 (100.0%). Anal. Calcd. (%) for $C_{12}H_9N_5S_2$: C, 50.16; H, 3.16; N, 24.37; S, 22.32. Found: C, 50.26; H, 3.36; N, 24.67; S, 22.66.

4-[1-(2-Furyl) methylidene] amino-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (3i)

M.p. 350-352°C, yield 47%; IR (KBr) ν_{max} (cm^{-1}): –1392 (C=S), 1461 (C=N), 3490 (NH). ^{1}H-NMR (DMSO-d_6): δ (ppm) 7.40-9.60 (7H, m, aromatic protons), 10.60 (1H, s, N=CH), 15.40 (1H, s, NH triazole). EI-MS *m/z*: 271.37 (M^+/1.8%), 272.06 (13.1%), 273.05 (4.8%). Anal. Calcd. (%) for $C_{12}H_9N_5OS$: C, 53.13; H, 3.34; N, 25.81; S, 11.82. Found: C, 53.43; H, 3.74; N, 25.90; S, 12.01.

General method for synthesis of 4-[1-4-substituted phenyl methylidene]amino-1-(morpholinomethyl)-3-(4-pyridyl)-4, 5-dihydro-1H-1, 2, 4-triazole-5-thione (4a-i)

The appropriate compounds **3a-i** (10 mmol) were dissolved in a mixture of ethanol and dioxane (2:1), followed by addition of formaldehyde (40%, 1.5 mL) and morpholine (1 mL). The reaction mixture was stirred for 1-2 h, and kept over night at room temperature. The separated solid was collected by filtration, dried, and crystallized from ethanol.

4-(Benzylidenamino)-2-(morpholinomethyl)-5-(pyridine-4-yl)-2H-1,2,4-triazole-(4H)-thione (4a)

M.p. 170-172°C, yield 75%; IR (KBr) ν_{max} (cm^{-1}): –1249 (C=S), 1693 (CH=N), 1422 (C=N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.20 (4H, d, CH_2), 4.00 (4H, d, CH_2), 5.70 (2H, s, CH_2), 8.10-9.50 (9H, m, aromatic protons), 11.10 (1H, s, CH). EI-MS *m/z*: 380.33 (M^+/1.6%), 381.10 (20.8%), 382.60 (5.2%), 99.56 (100%). Anal. Calcd. (%) for $C_{19}H_{20}N_6OS$: C, 59.98; H, 5.30; N, 22.09; S, 8.43. Found: C, 59.99; H, 5.33; N, 22.30; S, 8.49.

4-(4-Bromobenzylideneamino)-2-(morpholinomethyl)-5-(pyridine-4-yl)-2H-1,2,4-triazole-3(4H)-thione (4b)

M.p. 180-182°C, yield 85%; IR (KBr) ν_{max} (cm^{-1}): –1475 (C=N), 1272 (C=S), 1587 (C-N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.40 (4H, d, CH_2), 4.10 (4H, d, CH_2), 5.90 (2H, s, CH_2), 8.10-9.50 (8H, m, aromatic protons), 11.80 (1H, s, CH). EI-MS *m/z*: 459.36 (M^+/1.5%), 460.37 (M^++1/0.2%), 461.44 (M^++2/0.2%), 267.66 (1.7%), 180.67 (80.3%), 99.92 (100%). Anal. Calcd. (%) for $C_{19}H_{19}N_6OBrS$: C, 46.68; H, 4.17; N, 18.29; S, 6.98. Found: C, 46.98; H, 4.33; N, 18.34; S, 6.90.

4-[(4-Chlorophenyl)methylidene]amino-1-(morpholinomethyl)-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (4c)

M.p. 198-200°C, yield 80%; IR (KBr) ν_{max} (cm^{-1}): –1475 (C=N), 1272 (C=S), 1587 (C-N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.20 (4H, d, CH_2), 4.10 (4H, d, CH_2), 5.90 (2H, s, CH_2), 8.10-9.50 (8H, m, aromatic protons), 11.80 (1H, s, CH). EI-MS *m/z*: 414.85 (M^+/0.4%), 416.90 (M^++2/ 0.1%), 100.02 (100%). Anal. Calcd. (%) for $C_{19}H_{19}N_6OClS$: C, 55.00; H, 4.62; N, 20.25; S, 7.73. Found: C, 55.05; H, 4.75; N, 20.35; S, 7.87.

4-[4-Hydroxyphenyl) methylidene] amino-1-(morpholinomethyl)-3-(4-pyridyl)-4, 5-dihydro-1H-1, 2, 4-triazole-5-thione (4d)

M.p. 195-196°C, yield 80%; IR (KBr) ν_{max} (cm^{-1}): –1475 (C=N), 1272 (C=S), 1587 (C-N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 2.37 (4H, d, CH_2), 3.67 (4H, d, CH_2), 4.90 (2H, s, CH_2), 8.10-9.50 (8H, m, aromatic protons), 9.50 (1H, s, CH), 14.10 (1H, s, OH). EI-MS *m/z*: 396.20 (M^+/1.1%), 397.14 (21.7%), 398.13 (4.6%), 99.56 (100%). Anal. Calcd. (%) for $C_{19}H_{19}N_5O_2S$: C, 57.56; H, 5.08; N, 21.20; S, 8.09.

Found: C, 57.55; H, 5.35; N, 21.59; S, 8.30.

4-(4-Nitrobenzylideneamino)-2-(morpholinomethyl)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3(4H)-thione (4e)

M.p. 204-205°C, yield 80%; IR (KBr) ν_{max} (cm^{-1}): –1249 (C=S), 1693 (CH=N), 1422 (C=N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.20 (4H, d, CH_2), 4.20 (4H, d, CH_2), 5.70 (2H, s, CH_2), 7.90-9.60 (8H, m, aromatic protons), 11.90 (1H, s, CH). EI-MS *m/z*: 424.73 (M^+-1/0.2%), 325.75 (0.2%), 214.91 (0.1%). 99.9 (100%). Anal. Calcd. (%) for $C_{19}H_{19}N_7O_3S$: C, 53.64; H, 4.50; N, 23.04; S, 7.54. Found: C, 53.63; H, 4.60; N, 23.45; S, 7.65.

N,N-Dimethylamino-N'-[1-(morpholinomethyl)-3-(4-pyridyl)-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-4-yl] iminoformamide (4f)

M.p. 168-169°C, yield 90%; IR (KBr) ν_{max} (cm^{-1}): –1518 (C-N), 1273 (C=S), 1668 (CH=N), 1465 (CH_2-O). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.30 (6H, s, CH_3), 3.10 (4H, dd, CH_2), 4.00 (4H, dd, CH_2), 5.70 (2H, s, CH_2), 7.20-9.50 (8H, m, aromatic protons), 10.3 (1H, s, CH). EI-MS *m/z*: 423.54 (M^+/1.9%), 424.29 (23.0%), 425.18 (5.2%), 99.05 (100%). Anal. Calcd. (%) for $C_{21}H_{25}N_7OS$: C, 59.24; H, 5.95; N, 23.15; S, 7.57. Found: C, 59.34; H, 5.87; N, 23.50; S, 7.85.

4-[-1-(4-Methoxyphenyl)methylidene]amino-1-(morpholinomethyl)-3-(4-pyridyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (4g)

M.p. 138-140°C, yield 45%; IR (KBr) ν_{max} (cm^{-1}): –1249 (C=S), 1556 (C-N), 1687 (CH=N), 1422 (C=N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.20 (4H, d, CH_2), 3.90 (4H, d, CH_2), 4.20 (3H, s, OCH_3), 5.70 (2H, s, CH_2), 7.50-8.40 (4H, dd, phenyl proton), 8.60-9.50 (4H, dd, pyridine protons), 10.70 (1H, s, CH). EI-MS *m/z*: 409.86 (M^+-1/1.3%), 411.73 (0.1%), 310.69 (0.4%), 99.98 (100). Anal. Calcd. (%) for $C_{20}H_{22}N_6O_2S$: C, 58.52; H, 5.40; N, 20.47; S, 7.81. Found: C, 58.86; H, 5.55; N, 20.60; S, 7.95.

4-(Thienyl-2-yl)methyleneamino)-2-(morpholinomethyl)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3(4H)-thione (4h)

M.p. 143-145°C, yield 85%; IR (KBr) ν_{max} (cm^{-1}): –1249 (C=S), 1475 (C=N), 1595 (C-N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.01 (4H, dd, CH_2), 3.90 (4H, dd, CH_2), 5.60 (2H, s, CH_2), 7.60-8.20 (3H, m, CH thiophene), 8.50-9.40 (4H, m, CH, pyridine), 11.70 (1H, s, CH). EI-MS *m/z*: 386.33 (M^+/3.6%), 387.10 (22.4%), 388.09 (9.1%). Anal. Calcd. (%) for $C_{17}H_{18}N_6OS_2$: C, 52.83; H, 4.69; N, 21.74; S, 16.56. Found: C, 52.27; H, 4.36; N, 21.85; S, 16.75.

4-(Furan-2-yl)methyleneamino)-2-(morpholinomethyl)-5-(pyridin-4-yl)-2H-1,2,4-triazole-3(4H)-thione (4i)

M.p. 208-210°C, yield 40%; IR (KBr) ν_{max} (cm^{-1}): –1249 (C=S), 1475 (C=N), 1595 (C-N). ^{1}H-NMR ($CDCl_3$): δ (ppm) 3.30 (4H, dd, CH_2), 4.10 (4H, dd, CH_2), 5.60 (2H, s, CH_2), 7.60-8.40 (3H, m, CH furan), 8.50-9.40 (4H, m, CH, pyridine), 11.70 (1H, s, CH). EI-MS *m/z*: 370.34 (M^+/1.2%), 371.12 (21.4%), 372.12 (5.1%). Anal. Calcd. (%) for $C_{17}H_{18}N_6O_2S$: C, 55.12; H, 4.90; N, 22.69; S, 8.66. Found: C, 55.43; H, 4.95; N, 22.90; S, 8.96.

General method for synthesis of 1,2,4-triazolo-[3.4-b]-1,3,4-thiodiazole derivatives (6a-d)

To a mixture of corresponding 3-(4-pyridyl)-4-amino-5-mercapto-1,2,4-triazole, **1** (0.01 mol) and the appropriate carboxylic acid **5** (0.52 mL, 0.01 mol), phosphorus oxychloride (10 mL) was added and the reaction mixture was refluxed for 5 h. The mixture was concentrated under reduced pressure; ice-water was added with vigorous stirring. The precipitate was filtered, washed with 20% sodium bicarbonate solution and water, dried, and recrystallized from dimethylformamide/water mixture (1:1).

6-Methyl-3-(pyridine-4yl)-[1,2,4]triazole[3,4-b][1,3,4]thiadiazole (6a)

M.p. 257-258°C, yield 95%; IR (KBr) ν_{max} (cm^{-1}): –1559 (C=N), 1259 (C-S). ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.90 (3H, s, CH_3), 8.10-8.80 (4H, d, pyridine). EI-MS: *m/z:* 216.99 (M^+/100%), 176.98 (0.2%). 104.01 (51.8%). Anal. Calcd. (%) for $C_9H_7N_5S$: C, 49.76; H, 3.25; N, 32.24; S, 14.76. Found: C, 49.99; H, 3.33; N, 32.30; S, 14.49.

6-Phenyl-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6b)

M.p. 298-300°C, yield 60%; IR (KBr) ν_{max} (cm^{-1}): –1559 (C=N), 1259 (C-S). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.40-9.50 (9H, m, aromatic protons). EI-MS *m/z:* 278.99 (M^+/100), 255.75 (7.1%), 120.98 (78.1%). Anal. Calcd. (%) for $C_{14}H_9N_5S$: C, 60.20; H, 3.25; N, 25.07; S, 11.48. Found: C, 60.25; H, 3.26; N, 25.09; S, 11.55.

3,6-Di(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6c)

M.p. 290-292°C, yield 80%; IR (KBr) ν_{max} (cm^{-1}): –1559 (C=N), 1259 (C-S). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.20-9.00 (8H, m, aromatic protons). EI-MS *m/z:* 279.91 (M^+/1.0%), 255.62 (57.9%), 191.72 (14.4%), 121.99 (70.1%). Anal. Calcd. (%) for $C_{13}H_8N_6S$: C, 55.70; H, 2.88; N, 29.98; S, 11.44. Found: C, 55.89; H, 2.95; N, 29.99; S, 11.49.

6-(Pyridin-3-yl)-3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (6d)
M.p. 262-265°C, yield 85%; IR (KBr) ν_{max} (cm^{-1}): –1550 (C=N), 1259 (C-S). ^{1}H-NMR (DMSO-d_6): δ (ppm) 8.40-9.50 (8H, m, aromatic protons). EI-MS *m/z*: 279.67 (M^+/100%), 255.41 (4.6%), 121.84 (73.3%). Anal. Calcd. (%) for $C_{13}H_8N_6S$: C, 55.70; H, 2.88; N, 29.98; S, 11.44. Found: C, 55.95; H, 2.95; N, 30.00; S, 11.45.

General method for synthesis of 4-substitudphenyl-3-(pyridine-4-yl)-7H-(1,2,4)triazolo[3,4-b]-[1,3,4]thiadiazine (8a-f)

A solution of 3-(4-pyridyl)-4-amino-5-mercapto-1,2,4-triazole, **1** (0.005 mol) and phenacyl bromide **7** (0.005 mol) in absolute ethanol (30 mL) was heated under reflux for 2 h, cooled to room temperature, and then neutralized with ammonium hydroxide (35% v/v). The solid was separated by filtration, dried, and recrystallized from ethanol.

6-Phenyl-3-(pyridine-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadizaine (8a)
M.p. 268-270°C, yield 65%; IR (KBr) ν_{max} (cm^{-1}): 1690 C=N, 1249 C-S. ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.80 (2H, s, methylene protons), 8.20-9.40 (9H, m, aromatic protons). EI-MS *m/z*: 293.20 (M^+/2.85%), 259.24 (2.3%), 190.12 (7.9%). Anal. Calcd. (%) for $C_{15}H_{11}N_5S$: C, 61.42; H, 3.78; N, 23.87; S, 10.93. Found: C, 61.44; H, 3.88; N, 23.99; S, 10.99.

6-(4-Nitrophenyl)-3-(pyridine-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (8b)
M.p. 200-202°C, yield 60%; IR (KBr) ν_{max} (cm^{-1}): 1675 C=N, 1250 C-S. ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.80 (2H, s, methylene, protons), 8.70-9.80 (8H, m, aromatic protons). EI-MS *m/z*: 338.14 (M^+/3.1%), 339.15 (9.6%), 340.13 (0.2%), 293.21 (14.3%), 150.14 (100%). Anal. Calcd. (%) for $C_{15}H_{10}N_6O_2S$: C, 53.25; H, 2.98; N, 24.84; S, 9.48. Found: C, 53.36; H, 3.12; N, 24.95; S, 9.55.

6-(4-Chlorophenyl)-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (8c)
M.p. 219-220°C, yield 60%; IR (KBr) ν_{max} (cm^{-1}): 1675 C=N, 1250 C-S. ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.40 (2H, s, methylene, protons), 7.50-8.90 (8H, m, aromatic protons). EI-MS *m/z*: 327.27 (3.3%), 329.03 (36.8%), 150.34 (100.0%). Anal. Calcd. (%) for $C_{15}H_{10}N_5ClS$: C, 54.96; H, 3.07; N, 21.37; S, 9.78. Found: C, 54.99; H, 3.17; N, 21.56; S, 9.95.

6-(4-Bromophenyl)-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (8d)
M.p. 225-228°C, yield 70%; IR (KBr) ν_{max} (cm^{-1}): 1675 C=N, 1250 C-S. ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.40 (2H, s, methylene, protons), 7.60-8.90 (8H, m, aromatic protons). EI-MS *m/z*: 372.55 (1.8%), 374.98 (4.7%), 99.05 (100.0%). Anal. Calcd. (%) for $C_{15}H_{10}N_5BrS$: C, 48.40; H, 2.71; N, 18.81; S, 8.61. Found: C, 48.67; H, 2.95; N, 19.10; S, 8.72.

6-(4-Methoxyphenyl)-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (8e)
M.p. 215-227°C, yield 78%; IR (KBr) ν_{max} (cm^{-1}): 1675 C=N, 1250 C-S. ^{1}H-NMR (DMSO-d_6): δ (ppm) 3.90 (3H, s, OCH_3), 4.60 (2H, s, methylene, protons), 7.20-9.00 (8H, m, aromatic protons). EI-MS *m/z*: 323.55 (M^+/1.5%), 324.43 (17.5%), 325.58 (4.9%). Anal. Calcd. (%) for $C_{16}H_{13}N_5OS$: C, 59.43; H, 4.05; N, 21.66; S, 9.92. Found: C, 59.65; H, 4.35; N, 21.96; S, 10.10.

6-(4-Fluorophenyl)-3-(pyridin-4-yl)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine (8f)
M.p. 234-235°C, yield 80%; IR (KBr) ν_{max} (cm^{-1}): 1675 C=N, 1250 C-S. ^{1}H-NMR (DMSO-d_6): δ (ppm) 4.60 (2H, s, methylene, protons), 7.30-9.00 (8H, m, aromatic protons). EI-MS *m/z*: 311.46 (0.2%), 312.11 (16.3%), 313.06 (4.8%), 99.56 (100.0%). Anal. Calcd. (%) for $C_{15}H_{10}N_5FS$: C, 57.87; H, 3.24; N, 22.49; S, 10.30. Found: C, 57.99; H, 3.22; N, 22.49; S, 10.30.

Pharmacological screening

Male adult albino rats and mice were obtained from the animal house, Faculty of Medicine, Assiut University. Indomethacin (Liometacin® vial, Nile Company), carrageenan (Sigma), sodium carboxymethylcellulose (NaCMC) and normal saline were obtained from a local market. The test compounds and reference drug were suspended in 5% NaCMC in normal saline.

Animals were housed in separate cages, 6 animals each, in temperature-controlled rooms at 25 ± 1°C. Animals were allowed free access to food and water and maintained at a 12 h light/dark cycle. Work was conducted in accordance with the internationally accepted principles for laboratory animals' use and care as found in the European Community Guidelines (Tan, 2004).

Anti-inflammatory activity

The anti-inflammatory activity of 29 final compounds was determined according to the paw induced edema method (Nargund et al., 1994). Indomethacin was used as a comparator and reference drug. The test is based on the pedal inflammation in rat paws induced by subplantar injection of 0.2 mL carrageenan suspension (5% NaCMC) into the right hind paw of the rats.

Male adult albino rats (120-150 g) were divided into

groups of 4 animals each and fed *ad libitum* with rodent's chow and allowed free access to drinking water. The thickness of the rat paw was measured by a Veriner caliper (SMIEC) before and 1 h after injection of carrageenan to detect the inflammation induced by carrageenan.

Test compounds at doses of 10 mg/kg in 5% NaCMC were injected i.p. into 3 different groups of rats. The control group received a vehicle (5% NaCMC), and the reference group received indomethacin i.p. at 0.03 mmol/kg. The difference between the thicknesses of the 2 hind paws was taken as a measure of edema. The measurement was carried out at times of zero, 0.5, 1, 2, 3, 4 and 5 h after injection of the test compounds, reference drug, or the vehicle. Results of anti-inflammatory activity of the test compounds and the reference drug are listed in Table I.

Analgesic activity

Hot-plate test

The analgesic activity of **8a-f** was determined in mice using the hot-plate method (Boyle et al., 1986), in comparison with indomethacin as a reference drug. Male albino mice (30-35 g) were placed on a hot-plate apparatus for testing and the surface temperature was maintained at 55 ± 0.5°C. The reaction time in seconds was taken as the time period from the instant the animal reached the hot plate until the moment the animal licked its hind paw or jumped out within a Plexiglas cylinder placed on the hot-plate. The reaction time was taken as the end point and the increase in hot plate latency was taken as a measure of the analgesic activity.

Animals were randomly divided into groups of 4 mice each and each animal was used once. Solutions or suspensions of the test compounds and the reference drug in 5% NaCMC were injected i.p. at a dose level of 0.02 mmol/kg. Control animals were similarly treated with 5% NaCMC. Testing was done at 0.5, 1, 2, 3, and 5 h after the injection. Mean licking time ± S.E. was evaluated for each group and listed in Table II.

Ulcerogenic effect

The test was conducted according to a reported method (Boyle et al., 1986) using adult male albino rats. Male albino rats were fasted for 24 h. Tested compounds **8d**, **8e**, and **8f** or reference drug were administered orally to groups of 6 animals each. After 6 h, the animals were sacrificed, their stomachs were removed and gastric lesions on the mucosa were determined by using a stereoscopic microscope (XJP-XSC-195-40X). "Ulcer" was defined as at least 1 lesion that was 0.5 mm in length. All lesions of > 0.1 mm in length were summed to obtain the ulcer index and results are listed in Table III.

Acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of compounds **8e** and **8f** was determined in mice by the graphical Litchfield method (O'Neil et al., 2001). Groups of male adult albino mice, (25-30 g, 4 mice/group) were injected i.p. with graded doses of the test compound. The percentage mortality in each group of animals was determined 72 h after injection. Computation of LD_{50} was processed by a graphical method and was found to be 275 and 300 mg/kg, for compounds **8e** and **8f**, respectively, whereas the LD_{50} for indomethacin was 13 mg/kg (i.p.) (O'Neil et al., 2001).

Antibacterial

The antibacterial activity of compounds **1**, **3a-i**, **4a-i**, **6a-d**, and **8a-f** was investigated *in vitro* against methicillin resistant *Staphylococcus aureus* (MRSA), *Bacillus cereus*, *Escherichia coli*, and *Klebsiella pneumoniae* (clinical isolates obtained from Infection Control Unit, Assiut Univeristy Hospital, Faculty of Medicine, Assiut University) using the agar cup diffusion method for susceptibility screening, and the 2-fold dilution method (William, 1977) for MIC determination. Ampicillin was used as a reference drug, and DMSO as the solvent.

Agar cup diffusion method

Mueller-Hinton agar medium (MH) (Hi-Media, M 001), 38 g, was added to 1 L of distilled water, heated to boiling to dissolve the ingredients completely, and sterilized by autoclaving at 121°C for 30 min. High density inocula were prepared by diluting 3-5 well isolated colonies grown overnight on selective media in 5 mL of distilled water to prepare a suspension equivalent in density to 0.5 McFarland Barium Sulfate standard units with an average turbidity of 10^7 CFU/mL.

The sterile Petri dishes were seeded with 100 µL of the microorganism; a specified amount of the molten MH agar medium (45-50°C) was poured into the seeded Petri dishes to give a depth of 3-4 mm and allowed to solidify. Cylindrical plugs were removed from the agar using a sterile cork borer. Tested compounds (20 µL in DMSO), the blank solvent, and ampicillin sodium (20 µmol/mL in DMSO) were added to the wells in triplicate. The seeded plates were incubated at 37°C for 24 h the average diameters of the inhibition zones were measured in millimeters (Table IV).

Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the 2-fold dilution method (William, 1977) for compounds having moderate to strong antibacterial activity. The squares of inhibition zone diameters were plotted against log concentrations of the tested compounds. Extrapolation of the resulting straight line to intersect with the log concentration scale in the curve corresponded to log MIC, and MIC was obtained as the antilog (Table IV).

RESULTS AND DISCUSSION

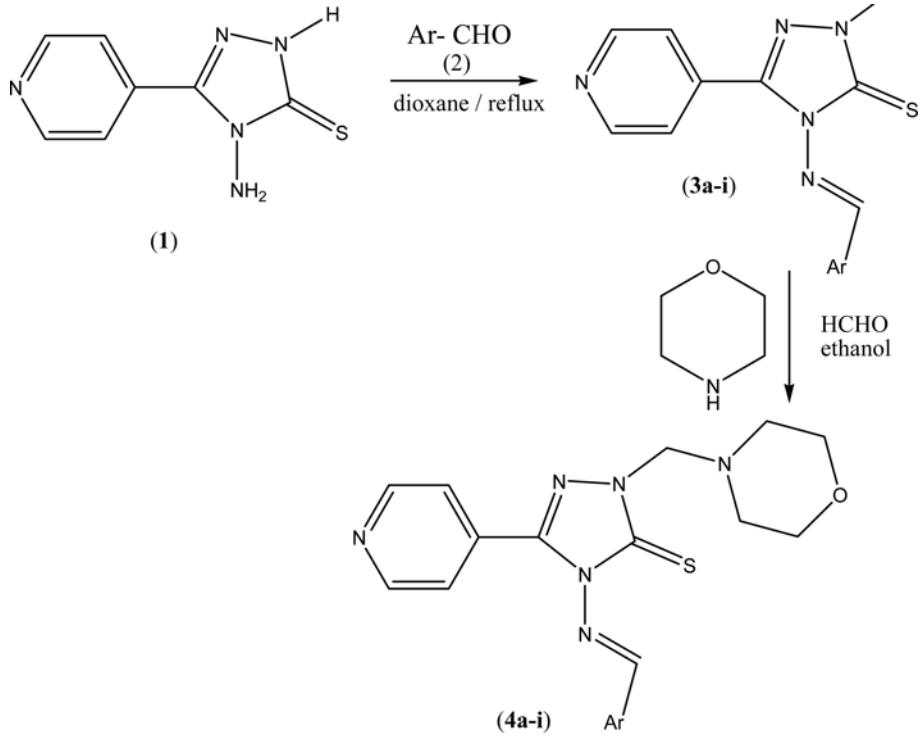
Chemistry

Recently, we have reviewed the chemistry of the 4-amino-1,2,4-triazole-3-thiones (Raafat and Ali, 2006). The amino and mercapto groups are ready-made nucleophilic centers for the synthesis of condensed heterocyclic rings. Therefore, these compounds can be considered as useful tools in fusing to triazolothiadiazoles or triazolothiadiazines. During this study, other groups reported the synthesis and characterization of Schiff base hydrazones bearing a 3-(4-pyridyl)-5-mercapto-1,2,4-triazoles moiety (Khanmohammadi et al., 2008).

Schiff bases **3a-i** were obtained by the condensation of 4-amino-3-(4-pyridine)-5-mercapto-1,2,4-triazole **1** with appropriate aldehydes in the presence of a catalytic amount of concentrated sulphuric acid in refluxing dioxane (Scheme 1). The structures of the synthesized compounds have been elucidated on the basis of IR, ^{1}H-NMR, mass spectral studies, and elemental analyses.

IR data for compounds **3a-i** revealed bands at 1344 cm^{-1} (C=S stretching) and the absence of an absorption in the region of ~2600-2550 cm^{-1} cited for an SH group, proving that these compounds were in the thionic form. In ^{1}H-NMR spectra of compounds **3a-i** in DMSO-d_6, the N=CH proton appeared at 10.75-15.40 ppm as a singlet. Furthermore, the molecular ion peak & base peak of the mass spectra of compounds **3a-i** were found to be in good agreement with the assigned structures.

Compounds **4a-i** were synthesized in a one pot multicomponent Mannich reaction involving **3a-i**, formaldehyde, and morpholine (Scheme 1). Assignment of the structures **4a-i** was based on their elemental analyses and spectroscopic data. The IR spectra of compounds **4a-i** showed an absorption band at 1587-1697 cm^{-1}, indicating the presence of –C=N, while



Ar = (a) C_6H_6, (b) 4-BrC_6H_4, (c) 4-ClC_6H_4, (d) 4-$CH_3OC_6H_4$, (e) 4-$NO_2C_6H_4$, (f) 4-HOC_6H_4, (g) 4-$(CH_3)2NC_6H_4$, (h) 2-theinyl, and (i) 2-furyl

Scheme 1. Synthesis of compounds **3a-i** and **4a-i**

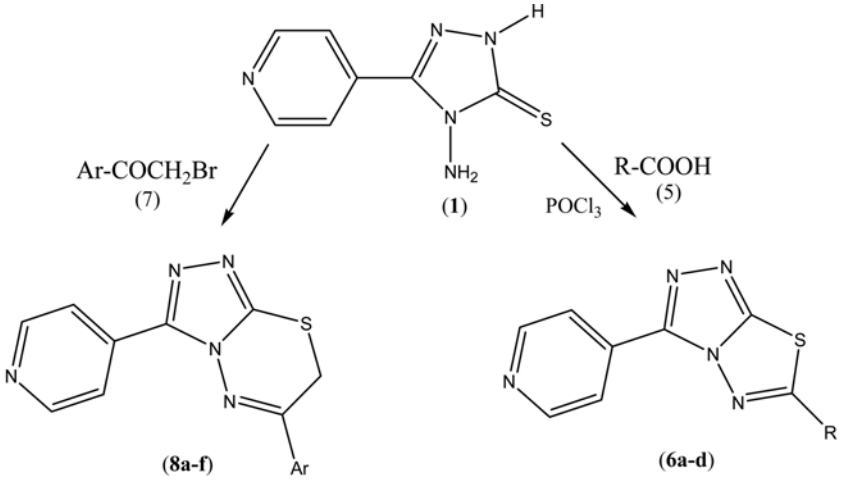
bands observed at 1249-1375 cm^{-1} could be attributed to the –C=S functional group. The ^{1}H-NMR spectrum of compound **4a** showed the -NCH_2N- protons resonated as a singlet at δ 5.70 integrating for two protons. The -CH_2-O-CH_2- protons of the morpholine residue appeared as a triplet at δ 4.10, while the -CH_2-N-CH_2- protons of the morpholine residue resonated as a triplet at δ 3.30 in addition to the aromatic protons at δ 8.10-9.50, and a singlet at δ 5.9 ppm (N=CH-). In MS data of compound **4a**, a peak observed at *m/z* 100 was explained due to the formation of a morpholinomethyl radical cation, thus confirming the formation of Mannich bases **4a-i**. The triazolothiadiazole derivatives **6a-d** have been synthesized by the condensation of compound **1** with the appropriate acids **5** in the presence of phosphorus oxychloride (Scheme 2). Structures of **6a-d** were confirmed by ^{1}H-NMR, IR, MS spectral data, and elemental analyses. IR spectra of **6a-d** are devoid of absorption bands due to –SH (-C=S) and $–NH_2$ stretching frequencies of parent compound **1** clearly indicated the formation of the target compounds. The ^{1}H-NMR spectrum of compound **6a**, for example, showed a singlet at δ 2.90 ppm (3H) of the (C_6-CH_3) of the triazolothiadiazole ring. On the other hand, treatment of **1** with 4-substituted phenacyl bromide in absolute ethanol containing potassium carbonate resulted in cyclocondensation, giving the corresponding triazolothiadiazines **8a-f** (Scheme 2). Structures of **8a-f** were established on the basis of elemental analyses and spectral data. IR data of **8a-f** showed no absorption bands assignable to NH_2 and NH groups. ^{1}H-NMR spectra of **8a-f** exhibited a singlet signal for SCH_2 at δ 4.8-4.6 ppm, in addition to aryl and pyridyl protons.

Anti-inflammatory activity

The synthesized compounds **1**, **3a-i**, **4a-i**, **6a-d**, and **8a-f** were evaluated for their *in vivo* anti-inflammatory effect by the carrageenan induced paw edema bioassay in rats using indomethacin as a reference drug (Nargund et al., 1994), and the results were presented as the time course and percentage of edema inhibition at a dose of 0.03 mmol/kg and time intervals of 0.5, 1, 2, 3, 4, and 5 h after injection (Table I).

The results showed that the anti-inflammatory activity of compounds **1** and **3a-i** revealed maximum activity at the 1 h post-injection time point, and the activity declined with time, indicating short acting compounds. Compounds **1** and **3a-i** exhibited ~50-111% edema inhibition compared to indomethacin, and compounds **3e**, **3g**, and **3i** were the most active among this series.

On the other hand, compounds **4a**, **4d**, **4e**, **4f**, **4h**, and **4i** showed maximum activity at 1 h post-injection, with ~50-130% edema inhibition compared to indomethacin. Furthermore, compounds **4b**, **4c**, and **4g** carrying Cl, Br, and NMe_2 moieties, respectively revealed maximum activity at 4 h post-injection and showed ~84-100% of the inhibition exhibited by indomethacin; a result that could be attributed to the structural similarities to that moieties in indomethacin. Compounds **6a-d** having CH_3, C_6H_5, 4-pyridyl, and 3-pyridyl, respectively showed anti-inflammatory activity of ~20-89% that of indomethacin at 5 h post-injection. In addition, results of compounds **8a-f** (R = H, NO_2, Cl, Br, OMe, and F) showed maximum activities at



R= (6a) CH_3, (6b) C_6H_5, (6c) 3-pyridyl, (6d) 4-pyridyl.

Ar = (8a) C_6H_5, (8b) 4-$NO_2C_6H_4$, (8c) 4-ClC_6H_4, (8d) 4-BrC_6H_4, (8e) 4-$CH_3OC_6H_4$, and (8f) 4-FC_6H_4.

Scheme 2. Synthesis of compounds **6a-d** and **8a-f**

Table I. Inhibitory effect of compounds **1**, **3a-i**, **4a-i**, **6a-d**, **8a-f**, and indomethacin on carrageenan induced paw edema in rats at 0.03 mmol/kg

Compound No.	% edema inhibition						
	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	–	–	–	–	–	–	–
Indomethacin	2.27	41.65	52.55	63.73	67.16	69.04	84.85
1	2.27	18.12	26.00	16.56	16.21	16.74	17.43
3a	0.25	6.82	48.90	40.21	32.70	37.66	27.87
3b	3.02	35.71	47.90	47.60	37.89	22.59	22.20
3c	3.25	36.71	48.80	48.00	38.11	28.30	28.26
3d	2.27	28.71	48.91	48.00	42.53	32.43	22.61
3e	5.54	48.24	58.51	50.88	22.11	22.59	23.24
3f	4.79	35.76	47.23	40.00	26.74	27.20	17.43
3g	2.77	42.12	58.30	53.67	48.21	17.15	17.84
3h	5.29	24.47	37.02	43.19	37.68	22.38	23.02
3i	2.77	42.12	58.30	53.67	48.21	17.15	17.84
4a	0.00	42.35	42.55	37.74	37.47	27.82	38.80
4b	4.27	47.65	44.91	46.69	47.79	58.58	48.55
4c	5.29	48.00	47.66	43.19	47.68	58.08	48.59
4d	2.27	41.65	68.51	63.73	63.58	63.81	58.92
4e	0.50	42.35	42.55	37.74	37.16	27.82	28.42
4f	2.27	18.12	26.80	16.56	16.21	16.74	17.43
4g	5.29	52.71	63.62	69.39	69.26	69.46	64.52
4h	2.27	29.41	48.00	36.17	36.84	21.00	21.58
4i	2.27	41.65	68.51	63.73	63.58	63.81	58.92
6a	0.76	53.89	64.04	69.81	74.95	75.10	75.31
6b	3.02	53.00	63.83	64.36	64.21	69.66	75.10
6c	4.03	53.41	53.00	48.43	64.00	58.58	48.96
6d	4.79	35.76	47.23	53.25	37.26	27.20	17.43
8a	3.53	52.48	68.51	53.25	53.05	63.81	64.11
8b	0.25	25.65	21.06	27.46	27.16	27.62	27.87
8c	4.79	38.12	57.02	58.10	63.58	63.81	68.51
8d	3.02	35.29	58.51	65.41	69.47	75.00	75.00
8e	2.27	41.65	52.55	63.73	67.16	69.04	84.85
8f	0.76	53.89	64.04	69.81	74.95	75.10	75.31

the 5 h interval, giving ~32-100% of the inhibition shown by indomethacin, while compounds **8d**, **8e**, and **8f** were the most active molecules with a long duration of action.

Analgesic activity

The potent long acting compounds with regard to their anti-inflammatory activity (**8a-f**) were tested for their analgesic activities using mean licking time in the hot-plate test, and indomethacin as a reference drug (Boyle et al., 1986). The effect was compared at the 0.02 mmol/kg dose level with a control group injected with the vehicle, and results were cited in Table II.

Results for the analgesic activity of **8a-f** (R_2=H, NO_2, Cl, Br, OMe, and F), indicated that **8a** and **8d-f** exhibited ~76-145% activity compared to indomethacin as a reference drug at the 5 h interval. Moreover, compounds **8c-e** were the most active compounds when they contained *p*-Cl, *p*-Br, or *p*-OMe moieties, respectively in their structures.

Ulcerogenic effect

The occurrence of gastrointestinal (GI) damage (bleeding and/or ulceration) is probably one of the most prevalent and serious side effects associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin (Nargund et al., 1994). Observation of the gastrointestinal mucosa for the presence of lesions following oral administration of

Table II. Analgesic activity of compounds (**8a-f**) and indomethacin at 0.02 mmol/kg

Compound No.	The average reaction time (sec) at different time intervals after compound administration				
	5 h	3 h	2 h	1 h	1/2 h
Control	7.0 ± 0.16	8.0 ± 1.29	9.0 ± 1.0	8.1 ± 0.74	8.6 ± 0.74
Indomethacin	29.2 ± 0.47**	25.8 ± 1.10**	20.7 ± 2.20**	14.6 ± 0.75*	10.6 ± 0.75**
8a	22.2 ± 0.90**	21.4 ± 1.90**	18.6 ± 2.20**	13.8 ± 1.30**	9.8 ± 0.18**
8b	22.2 ± 0.90**	26.6 ± 1.90**	21.6 ± 2.20**	17.8 ± 1.30**	11.8 ± 0.80**
8c	24.2 ± 1.48**	28.21 ± 1.4**	24.1 ± 1.10**	18.5 ± 0.70**	12.3 ± 0.80**
8d	38.1 ± 0.70**	32.3 ± 1.70**	26.3 ± 1.70**	21.0 ± 1.30**	14.3 ± 0.90**
8e	42. 5± 0.40*	34.9 ± 0.90**	34.5 ± 0.90**	22.2 ± 0.10**	15.2 ± 1.80**
8f	23.2 ± 0.48**	22.2 ± 1.40**	19.1 ± 1.10**	14.5 ± 0.70**	10.2 ± 0.8**

Values are the mean ± S.E. of 5 observations
*Significant difference at $p < 0.05$ *vs* control value (Student's-test)
**Significant difference at $p < 0.01$ *vs* control value (Student's-test)
Note: In this Table, a space on both sides of the ± sign in the data was inserted.

graded doses (10, 30, and 50 mg/kg) of the test compounds as well as the reference drug was conducted to examine for ulcerogenic effects in rats (Boyle et al., 1986). Both the frequency of ulceration (expressed as a ratio of ulcerated animals) and the severity of ulceration (expressed as an ulcer index) were used for comparison of the tested compounds and indomethacin.

Compounds **8d**, **8e**, and **8f**, that exhibited potent analgesic and anti-inflammatory profiles in the prementioned animal models, were evaluated for their ulcerogenic effects in rats. Results are recorded in Table III.

The tested compounds **8e** and **8f** showed superior GI safety profiles, because they provided 100% protection in the population of the tested animals at an oral dose of 10 mg/kg. At doses of 30 and 50 mg/kg, they elicited 83% and 50% protection, respectively.

Table III. Ulcerogenic effects of compounds **8d**, **8e**, and **8f** in comparison to indomethacin

Compound. No.	Dose mg/kg	Ratio of ulcerated animals	Ulcer index (mean ± S.E.)
Indomethacin	10	4/6	1.75 ± 0.23
	30	6/6	2.95 ± 0.16
	50	Not tested	–
8d	10	2/6	1.10 ± 0.35
	30	4/6	1.35 ± 0.58
	50	6/6	4.00 ± 0.35
8e	10	0/6	0.00
	30	1/6	0.95 ± 0.25
	50	3/6	1.75 ± 0.17
8f	10	0/6	0.00
	30	1/6	1.15 ± 0.25
	50	3/6	1.35 ± 0.34

However, compound **8d** was found to cause 33%, 67%, and 100% ulceration at doses of 10, 30 and 50 mg/kg, respectively when compared to indomethacin which showed 67% and 100% ulceration at doses of 10 and 30 mg/kg, respectively.

It should be mentioned that compound **8e**, which contained a triazolothiadiazine (*p*-Cl) in its structure, was the most active derivative with regards to the anti-inflammatory and analgesic activities, and displayed the highest safety due its structural similarity to indomethacin.

Acute toxicity (LD_{50})

The median lethal dose (LD_{50}) of compounds **8a** and **8e** was also determined in mice according to a reported method (Boyle et al., 1986), and found to be 275 and 300 mg/kg (i.p.) respectively, whereas that of indomethacin was 13 mg/kg (i.p.) (O'Neil et al., 2001).

Antibacterial activity

Compounds **1**, **3a-h**, **4a-h**, **6a-d**, and **8a-f** were tested for their antibacterial activity *in vitro* against MRSA, *Bacillus cereus* as representatives of the Gram positive strains, and *Escherichia coli* and *Klebsiella pneumoniae* as representatives of the Gram negative strains (clinical isolates obtained from Infection Control Unit, Assiut University Hospital, Faculty of Medicine, Assiut University) using the agar cup diffusion method (William, 1977) for susceptibility screening, and the 2-fold dilution method for MIC determination. Ampicillin was used as a reference drug (20 μmol/mL), and DMSO was used as a solvent control.

Results from the tested compounds **1**, **3a-i**, **4a-i**, **6a-d**, and **8a-f**, showed higher antibacterial activity than ampicillin against the organisms used, and especially

Table IV. Antibacterial activity of the test compounds **1**, **3a-i**, **4a-i**, **6a-d**, **8a-f**, and ampicillin (20 μmol/mL)

Compound	*In vitro* activity-inhibition zone in mm (MIC)			
	Staphylococcus aureus	*Bacillus cereus*	*Escherichia coli*	*Klebsiella pneumoniae*
1	19	20	18	20
3a	23 (65)	31 (20)	28 (25)	18 (79)
3b	–	–	10	12
3c	29 (23)	18 (70)	21 (60)	18 (79)
3d	–	12	13	11
3e	31 (20)	16 (135)	23 (65)	16 (130)
3f	–	11	11	13
3g	–	–	–	–
3h	28 (25)	26 (40)	29 (25)	25 (50)
3i	–	17	15	12
4a	–	12	14	15
4b	10	15	11	7
4c	–	12	15	10
4d	–	12	13	15
4e	–	16	12	14
4f	30 (20)	17 (130)	20 (70)	17 (120)
4g	–	22	16	15
h4	–	16	11	10
4i	–	15	10	12
6a	–	16	17	15
6b	30 (20)	20 (70)	17 (125)	17 (120)
6c	30 (20)	20 (70)	17 (125)	17 (120)
6d	28 (23)	16 (70)	20 (50)	17 (70)
8a	33 (20)	33 (30)	30 (25)	31 (30)
8b	32 (25)	32 (33)	32 (23)	32 (30)
8c	34 (25)	31 (30)	33 (20)	33 (20)
8d	33 (20)	33 (30)	30 (25)	31 (30)
8e	32 (25)	32 (33)	32 (23)	32 (30)
8f	30 (25)	30 (27)	30 (23)	32 (30)
Ampicillin	20 (69)	22 (60)	23 (50)	20 (70)
DMSO	–	–	–	–

(–): no antibacterial activity at the studied concentration

for compounds **6b-d** and **8a-f** (Table IV). Also, it was observed that compounds **8a** and **8e** were the most active derivatives against all used species. Meanwhile, compounds **3d**, **3f**, **3i**, **4a**, **4c-4e**, **4g-i**, and **6a** were inactive against *Staphylococcus aureus* (MRSA); while compound **3b** was inactive against MRSA and *Bacillus cereus*. On the other hand, compound **3g** was completely inactive against the tested organisms.

Results from the compounds tested for MIC were in accordance with the data of the inhibition zone, where compounds **6b-d** and **8a-f** carrying methyl, phenyl, isonicotinic acid, nicotinic acid, or *p*-substituted phenyl triazolothiadiazine moieties in their structures gave ~73-165% the activity of ampicillin and were the most active against the tested organisms.

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