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Original article

N-Acylated and *N*,*N*′-diacylated imidazolidine-2-thione derivatives and *N*,*N*′-diacylated tetrahydropyrimidine-2(1*H*)-thione analogues: Synthesis and antiproliferative activity

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Abstract

Fifty-one acylthioureas (ATUs) incorporating imidazolidine-2-thione or its upper cyclohomologue were prepared by parallel synthesis and evaluated against a high number of human cancer cell lines for antiproliferative activity. ATUs **1o** (3,5-dichlorobenzoyl), **1s** (2-furoyl), **3s** (2-furoyl) and **1t** (2-thenoyl) displayed activity against leukemia, melanoma LOX IMVI, non-small cell lung NCI-H522, renal 786-0, CAKI-1, SN12C, UO-31 and breast MCF7, MDA-MB-435, T-47D cancer cell lines in the 0.3–9.7 μM concentration range. Compound **14s** exhibited selectivity for melanoma SK-MEL-5 ($GI_{50} < 5$ nM); **1s** for leukemia MOLT-4 (GI_{50}: 300 nM); **1q**, **3b** and **3q** for renal cancer UO-31 (GI_{50}: 70–200 nM); **8s**, **9s** for non-small cell lung cancer EKVX (GI_{50}: 300, 10 nM) and **3j** for HOP-92 (GI_{50}: 700 nM) cell line.

Keywords: Acylthioureas; Antiproliferative activity; Parallel synthesis

1. Introduction

Acylthioureas (ATUs) have been reported to display a wide range of biological activities, such as antiviral [1], antibacterial [2], tubercolostatic [3], fungicidal [4–6], herbicidal [7,8], plant growth regulating [7,9], anticonvulsant [10], antiaggregating [11–13], antiarrythmic [13], analgesic [13], antihyperlipidemic [13], local anaesthetic [13], thyreostatic [14], central nervous system (CNS) depressant [15] and antiproliferative [13,16–19]. In particular, some benzoylphenylthioureas have been recently described as potent antitumor agents inhibiting tubulin polymerization [19] and some quinoline and quinazoline-acylthiourea derivatives have been identified as potent and selective inhibitors of the autophosphorylation (tyrosine kinase activity) of platelet-derived growth factor (PDGF) receptor, involved in cell-proliferation processes [20].

In the past, the pharmacological potential of this chemical class had attracted our attention and had led some of us to synthesize a number of ATUs endowed with various biological activities [11–13]. Recently, the *N*,*N*′-bis(4-chlorobenzoyl) derivatives of imidazolidine-2-thione (**1j**, Fig. 1) revealed a significant cytotoxicity in MT-4 cell-based assays ($IC_{50} = 9.9$ μM). In order to investigate the influence of the acyl portion on the antiproliferative activity, we prepared in parallel a series of symmetric analogues of **1j** (Table 1) in which the 4-chlorobenzoyl moiety was replaced with an acetyl (**1a**), a 1-naphthoyl (**1r**) or an heteroaroyl (2-furoyl **1s**, 2-thienoyl **1t**). Besides, the substitution on **1j** phenyl ring was varied by shifting the chlorine atom to the *meta* (**1i**) and *ortho* (**1h**) position, or by replacing it with a more electron-withdrawing

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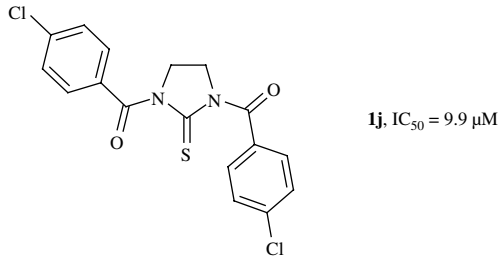
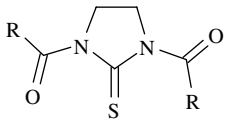


1j, IC_{50} = 9.9 μM

Fig. 1. ATU lead compound.

(3-nitro **1k**) or an electron-donating (2- and 4-methyl **1e**, **1f**; 4-methoxy **1l**) or a more sterically demanding (4-*t*-butyl **1g**) substituent. Also the phenyl ring unsubstitution (**1d**), dichlorosubstitution [patterns 2,4 (**1m**), 3,4 (**1n**) and 3,5 (**1o**)] and polysubstitution (4-chloro-3-nitro **1p**; 3,4,5-trimethoxy **1q**) were considered. With the aim to further expand the structure–activity relationship (SAR) study, we synthesized the superior cyclohomologues **2** (Table 2) and the asymmetric analogues **3** (Table 3) in which one of the two acyl moiety was replaced by a formyl group. Successively, we replaced one of the benzoyl functions of ATU **1d** with the electron-poor α,β-unsaturated system *N*-methylene(malononitrile) (**4d**: Y = W = cyano, Table 4). The significant antiproliferative activity of **4d** prompted us to explore the influence of the nature of the two electron-withdrawing groups Y and W on the activity. Thus, we synthesized a series of analogues of **4d** (ATU **4s–14s**, Table 4) keeping constant the *N*-furoyl portion, which had given the best results in series **1–3**, and varying Y and W (Y = W or Y ≠ W; Y, W: cyano, acetyl, pivaloyl, benzoyl, methoxycarbonyl, ethoxycarbonyl, aminocarbonyl and *p*-chlorophenylaminocarbonyl). Finally, we prepared three urea-isosters (**15s–17s**, Table 4).

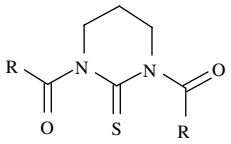
Table 1
Antiproliferative activity of ATUs **1a**, **1d–t** against MT-4 cells[a]



Compound	RCO	IC_{50}[b] (μM)
1a	Acetyl	>100
1d	Benzoyl	94
1e	2-Toluoyl	>100
1f	4-Toluoyl	35
1g	4-*t*-Butylbenzoyl	>100
1h	2-Chlorobenzoyl	54
1i	3-Chlorobenzoyl	35
1j	4-Chlorobenzoyl	9.9
1k	3-Nitrobenzoyl	>100
1l	4-Methoxybenzoyl	>100
1m	2,4-Dichlorobenzoyl	39
1n	3,4-Dichlorobenzoyl	4.6
1o	3,5-Dichlorobenzoyl	13
1p	4-Chloro-3-nitrobenzoyl	11
1q	3,4,5-Trimethoxybenzoyl	7.0
1r	1-Naphthoyl	13
1s	2-Furoyl	7.4
1t	2-Thenoyl	11

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration required to reduce MT-4 cell growth by 50% in comparison with untreated controls, as measured by the MTT method.

Table 2
Antiproliferative activity of ATUs **2d**, **2f**, **2h**, **2j**, **2m–o**, **2q**, **2s** and **2t** against MT-4 cells[a]



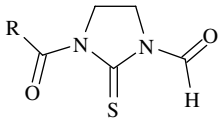
Compound	RCO	IC_{50}[b] (μM)
2d	Benzoyl	>100
2f	4-Toluoyl	>100
2h	2-Chlorobenzoyl	>100
2j	4-Chlorobenzoyl	38
2m	2,4-Dichlorobenzoyl	>100
2n	3,4-Dichlorobenzoyl	72
2o	3,5-Dichlorobenzoyl	30
2q	3,4,5-Trimethoxybenzoyl	>100
2s	2-Furoyl	36
2t	2-Thenoyl	>100

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration required to reduce MT-4 cell growth by 50% in comparison with untreated controls, as measured by the MTT method.

2. Chemistry

The title compounds and three urea-isosters were prepared (Scheme 1) by reacting (thio)ureas **I**, **II**, **IV–XVIII** (Fig. 2a) with the suitable acylating reagent [acetic anhydride (**a**) and acyl chlorides (**b–t**)] (Fig. 2b). Thioureas **I** and **II** and the acylating reagents were commercially available, while thioureas **IV–XV** and ureas **XVI–XVIII** were prepared according to one-pot procedures (Scheme 1b) previously described by

Table 3
Antiproliferative activity of ATUs **3a–d**, **3f**, **3j**, **3m–o**, **3q** and **3s** against MT-4 cells[a]



Compound	RCO	IC_{50}[b] (μM)
3a	Acetyl	>100
3b	Pivaloyl	43
3c	*trans*-Cinnamoyl	>100
3d	Benzoyl	12
3f	4-Toluoyl	31
3j	4-Chlorobenzoyl	27
3m	2,4-Dichlorobenzoyl	>100
3n	3,4-Dichlorobenzoyl	41
3o	3,5-Dichlorobenzoyl	15
3q	3,4,5-Trimethoxybenzoyl	13
3s	2-Furoyl	9.0

[a] Data mean values for three separate experiments. Variation among triplicate samples was less than 10%.
[b] Compound concentration required to reduce MT-4 cell growth by 50% in comparison with untreated controls, as measured by the MTT method.