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Synthesis and in Vitro Antimicrobial Activity of 6-Substituted 2*H*-1,3,5-Thiadiazine-2,4(3*H*)-diones

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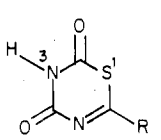
A series of 6-substituted 2*H*-1,3,5-thiadiazine-2,4(3*H*)-diones (**1a–m**) was prepared by treatment of alkyl, aryl, and heterocyclic primary thioamides with phenoxycarbonyl isocyanate to give *N*-(phenoxycarbonyl)-*N'*-thioacylureas, which gave **1** upon heating in refluxing xylene solution or upon treatment with aqueous sodium carbonate solution followed by acidification. ^{1}H NMR and infrared spectral evidence indicates that the 6-alkyl derivatives **1a,b,l,m** exist predominately in the exocyclic alkylidene tautomeric form. The major product obtained from alkaline and acid hydrolysis of the 6-phenyl derivative **1c** was found to be benzoic acid and benzoylurea, respectively. The majority of compounds **1a–m** exhibited in vitro antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes*. Several derivatives, **1b–d,h,j**, displayed minimum inhibitory concentration values below 2 μg/mL against *Trichophyton mentagrophytes*. Four derivatives, **1c,e,g,h**, inhibited the growth of *Seratia marcesens*, *Staphylococcus aureus*, and *Staphylococcus epidermis* in an in vitro sensitivity disk assay. 2-Furyl derivative **1h** displayed antileukemic activity against P-388 lymphocytic leukemia.

Heteroatom-modified analogues of uracil and its 5- or 6-substituted derivatives have represented a productive source of compounds exhibiting antimicrobial, cytostatic, or virostatic activities, presumably involving antimetabolic mechanisms of action.[2] 3-Oxauracils,[3] 5,6-dihydro-6-oxauracil,[4] 5- and 6-azauracil,[5,6] 6-azathymine,[7] and 5-azaorotate[8] are examples of such analogues displaying one or more of the above activities. Our interest in isoconjugate analogues of biologically important pyrimidine and purine derivatives[9-11] has prompted the investigation of 2*H*-1,3,5-thiadiazine-2,4(3*H*)-diones, **1**, which may be regarded as 6-substituted 5-thiauracils. The synthesis of **1**, examination of its aqueous stability, and a preliminary evaluation of in vitro antimicrobial properties are described for 14 derivatives in this class.



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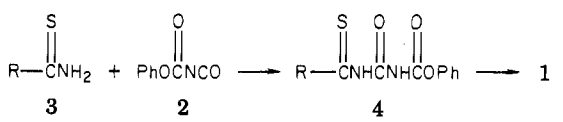
Chemistry. Previously reported[12,13] syntheses of 3-aryl or 3-thiocarbonyl derivatives of **1** are unsuitable for the preparation of the desired 3-unsubstituted derivatives, since the heterocyclic ring is unlikely to survive conditions necessary for the removal of the 3-substituent. Uracils have been synthesized by the reaction of enamines with phenoxycarbonyl isocyanate[14] (**2**), which has also been shown to be useful in the preparation of mesoionic xanthine and uracil analogues.[15,16] The reaction of primary thioamides **3** with **2** in toluene gives *N*-(phenoxycarbonyl)-*N'*-thioacylureas, **4** (Scheme I).

Scheme I



3 **2** **4**

Table I. *N*-(Phenoxycarbonyl)-*N'*-thioacylureas

no.	R	mp,[a] °C	formula[b]	yield, %
4a	*i*-C_3H_7	155-157	$C_{12}H_{14}N_2O_3S$	71
4b	$CH_2C_6H_5$	129-130	$C_{16}H_{14}N_2O_3S$	70
4c	C_6H_5	159-161	$C_{15}H_{12}N_2O_3S$	85
4d	4-$CH_3C_6H_4$	158-160	$C_{16}H_{14}N_2O_3S$	76
4e	4-ClC_6H_4	133-135	$C_{15}H_{11}ClN_2O_3S$	78
4f	4-$(CH_3)_2NC_6H_4$	162-164	$C_{17}H_{17}N_3O_3S$	67
4g	2-thienyl	165-167	$C_{13}H_{10}N_2O_3S_2$	70

[a] All compounds were recrystallized from $CHCl_3$-petroleum ether (bp 40-60 °C) and melted with decomposition. [b] All compounds analyzed for C, H, and N within ±0.4% of theoretical values.

In a number of cases, **4a–g**, these intermediates were isolated and characterized (Table I). In one case, where R = *tert*-butyl, the products of this reaction were phenyl

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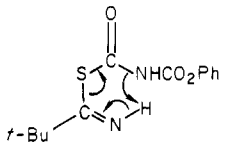
carbamate and pivalonitrile which may result from initial nucleophilic attack by sulfur, leading to intermediate **5**.



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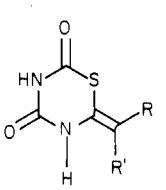
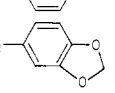
This intermediate, which cannot be stabilized by either conjugation with the substituent group or by enamine formation prior to S to N acyl group migration, may fragment to give the observed products.

Intermediate ureas **4** were routinely converted, without purification to the desired products **1a–m** (Table II), by heating in refluxing xylene solution containing a small amount of pyridine (method A) or by the use of aqueous sodium carbonate solution (method B), which reduced the reaction time from several hours to 10–20 min, improved yields, and gave products of higher initial purity.

Structure assignment for compounds **1a–m** is based upon elemental analyses, spectral properties, and the observation of parent molecular ions in their mass spectra. The N–H stretching region of their infrared spectra exhibit complex absorption patterns similar to those of uracils, with characteristic peaks at 2850, 3040, and 3140 cm^{-1}. Two carbonyl bands are observed at 1690–1725 and 1660–1690 cm^{-1}, as well as strong carbon–nitrogen double-bond absorption at 1540–1570 cm^{-1}. This latter band is replaced in **1a,b,l,m** by a band at 1625 cm^{-1} assigned to carbon–carbon double bond absorption, indicating that the preferred tautomer in these cases is the exocyclic alkylidene structure **6a–d**. ^1H NMR evidence supporting this



6a, R = R' = CH_3
b, R = H; R' = CH_2Ph
c, R = H; R' = 
d, R = H; R' = 

structure is the low-field resonance of the methyl groups of **6a** appearing as singlets at δ 1.72 and 1.79 and the presence of singlets in the vinyl region (δ 6.20) for **6b–d**.

The thiadiazinediones **1** are weakly acidic, dissolving in aqueous buffers in the range of pH 9–10. In contrast to the stability of uracils, analogues **1** undergo aqueous hydrolysis. Although this hydrolysis is slow ($t_{1/2}$ > 48 h) in the pH range 7–8, it becomes rapid at pH >10. The major alkaline hydrolysis product of **1c** was found to be benzoic acid, which probably arises from alkaline labile benzoylurea. The stability of **1** was also found to be solvent dependent. Although thiadiazinediones **1** could be purified by recrystallization from glacial acetic acid, solutions of **1** in dry dimethylformamide or dimethyl sulfoxide undergo rapid discoloration at room temperature.

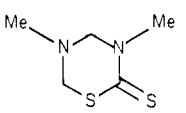
Antimicrobial Activity. In a disk sensitivity assay **1a–m** were inactive against *Aspergillus niger*, while only **1c,h,j** displayed activity against *Saccharomyces cerevisiae*. Minimum inhibitory concentrations (MIC) were determined in Sabouraud dextrose broth by tube dilution (Table II) for *Candida albicans* and *Trichophyton mentagrophytes*. Although only moderately active against *C. albicans*, a number of compounds (**1b–d,g,h,j**) inhibited the growth of *T. mentagrophytes* at 2 $\mu g/mL$. Of these, **1b,d,h,j** appeared to be fungicidal at 2 $\mu g/mL$, since dilution or replating of these tubes failed to produce growth of the microorganism. Two compounds that were more active against both fungi, **1c** and **h**, were also found to inhibit the growth of *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermis* in a sensitivity disk assay. Compounds **1e** and **1g** also inhibited the growth of these three bacteria in this assay, while the remaining compounds were inactive. Compounds **1b,d,e** inhibited the growth of *E. coli*, while the others were inactive.

Discussion

The minimum inhibitory concentrations of the hydrolysis products of **1c** (benzonitrile, benzoic acid, thiobenzamide, and benzoylurea) were found to be >50 $\mu g/mL$ against *C. albicans*, indicating that the observed activity of **1c** does not arise from these products. A previously reported[12] 3-substituted derivative of **1c**, 3,6-diphenyl-1,3,5-thiadiazine-2,4-dione, was found to be devoid of inhibitory activity in the sensitivity disk and tube dilution assays.

Compounds with alkyl or with the more electron-donating phenyl 6-substituents, i.e., **1d,f,i,k**, appear generally less active than those with phenyl (**1c**), 4-chlorophenyl (**1e**), 4-acetylphenyl (**1j**), and 2-furyl (**1h**) substituents. Among this group of derivatives, there is no apparent correlation of activity with substituent hydrophobicity.

A large number of tetrahydro-1,3,5-thiadiazines, represented by mylone (**7**), have been reported to exhibit in vitro antifungal and antibacterial activities.[17] With the exception of their reactivity toward nucleophiles, these tetrahydrothiadiazines share only limited structural and chemical properties with those of **1**.



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The biological data reported here are insufficient to reveal information concerning the mechanism of fungitoxicity. The formation of 6-substituted uridine analogues by ribityl alkylation of N-3 would result in the formation of a cationic or betaine-type structure. This would suggest that the activity of **1** may be related to its reactivity toward nucleophiles rather than as an antimetabolic function.

In a preliminary screen for antileukemic activity[18] (P-388 lymphocytic leukemia), determined under the auspices of the National Cancer Institute, compound **1h** produced a % T/C value of 124 at the 100 mg/kg dose level. This compound has been selected for evaluation against the Division of Cancer Treatment (NCI) panel of experimental murine neoplasias.

Experimental Section

Melting points were determined with a Fisher-Johns hot-stage apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, and determined values are within 0.4% of theoretical values. Infrared spectra (KBr disks) were obtained with a Perkin-Elmer 727B spectrophotometer and a Nicolet 7199 FT interferometer. ^1H NMR spectra were

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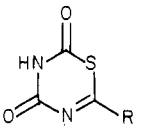
Table II. 6-Substituted 1,3,5-Thiadiazine-2,4(3*H*)-diones



no.	R	mp,[a] °C	yield, % method A[b]	yield, % method B[c]	formula[d]	MIC[e] *C. albicans*	MIC[e] *T. mentagrophytes*
1a	i-C_3H_7	189-191	65		$C_6H_8N_2O_2S$	81	15
1b	$CH_2C_6H_5$	184-186	57	82	$C_{10}H_8N_2O_2S$	125	<2
1c	C_6H_5	194-196	58	90	$C_9H_6N_2O_2S$	8	<2
1d	4-$CH_3C_6H_4$	210-213	78	87	$C_{10}H_8N_2O_2S$	>250	<2
1e	4-ClC_6H_4	204-206	85	83	$C_9H_5ClN_2O_2S$	15	4
1f	4-$(CH_3)_2NC_6H_4$	209-211	76		$C_{11}H_{11}N_3O_2S$	>125	62
1g	2-thienyl	164-166	73		$C_7H_4N_2O_2S_2$	62	2
1h	2-furyl	212-215		91	$C_7H_4N_2O_2S$	15	<2
1i	4-$CH_3OC_6H_4$	210-212	15		$C_{10}H_8N_2O_3S$	125	8
1j	4-$CH_3COC_6H_4$	203-205	46		$C_{11}H_8N_2O_3S$	31	<2
1k	3,4-$(CH_2O_2)C_6H_3$	219-221	58		$C_{10}H_6N_2O_4S$	>250	125
1l	3,4-$(CH_2O_2)C_6H_3CH_2$	198-203	20		$C_{11}H_8N_2O_4S$	125	4
1m	3-pyridylmethyl	>300	46		$C_9H_7N_3O_2S$	62	8
5-chloro-7-iodo-8-hydroxyquinoline						1	4

[a] All compounds melted with decomposition. [b] Method A, refluxing xylene with pyridine. [c] Method B, aqueous sodium carbonate solution. [d] All compounds analyzed for C, H, and N within ±0.4% of theoretical values. [e] Minimum inhibitory concentration, in micrograms per milliliter.

recorded with Varian T-60A and FT-80 spectrometers using 1% (v/v) Me_4Si as an internal standard. UV spectra were recorded with a Varian-Cary 118 spectrophotometer.

Thioamides not commercially available were prepared by the treatment of an ethanolic solution of the corresponding nitrile with ammonia and hydrogen sulfide.[19] The following procedures illustrate the preparation of the *N*-(phenoxycarbonyl)-*N'*-thioacylureas **4** and 6-substituted-1,3,5-thiadiazine-2,4-diones **1**.

***N*-(Phenoxycarbonyl)-*N'*-(phenylthiocarbonyl)urea (4c).** To a solution of 1.37 g (10 mmol) of thiobenzamide in 50 mL of dry toluene was added 1.95 g (12 mmol) of phenoxycarbonyl isocyanate (**2**).[20,21] The mixture was stirred overnight at room temperature and then was chilled to 5 °C. The resulting precipitate was collected and recrystallized from $CHCl_3$–petroleum ether (bp 40–60 °C) to give **4c** as violet crystals: mp 159–161 °C dec; yield 2.55 g (85%).

2*H*-6-Phenyl-1,3,5-thiadiazine-2,4(3*H*)-dione (1c). Method A. A solution of 1.5 g (5 mmol) of **4c** in 40 mL of dry xylene, containing 5 drops of pyridine, was refluxed for 3 h. After the solution was cooled to room temperature, the product was collected by filtration. Recrystallization from chlorobenzene gave 0.59 g (57%) of **1c** as white crystals: mp 194–196 °C dec; IR (KBr) 3120, 3020, 2850, 1690, 1655 cm^{-1}; UV (EtOH) λ_{max} 260 nm (log ϵ 3.56); ^1H NMR (Me_2SO-d_6) δ 7.70 (m, 3 H, aromatic), 8.15 (m, 2 H, aromatic); mass spectrum, m/e 206 (M^+).

Method B. To 1.5 g (5 mmol) of **4c** was added 20 mL of 5% aqueous potassium carbonate solution. The mixture was stirred at room temperature until the color of the starting material was completely discharged (15 min) and then was acidified (pH 2) with 6 N sulfuric acid. After the mixture was cooled to 5 °C, the product was collected by filtration and recrystallized from chlorobenzene to give 0.93 g (90%) of **1c**: mp 194–196 °C dec.

Aqueous Hydrolysis of 6-Substituted 2*H*-1,3,5-Thiadiazine-2,4(3*H*)-diones (1). The longest wavelength (260–310 nm) ultraviolet absorption band of **1b,c,h** in aqueous phosphate buffers, pH 7 and 9, was monitored over a 48-h period. Typical decreases in absorbance values in the range of 10–20% of initial readings were observed. At pH 11 the half-life of **1h** was estimated to be 20 h.

A solution of **1c** (0.25 g, 1.2 mmol) in 100 mL of 1 N sodium hydroxide solution was allowed to stand for 1 h at 25 °C. This solution was extracted with three 20-mL portions of chloroform, and the combined extract was evaporated. Addition of petroleum ether (bp 40–60 °C) to the residue resulted in crystallization of thiobenzamide (21 mg, 14%), mp 122–124 °C. Evaporation of the mother liquor gave benzonitrile (10 mg, 8%), identified by comparison of its IR spectrum to that of an authentic sample. Acidification of the alkaline reaction mixture and extraction with four 25-mL portions of ether gave benzoic acid (97 mg, 65%) following evaporation of the combined extracts. Both benzoic acid and thiobenzamide were identified by comparison of their IR spectra with those of authentic samples.

Microbiological Methods. The cultures employed were *Escherichia coli* ATCC 25922, *Serratia marsesens* ATCC 8100, *Staphylococcus aureus* ATCC 12600, *Staphylococcus epidermis* ATCC 12228, *Asperigillus niger* ATCC 16888, *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9763, and *Trichophyton mentagrophytes* ATCC 9129. In the sensitivity disk assay a lawn was prepared on Trypticase Soy agar plates using 1 mL of a 24-h growth of the test bacteria in trypticase soy broth. Fungi were grown in Sabouraud dextrose broth and plated on Sabouraud dextrose agar. Paper disks (6 mm), impregnated with 1 mg of test compound, were placed on the agar and incubated for 24 h at 37 °C for bacteria or for 3 days at 26 °C for fungi. Clear zones greater than 8 mm in diameter, including disk, were evidence of growth inhibition. In the tube dilution studies, 2-fold dilutions were employed in triplicate starting from 250 μg/mL or the highest soluble concentration in Sabouraud dextrose broth. Incubations were conducted in a shaker bath at 26 °C for 7 days.

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(21) Observed bp 70–75 °C (2.5 mm) [lit.[13] bp 70–75 °C (0.25 mm)].