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Short communication

New triazole and triazolothiadiazine derivatives as possible antimicrobial agents

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Abstract

Triazole and triazoles fused with six-membered ring systems are found to possess diverse applications in the fields of medicine, agriculture and industry. The new 1,2,4-triazole and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine derivatives were synthesized as novel antimicrobial agents. The reaction of 1*H*-indol-3-acetic acid with thiocarbohydrazide gave the 4-amino-3-mercapto-5-[(1*H*-indol-3-yl)methyl]-4*H*-1,2,4-triazole. The reaction of triazole with arylaldehydes in ethanol gave the 4-arylideneamino-3-mercapto-5-[(1*H*-indol-3-yl)methyl]-4*H*-1,2,4-triazoles (**I**). The 3-[(1*H*-indol-3-yl)methyl]-6-aryl-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines (**II**) were obtained by condensing triazole with phenacyl bromides in absolute ethanol . The chemical structures of the compounds were elucidated by IR, ^{1}H NMR and FAB$^+$-MS spectral data. Their antimicrobial activities against *Micrococcus luteus* (NRLL B-4375), *Bacillus cereus* (NRRL B-3711), *Proteus vulgaris* (NRRL B-123), *Salmonella typhimurium* (NRRL B-4420), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (NRRL B-3704), *Candida albicans and Candida glabrata* (isolates obtained from Osmangazi University, Faculty of Medicine) were investigated and significant activity was obtained.

Keywords: Indole; Triazole; Triazolothiadiazine; Antimicrobial activity

1. Introduction

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is real perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant. Through the various molecules designed and synthesized for this aim, it was demonstrated that 1,2,4-triazoles and their derivatives could be considered as possible antimicrobial agents [1–7].

The biological activities of various 1,2,4-triazole derivatives and their N-bridged heterocyclic analogs have also been extensively studied. Triazole fused six-membered ring system is also found to possess diverse applications in the field of medicine [8–11]. The commonly known systems are triazole fused with pyridines [8], pyridazines [9], pyrimidines [10], pyrazines [11] and triazines [10]. The literature survey reveals that there are not many examples of triazoles fused with thiadiazines. Those incorporating the N–C–S linkage as in the skeleton of 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazine exhibit a broad spectrum of antimicrobial activity [12–18].

4-Amino-1,2,4-triazol-3-thiones can be considered as useful tools in fusing to triazolothiadiazines. The amino and

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mercapto groups are ready-made nucleophilic centers for the synthesis of condensed heterocyclic rings [3,19,20]. Besides, the amino group of this structure is of importance for obtaining various Schiff base derivatives with well-known antimicrobial properties [21–25].

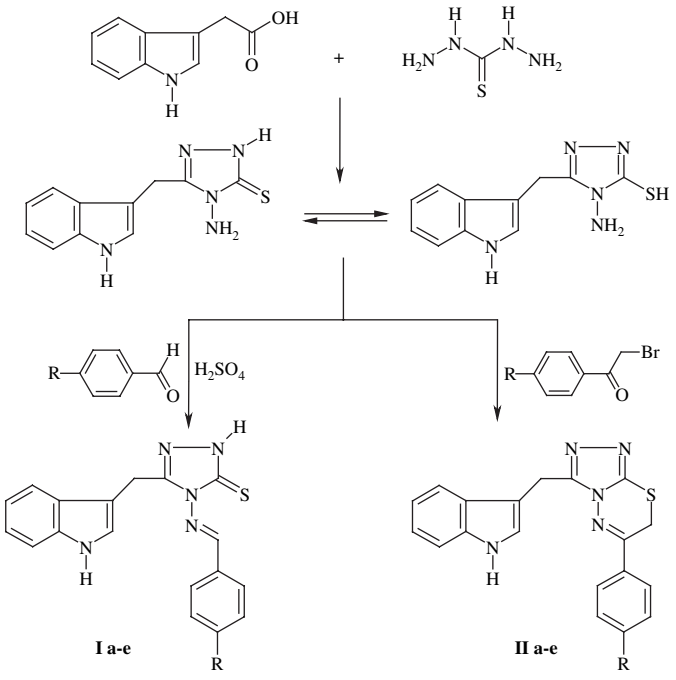
On the other hand, antimicrobial properties have also been reported to be associated with the indolic nucleus [5,26].

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles.

In the present study, prompted by these observations, the synthesis and antimicrobial screening of new Schiff bases of 1,2,4-triazole derivatives and 1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines as hybrid molecules including different pharmacophores are aimed at.

2. Chemistry

In the present work, 4-amino-3-mercapto-5-[(1*H*-indol-3-yl) methyl]-1,2,4-triazole has been synthesized [27] by heating thiocarbohydrazide with 1*H*-indol-3-acetic acid. The reaction of triazole with arylaldehyde in presence of concentrated sulfuric acid in ethanol gave the Schiff bases (5-[(1*H*-indol-3-yl)methyl]-4-arylideneamino-3-mercapto-1,2,4-triazoles) (**Ia–e**). 3-[(1*H*-Indol-3-yl)methyl]-6-aryl-7*H*-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**IIa–e**) were obtained by condensing triazole with phenacyl bromides in absolute ethanol (Scheme 1).



R: **a**=H, **b**=Cl, **c**=CH_3, **d**=NO_2, **e**=$N(CH_3)_2$

Scheme 1.

3. Biology

3.1. Antimicrobial activity

Antimicrobial activities of compounds were tested using microbroth dilution method [28,29]. Tested microorganism strains were; *Micrococcus luteus* (NRLL B-4375), *Bacillus cereus* (NRRL B-3711), *Proteus vulgaris* (NRRL B-123), *Salmonella typhimurium* (NRRL B-4420), *Staphylococcus aureus* (NRRL B-767), *Escherichia coli* (NRRL B-3704), *Candida albicans and Candida glabrata* (isolates obtained from Osmangazi University, Faculty of Medicine). The observed data on the antimicrobial activity of the compounds and control drugs are given in Table 1.

4. Results, discussion and conclusion

In this present work, a series of 10 new compounds were synthesized. Scheme 1 illustrates the way used for the preparation of target compounds. As a starting material, thiocarbohydrazide and 1*H*-indol-3-acetic acid were used to produce triazole. The structure of the compounds was elucidated by IR, ^{1}H NMR, mass spectral data and elemental analysis. In the IR spectra of all compounds C=N and C=C bands were observed at about 1630–1430 cm^{-1} region. According to the IR spectroscopic data of the compounds **Ia–e** which have triazoline-3-thione structure, the observation of C=S stretching bands at 1380–1365 cm^{-1} and the absence of an absorption at about 2600–2550 cm^{-1} region cited for SH group have proved that these compounds were in the thionic form.

In the ^{1}H NMR spectra of compounds (**Ia–e** and **IIa–e**) that are taken in DMSO-d_6, NH proton of the indole ring was seen as singlet at about 10.90–11.30 ppm. The signal due to indol-CH_2 methylene protons, present in all compounds, appeared at 4.20–4.60 ppm, as singlet or multiplets. The N=CH proton of compounds **Ia–e** appeared at 10.20–10.30 ppm as singlet. All the other aromatic and aliphatic protons were observed at the expected regions. Mass spectra (MS (FAB)) of compounds showed M + 1 peaks, in agreement with their molecular formula.

All compounds were evaluated for their antimicrobial properties. MICs were recorded as the minimum concentration of compound, which inhibits the growth of tested microorganisms. Compound **IIc** showed similar antifungal activity against *C. globrata,* when compared with ketoconazole. When compared with chloramphenicol, compound **IIa** showed similar antibacterial activity against *S. aureus,* and *B. cereus*; also compounds **Ic**, **IIb** and **IIe** showed similar antibacterial activities against *B. cereus* (Table 1).

In conclusion, a series of novel 4-arylideneamino-3-mercapto-5-[(1*H*-indol-3-yl)methyl]-4*H*-1,2,4-triazoles (**I**) and 3-[(1*H*-indol-3-yl)methyl]-6-aryl-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines (**II**) were synthesized and their antimicrobial activities have been evaluated. Among these series compound **IIc** including the methyl on phenyl showed significant antifungal activitiy. On the other hand compounds **Ic**, **IIa**, **IIc**, **IIe** also showed significant antibacterial activities. As a result,

Table 1
Antibacterial and antifungal activities of the compounds as MIC values (μg/ml)

Comp.	A	B	C	D	E	F	G	H
Ia	250	250	62.5	62.5	125	125	125	125
Ib	62.5	250	125	62.5	125	125	125	250
Ic	62.5	125	62.5	125	125	250	250	125
Id	125	250	125	125	250	125	125	125
Ie	125	125	125	62.5	250	125	250	250
IIa	62.5	125	62.5	62.5	62.5	250	125	125
IIb	62.5	125	250	125	125	125	125	250
IIc	62.5	250	125	62.5	125	125	250	62.5
IId	125	250	125	250	125	125	125	125
IIe	62.5	125	125	125	250	125	125	250
Reference 1	1.95	125	0.97	0.97	62.5	62.5	–	–
Reference 2	–	–	–	–	–	–	62.5	62.5

A: *Micrococcus luteus* (NRLL B-4375), B: *Bacillus cereus* (NRRL B-3711), C: *Proteus vulgaris* (NRRL B-123), D: *Salmonella typhimurium* (NRRL B-4420), E: *Staphylococcus aureus* (NRRL B-767), F: *Escherichia coli* (NRRL B-3704), G: *Candida albicans* (isolates obtained from Osmangazi University, Faculty of Medicine), H: *Candida glabrata* (isolates obtained from Osmangazi University Faculty of Medicine).
Reference 1: chloramphenicol, reference 2: ketoconazole.

we can say that the triazolothiadiazine derivatives are more active than triazoles when their antimicrobial activity is compared.

5. Experimental

All reagents were used as purchased from commercial suppliers without further purification. Melting points were determined by using a Gallenkamp apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed with glass plates (0.25 mm) precoated with Merck silica gel 60 F_{254}, and flash chromatography separations (FC) were carried out with Merck silica gel 60 (200e 450 mesh), using 40:60 EtOAc/petroleum benzine as eluents. Spectroscopic data were recorded by the following instruments. IR: Shimadzu IR-435 spectrophotometer; ^{1}H NMR: Bruker 250 MHz spectrometer; MS: fast atom bombardment mass spectra (FAB-MS) were obtained by VG Quattro mass spectrometer. Microanalytical data were obtained by the Microanalytical Section of Service Center (CNRS, Ecole Normale de Chimie de Montpellier, France).

5.1. General procedure for the synthesis of the compounds

5.1.1. 4-Amino-3-mercapto-5-[(1H-indol-3-yl)methyl]-1,2,4-triazole

Equimolar mixture of thiocarbohydrazide (0.1 mol) and 1*H*-indol-3-acetic acid was heated in an oil-bath at 160–170 °C for 2 h. The fused mass thus obtained was dispersed with hot water to obtain the triazole. The product was recrystallized from methanol.

*5.1.2. 5-[(1H-Indol-3-yl)methyl]-4-arylideneamino-3-mercapto-1,2,4-triazoles (**Ia–e**)*

To a suspension of arylaldehyde (0.005 mol) in ethanol (10 ml), was added an equimolar amount of triazole. The suspension was heated until a clear solution was obtained. A few drops of conc. sulfuric acid were added as a catalyst and the solution was refluxed for 3 h on a water bath. The precipitated solid was filtered off and recrystallized from ethanol.

*5.1.3. 3-[(1H-Indol-3-yl)methyl]-6-aryl-7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazines (**IIa–e**)*

A solution of triazole (0.005 mol) and phenacyl bromide (0.005 mol) in absolute ethanol (30 ml) was heated under reflux for 1 h, cooled to room temperature and then neutralized with ammonium hydroxide. The product thus obtained was recrystallized from ethanol.

Compound **Ia**: yield 65%, m.p. 186–188 °C. IR (KBr) ν_{max} (cm^{-1}): 1580–1432 (C=C and C=N), 1375 (C=S). ^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.40 (2H, s, CH_2), 6.90–8.05 (10H, m, aromatic protons), 10.20 (1H, s, N=CH), 11.05 (1H, s, indole NH), 13.80 (1H, s, triazole NH). MS (FAB) *m/z*: 334 [M + 1]. Anal. Calc. for $C_{18}H_{15}N_5S$: C, 64.84; H, 4.53; N, 21.00. Found: C, 64.88; H, 4.55; N, 20.96.

Compound **Ib**: yield 70%, m.p. 194–196 °C. IR (KBr) ν_{max} (cm^{-1}): 1601–1455 (C=C and C=N), 1369 (C=S). ^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.35 (2H, s, CH_2), 7.05–7.45 (5H, m, indole protons), 8.00–8.45 (4H, m, phenyl protons), 10.25 (1H, s, N=CH), 11.10 (1H, s, indole NH), 13.70 (1H, s, triazole NH). MS (FAB) *m/z*: 368 [M + 1]. Anal. Calc. for $C_{18}H_{14}ClN_5S$: C, 58.77; H, 3.84; N, 19.04. Found: C, 58.74; H, 3.88; N, 19.01.

Compound **Ic**: yield 60%, m.p. 207–209 °C. IR (KBr) ν_{max} (cm^{-1}): 1585–1430 (C=C and C=N), 1380 (C=S). ^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.25 (3H, s, CH_3), 4.45 (2H, s, CH_2), 7.00–8.10 (9H, m, aromatic protons), 10.30 (1H, s, N=CH), 11.00 (1H, s, indole NH), 13.90 (1H, s, triazole NH). MS (FAB) *m/z*: 348 [M + 1]. Anal. Calc. for $C_{19}H_{17}N_5S$: C, 65.68; H, 4.93; N, 20.16. Found: C, 65.70; H, 4.95; N, 20.19.

Compound **Id**: yield 75%, m.p. 238–239 °C. IR (KBr) ν_{max} (cm^{-1}): 1595–1460 (C=C and C=N), 1372 (C=S). ^{1}H NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.25 (2H, s, CH_2), 6.90–7.55 (5H, m, indole protons), 8.10–8.40 (4H, dd,

$J = 8.77$ Hz and 8.70 Hz, phenyl protons), 10.25 (1H, s, N=CH), 10.90 (1H, s, indole NH), 13.80 (1H, s, triazole NH). MS (FAB) *m/z*: 379 [M + 1]. Anal. Calc. for $C_{18}H_{14}N_6O_2S$: C, 57.13; H, 3.73; N, 22.21. Found: C, 57.15; H, 3.75; N, 22.20.

Compound **Ie**: yield 65%, m.p. 200–202 °C. IR (KBr) ν_{max} (cm^{-1}): 1565–1465 (C=C and C=N), 1365 (C=S). ^1H NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.10 (6H, s, $N(CH_3)_2$), 4.20 (2H, s, CH_2), 6.80–7.80 (9H, m, aromatic protons), 10.20 (1H, s, N=CH), 10.95 (1H, s, indole NH), 13.70 (1H, s, triazole NH). MS (FAB) *m/z*: 377 [M + 1]. Anal. Calc. for $C_{20}H_{20}N_6S$: C, 63.81; H, 5.35; N, 22.32. Found: C, 63.83; H, 5.32; N, 22.30.

Compound **IIa**: yield 45%, m.p. 162–164 °C. IR (KBr) ν_{max} (cm^{-1}): 1610–1440 (C=C and C=N). ^1H NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.40–4.60 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 6.90–8.05 (10H, m, aromatic protons), 10.90 (1H, s, indole NH). MS (FAB) *m/z*: 346 [M + 1]. Anal. Calc. for $C_{19}H_{15}N_5S$: C, 66.07; H, 4.38; N, 20.27. Found: C, 66.10; H, 4.30; N, 20.30.

Compound **IIb**: yield 55%, m.p. 220–222 °C. IR (KBr) ν_{max} (cm^{-1}): 1602–1470 (C=C and C=N). ^1H NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.35–4.45 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 6.95–8.20 (9H, m, aromatic protons), 11.20 (1H, s, indole NH). MS (FAB) *m/z*: 380 [M + 1]. Anal. Calc. for $C_{19}H_{14}ClN_5S$: C, 60.08; H, 3.71; N, 18.44. Found: C, 60.11; H, 3.75; N, 18.40.

Compound **IIc**: yield 45%, m.p. 107–109 °C. IR (KBr) ν_{max} (cm^{-1}): 1622–1461 (C=C and C=N). ^1H NMR (250 MHz) (DMSO-d_6) δ (ppm): 2.35 (3H, s, CH_3), 4.30–4.50 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 6.80–8.00 (9H, m, aromatic protons), 11.10 (1H, s, indole NH). MS (FAB) *m/z*: 360 [M + 1]. Anal. Calc. for $C_{20}H_{17}N_5S$: C, 66.83; H, 4.77; N, 19.48. Found: C, 66.85; H, 4.79; N, 19.50.

Compound **IId**: yield 50%, m.p. 224–226 °C. IR (KBr) ν_{max} (cm^{-1}): 1619–1451 (C=C and C=N). ^1H NMR (250 MHz) (DMSO-d_6) δ (ppm): 4.35–4.55 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 7.05–8.15 (9H, m, aromatic protons), 11.30 (1H, s, indole NH). MS (FAB) *m/z*: 391 [M + 1]. Anal. Calc. for $C_{19}H_{14}N_6O_2S$: C, 58.45; H, 3.61; N, 21.53. Found: C, 58.49; H, 3.60; N, 21.50.

Compound **IIe**: yield 40%, m.p. 215–217 °C. IR (KBr) ν_{max} (cm^{-1}): 1630–1481 (C=C and C=N). ^1H NMR (250 MHz) (DMSO-d_6) δ (ppm): 3.10 (6H, s, $N(CH_3)_2$), 4.35–4.45 (4H, m, CH_2 and C_7 protons of triazolothiadiazine), 7.00–8.05 (9H, m, aromatic protons), 11.20 (1H, s, indole NH). MS (FAB) *m/z*: 389 [M + 1]. Anal. Calc. for $C_{21}H_{20}N_6S$: C, 64.93; H, 5.19; N, 21.63. Found: C, 64.95; H, 5.21; N, 21.60.

5.2. Microbiology

Microdilution broth susceptibility assay was used for the antimicrobial evaluation of the compounds, whereas antifungal susceptibility of *C. albicans* was examined according to NCCLS reference method for broth dilution antifungal susceptibility testing of yeasts [28,29]. Chloramphenicol was used as standard antibacterial agent and ketoconazole was used as antifungal agent. Both are prepared as described in the related references.

Tested microorganism strains were *M. luteus* (NRLL B-4375), *B. cereus* (NRRL B-3711), *P. vulgaris* (NRRL B-123), *S. typhimurium* (NRRL B-4420), *S. aureus* (NRRL B-767), *E. coli* (NRRL B-3704), *C. albicans* and *C. glabrata* (isolates obtained from Osmangazi University, Faculty of Medicine) (Table 1).

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