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Short Communication

Synthesis of some new biologically active thiadiazolotriazinones–Part III☆

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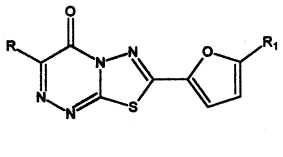
Abstract

4-Amino-6-arylmethyl/*tert*-butyl-3-mercapto-1,2,4-triazin-5(4H)-ones (**1**) were condensed with arylfuroic acids (**2**) to yield 7-(5-aryl-2-furyl)-3-arylmethyl/*tert*-butyl-4H-1,3,4-thiadiazolo[2,3-c]-1,2,4-triazin-4-ones (**3**). The newly synthesized compounds exhibited antibacterial activity comparable to that of nitrofurazone. In addition, two compounds displayed in vitro antitumor activity with moderate growth inhibition against a panel of 60 tumor cell lines.

Keywords: Arylmethyltriazinones; Arylfuroicacid; Thiadiazolotriazinones; Antibacterial activity; Antitumor activity

1. Introduction

In continuation of our studies on the synthesis of biologically active thiadiazolotriazinones [1,2] and in an attempt to significantly improve antibacterial activity, we considered substitution at C7 by furyl moieties. It was hoped that these compounds (general structure I), in addition to retaining good properties of previous 7-aryl thiadiazolotriazinones, would benefit from the incorporation of great part of the furaldehyde semicarbazone pattern(O=C–N–N=C-furyl) characteristic of nitrofuran antibiotics. A similar strategy on the triazolothiadiazole template produced antibacterials comparable or superior to nitrofurazone; actually, we found that replacement of nitro by halophenyls as the 2-furyl substituent (e.g. Ib versus IIa) was advantageous [3]. Following these results, and with a view to avoid long term toxicity of nitrofuran drugs, halophenylfurans were selected as C7 moieties for the present work.



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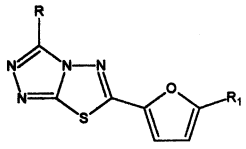

IIa R_1=NO_2, IIb R_1=2,5-dichlorphenyl

Moreover selection of C3 substituents was made after previous results in this template, favoring alkyl [2], benzyl and halo-benzyl groups. Screening of these novel thiadiazolotriazinones was expanded to include antitumor activity.

2. Chemistry

For the present work, three 6-arylmethyl-4-amino-3-mercapto-1,2,4-triazin-5(4H)-ones (R = benzyl, 4-chlorobenzyl and 2,4-dichlorobenzyl) were prepared by condensing the respective azalactones with thiocarbohydrazide [4]. The 6-*tert*-butyl-4-amino-3-mercapto-1,2,4-triazin-5(4H)-one was obtained commercially and used after recrystallization from ethanol. The 5-aryl-2-furoic acids (**2**) were synthesized through Meerwein reaction [5]. The triazinones **1** were then condensed

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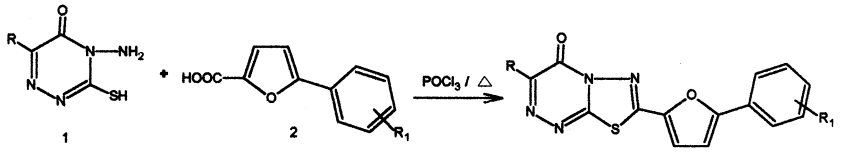
Table 1
Data of 7-substituted-3-arylmethyl/*tert*-butyl-1,3,4-thiadiazolo-[2,3-c]-1,2,4-triazin-4-ones (**3**)



Compound No.	R	R_1	m.p (°C)	Yield (%)	Molecular formula
3a	tert-Butyl	2,4-Dichloro	233-35	85	$C_{18}H_{14}Cl_2N_4O_2S$
3b	tert-Butyl	3-Chloro-4-fluoro	238-40	81	$C_{18}H_{14}ClFN_4O_2S$
3c	tert-Butyl	4-Bromo	223-25	82	$C_{18}H_{15}BrN_4O_2S$
3d	Benzyl	2,4-Dichloro	182-84	78	$C_{21}H_{12}Cl_2N_4O_2S$
3e	Benzyl	3-Chloro-4-fluoro	190-92	81	$C_{21}H_{12}ClFN_4O_2S$
3f	Benzyl	4-Bromo	225-27	80	$C_{21}H_{13}BrN_4O_2S$
3g	4-Chlorobenzyl	2,4-Dichloro	190-92	76	$C_{21}H_{11}Cl_3N_4O_2S$
3h	4-Chlorobenzyl	3-Chloro-4-fluoro	152-58	73	$C_{21}H_{11}Cl_2FN_4O_2S$
3i	4-Chlorobenzyl	4-Bromo	165-67	79	$C_{21}H_{12}BrClN_4O_2S$
3j	2,4-Dichlorobenzyl	2,4-Dichloro	242-44	73	$C_{21}H_{10}Cl_4N_4O_2S$
3k	2,4-Dichlorobenzyl	3-Chloro-4-fluoro	236-38	70	$C_{18}H_{14}Cl_2N_4O_2S$
3l	2,4-Dichlorobenzyl	4-Bromo	220-22	68	$C_{21}H_{11}BrCl_2N_4O_2S$

All the compounds were analyzed satisfactorily for their N content (± 0.3%). IR (KBr, γ_{max} cm^{-1}): **3f**, 3111(C–H), 1699(C=O), 1537(C=N), 1519(C=C); **3h**, 3107(C–H), 1698(C=O), 1498(C=C), 1082(C–F), 736(C–Cl); **3j**, 1694(C=O), 1588 (C=N), 1518(C=C), 734(C–Cl). ^{1}H NMR (300 MHz, $CDCl_3$): **3a**, δ, 1.48 (s, 9H, $C(CH_3)_3$), 6.91 (d, 1H, furan H, $J = 3.7$ Hz), 7.36–7.51 (m, 1H, Ar–H), 7.53 (d, 1H, furan H, $J = 3.7$ Hz), 7.71 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.83 (d, 1H, Ar–H, $J = 8.7$ Hz); **3e**, δ, 4.29 (s, 2H, $-CH_2-$), 6.88 (d, 1H, furan H, $J = 3.7$ Hz), 7.45 (d, 1H, furan H, $J = 3.7$ Hz), 7.21–7.54 (m, 5H, Ar–H), 7.72 (d, 1H, Ar–H, $J = 8.7$ Hz), 7.83 (d, 1H, Ar–H, $J = 8.7$ Hz); **3f**, δ, 4.23(s, 2H, $-CH_2-$), 7.17 (d, 1H, furan H, $J = 3.6$ Hz), 7.13–7.49 (m, 6H, Ar–H), 7.55 (d, 1H, furan H, $J = 3.6$ Hz), 7.62(d, 1H, Ar–H, $J = 7.5$ Hz), 7.74 (d, 1H, Ar–H, $J = 7.5$ Hz), 7.99 (d, 1H, Ar–H, $J = 10.2$ Hz); **3g**, δ, 4.29 (s, 2H, $-CH_2-$), 6.88 (d, 1H, furan H, $J = 3.7$ Hz), 7.16–7.59 (m, 5H, Ar–H), 7.46 (d, 1H, furan H, $J = 3.7$ Hz), 7.7 (d, 1H, Ar–H, $J = 8.5$ Hz), 7.82 (d, 1H, Ar–H, $J = 8.5$ Hz); **3j**, δ, 4.43(s, 2H, $-CH_2-$), 6.9 (d, 1H, furan H, $J = 3.7$ Hz), 7.20–7.55 (m, 4H, Ar–H), 7.51 (d, 1H, furan H, $J = 3.7$ Hz), 7.72 (d, 1H, Ar–H, $J = 8.6$ Hz), 7.86 (d, 1H, Ar–H, $J = 8.6$ Hz); **3k**, δ, 4.43 (s, 2H, $-CH_2-$), 6.88 (d, 1H, furan H, $J = 3.7$ Hz), 7.21–741 (m, 2H, Ar–H), 7.35–4.41 (m, 2H, Ar–H), 7.51 (d, 1H, furan H, $J = 3.7$ Hz), 7.66 (d, 1H, Ar–H), 7.82 (d, 1H, Ar–H, $J = 8.9$Hz). MS: 3a, m/z 420 (24%, M^+), 392 (14%, M^+–CO), 237 (12%, 5-(2,4-dichlorophenyl)-2-furonitrile), 183 (100%, M^+-237), 83 (10%, $(CH_3)_3CCN^+$); **3e**, m/z 438 (8%, M^+), 217 (33%, M–5(3-chloro-4-fluorophenyl)-2-furonitrile), 167 (8%, (3-chloro-4-fluorophenyl)cyclopropylcation), 117 (30%, $C_6H_5CH_2CN^+$), 91 (89%, $C_6H_5CH_2^+$); **3f**, m/z 464 (100%, M^+), 438 (38% M–CO), 266 (26%, 5-(4-bromophenyl)furo-2-thiocarbonyl cation), 91 (28%, $C_6H_5CH_2^+$); **3j**, m/z 522 (5%, M^+), 489 (18%, M–Cl), 237 (32%, 5-(2,4-dichlorophenyl)-2-furonitrile).

with 5-aryl-2-furoic acids (**2**) in the presence of phosphorus oxychloride to afford 7-(5-aryl-2-furyl)-3-arylmethyl/*tert*-butyl-4H-1,3,4-thiadiazolo[2,3-c]-1,2,4-triazin-4-ones (**3**) in good yields. Formation of these thiadiazolotriazinones **3** was confirmed by elemental analysis, IR, ^{1}H NMR and mass spectral data. The characterization data of these compounds **3** are given in Table 1. The key spectral features are as reported by us previously.

2.1. Antibacterial activity

All the newly synthesized compounds were screened for their in vitro antibacterial activity against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphilococus aureus* Smith according to the serial dilution technique [6]. Nitrofurazone was used as a standard drug. All the tested compounds (Table 2) showed moderate activities against tested bacterial straines. Comparing the antibacterial activities of the compounds reported in Part I and II of the series, it is evident that the introduction of arylfuryl moieties at C7

Table 2
Antibacterial activity [a] of thiadiazolotriazinones (MIC, μg ml^{-1})

Comp. No.	*S. aureus*	*E. coli*	*P. aeruginosa*	*B. subtilis*
3a	12.5	6	6	12.5
3b	12.5	25	12.5	12.5
3c	25	6	12.5	6
3d	12.5	12.5	6	25
3e	12.5	12.5	6	12.5
3f	12.5	12.5	25	6
3g	6	25	12.5	6
3h	6	12.5	12.5	12.5
3I	6	6	25	12.5
3j	12.5	12.5	6	12.5
3k	12.5	12.5	12.5	12.5
3l	12.5	12.5	12.5	6
Nitrofurazone	12.5	6	12.5	12.5

[a] *S. aureus*, *Staphillococcus aureus* Smith; *E. coli*, *Escherichia coli*; *P. aeruginosa*, *Pseudonmonas aeruginosa*; *B. subtilis*, *Bacillus subtilis*.

Table 3
Preliminary in vitro antitumor screening [a] data of thiadiazolotriazinones **3a**, **3b**, **3g** and **3h**

Comp. No.	Growth percentage [b]			
	NCI-H 460 (Lung)	MCF-7 (Breast)	SF 268 (CNS)	Activity [c]
3a	42	34	64	Inactive
3b	35	46	72	Inactive
3g	39	27	40	Active
3h	30	12	43	Active

[a] Fixed concentration assay (100 μM; standard NCI protocol).
[b] Percent cell growth reduction following 48-h incubation with test compounds (optical density, sulforhodamine procedure) [6].
[c] Active when growth percentage is $<32\%$ for any of the three cell lines.

did not enhance antibacterial activities noticeably. However, among tested compounds the best (**3a**) and worst compounds overall (**3b**) were characterized by *tert*-butyl at C3, while differing for 2,4-diclorophenyl versus 3-chloro 4-flurophenyl as the furan substituent.

2.2. Antitumor activity

Four newly synthesized compounds were screened for their antitumor activities at NIH, Bethesda, Maryland, USA under the Drug Discovery Programme of NCI according to the procedure suggested by Boyd and Paull [7] in a primary three cell line–one dose anticancer assay against NCI-H 460 (Lung), MCF 7 (Breast) and SF 268 (CNS). In the current protocol each cell line is inoculated on a preincubated microtiter plate. The test agents are added at a single concentration and the culture is incubated for 48 h. End point determinations are made with Sulforhodamine B, a protein binding dye. Results for each test agents are reported as the percent growth of the treated cells when compared with the untreated control cells. Compounds which reduce the growth of any one of the cell lines to 32% or less (negative numbers indicate cell kill) are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range. In the present screening program (Table 3) compounds **3a** and **3b** (*tert*-butyl substitution at C3) were found to be inactive while the compounds **3g** and **3h** (4-chlorobenzyl substitution at C3) possessed growth percentage to less than 32% against Breast (MCF-7) cell lines and are regarded as active compounds. These two compounds **3g** and **3h** were then passed on for evaluation in the full panel of 60 cell lines derived from seven cancer types namely, Lung, Colon, Melanoma, Renal, Ovarian, CNS and Leukemia (Table 4). These compounds showed antiproliferative activity on the whole cell panel, although they did not prove cytotoxic or cytostatic at the maximum tested concentration (100 μM). Compound **3g** showed

Table 4
Sixty cell line in vitro antitumor screening [a] (GI_{50}, μM) thiadiazolotriazinones **3g** and **3h**

Panel/cell line	**3g**	**3h**
Leukemia		
CCRF-CEM	61.7	45.8
HL-60 (TB)	23.2	43.6
K-562	89.9	37.6
MOLT-4	43.6	43.6
Non-small cell lung Cancer		
A549/AATCC	80.5	51.2
EKVX	29.4	24.6
HOP-62	43.1	
HOP-92	38.4	58.8
NCI-H23	66.5	
NCIH322M	31.1	
NCI-H460	59.6	54.9
NCI-H522	52.8	62.1
Colon cancer		
COLO205	72.2	57.2
HCC-2998	49.6	29.7
HCT-116	27.6	34.9
HCT-15	41.4	50.4
HT29	64.1	91.2
KM12	76.5	54.5
CNS cancer		
SNB-19	33.4	76.5
U251	31.0	88.9
Melanoma		
LOXIMVI	47.1	70.5
M14		64.5
SKMEL-2	36.7	51.4
UACC-62	63.1	53.2
Ovarian cancer		
OVCAR-3	34.1	72.2
OVCAR-4	42.8	42.1
OVCAR-5	94.8	56.2
OVCAR-8	47.8	58.8
Renal cancer		
786-O	48.0	71.0
A498	41.5	42.1
ACHN	52.0	35.7
CAK-I	73.5	42.9
RXF393	53.8	
SN12C	44.6	42.7
UO-31	57.8	33.6
Prostate cancer		
PC-3	79.8	57.1
Du-145	69.9	
Breast cancer		
MCF7	30.5	32.2
NCI/ADR-RES	80.6	56.8
MDA-MB-435	93.3	82.0
MDA-N	47.9	95.4
BT-549		78.2
T-47D	74.5	80.2

[a] The other two standard parameters, TGI and LC_{50} were above 100 μM (maximum tested concentration).

highest activity against Leukemia HL-60 (TB) cell line ($GI_{50} = 23$ μM) while **3h** showed highest activity against non-small cell lung cancer EKVX cell line ($GI_{50} = 24.6$ μM).

3. Experimental

Melting points were taken in open capillary tubes and are uncorrected. The IR spectra in KBr disc were recorded either on a Shimadzu FT IR or a JASCO FT IR spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$–DMSO-d_6 either on a Bruker AC-300F (300 MHz) or a 400 MHz NMR spectrometer using TMS as an internal standard. The mass spectra were recorded either on a JEOL-JMS D-300 or on a VG 70S mass spectrometer operating at 70 eV. Purity of the compounds was checked by thin-layer chromatography (TLC) on silica gel plates using a toluene:acetone (8:2) solvent system. Iodine was used as the visualizing agent. Triazinones **1** was prepared according to the method reported by us earlier.

3.1. General procedure for the preparation of 5-aryl-2-furoic acids (2)

A mixture of substituted aniline (0.1 mol), dilute hydrochloric acid (15%, 60 ml) and water (90ml) was heated to get clear solution. The solution was cooled to 0 °C and was diazotized by the addition of sodium nitrite solution (30%, 24 ml). After filtration, the cold clear solution of diazonium salt was treated with furoic acid (0.1 mol) and water (50 ml). To this, an aqueous solution of cupric chloride (2.5 g in 10 ml of water) was added drop wise with stirring. Stirring was continued for 4 h and the precipitated solid was collected by filtration. The crude acid was recrystallized from a mixture of ethanol and dioxane to yield pure 5-aryl-2-furoic acid.

The compounds synthesized using this procedure are as follows.

Compound **2a**: 5-(2,4-Dichlorophenyl)-2-furoic acid, m.p. 199 °C, yield 72%. IR: (KBr disc, γ_{max} cm^{-1}): 3431(O–H str.), 3135(C–H), 1694(C=O), 1585(C=C), 726(C–Cl). ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ, 7.22 [d, 1H ($J = 3.7$ Hz), furan H], 7.26[d, 1H ($J = 3.7$ Hz), furan H], 7.39 [d, 1H, ($J = 9.0$ Hz), Ar–H], 7.51 [d, 1H ($J = 2.2$ Hz), Ar–H], 7.95 [d, 1H ($J = 9.0$ Hz), Ar–H].

Compound **2b**: 5-(3-Chloro-4-fluorophenyl)-2-furoic acid acid, m.p. 183 °C, yield 66%. IR: (KBr disc, γ_{max} cm^{-1}): 3405(O–H str.), 3090(C–H), 1687(C=O), 1567(C=C), 1068(C–F), 726(C–Cl). ^{1}H NMR (400 MHz, $CDCl_3 + (CD_3)_2CO$): δ, 6.76 [d, 1H, ($J = 3.7$ Hz), furan H], 7.22–7.31 (m, 1H, Ar–H), 7.38 (d, 1H ($J = 3.7$ Hz), furan H), 7.68 (m, 1H, Ar–H), 7.87 (d, 1H, ($J = 9.0$ Hz), Ar–H).

Compound **2c**: 5-(4-Bromophenyl)-2-furoic acid, m.p. 178 °C (Lit. [8] m.p. 178 °C), yield 68%. IR: (KBr disc, γ_{max} cm^{-1}): 3420(O–H str.), 3080(C–H), 1668(C=O).

3.2. General procedure for the preparation of 7-(5-aryl-2-furyl)-3-arylmethyl/tert-butyl-4H-1,3,4-thiadiazolo[2,3-c]-1,2,4-triazin-4-ones (3)

A mixture of triazinone **1** (0.01 mol), aryl furoic acid **2** (0.01 mol) and phosphorus oxychloride (10 ml) was refluxed on a water bath for about 5 h. Excess of phosphorus oxychloride was removed under reduced pressure. The reaction mixture was cooled and poured onto crushed ice (200 g). The resulting solid product was filtered, washed with sodium bicarbonate solution (2%), followed by distilled water. It was dried and recrystallized from a mixture of ethanol and dioxane. The yield and characterization data of thiadiazolotriazinones (**3**) prepared according to this method are given in Table 1.

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