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Synthesis and antimicrobial evaluation of 3-(4-*tert*-amino-2-butynyl)thio and alkyl/alkenylthio-4,5-disubstituted-4*H*-1,2,4-triazoles

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Introduction

Triazoles and, in particular, substituted-1,2,4-triazoles, are among the various heterocycles that have received the most attention during the last 2 decades as potential antimicrobial agents [1–9]. Substitutions have been carried out primarily at the 3-position of the 1,2,4-triazole ring, and include thio [10–12], alkylthio and alkenylthio [13, 14] derivatives. In view of these and other structural modifications, it was of interest to investigate the importance of inserting various degrees of unsaturation on the antimicrobial activity of the triazole ring. Therefore, we have synthesized the following new series of 3-(4-*tert*-amino-2-butynyl)thio-4,5-disubstituted-4*H*-1,2,4-triazoles and 3-(alkyl/alkenyl)thio-4,5-disubstituted-4*H*-1,2,4-triazoles, and have screened them for antimicrobial activity.

Chemistry

The title compounds **9–36** (tables I and II) were prepared by the methods shown in scheme 1. Benzoic acid hydrazide **1** was prepared from the reaction of ethylbenzoate with hydrazine hydrate [15]. Reaction of **1** with the corresponding isothiocyanate yielded 1-benzoyl-4-substituted thiosemicarbazides **2** and **3** [16–18]. Compounds **2** and **3** were cyclized to 4,5-disubstituted-4*H*-1,2,4-triazol-3-yl-thiols **4** and **5** in the presence of 8% aqueous sodium hydroxide [19–21]. Alkylation of **4** and **5** with propargylbromide in alcoholic potassium hydroxide afforded the allylic rearrange product 3-allenylthio-4,5-diphenyl-4*H*-1,2,4-triazole **8** and the expected alkylated product 3-(2-propynyl)thio-4-cyclohexyl-5-phenyl-4*H*-1,2,4-triazole **7**, respectively [22, 23]. Similar rearrangements have been reported in the literature [24–26]. The 3-(2-propynyl)thio-4,5-diphenyl-4*H*-1,2,4-triazole **6** was prepared by stirring the potassium salt of **4** in THF with propargyl bromide at 0°C. The Mannich reaction of the acetylenic triazoles **6** and **7** with paraformaldehyde and the selected secondary amines in peroxide-free dioxane in the presence of catalytic amounts of cuprous chloride yielded the 3-(4-*tert*-amino-2-butynyl)thio-4,5-disubstituted-4*H*-1,2,4-triazoles **9–24** (table I). The IR, NMR spectra and elemental analyses were consistent with the assigned structures. It is interesting to note from the NMR analyses that the presence of a phenyl group at the 4-position of the triazole ring shifted the signals of S-CH_2 to a higher field (δ, 3.9–4.0) in compounds **6** and **9–17** relative to (δ, 4.1–4.19) in compounds **7** and **18–24** where a cyclohexyl was the substituent at the 4-position of the ring. This shielding effect may be attributed to the diamagnetic anisotropy of the phenyl ring (ring current effect) [27]. The 3-(alkyl/alkenyl)thio derivatives **25–36** were prepared by the addition of the desired alkyl/alkenyl halides into the alcoholic potassium salt of 4,5-disubstituted-4*H*-1,2,4-triazol-3-yl-thiols **4** and **5**. The physical constants of the compounds prepared are listed in table II. The IR and NMR spectra were consistent with the assigned structures.

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Table I. 3-(4-*tert*-Amino-2-butynyl)thio-4,5-disubstituted-4*H*-1,2,4-triazoles. All compounds were crystallized from ethanol-water and were analyzed for C, H and N; the results had a maximum deviation of ± 0.4% from theoretical values.

Compound	*R*	*Am*	*mp °C*	*Yield %*	*Formula*
9	phenyl	piperidino	97–99	82	$C_{23}H_{24}N_4S$
10	phenyl	pyrrolidino	119–120	42	$C_{22}H_{22}N_4S$
11	phenyl	N-butylmethylamino	62–64	60	$C_{23}H_{26}N_4S$
12	phenyl	Morpholino	154–156	38	$C_{22}H_{22}N_4OS$
13	phenyl	2-Methylpiperidino	99–100	55	$C_{24}H_{26}N_4S$
14	phenyl	2,6-dimethylpiperidino[a]	102–104	58	$C_{26}H_{29}N_4S$
15	phenyl	perhydroazepino	96–97	74	$C_{24}H_{26}N_4S$
16	phenyl	perhydroazocino	105–107	72	$C_{25}H_{28}N_4S$
17	phenyl	N-methylpiperazine	121–123	40	$C_{23}H_{30}N_4S$
18	cyclohexyl	piperidino	68–70	55	$C_{23}H_{30}N_4S$
19	cyclohexyl	pyrrolidino	84–86	42	$C_{22}H_{28}N_4S$
20	cyclohexyl	N-butylmethyl amino	64–65	58	$C_{23}H_{32}N_4OS$
21	cyclohexyl	Morpholino	58–60	41	$C_{22}H_{28}N_4S$
22	cyclohexyl	2-methylpiperidino	98–99	65	$C_{24}H_{32}N_4S$
23	cyclohexyl	2,6-dimethylpiperidino[a]	120–122	80	$C_{25}H_{35}N_4S$
24	cyclohexyl	perhydroazepino	90–92	74	$C_{24}H_{32}N_4S$

[a]The 2,6-dimethyl in 14 and 23 is *cis* (diequatorial).

Table II. 3-Alkyl/alkenylthio-4,5-disubstituted-4H-1,2,4-triazoles. All compounds were crystallized from ethanol-water and were analyzed for C, H and N; the results had a maximum deviation of ± 0.4% from theoretical values.

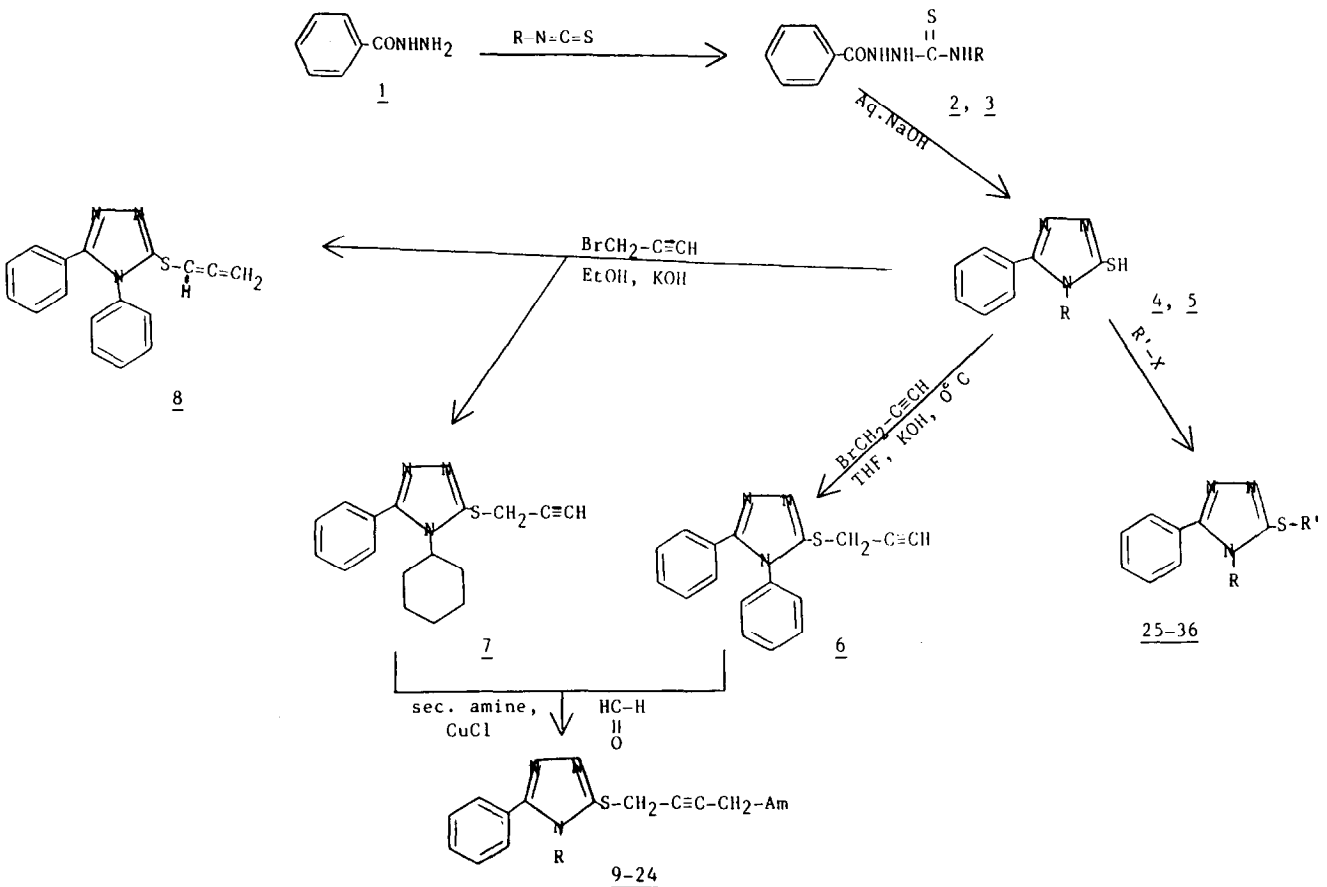
Compound	*R*	*R1*	*mp °C*	*Yield %*	*Formula*
25	phenyl	methyl	159–160	86	$C_{15}H_{13}N_3S$
26	phenyl	ethyl	140–142	68	$C_{16}H_{15}N_3S$
27	phenyl	isopropyl	148–150	90	$C_{17}H_{17}N_3S$
28	phenyl	butyl	118–120	75	$C_{18}H_{19}N_3S$
29	phenyl	benzyl	150–151	88	$C_{21}H_{17}N_3S$
30	phenyl	allyl	108–109	87	$C_{17}H_{15}N_3S$
31	cyclohexyl	methyl	110–111	52	$C_{15}H_{19}N_3S$
32	cyclohexyl	ethyl	96–97	73	$C_{16}H_{21}N_3S$
33	cyclohexyl	isopropyl	103–105	66	$C_{17}H_{23}N_3S$
34	cyclohexyl	butyl	88–90	93	$C_{18}H_{25}N_3S$
35	cyclohexyl	benzyl	132–133	68	$C_{21}H_{23}N_3S$
36	cyclohexyl	allyl	80–81	79	$C_{17}H_{21}N_3S$

Antimicrobial activity

The antimicrobial activity of compounds **4–36** were tested against various Gram-positive organisms (table III), the Gram-negative bacilli *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella sonnei*, and the *Candida* species·*C albicans* and *C pseudotropicalis*. Streptomycin was used as a reference compound against these organisms. All compounds tested were inactive against the Gram-negative bacilli and the *Candida* species (data not shown). This lack of inhibition may be attributed to the complex nature of the cell envelope of these organisms which prevents the penetration of the compounds, and therefore the inability to attain inhibitory concentrations within the cell.

It is apparent from table III that the 4,5-disubstituted-4*H*-1,2,4-triazol-3-yl-thiols **4** and **5**, the acetylenic derivatives **6** and **7** and the alkyl/alkenylthio ethers **25–36** were inactive against Gram-positive bacteria, whereas most of the Mannich reaction products **11–24** were active.

The minimum inhibitory concentrations (MIC) of selected derivatives against the 3 Gram-positive organisms are presented in table IV. The MIC of the Mannich products with cyclohexyl substituents at the 4-position of the triazole ring, compounds **20** and **24**, were lower than that of the corresponding compounds with a phenyl group at the 4-position, compounds **11** and **15**. This difference in activity may be associated with the changes in the lipophilic properties of the more effective compounds.



Scheme 1.

In conclusion we may state that variation in the degree of unsaturation of the thioethers as in alkyl, alkenyl and alkyne derivatives of 4,5-disubstituted-4*H*-1,2,4-triazol-3-yl-thiol did not result in formation of active compounds. However, conversion of the terminal acetylenic proton into corresponding Mannich products frequently resulted in compounds with antimicrobial activity against Gram-positive organisms. These results suggest the importance of the amino functional group or its ability to generate a cation at physiological pH with respect to the antimicrobial activity.

Experimental protocols

Chemical methods

Melting points were determined by using a calibrated Thomas Hoover melting point apparatus. IR spectra were recorded using a Unicam SP-300 spectrophotometer. NMR spectra were determined on a Varian FT80 A spectrometer. Microanalyses were performed in the laboratories of H Malissa and G Reuter, FRG. The analyses are indicated only by symbols of the elements analyzed. The results obtained had a maximum deviation of ± 0.4% from the theoretical value.

Starting material

Benzoic acid hydrazide **1** was prepared from ethylbenzoate and hydrazine hydrate as previously described by Gatterman and Wieland [15]. 1-Benzoyl-4-substituted thiosemicarbazides **2** and **3** were synthesized from the reaction of **1** with the corresponding isothiocyanates according to the methods described in the literature [16–18].

*4,5-Diphenyl-4*H*-1,2,4-triazol-3-yl-thiol* ***4***

1-Benzoyl-4-phenylthiosemicarbazide (0.4 mol) was refluxed in 100 ml 8% aqueous sodium hydroxide solution for 8 h. The mixture was cooled to room temperature, filtered and the filtrate was neutralized with 10% acetic acid. The crude product was collected and crystallized from aqueous ethanol, affording **4** in 90% yield, mp 278–280°C. Anal $C_{14}H_{11}N_3S$ (C, H, N, S).

Table III. Antimicrobial activity of compounds **4–36**. –: no growth; +: 11–15 mm diameter of inhibition zone; ++: 16–20 mm diameter of growth inhibition zone; NT: not tested.

Compound	*Diameter of inhibition zone*		
	S aureus	B subtilis	S faecalis
4	–	–	–
5	–	–	–
6	–	–	–
7	–	–	–
9	–	–	–
10	–	–	–
11	+	+	+
12	–	–	–
13	–	–	–
14	NT	NT	NT
15	+	+	+
16	–	–	–
17	+	+	+
18	+	+	+
19	–	–	–
20	+	++	+
21	–	–	–
22	+	+	+
23	+	+	+
24	+	++	+
25–36	–	–	–
Streptomycin	+	+	+

Table IV. Minimum inhibitory concentration (MIC) of compounds **11**, **15**, **17**, **18**, **20**, **21**, **22** and **24**. –: no growth; +: growth.

Compd	*Conc μg/ml*	S aureus	B subtilis	S faecalis
11	1000	–	–	–
	500	+	+	+
15	1000	–	–	–
	500	+	+	+
17	1000	–	–	–
	500	+	+	+
18	1000	–	–	–
	500	+	+	+
20	1000	–	–	–
	500	–	–	–
	250	–	–	–
	125	+	+	+
21	1000	–	–	–
	500	+	+	+
22	1000	–	–	–
	500	+	+	+
24	1000	–	–	–
	500	–	–	–
	250	+	+	+

*4-Cyclohexyl-5-phenyl-4*H*-1,2,4-triazol-3-yl-thiol* ***5***

This compound was prepared by the same procedure indicated for **4** and was obtained in 81% yield, mp 179–180°C, Anal $C_{14}H_{17}N_3S$ (C, H, N, S).

*3-(2-Propynyl)thio-4,5-diphenyl-4*H*-1,2,4-triazole* ***6***

To a stirred solution of 4,5-diphenyl-4*H*-1,2,4-triazol-3-yl-thiol **4** (0.10 mol) in 600 ml THF was added 0.10 mol potassium hydroxide with continuous stirring. The reaction mixture was cooled to 0°C, then 0.11 mol cold propargyl bromide was added dropwise. The reaction mixture was stirred for 1 h at 0°C, filtered and the filtrate poured into 1 000 ml cold water. The precipitate was crystallized from aqueous ethanol in 88% yield, mp = 128–130°C. Anal $C_{17}H_{13}N_3S$ (C, H, N, S). The IR spectrum showed the following characteristic absorption bands (KBr, cm^{-1}), 3280 (≡CH), 3060 (CH, ArH), 2810 (CH_2), 2100 (weak C≡C), 1580 (C=N), and 1500, 1450 (C⋯C, Ar). The NMR spectrum provided the following characteristic chemical shifts ($CDCl_3$, δ) 4.01 (doublet, 2H, S-CH_2, J = 2.6 Hz) and 2.25 (triplet IH, =CH, J = 2.6 Hz). Other signals in the NMR spectra were consistent with the various protons in the aromatic rings.

*3-(2-Propynyl)thio-4-cyclohexyl-5-phenyl-4*H*-1,2,4-triazole 7*

To a stirred solution of 4-cyclohexyl-5-phenyl-4*H*-1,2,4-triazol-3-yl-thiol **5** (0.10 mol) in 500 ml absolute ethanol was added a solution of 0.10 mol potassium hydroxide in 50 ml ethanol. Propargyl bromide (0.11 mol) was added dropwise. The reaction mixture was refluxed for 1 h. After cooling to room temperature, the solution was filtered and the filtrate was poured into 1 000 ml cold distilled water. The crude product was crystallized from ethanol–water, affording **7** in 88% yield, mp: 126–128°C. Anal $C_{17}H_{19}N_3S$ (C, H, N, S). The IR spectrum showed the following characteristic absorption bands (KBr, cm^{-1}), 3280 (≡CH), 3030 (ArH), 2940, 2850 (CH_2), 2100 (weak, C≡C), 1850 (C=N), 1500, 1450 (C⋯C, Ar). NMR spectra demonstrated the following chemical shifts ($CDCl_3$, δ), 4.15 (doublet, 2H, S-CH_2, J = 2.6 Hz) and 2.26 (triplet, 1H, CH, J = 2.6 Hz). Other signals in the NMR spectrum were consistent with the various protons in the aromatic and cyclohexyl rings.

*3-Allenylthio-4,5-diphenyl-4*H*-1,2,4-triazole* ***8***

This compound was obtained when 4,5-diphenyl-4*H*-1,2,4-triazol-3-yl-thiol **4** was treated with propargyl bromide by the same procedure described for preparation of **7**. Compound **8** was obtained in 84% yield with a mp = 112–113°C. The IR spectrum showed the following characteristic absorption bands (KBr, cm^{-1}), 3040 (ArH), 1940 (Strong, C=C=C). NMR spectrum revealed the following chemical shifts 7.32 (multiplet, 10H, ArH), 6.24 (triplet, 1H, S-CH=C-), 4.92 (doublet, 2H, C=CH_2).

*3-(4-*tert*-Amino-2-butynyl)thio-4,5-disubstituted-4*H*-1,2,4-triazoles* ***9–24***

A mixture of 0.003 mol 3-(2-propynyl)thio-4,5-disubstituted-4*H*-1,2,4-triazole **6** or **7**, 0.003 mol *para*formaldehyde, 0.003 mol of the appropriate secondary amine and cuprous chloride (catalytic amount) in 10 ml peroxide-free dioxane was heated at 70°C for 3 h. After cooling, each reaction mixture was filtered and ice water (25 ml) was added to the filtrate. The crude products were collected and crystallized from

ethanol–water. Yields, melting points and elemental analyses are listed in table I. The IR spectra showed the following absorption bands (KBr, cm^{-1}), 3040 (ArH), 2100 (weak, C≡C), 1580 (C=N), 1500, 1450 (C=C, Ar). The NMR spectra indicated the following range of chemical shifts ($CDCl_3$, δ), 3.90–4.19 (triplet, 2H, S-CH_2, J = 2.2 Hz), 3.15–3.25 (triplet, 2H, ≡C-CH_2-N, J = 2.2 Hz). Other signals in the NMR spectra were consistent with the various protons in the aromatic and cyclohexyl rings and the secondary amines.

*3-(Alkyl/alkenyl)-thio-4,5-disubstituted-4*H*-1,2,4-triazoles* ***25–36***

To a stirred solution of 0.05 mol 4,5-disubstituted-4*H*-1,2,4-triazol-3-yl-thiol **4** and **5** in 200 ml absolute ethanol was added 0.05 mol potassium hydroxide. The appropriate alkyl or alkenyl halide (0.05 mol) was added dropwise, and the mixtures were refluxed for 1 h. After pouring into ice-cold water (500 ml), each crude product was crystallized from ethanol. Yields, melting points and elemental analyses are listed in table II. The IR spectra showed the following characteristic absorption bands (KBr, cm^{-1}), 3080 (=CH), 3040 (ArH), 2950, 2850 (CH_2), 1660 (C=C), 1580 (C=N), 1500, 1450 (C—C, Ar), and 1385 (CH_3-C-CH_3). The NMR spectra provided the following characteristic chemical shifts ($CDCl_3$, δ), 7.50, 7.30 (m, ArH), 5.60 (m, 1H, =CH), 5.20 (m, 2H, =CH_2), 3.70 or 3.60 (m, 2H, CH_2-C=C), 3.35 or 3.25 (m, 1H, S-CH), 3.15 or 3.00 (q, 2H, S-CH_2), 2.90 or 2.70 (S, 3H, S-CH_3), 1.42 (t, 3H, S-C-CH_3), 1.40 (d, 6H, S-C-$(CH_3)_2$), and 2.01, 1.75, 1.25 (m, 11H, cyclohexyl).

Microbiological methods

Test organisms and culture media

Staphylococcus aureus ATCC25923, *Streptococcus faecalis* ATCC19433, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Shigella Sonnei* ATCC25931 were cultivated in Trypton soya broth and agar (Oxoid), while *Candida albicans* (local isolate) and *Candida pseudotropicalis* ATCC612 were grown in yeast glucose (5 g yeast extract, 30 g glucose in 1 liter distilled water) and yeast glucose agar prepared by addition of 2% agar (Oxoid).

Antimicrobial assay

Screening for anti-microbial activity of all compounds was initially performed employing the well-agar diffusion technique [23]. Each compound was tested at a concentration of 1 mg/ml in dimethylsulfoxide (DMSO)/methanol (1/9). The zones of inhibition were measured after 18 h incubation at 37°C. The compounds which exhibited anti-microbial activity were then subjected to a secondary assay to determine their minimum inhibitory concentrations (MIC). Each compound was suspended in Tween 80 and 2-fold serial dilutions in molten agar were prepared with final concentrations ranging from 1 000 to 62.5 μg/ml [28]. The agar suspensions were poured into sterile plates, and after solidification 10 μl (containing 10^4-10^5 cells) of each susceptible bacterial suspension was applied as a drop on the surface of the agar. The drops were allowed to dry without spreading. The plates were then incubated at 37°C and examined after 18 h for the presence of a growth. The MIC was defined as the lowest concentration inhibiting visible growth. In each assay, a control test which contained the solvent system only was also carried out and showed that neither DMSO: methanol not Tween 80 suspension exhibited growth inhibitory effects. Streptomycin sulfate (0.5 mg/ml) and phenol (40 mg/ml) were used as a standard antimicrobial compounds against bacterial and candidal species, respectively. All experiments were performed in triplicate.

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