Synthesis and Antimicrobial Properties of New 4-(Alkylidene/arylidene)-amino-5-(2-furanyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones and 6-Aryl-3-(2-furanyl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4]thiadiazines

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Summary

A series of 4-(alkylidene/arylidene)amino-5-(2-furanyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (2) and 6-aryl-3-(2-furanyl)-7*H*-1,2,4-triazolo[3,4-*b*][1,3,4] thiadiazines (3) were synthesized. The configuration of 2g was assigned on the basis of ^{1}H-NMR data. Of the new derivatives tested, only 2b, 2g, and 4f were found to be active against *Staphylococcus aureus* and/or *Staphylococcus epidermidis* (MIC 125-1.95 µg/ml), whereas all exhibited varying degrees of antifungal activity (MIC 25-0.8 µg/ml).

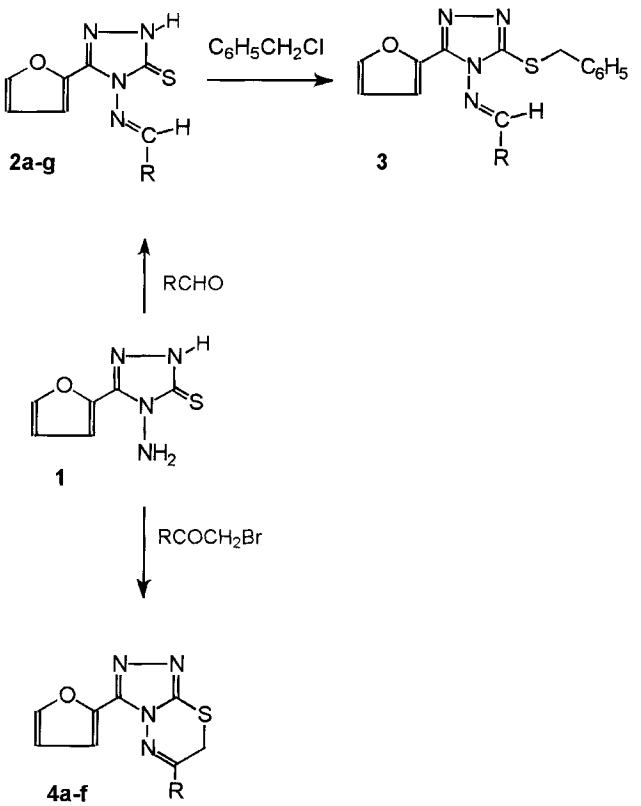
Introduction

The prevalence of resistant infections in spite of existing useful antimicrobial agents decreases the chemotherapeutic and chemopreventive alternatives and stimulates the search for new compounds. The 1,2,4-triazole nucleus and N-bridged heterocycles derived from it have recently been incorporated into a variety of compounds with antibacterial[1], antifungal[2] and antiparasitic[3] properties. As part of a synthethic effort directed toward the synthesis of antimicrobial agents[4], 4-amino-5-(2-furanyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**1**)[5], a versatile substrate for mono- and difunctional electrophiles by virtue of its vicinal amino and thione/thiole groups, has been condensed with aldehydes and ω-bromoacetophenones to afford 4-(alkylidene/arylidene)amino-5-(2-furanyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**2a-g**) and 6-aryl-3-(2-furanyl)-7*H*-1,2,4-triazolo-[3,4-*b*][1,3,4]thiadiazines (**4a-f**), respectively.

Results and Discussion

Chemistry

The key intermediate **1** was prepared from furan-2-carbohydrazide following the method of Reid and Heindel[6]. Condensation of **1** with appropriate aldehydes in ethanolic medium furnished **2a-g** in good yields (Scheme 1 and Table 1). **2a-g** are capable of existing in thiole or thione forms either as the *E* or *Z* isomers. The low field NH (δ 14.1 ppm)[7] and N=CH resonances (δ 9.84–10.01 ppm)[1] observed in the ^{1}H-NMR spectra indicated the presence of the thione form and only one isomer, the *E* isomer. The paramagnetic shift observed for the N=CH proton was attributed to the anisotropy of the thiocarbonyl group which could be effective only in the *E* form. To confirm this assignment **2a** was reacted with $C_6H_5CH_2Cl$ in the presence of K_2CO_3 to yield the thioether derivative **3**. As expected, the ^{1}H-NMR spectrum of **3** displayed the N=CH proton at δ 8.73 ppm[8]. **2g** existed as the *E* configurated species about the ethylenic bond as reflected by the 16 Hz coupling constant[9] observed in the ^{1}H-NMR



Scheme 1

  0365-6233/96/0809-0427 $5.00 + .25/0

Table 1. Formula, % yields and melting points of **2–4**.

Comp.	R	Mp [°C]	Yield [%]	Formula [a] (Molecular mass)
2a	4-$CH_3C_6H_4$	224–225	76	$C_{14}H_{12}N_4OS$ (284.34)
2b	4-BrC_6H_4	219–220	67	$C_{13}H_9BrN_4OS$ (349.21)
2c	2,5-(OH)BrC_6H_3	218–219	95	$C_{13}H_9BrN_4O_2S$ (365.21)
2d	2,6-$Cl_2C_6H_3$	234	70	$C_{13}H_8Cl_2N_4OS$ (339.19)
2e	4-COOH C_6H_4	240–241	79	$C_{14}H_{10}N_4O_3S$ (314.32)
2f	2- $NO_2C_6H_4$	233–234	90	$C_{13}H_9N_5O_3S$ (315.31)
2g	2-(5-nitro-2-furyl)-ethenyl	219–220	87	$C_{13}H_9N_5O_4S$ (331.31)
3	4-$CH_3C_6H_4$	98–99	73	$C_{21}H_{18}N_4OS$ (374.12)
4a	C_6H_5	163–165	75	$C_{14}H_{10}N_4OS$ (282.32)
4b	4-$CH_3C_6H_4$	185–186	89	$C_{15}H_{12}N_4OS$ (296.36)
4c	4-$OCH_3C_6H_4$	172–173	96	$C_{15}H_{12}N_4O_2S$ (312.36)
4d	4-ClC_6H_4	220–221	81	$C_{14}H_9ClN_4OS$ (316.76)
4e	4-BrC_6H_4	234–235	88	$C_{14}H_9BrN_4OS$ (361.22)
4f	4-$NO_2C_6H_4$	>300	91	$C_{14}H_9N_5O_3S$ (327.32)

[a] Satisfactory analyses were obtained.

spectrum. Since *s-trans* conformation (about the sp^3 C-C bond) has been reported to be the more stable form[10] the structure of **2g** is proposed as *s-trans*. The structures were quite stable under EI and provided abundant molecular ions. The major fragmentation pattern observed in the EIMS involved N-N bond cleavage at position 4- of the 1,2,4-triazole system furnishing $C_6H_5N_3OS^{+\cdot}$ (*m/z* 167) and $RC_7H_4N^{+\cdot}$ which subsequently led to fragments characteristic for the 1,2,4-triazole and aldehyde moieties[11,12]. Additional spectral characteristics are presented in the experimental.

Reaction of **1** with substituted ω-bromoacetophenones in absolute ethanol yielded **4a-f** (Scheme1 and Table 1). The literature method[3] was slightly modified and the neutralization step was omitted since the products before and after neutralization were identical (TLC, m.p., IR). Absence of the N-H absorptions in both IR and ^{1}H-NMR spectra and observation of additional resonances assigned to the SCH_2 (δ 4.00–4.07 ppm) and to the aryl protons (δ 6.94–8.42 ppm) at the 6-position in the ^{1}H-NMR spectra confirmed cyclocondensation to **4a-f**[13]. Further verification for ring closure was obtained from EIMS where the molecular ions were the base peaks. The major fragmentation route involved concomitant breaking of the N–N and C–C bonds of the 1,3,4-thiadiazine system, yielding fragments $C_7H_5N_3OS^{+\cdot}$ (*m/z* 179) and $RC_7H_4N^{+\cdot}$, which was consistent with the literature[3]. Additional spectral details are presented in the experimental section.

Microbiology

2a–g and **4a–f** were evaluated for *in vitro* antimicrobial action against some bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* UC 57, *Shigella flexneri*, *Pseudomonas aeruginosa* ATCC 1539, *Proteus mirabilis* and *Salmonella typhi*) and fungi (*Trichophyton tonsurans* NCPF 245, *Trichophyton mentagrophytes* var. *erinacei* ATCC 375, *Trichophyton mentagrophytes*, *Microsporum gypseum* NCPF 580, *Microsporum canis*, *Microsporum audouinii*) using the microdilution method[14,15]. Mueller Hinton Broth, Yeast Nitrogen Base (YNB Difco) and Nutrient Broth media were used in the tests. The substances and the standards – nifuroxazide, miconazole and clotrimazole – were dissolved in DMSO to give serial dilutions of 250–0.49 or 234–0.49 μg/ml and 25–0.20 μg/ml for the antibacterial and antifungal activity tests, respectively. Only **2b**, **2g**, and **4f** showed antibacterial activity whereas all the compounds showed varying degrees of antifungal activity against the microorganisms used (Table 2).The correlation between structure and antifungal activity in these series was not straightforward, however, some trends could be observed. As can be seen in Table 2 compounds **2** were more active than compounds **4**. Among **2**, the 4-substituted derivatives were more active than other entries with substituents at the 2- and/or 5-positions where there was no preference for the electronic nature of the substituent. The most active compound was the 4-Br substituted derivative **2b** followed by the 4-CH_3 and 4-COOH substituted derivatives **2a** and **2e**. Potency was insensitive to the electronic nature of the substituent on the phenyl ring in **4** also, where the most active

Table 2. Antibacterial and antifungal activity of **2** and **4** (MIC μg/ml).

Comp.	Microorganism							
	A	B	C	D	E	F	G	H
2a			6	12.5	6	6	12.5	6
2b	117	125	0.8	12.5	6.2	12.5	6.2	3
2c			12.5	12.5	12.5	12.5	25	6.2
2d			25	25	25	25	25	25
2e			3	12.5	12.5	12.5	0.8	12.5
2f			25	>25	25	25	>25	25
2g	3.66	1.95	12.5	12.5	12.5	12.5	25	6.2
4a			3	25	25	25	25	25
4b			25	>25	12.5	25	25	25
4c			>25	>25	6.2	6.2	>25	>25
4d			25	>25	>25	25	>25	>25
4e			25	>25	25	25	25	25
4f		7.81	25	>25	6.2	12.5	>25	25
Miconazole			0.2	0.2	0.2	0.2	0.2	0.2
Clotrimazole			0.2	0.2	0.2	0.2	0.2	0.2
Nifuroxazide	0.97	0.97						

A = *S.aureus* ATCC 6538, B = *S. epidermidis* ATCC 12228, C = *T. tonsurans* NCPF 245, D = *T. mentagrophytes* var. *erinacei* ATCC 375, E = *T. mentagrophytes*, F = *M. gypseum* NCPF 580, G = *M. canis*, H = *M. audouinii*

derivative was the unsubstituted compound **4a** followed by the 4-CH_3 and 4-NO_2 substituted entries **4b** and **4f**.

Experimental

M.p.'s: Büchi (Tottoli) melting point apparatus, uncorrected.– UV spectra: Shimadzu UV 2100S.– IR spectra: Perkin Elmer 1600 FT-IR (KBr).– ^{1}H-NMR spectra: Bruker AC 200(200 MHz) (TMS/$CDCl_3$ or ([D6]DMSO).– EIMS: Pennsylvania State University (USA) or VG Zab Spec (70 eV)– TLC: precoated plates 60 F_{254} (Merck Art. 5735) ($CHCl_3$:$C_2H_5COCH_3$, 1:4 or CH_3COCH_3:C_6H_6, 3:7).– Elemental analysis: Carlo Erba 1106.

*4-(Alkylidene/arylidene)amino-5-(2-furanyl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones (**2a-g**)*

A mixture of **1** (0.01 mol) and an appropriate aldehyde (0.01 mol) in C_2H_5OH (150 ml) was refluxed for 3h. The crude product that solidified on cooling was filtered, dried and recrystallized from C_2H_5OH.

2a UV (C_2H_5OH): λ max nm (log ε) = 287.8 (4.300), 254.4 (4.433), 215.4 (4.250).– IR (KBr): ν cm^{-1} = 3085 (N-H), 1625, 1598, 1564, 1525, 1503 (C=N, C=C).– ^{1}H-NMR ([D6]DMSO): δ = 14.16 (s, 1H, NH), 9.84 (s, 1H, N=CH), 7.95 (s, 1H, furan H5), 7.85 (d, *J* = 8.4 Hz, 2H, ar), 7.49 (d, *J* = 8.4 Hz, 2H, ar.), 7.10 (d, $J_{3,4}$ = 3.4 Hz, 1H furan H3), 6.72 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H, furan H4), 2.49 (s, 3H, CH_3).– MS (70 eV): *m/z* (%) = 284 (100) [M^+], 256(6) [$M^+ - N_2$], 252(2) [M^+–S], 246 (3), 193 (6), 167 (64), 151(8), 138 (11), 117 (68), 108 (78), 102 (25), 94 (32), 93 (46), 79 (34), 63 (41).

2e UV (C_2H_5OH): λ max nm (log ε) = 289.4 (4.328), 254.6 (4.518).– IR (KBr): ν cm^{-1} = 3354, 3100 (O-H/N-H), 1689 (C=O), 1630, 1612, 1564, 1558, 1529 (C=N, C=C).– ^{1}H-NMR ([D6]DMSO): δ = 14.12 (s, 1H, NH), 13.20 (bs, 1H, OH), 10.01 (s, 1H, N=CH), 8.13 (d, *J* = 8.3 Hz, 2H, ar.), 8.06 (d, *J* = 8.3 Hz, 2H, ar.), 7.96 (s, 1H, furan H5), 7.15 (d, $J_{3,4}$ = 3.4 Hz, 1H, furan H3) 6.72 (dd, $J_{3,4}$ = 3.4 Hz, $J_{4,5}$ = 1.5 Hz, 1H, furan H4). ;MS (70 eV): *m/z* (%) = 314(79) [M^+], 282 (4) [M^+–S], 269 (4) [M^+–COOH], 245 (3), 221 (3), 203 (4), 193 (3), 192 (3), 170 (4), 167 (100), 147 (6), 130 (10), 108 (38), 102 (6), 94 (17), 93 (10), 39 (22).

*3-(Benzylthio)-5-(2-furanyl)-4-(4-methylbenzylidene)amino-4H-1,2,4-triazole (**3**)*

A mixture of **2a** (0.005 mol), $C_6H_5CH_2Cl$ (0.005 mol) and K_2CO_3 (0.01 mol) in CH_3COCH_3 (20 ml), was refluxed for 3h. Excess of solvent was distilled off to leave a viscous oil which solidified on trituration with petroleum ether. The solid thus obtained was recrystallized from C_2H_5OH to afford **3**.

3 UV (C_2H_5OH): λ max nm (log ε) = 267.5 (4.527).– IR (KBr): ν cm^{-1} = 1599, 1562, 1514, 1495 (C=N, C=C).– ^{1}H-NMR ([D6]DMSO): δ = 8.73 (s, 1H, N=CH), 7.87 (s, 1H, furan H5), 7.81 (d, *J* = 8.0 Hz, 2H, ar.), 7.39 (d, *J* = 8.0 Hz, 2H, ar.), 7.33–7.25(m, 5H, ar.), 6.92 (d, $J_{3,4}$ = 3.2 Hz, 1H, furan H3), 6.66 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 1.5 Hz, 1H, furan H4), 4.41(s, 2H, SCH_2), 2.40(s, 3H, CH_3).

*6-Aryl-3-(2-furanyl)-7H-1,2,4-triazolo[3,4-b][1,3,4]thiadiazines (**4a-f**)*

To a solution of **1** (0.01 mol) in absolute C_2H_5OH was added an appropriate ω-bromoacetophenone (0.01 mol). The mixture was refluxed for 5 h. The solid obtained during reflux or on cooling was filtered, dried and recrystallized from C_2H_5OH.

4e UV (C_2H_5OH): λ max nm (log ε) = 302.2(4.337), 261.4(4.414).– IR (KBr): ν cm^{-1} = 1621, 1584, 1557, 1515, 1490 (C=N, C=C).– ^{1}H-NMR($CDCl_3$): δ = 7.81 (d, *J* = 8.7 Hz, 2H, ar.), 7.69 (d, *J* = 8.7 Hz, 2H, ar.), 7.65 (s, 1H , furan H5), 7.16 (d, $J_{3,4}$ = 3,3 Hz, 1H, furan H3), 6.58 (dd, $J_{3,4}$ = 3,3 Hz, $J_{4,5}$ = 1.5 Hz, 1H, furan H4), 4.00 (s, 2H, SCH_2).– MS (70 eV): *m/z* (%) = 360, 362 (100, 98) [M^+, $(M+2)^+$], 281 (5) [M^+–Br], 253(6), 225, 227 (7) (only ^{79}Br is given), 208, 210 (7), 195, 197 (49), 188 (10), 181, 183

(62), 179 (75), 160 (13), 155, 157 (32), 151 (34), 137 (21), 121 (62), 116 (10), 109 (65), 102 (89), 93 (78), 90 (5), 89 (17), 83 (27), 76 (60), 64 (32).

4f UV (C_2H_5OH): λ max nm (log ε) = 305.4 (4.540), 266.1 (4.605).– IR(KBr): ν cm^{-1} = 1618, 1607, 1598, 1523, 1514 (C=C, C=N, NO_2), 1346 (NO_2).– ^{1}H-NMR($CDCl_3$): δ = 8.42 (d, *J* = 8.8 Hz, 2H, ar.), 8.12 (d, *J* = 8.8 Hz, 2H, ar.), 7.68 (s, 1H, furan H5), 7.16 (d, $J_{3,4}$ = 3.4 Hz, 1H furan H3), 6.61 (s (distorted singlet), 1H, furan H4), 4.07 (s, 2H, SCH_2).– MS (70 eV): *m/z* (%) = 327 (100) [M^+], 297 (14) [M^+–NO], 281 (8) [M^+–NO_2], 179 (25), 162 (18), 160 (5), 151 (10), 148 (50), 137 (18), 121 (44), 119 (22), 118 (19), 109 (34), 102 (37), 93 (55), 90 (26), 89 (26), 83 (8), 76 (33), 64 (25), 58 (66).

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