Eur. J. Med. Chem. 35 (2000) 1037–1040
© 2000 Éditions scientifiques et médicales Elsevier SAS. All rights reserved
PII: S0223-5234(00)01178-8/FLA

Laboratory Note

Synthesis and antimicrobial activities of some 3-arylamino-5-[2-(substituted 1-imidazolyl)ethyl]-1,2,4-triazole derivatives

Şeref Demirayak[a], Kadriye Benkli[a]*, Kıymet Güven[b]

[a]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Anadolu, 26470 Eskişehir, Turkey
[b]Department of Microbiology, Faculty of Science, University of Anadolu, 26470 Eskişehir, Turkey

Received 17 November 1999; revised 6 June 2000; accepted 8 June 2000

Abstract – In this study, some 3-arylamino-5-[2-(substituted imidazol-1-yl or benzimidazol-1-yl)ethyl]-1,2,4-triazole derivatives were synthesised by reacting 3-(substituted imidazol-1-yl)propionyl hydrazides, with *S*-methyl-*N'*-arylisothiouronium iodide salts. Their structures were elucidated by IR, ^{1}H-NMR and mass spectroscopic data and elemental analyses results. Antimicrobial activities of the compounds were observed against *Staphylococcus aureus* NRRL B-767, *Micrococcus luteus* NRRL B-4375, *Escherichia coli* B, *Pseudomonas aeroginosa* NRRL B-23 and the fungi *Candida albicans* and *Candida glabrata* by using the tube dilution technique. Remarkable activity was obtained. © 2000 Éditions scientifiques et médicales Elsevier SAS

3-amino[1,2,4]triazole / benzimidazol / imidazol / antimicrobial activity

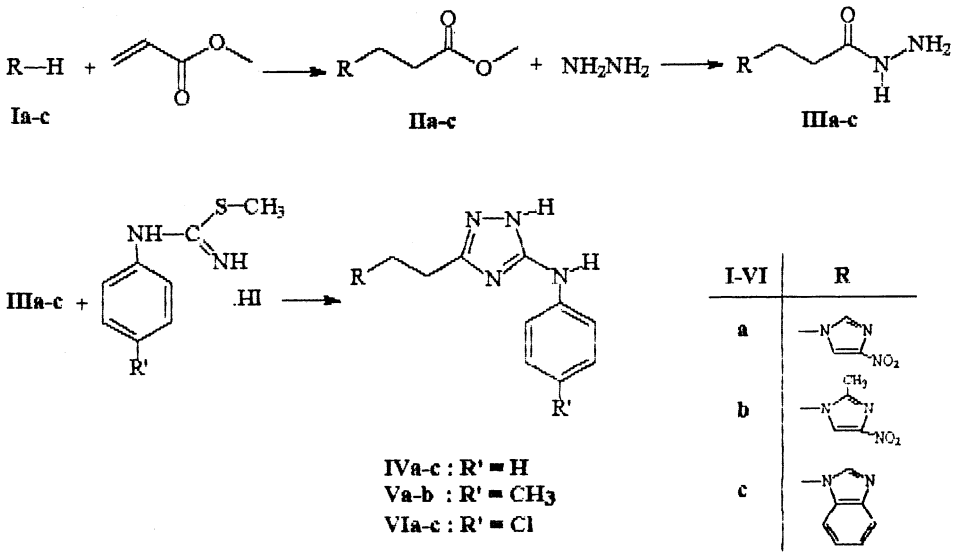
1. Introduction

Triazole moiety may be considered as a bioisostere of imidazole, which is a part of the azole group of antifungal drugs (i.e. fluconazole). In this study triazole has been used instead of imidazole [1–3]. As an extension of our previous work on 3-arylamino-5-aryloxymethyl-1,2,4-triazole derivatives which showed appreciable antibacterial and antifungal activities [4], some new triazole derivatives were synthesised using substituted nitroimidazolyl-ethyl and benzimidazolyl-ethyl residues instead of aryloxymethyl groups. Antibacterial and antifungal activities of the compounds were investigated.

2. Chemistry

To obtain 3-aryl-5-[2-(substituted imidazol-1-yl or benzimidazol-1-yl)-ethyl]-1,2,4-triazole derivatives **IVa–c, Va,b, VIa–c**, *S*-methyl-*N'*-arylisothiouronium iodide salts and 3-(substituted imidazol-1-yl or benzimidazol-1-yl)propionyl hydrazides were reacted by heating in pyridine. Pyridine behaves both as a solvent and a base in this reaction. The structures of the compounds were elucidated by spectral data and elemental analyses. In the IR spectra, the bands due to N–H group, which is present in all studied compounds, were observed at about 3260–3230 cm^{-1}. The bands at about 1050 cm^{-1} were characteristic for the triazole derivatives [5]. In ^{1}H-NMR spectra, the peaks of ethylene protons were observed at about 3.40 ppm as broad singlets and at 4.40 ppm as triplets. The methylene protons were observed as broad singlets instead of triplets probably due to the effect of DMSO used as a solvent [6]. Aromatic protons were observed at about 6.70–7.60 ppm as doublets, triplets or multiplets depending on substitution. The peaks of N–H protons of arylamino and triazole residue were obtained as unequal size doublets, probably due to the tautomeric form of the compounds. The integral values of these doublets correspond to one proton for N–H groups. In MS spectra, molecular ion peaks or M + 1 peaks were obtained from EI-MS or ES-MS and FAB-MS spectra, respectively [6] (*scheme 1*).

* Correspondence and reprints:
E-mail address: kbenkli@anadolu.edu.tr (K. Benkli).



Scheme 1.

3. Results and discussion

In one of our recent works [4], we found out that some of the 3-arylamino-5-aryloxymethyl-1,2,4-triazole derivatives were highly effective against aerobic bacteria. We performed a pharmaco modulation on these compounds, replacing the 5-aryloxymethyl group by a (2-substituted-1-imidazolyl)ethyl group. The compounds, which were synthesised, exhibited noticeable antibacterial and antifungal activities, as expected (*table I*). The in vitro antimicrobial activity results showed that the most sensitive micro-organism to the control antibiotic, Chloramphenicol Succinate, is *S. aureus* and to the control antifungal, Ketoconazol, the most sensitive organism is *C. glabrata*. MIC values of these standards were lower than expected. This situation may be attributed to the resistance gained by the bacteria strains by time. In consideration of the results, we may conclude that some of the tested products have noticeable antibacterial and antifungal activities. Some of the compounds which possess lower MIC values (for example, 31.25 μg/mL for **VIb** against *S. aureus*) may be considered as highly potential antibacterial. Most of the compounds have 62.5 or 125 μg/mL MIC values against these micro-organisms. Unfortunately, no correlation between the substituents and biological activities of the compounds was noticeable. Compounds **IVa** and **VIb** showed higher antimicrobial activity than the other compounds against *E. coli*, *M. lutea* and *S. aureus*. These better activities, observed for **IVa** and **VIb**, could be interpreted as a consequence of the presence of a nitroimidazole moiety in their structure. It seems that, compounds **IVa**, **Vb** and **VIb** have higher anti *E. coli* B activities and the compounds **IVa** and **Vb** have higher anti *M. luteus* activities than Chloramphenicol Succinate. However, compounds **IVa**, **Va** and **VIb** have lower anti *S. aureus* activities than Chloramphenicol Succinate. No difference was observed between the anti *C. albicans* activities of Ketoconazol

Table I. Antibacterial activity expressed as MIC values in μg/mL[a].

Comp.	E.c.	M.l.	P.a.	S.a.	C.a.	C.g.
IVa	125	62.5	125	62.5	500	125
IVb	250	125	125	125	500	125
IVc	250	125	250	125	500	125
Va	250	125	125	62.5	500	125
Vb	125	125	125	250	500	125
VIa	250	250	125	250	500	125
VIb	125	62.5	250	31.25	500	125
VIc	250	125	250	125	500	125
A	250	125	125	7.81	500	62.5
B	250	125	125	7.81	500	250

[a] **A**: Ketoconazol, **B**: Chloramphenicol succinate, E.c.: *E. coli* B, M.l.: *M. luteus* NRRL B-4375, P.a.: *P. aeroginosa* NRRL B-23, S.a.: *S. aureus* NRRL B-767, C.a.: *C. albicans*, C.g.: *C. glabrata*.

and the compounds tested. *C. glabrata* was more sensitive to both Ketoconazol and the compounds tested than *C. albicans*, and all the compounds gave the same MIC values for *C. glabrata*. Hence, the tested compounds may be regarded as highly active antifungal substances against *C. glabrata* and less active against *C. albicans*.

4. Experimental protocols

4.1. Chemistry

Melting points were determined using a Gallenkamp apparatus and are uncorrected. Spectroscopic data were recorded on the following instruments: IR: Shimadzu 435 IR spectrophotometer; ^{1}H-NMR: DPX 400 NMR spectrometer, Jeol JNM-EX 90A FT NMR spectrometer; MS: VG Platform mass spectrometer. Microanalyses: Leco CHNS elemental analyses apparatus. C, H, N values were within $\pm 0.4\%$ of the theoretical values. Methyl 3-substituted propionate derivatives (**IIa–c**) [7, 8] and *S*-methylisothiouronium salts [9] were prepared using previously reported methods.

*4.1.1. General procedure for compounds **III***

A mixture of suitable **II** (50 mmol) and hydrazine hydrate (100 mmol) in methanol (50 mL) was refluxed for 3 h. The mixture was allowed to cool to crystallise. The title compounds crystallised by cooling of the reaction mixture.

*4.1.1.1. 3-(4-Nitroimidazol-1-yl)-propionylhydrazide **IIIa***

Yield: 76%; m.p.: 230–234 °C. Anal. Calcd. for $C_6H_9N_5O_3$: C, 36.18; H, 4.52; N, 35.17. Found: C, 36.22; H, 4.51; N, 35.20.

*4.1.1.2. 3-(2-methyl-4-nitroimidazol-1-yl)-propionylhydrazide **IIIb***

Yield: 62%; m.p.: 146–149 °C. Anal. Calcd. for $C_7H_{11}N_5O_3$: C, 39.44; H, 5.16; N, 32.86. Found: C, 39.47; H, 5.18; N, 32.88.

*4.1.1.3. 3-(benzimidazol-1-yl)-propionylhydrazide **IIIc***

Yield: 66%; m.p.: 264–266 °C. Anal. Calcd. for $C_{10}H_{122}N_4O$: C, 58.82; H, 5.88; N, 27.45. Found: C, 58.87; H, 5.85; N, 27.46.

*4.1.2. General procedure for compounds **IV–VI***

A mixture of the suitable **III** (10 mmol) and *S*-methyl-*N*′-arylisothiouronium iodide salt (10 mmol) in pyridine (10 mL) was refluxed for 6 h. The cooled mixture was poured into crushed ice. The precipitate was collected by filtration and the raw product was recrystallised from ethanol.

*4.1.2.1. 3-Phenylamino-5-[2-(4-nitroimidazol-1-yl)-ethyl]1,2,4-triazole **IVa***

Yield: 52%; m.p.: 192 °C. IR (KBr) ν_{max} (cm^{-1}): 3252 (N–H), 1605–1547 (C=N, C=C), 1495, 1309 (N–O), 1056 (1,2,4 triazole). ^{1}H-NMR σ (ppm): 3.44 (2H, bs), 4.45 (2H, t, $J = 6.71$ Hz), 6.7–6.85 (1H, m), 7.15 (2H, t, $J = 7.58$ Hz), 7.43 (2H, d, $J = 7.75$ Hz), 7.65 (1H, s), 8.20 (1H, s), 12.25, 12.87 (1H, bs). MS ES (M + 1) m/z: 300.3; EI: 299.4 (M), 186.4, 118.3, 104.3, 77.3 (100%). Anal. Calcd. for $C_{13}H_{13}N_7O_2 \cdot 1/2H_2O$: C, 50.64; H, 4.57; N, 31.80. Found: C, 50.67; H, 4.51; N, 31.46.

*4.1.2.2. 3-Phenylamino-5-[2-(2-methyl-4-nitroimidazol-1-yl)-ethyl]1,2,4-triazole **IVb***

Yield: 54%; m.p.: 238 °C. IR (KBr) ν_{max} (cm^{-1}): 3260 (N–H), 1607–1550 (C=N, C=C), 1481, 1327 (N–O), 1062 (1,2,4-triazole). ^{1}H-NMR σ (ppm): 2.29 (3H, s), 3.15 (2H, bs), 4.35 (2H, t, $J = 6.45$ Hz), 6.80 (1H, t, $J = 6.85$ Hz), 7.20 (2H, t, $J = 7.25$ Hz), 7.49 (2H, d, $J = 8.04$ Hz), 8.35 (1H, s), 9.1 (1H, s), 12.9 (1H, s). MS FAB: 314 (M + 1). Anal. Calcd. for $C_{14}H_{15}N_7O_2$: C, 53.67; H, 4.83; N, 31.29. Found: C, 53.59; H, 4.75; N, 30.75.

*4.1.2.3. 3-Phenylamino-5-[2-(benzimidazol-1-yl)-ethyl]1,2,4-triazole **IVc***

Yield: 73%; m.p.: 201 °C. IR (KBr) ν_{max} (cm^{-1}): 3239 (N–H), 1618–1553 (C=N, C=C), 1496, 1291 (N–O), 1041 (1,2,4-triazole). ^{1}H-NMR σ (ppm): 3.46 (2H, bs), 4.63 (2H, t, $J = 6.71$ Hz), 6.70–6.85 (1H, m), 7.18–7.27 (4H, m), 7.46 (2H, d, $J = 8.13$ Hz), 7.58–7.65 (2H, m), 8.12 (1H, s), 9.2 (1H, bs), 12.9 (1H, bs). MS ES (M + 1) m/z: 305.2 (M + 1); EI: 305.5 (M + 1), 304.5 (M), 186, 172, 131 (100%). Anal. Calcd. for $C_{17}H_{16}N_6 \cdot 1/2H_2O$: C, 65.15; H, 5.47; N, 26.82. Found: C, 65.92; H, 5.83; N, 26.81.

*4.1.2.4. 3-(4-Methyl)phenylamino-5-[2-(4-nitroimidazol-1-yl)-ethyl]1,2,4-triazole **Va***

Yield: 48%; m.p.: 186 °C. IR (KBr) ν_{max} (cm^{-1}): 3230 (N–H), 1625–1550 (C=N, C=C), 1495, 1290 (N–O), 1040 (1,2,4 triazole). ^{1}H-NMR σ (ppm): 2.18 (3H, s), 3.23 (2H, bs), 4.34 (2H, t, $J = 6.86$ Hz), 6.95 (2H, d, $J = 8.50$ Hz), 7.42 (2H, d, $J = 8.75$ Hz), 8.21 (1H, s), 9.20 (1H, bs), 12.9 (1H, bs). MS FAB: 314 (M + 1). Anal. Calcd. for $C_{14}H_{15}N_7O_2$: C, 53.67; H, 4.83; N, 31.29. Found: C, 53.80; H, 4.70; N, 30.95.

4.1.2.5. 3-(4-Methyl)phenylamino-5-[2-(2-methyl 4-nitroimidazol-1-yl)-ethyl]1,2,4-triazole ***Vb***

Yield: 55%; m.p.: 228 °C. IR (KBr) ν_{max} (cm^{-1}): 3233 (N–H), 1628–1557 (C=N, C=C), 1495, 1294 (N–O), 1045 (1,2,4 triazole). ^{1}H-NMR σ (ppm): 2.22 (3H, s), 2.32 (3H, s), 3.31 (2H, bs), 4.44 (2H, t, $J = 6.35$ Hz), 7.01 (2H, d, $J = 8.69$ Hz), 7.36 (2H, d, $J = 8.86$ Hz), 8.41 (1H, s), 8.89 (1H, bs), 12.69 (1H, bs). MS FAB: 328 (M + 1). Anal. Calcd. for $C_{15}H_{17}N_7O_2$: C, 54.99; H, 5.19; N, 29.94. Found: C, 54.96; H, 5.24; N, 30.11.

4.1.2.6. 3-(4-Chloro)phenylamino-5-[2-(4-nitroimidazol-1-yl)-ethyl]1,2,4-triazole ***VIa***

Yield: 50%; m.p.: 300 °C. IR (KBr) ν_{max} (cm^{-1}): 3250 (N–H), 1615–1549 (C=N, C=C), 1498, 1312 (N–O), 1043 (1,2,4 triazole). ^{1}H-NMR σ (ppm): 3.33 (2H, bs), 4.44 (2H, t, $J = 6.63$ Hz), 7.24 (2H, t, $J = 6.25$ Hz), 7.49 (2H, d, $J = 8.65$ Hz), 7.81 (1H, s), 8.12 (1H, s), 8.41, 8.45 (1H, bs), 12.48, 13.41 (1H, bs). MS FAB: 344 (M + 1). Anal. Calcd. for $C_{13}H_{12}ClN_7O_2 \cdot 1/2H_2O$: C, 45.55; H, 3.82; N, 28.61. Found: C, 45.25; H, 3.78; N, 29.01.

4.1.2.7. 3-(4-Chloro)phenylamino-5-[2-(2-methyl 4-nitroimidazol-1-yl)-ethyl]1,2,4-triazole ***VIb***

Yield: 58%; m.p.: 242 °C. IR (KBr) ν_{max} (cm^{-1}): 3264 (N–H), 1613–1542 (C=N, C=C), 1468, 1337 (N–O), 1067 (1,2,4-triazole), ^{1}H-NMR σ (ppm): 2.28 (3H, s), 3.44 (2H, bs), 4.34 (2H, t, $J = 6.54$ Hz), 7.14 (2H, d, $J = 8.91$ Hz), 7.49 (2H, d, $J = 8.68$ Hz), 8.22 (1H, s), 9.06, 9.35 (1H, bs), 12.25, 12.50 (1H, bs). MS FAB: 348 (M + 1). Anal. Calcd. for $C_{14}H_{14}ClN_7O_2$: C, 48.35; H, 4.05; N, 28.19. Found: C, 47.97; H, 3.85; N, 28.55.

4.1.2.8. 3-(4-Chloro)phenylamino-5-[2-(benzimidazol-1-yl)-ethyl]1,2,4-triazole ***VIc***

Yield: 68%; m.p.: 194 °C. IR (KBr) ν_{max} (cm^{-1}): 3241 (N–H), 1619–1555 (C=N, C=C), 1497, 1293 (N–O), 1045 (1,2,4-triazole). ^{1}H-NMR σ (ppm): 3.20 (2H, bs), 4.64 (2H, t, $J = 6.85$ Hz), 7.18–7.27 (4H, m), 7.41 (2H, d, $J = 8.84$ Hz), 7.59–7.65 (2H, m), 8.13 (1H, s), 9.11 (1H, bs), 12.87 (1H, bs). MS FAB: 340 (M + 1). Anal. Calcd. for $C_{17}H_{15}ClN_6$: C, 60.26; H, 4.46; N, 24.80. Found: C, 59.88; H, 4.28; N, 24.35.

4.2. Microbiology

Antimicrobial activities of the synthesised compounds against two Gram-positive bacteria (*Staphylococcus aureus* NRRL B-767 and *Micrococcus luteus* NRRL B-4375), two Gram-negative bacteria (*Escherichia coli* B and *Pseudomonas aeroginosa* NRRL B-23) and the fungi (*Candida albicans* and *Candida glabrata)* were expressed as the minimum inhibitory concentration (MIC) values. The MIC values were determined by the tube dilution technique [10–12]. MIC values of the synthesised compounds and the control drugs, Ketoconazol and Chloramphenicol Succinate, were compared. The stock solutions of the compounds were prepared in DMSO. The standard bacteria strains were obtained from the U.S. Department of Agricultural Research Service (Midwest Area), Northern Regional Research Center (1815 North University Street, Peoria, IL 61604 USA), and the University of East Angelia (School of Biological Sciences, UK). *Candida albicans* and *Candida glabrata* strains were isolated from patients in Osmangazi University Hospital in Turkey. The MIC values, in μg/mL, are given in *table I*.

References

[1] Ram V.J., Pandey H.N., Chem. Pharm. Bull. 22 (1974) 2778–2783.

[2] Wolf M.E., Burger's Medicinal Chemistry, Part II, 4th Edition, John Wiley and Sons, New York, 1979.

[3] Foye W.O., Lemke T.L., Williams D.A., Principles of Medicinal Chemistry, 4th Edition, Williams and Wilkins, Baltimore, MD, 1995.

[4] Demirayak S., Benkli K., Güven K., Pharm. Acta Helv. 72 (1998) 285–290.

[5] Jennings A.L., Boggs J.E., J. Org. Chem. 29 (1964) 2065–2066.

[6] Silverstein R.M., Bassler G.C., Morrill T.C., Spectrometric Identification of Organic Compounds, John Wiley and Sons, New York, 1991.

[7] Bhujanga Rao A.K.S., Gundu Rao C., Singh B.B., J. Org. Chem. 55 (1990) 3702–3704.

[8] Efros L.S., Porai-Koshits B.A., Zh. Obshch. Khim. 23 (1953) 697–705 C.A.: 48 7603h.

[9] Rasmussen, C.R., Villani, F.J., Raynolds B.E., et al., Synthesis (1988) 460–466.

[10] Finegold S.M., Martin W.J., Scott E.G., Bailey and Scott's Diagnostic Microbiology, The C.V. Mosby Company, Saint Louis, 1978.

[11] McGinnis M.R., Rinaldi M.G., Antifungal drugs: mechanism of action, drug resistance, susceptibility testing and assays of activity in biological fluids, in: Lorian V. (Ed.), Antibiotics in Laboratory Medicine, 3rd Edition, Williams and Wilkins, London, 1991, p. 198.

[12] Rodetsky M., Wheeler R.C., Roe M.H., Todd J.K., J. Clin. Microbiol. 24 (1986) 600–606.