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European Journal of Medicinal Chemistry 44 (2009) 1106–1118
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
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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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Received 19 May 2008; received in revised form 17 June 2008; accepted 18 June 2008
Available online 24 June 2008

**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.
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*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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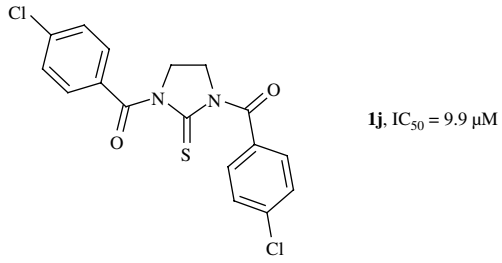
doi:10.1016/j.ejmech.2008.06.010

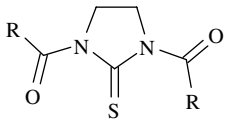



**1j**, $IC_{50}$ = 9.9 μM

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).

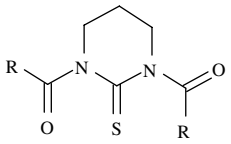
Table 1
Antiproliferative activity of ATUs **1a**, **1d–t** against MT-4 cells[a]




| 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]



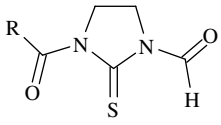

| Compound | RCO | $IC_{50}$[b] (μM) |
|---|---|---|
| **2d** | Benzoyl | >100 |
| **2f** | 4-Toluoyl | >100 |
| **2h** | 2-Chlorobenzoyl | >100 |
| **2j** | 4-Chlorobenzoyl | 38 |
| **2m** | 2,4-Dichlorobenzoyl | >100 |
| **2n** | 3,4-Dichlorobenzoyl | 72 |
| **2o** | 3,5-Dichlorobenzoyl | 30 |
| **2q** | 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

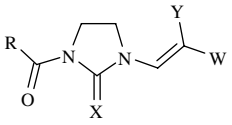
Table 3
Antiproliferative activity of ATUs **3a–d**, **3f**, **3j**, **3m–o**, **3q** and **3s** against MT-4 cells[a]




| 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.

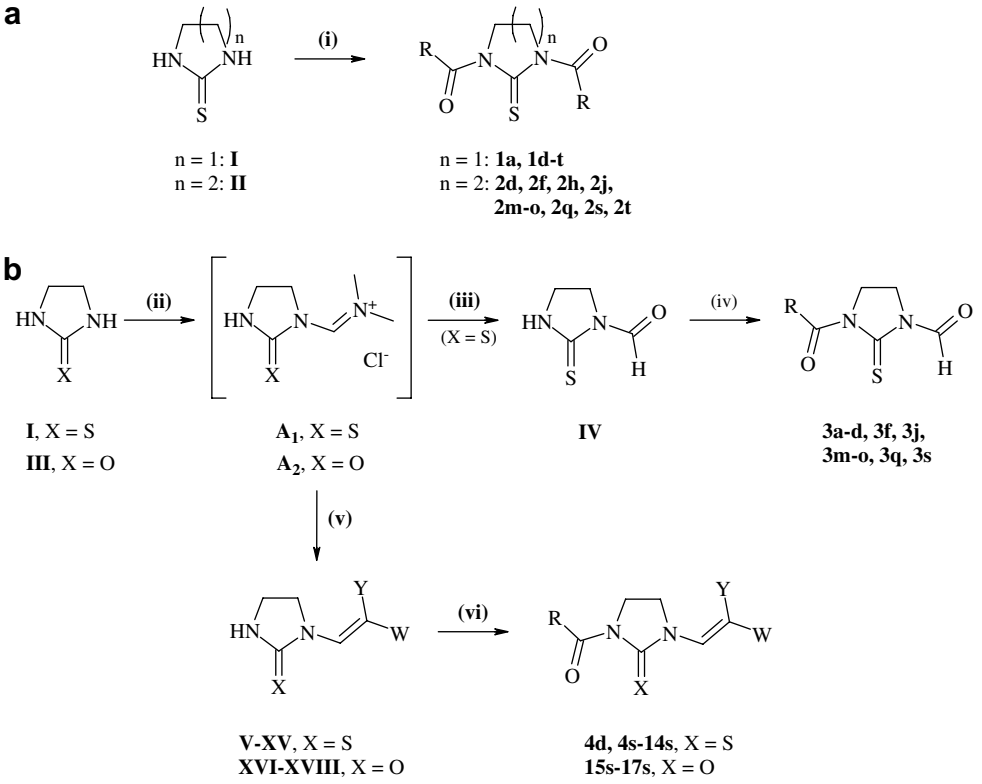
Table 4
Antiproliferative activity of acyl(thio)ureas **4d** and **4s–17s** against MT-4 cells[a]



| Compound | RCO | X | Y | W | $IC_{50}$[b] (μM) |
|---|---|---|---|---|---|
| **4d** | Benzoyl | S | CN | CN | 11 |
| **4s** | 2-Furoyl | S | CN | CN | 4.4 |
| **5s** | 2-Furoyl | S | CN | $COOCH_3$ | 5.0 |
| **6s** | 2-Furoyl | S | CN | $COC_6H_5$ | 3.0 |
| **7s** | 2-Furoyl | S | CN | $COC(CH_3)_3$ | 14.0 |
| **8s** | 2-Furoyl | S | CN | $CONH_2$ | 4.0 |
| **9s** | 2-Furoyl | S | CN | $CONH(4\text{-}Cl\text{-}C_6H_4)$ | 6.5 |
| **10s** | 2-Furoyl | S | $COCH_3$ | $COCH_3$ | 4.0 |
| **11s** | 2-Furoyl | S | $COC_6H_5$ | $COCH_3$ | 5.0 |
| **12s** | 2-Furoyl | S | $COOCH_3$ | $COCH_3$ | 7.0 |
| **13s** | 2-Furoyl | S | $COC_6H_5$ | $COC_6H_5$ | 7.0 |
| **14s** | 2-Furoyl | S | $COC_6H_5$ | $COOCH_2CH_3$ | 4.0 |
| **15s** | 2-Furoyl | O | CN | CN | >100 |
| **16s** | 2-Furoyl | O | $COC_6H_5$ | $COCH_3$ | >100 |
| **17s** | 2-Furoyl | O | $COC_6H_5$ | $COOCH_2CH_3$ | 43 |

[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.

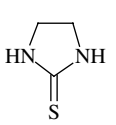
some of us [21]. Briefly, thiourea **I** and urea **III** were converted into the corresponding *N*-methyleniminium salts $\mathbf{A_1}$ and $\mathbf{A_2}$ by reaction with the DMF–benzoyl chloride complex. Then, the one-pot hydrolysis of $\mathbf{A_1}$ led to the *N*-formyl thiourea **IV**, whereas the one-pot condensation of $\mathbf{A_1}$ and $\mathbf{A_2}$ with active methylene reagents ($Y-CH_2-W$) gave the corresponding Knoevenagel-type derivatives **V–XVIII** as single geometric isomers. The reactions of (thio)urea acylation were performed in parallel (except for **3a** and **4d**) by using ordered arrays of reaction vessels (Carousel-6 and −12 Reaction Stations™). A suitable parallel acylation method (Scheme 1a and b) was set-up for each type of (thio)urea (**I** and **II**, **IV**, **V–XVIII**) due to their different reactivities. Thus, all reactions were performed in pyridine, in the presence of a small excess of acylating reagent, but at different reaction temperatures and times: 90 °C for 30 min for the *N*,*N*′-diacylation of **I** and **II** (ATUs **1** and **2**, respectively); rt for 12 h for the *N*-acylation of **IV** (ATUs **3**); 65 °C for 4 h for the *N*-acylation of **V–XVIII** with 2-furoyl chloride (ATUs **4s–14s** and acylureas **15s–17s**). In the last case, the addition of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA) increased the yields. The acylation of **IV** with acetic anhydride (ATU **3a**) required higher temperature than the other acylations of **IV**, whereas the benzoylation of **V** was carried out at rt for 2 h (ATU **4d**).



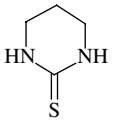

Scheme 1. (a) (i) RCOCl [for **1a**: acetic anhydride] (2.2 equiv.), pyridine, 90 °C, 30 min. (b) (ii) dry DMF, 80 °C, then PhCOCl (1 equiv.), rt, 15 min; (iii) $H_2O$, 80 °C, 5 min; (iv) RCOCl (1.1 equiv.), pyridine, rt, 12 h [for **3a**: acetic anhydride (large excess), pyridine, 100 °C, 3 h]; (v) $Y-CH_2-W$ (1 equiv.), $Et_3N$ (1.5 equiv.), DMF, 120 °C, 30 min (for **V**, **VII** and **XVI**: without $Et_3N$, 40 min); (vi) RCOCl (1.1 equiv.), TMEDA (1.1 equiv.), pyridine, 65 °C, 4 h [for **4d**: rt, 2 h]. For the structure list of (thio)ureas **V–XVIII** and acyl chlorides RCOCl (**b–t**), see Fig. 2.

**a**

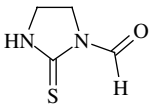
(Thio)ureas **I**, **II**, **IV-XVIII**



I

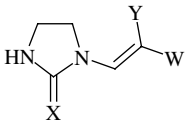


II



IV

| X=S | X=O | Y | W |
|---|---|---|---|
| **V** | **XVI** | CN | CN |
| **VI** | | CN | $COOCH_3$ |
| **VII** | | CN | $COC_6H_5$ |
| **VIII** | | CN | $COC(CH_3)_3$ |
| **IX** | | CN | $CONH_2$ |
| **X** | | CN | $CONH(4\text{-}Cl\text{-}C_6H_4)$ |
| **XI** | | $COCH_3$ | $COCH_3$ |
| **XII** | **XVII** | $COC_6H_5$ | $COCH_3$ |
| **XIII** | | $COOCH_3$ | $COCH_3$ |
| **XIV** | | $COC_6H_5$ | $COC_6H_5$ |
| **XV** | **XVIII** | $COC_6H_5$ | $COOC_2H_5$ |



**V-XVIII**

**b**

Acylating reagents: acetic anhydride (**a**) and acyl chlorides RCOCl (**b-t**)

| | RCO |
|---|---|
| **b** | pivaloyl |
| **c** | *trans*-cinnamoyl |
| **d** | benzoyl |
| **e** | 2-toluoyl |
| **f** | 4-toluoyl |
| **g** | 4-*t*-butylbenzoyl |
| **h** | 2-chlorobenzoyl |
| **i** | 3-chlorobenzoyl |
| **j** | 4-chlorobenzoyl |
| **k** | 3-nitrobenzoyl |

| | RCO |
|---|---|
| **l** | 4-methoxybenzoyl |
| **m** | 2,4-dichlorobenzoyl |
| **n** | 3,4-dichlorobenzoyl |
| **o** | 3,5-dichlorobenzoyl |
| **p** | 4-chloro-3-nitrobenzoyl |
| **q** | 3,4,5-trimethoxybenzoyl |
| **r** | 1-naphthoyl |
| **s** | 2-furoyl |
| **t** | 2-thenoyl |

Fig. 2. Building blocks used (a) (thio)ureas **I**, **II**, **IV–XVIII** (b) acylating reagents: acetic anhydride (**a**) and acyl chlorides RCOCl (**b–t**).

The work-up procedures simply required quenching with water or 2 N HCl solution, followed by filtrations or extractions. The final products were purified by crystallization. The yields ranged from 44 to 99% (see Section 5).

## 3. Results and discussion

The fifty-four acyl(thio)ureas synthesized were tested for antiproliferative activity against MT-4 lymphoblastoid T cells. The results are expressed as $IC_{50}$ values (Tables 1–4). Twenty-six ATUs were also evaluated at National Cancer Institute (NCI) (Bethesda, MD, USA) for their in vitro anticancer activity against subpanels of nine different types of cell lines derived from human tumors (Tables 5–7). The results are expressed as $GI_{50}$, TGI and $LC_{50}$ values. Table 5 shows the number of cell lines against which each compound was screened (47–60), the number of lines against which it gave a positive $GI_{50}$, or TGI or $LC_{50}$ value (inferior to 100 μM) and the corresponding concentration range. The $GI_{50}$ values of the twenty-two most active molecules are reported in Tables 6 and 7.

Table 5
Anticancer activity of **1m–o**, **1q**, **1s**, **1t**, **2s**, **2t**, **3b**, **3j**, **3m–3o**, **3q**, **3s**, **4d**, **4s–6s** and **8s–14s**[a]

| Compound | Number (No) of human tumor cell lines[b] | | | | | | |
|---|---|---|---|---|---|---|---|
| | Investigated | Giving positive $GI_{50}$, TGI and $LC_{50}$ | | | | | |
| | | $GI_{50}$ (μM)[c] | | TGI (μM)[d] | | $LC_{50}$ (μM)[e] | |
| | | No | Range | No | Range | No | Range |
| **1m** | 57 | 57 | 7.8–41.0 | 53 | 28.5–98.9 | 30 | 55.5–99.6 |
| **1n** | 57 | 57 | 4.5–34.4 | 55 | 5.6–73.5 | 42 | 48.4–97.5 |
| **1o** | 59 | 59 | 1.5–31.7 | 57 | 3.4–66.8 | 49 | 14.1–93.9 |
| **1q** | 57 | 57 | 0.2–27.2 | 55 | 12.8–74.5 | 39 | 41.1–99.5 |
| **1s** | 60 | 60 | 0.3–28.7 | 58 | 4.3–83.3 | 46 | 29.5–94.7 |
| **1t** | 60 | 60 | 1.2–19.1 | 58 | 3.9–59.3 | 52 | 12.7–94.3 |
| **2s** | 52 | 12 | 2.3–82.1 | 1 | 93.3 | – | – |
| **2t** | 52 | 4 | 26.2–84.7 | 1 | 66.2 | – | – |
| **3b** | 58 | 40 | 0.07–92.0 | 7 | 52.9–97.6 | – | – |
| **3j** | 57 | 57 | 0.7–49.5 | 55 | 11.0–80.0 | 42 | 40.2–84.8 |
| **3m** | 57 | 26 | 20.4–85.6 | 4 | 46.8–98.7 | – | – |
| **3n** | 56 | 56 | 2.8–39.6 | 53 | 7.7–58.0 | 48 | 42.9–95.6 |
| **3o** | 57 | 57 | 2.4–28.9 | 57 | 5.6–97.5 | 47 | 39.8–89.1 |
| **3q** | 58 | 58 | 0.07–48.5 | 50 | 3.4–97.6 | 26 | 26.4–97.0 |
| **3s** | 58 | 58 | 1.5–43.8 | 57 | 8.3–75.1 | 39 | 37.2–95.6 |
| **4d** | 52 | 46 | 3.5–89.2 | 22 | 11.4–98.3 | – | – |
| **4s** | 47 | 47 | 2.0–79.7 | 27 | 6.6–89.4 | 4 | 67.4–92.2 |
| **5s** | 50 | 50 | 2.1–54.9 | 42 | 5.0–79.6 | 22 | 9.9–98.5 |
| **6s** | 52 | 52 | 2.2–68.2 | 28 | 5.4–93.3 | 7 | 49.6-92.3 |
| **8s** | 53 | 51 | 0.3–43.6 | 34 | 1.0–98.2 | 4 | 65.0-91.6 |
| **9s** | 53 | 51 | 0.01–41.2 | 38 | 21.3–48.3 | – | – |
| **10s** | 49 | 49 | 1.5–38.4 | 44 | 3.4–71.5 | 25 | 11.5–96.3 |
| **11s** | 47 | 47 | 1.1–28.0 | 35 | 3.6–46.7 | 13 | 22.7–47.5 |
| **12s** | 49 | 49 | 1.6–41.7 | 43 | 4.0–98.4 | 25 | 10.0–91.8 |
| **13s** | 49 | 48 | 1.2–41.8 | 37 | 4.1–44.2 | 19 | 28.3–48.7 |
| **14s** | 47 | 46 | <46.0 | 23 | <48.4 | 8 | 14.3–48.2 |

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell lines screen.
[b] The table shows the number of cell lines against which each compound was screened, the number of lines against which it gave a positive $GI_{50}$, or TGI or $LC_{50}$ value (<100 μM) and the corresponding concentration range.
[c] Compound concentration that produces 50% growth inhibition.
[d] Compound concentration that produces total growth inhibition.
[e] Compound concentration that produces 50% cytocidal effect.

Data of Tables 1–3 display that ATUs **1** (Table 1) were more active than the corresponding more conformationally flexible six-membered ring homologues (ATUs **2**, Table 2) (**1d** vs **2d**; **1f** vs **2f**; **1h** vs **2h**; **1j** vs **2j**; **1m** vs **2m**; **1n** vs **2n**; **1o** vs **2o**; **1q** vs **2q**; **1s** vs **2s**; **1t** vs **2t**) and in general more effective than the corresponding *N*-acyl-*N*′-formyl congeners (series **3**, Table 3) (**1j** vs **3j**; **1m** vs **3m**; **1n** vs **3n**; **1o** vs **3o**; **1q** vs **3q**; **1s** vs **3s**). The most active derivatives (**1j**, **1n–t**, **3d**, **3o**, **3q** and **3s**) exhibited an $IC_{50}$ value range of 4.6–15 μM. Data show evidence that the nature of the acyl portion greatly influences the antiproliferative properties of the compounds. Thus, in series **1–3** (with the exception of compound **2t**), the replacement of the benzoyl with the more encumbering 1-naphthoyl or the heteroaromatic 2-furoyl or

Table 6
$GI_{50}$ values of **1m–o**, **1q**, **1s**, **1t**, **3j**, **3n**, **3o**, **3q** and **3s** against different cancer cell lines in culture[a]

| Panel | Compound $GI_{50}$ (μM)[b] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell line | **1m** | **1n** | **1o** | **1q** | **1s** | **1t** | **3j** | **3n** | **3o** | **3q** | **3s** |
| Leukemia | | | | | | | | | | | |
| CCRF–CEM | 19.5 | 4.5 | 3.3 | 12.2 | 1.2 | 2.1 | 12.8 | 7.1 | 5.4 | 18.3 | 3.6 |
| HL-60 (TB) | 16.6 | 12.7 | 3.3 | 16.0 | 4.0 | 2.2 | 23.3 | 24.6 | 10.3 | 23.4 | 2.8 |
| K-562 | 31.4 | 16.5 | 4.8 | 24.9 | 1.0 | 3.5 | 12.9 | 13.8 | 14.8 | 30.8 | 2.6 |
| MOLT-4 | 20.5 | 15.7 | 3.4 | 23.5 | 0.3 | 3.2 | 19.2 | 22.8 | 15.0 | 29.4 | 4.6 |
| RPMI-8226 | 12.9 | 1.8 | 1.5 | 3.7 | 2.0 | 1.2 | 3.4 | 2.8 | 2.4 | 7.3 | 1.8 |
| SR | n.t.[c] | n.t.[c] | 3.3 | n.t.[c] | 1.8 | 3.4 | n.t.[c] | n.t.[c] | n.t.[c] | n.t.[c] | 2.1 |
| Non-small cell lung cancer | | | | | | | | | | | |
| A549/ATCC | 20.1 | 27.8 | 18.4 | 19.9 | 9.8 | 4.0 | 14.8 | 17.8 | 20.8 | 33.2 | 12.5 |
| EKVX | 20.5 | 20.8 | 17.6 | n.d.[d] | 13.4 | 15.9 | 15.5 | 17.5 | 18.0 | 31.8 | 13.7 |
| HOP-62 | 23.1 | 17.6 | 14.3 | 15.2 | 8.7 | 6.7 | 19.1 | 16.1 | 15.2 | 20.0 | 12.0 |
| HOP-92 | 7.8 | 20.0 | 3.2 | 16.1 | 12.7 | 7.2 | 0.7 | n.d.[c] | 17.9 | 9.3 | 11.5 |
| NCI-H226 | 40.9 | 19.0 | 23.7 | 19.5 | 28.7 | 15.9 | 43.4 | 20.7 | 14.7 | 35.9 | 28.5 |
| NCI-H23 | 19.8 | 22.0 | 14.8 | 18.7 | 19.5 | 10.6 | 23.0 | 20.9 | 17.2 | 25.9 | 16.7 |
| NCI-H322M | 18.1 | 19.8 | 16.8 | 17.4 | 15.7 | 15.5 | 15.8 | 16.8 | 19.7 | 24.8 | 18.5 |
| NCI-H460 | 35.4 | 22.0 | 20.8 | 27.2 | 3.3 | 18.7 | 21.6 | 19.2 | 22.5 | 34.0 | 7.3 |
| NCI-H522 | 18.2 | 6.1 | 3.7 | 3.7 | 3.1 | 3.8 | 14.8 | 4.9 | 3.5 | 5.8 | 4.0 |
| Colon cancer | | | | | | | | | | | |
| COLO 205 | 21.0 | 17.7 | 18.3 | 19.4 | 9.4 | 7.7 | 20.7 | 20.0 | 17.2 | 20.2 | 8.7 |
| HCC-2998 | 22.0 | 19.9 | 16.7 | 19.0 | 12.4 | 8.0 | 19.8 | 18.4 | 17.6 | 19.8 | 13.4 |
| HCT-116 | 23.5 | 18.1 | 16.9 | 18.2 | 7.3 | 11.4 | 18.0 | 16.2 | 18.6 | 35.6 | 10.2 |
| HCT-15 | 22.1 | 19.6 | 13.5 | 20.2 | 4.1 | 5.3 | 21.3 | 19.3 | 17.0 | 25.6 | 5.6 |
| HT29 | 20.4 | 24.2 | 22.2 | 22.7 | 5.0 | 11.9 | 17.2 | 19.4 | 24.3 | 26.7 | 7.5 |
| KM12 | 15.3 | 16.1 | 10.1 | 14.2 | 4.0 | 3.4 | 12.7 | 15.7 | 14.4 | 14.4 | 5.3 |
| SW-620 | 28.2 | 21.3 | 11.1 | 20.3 | 5.5 | 4.8 | 18.0 | 19.4 | 19.0 | 26.8 | 5.5 |
| CNS cancer | | | | | | | | | | | |
| SF-268 | 24.5 | 16.8 | 14.1 | 11.5 | 2.7 | 8.2 | 19.2 | 22.2 | 16.2 | 21.4 | 3.9 |
| SF-295 | 22.9 | 18.5 | 19.5 | 19.0 | 16.9 | 16.7 | 26.9 | 18.0 | 17.7 | 29.3 | 21.4 |
| SF-539 | 27.2 | 15.1 | 12.8 | 14.5 | 3.0 | 1.8 | 26.6 | 20.6 | 20.2 | 21.3 | 2.5 |
| SNB-19 | 24.9 | 21.4 | 19.0 | 18.5 | 25.4 | 14.4 | 15.6 | 17.5 | 20.5 | 28.5 | 24.6 |
| SNB-75 | 22.3 | 18.9 | 14.1 | 7.1 | 10.4 | 1.4 | 20.0 | 17.5 | 13.3 | 10.9 | 8.6 |
| U251 | 17.8 | 16.4 | 12.9 | 16.3 | 9.0 | 7.9 | 14.4 | 12.5 | 17.0 | 16.4 | 14.1 |
| Melanoma | | | | | | | | | | | |
| LOX IMVI | 17.1 | 7.6 | 3.0 | 15.4 | 3.4 | 2.9 | 11.7 | 9.3 | 7.1 | 16.4 | 3.4 |
| MALME-3M | 25.4 | 14.9 | 9.8 | 13.4 | 3.7 | 10.3 | 24.5 | 20.4 | 3.1 | 24.3 | 8.3 |
| M14 | 16.3 | 19.6 | 11.9 | 16.8 | 7.7 | 3.6 | 14.6 | 16.8 | 15.6 | 20.6 | 10.7 |
| SK-MEL-2 | 18.4 | 18.4 | 16.1 | 16.3 | 14.2 | 12.6 | 16.3 | 15.3 | 16.9 | 19.9 | 16.5 |
| SK-MEL-28 | 18.4 | 30.6 | 12.2 | 17.2 | 5.5 | 3.8 | 19.1 | 18.2 | 18.4 | 29.5 | 26.2 |
| SK-MEL-5 | n.t.[c] | n.t.[c] | 16.1 | n.t.[c] | 10.1 | 4.6 | n.t.[c] | n.t.[c] | n.t.[c] | n.t.[c] | 12.7 |
| UACC-257 | 28.2 | 17.3 | 15.5 | 21.7 | 4.6 | 7.5 | 17.5 | 17.7 | 17.4 | 19.9 | 11.7 |
| UACC-62 | 17.3 | 18.1 | 15.6 | 14.9 | 3.2 | 12.3 | 15.9 | 16.0 | 15.8 | 15.8 | 5.0 |
| Ovarian cancer | | | | | | | | | | | |
| IGROV1 | 21.0 | 19.7 | 15.3 | 24.7 | 6.7 | 9.6 | 14.2 | 15.1 | 18.5 | 21.9 | 7.5 |
| OVCAR-3 | 19.7 | 17.1 | 10.2 | 15.2 | 2.7 | 3.5 | 15.4 | 15.0 | 14.8 | 16.9 | 4.2 |
| OVCAR-4 | 25.8 | 16.8 | 18.0 | 16.9 | 15.6 | 18.8 | 17.3 | 18.2 | 17.1 | 24.1 | 18.6 |
| OVCAR-5 | 21.6 | 24.3 | 22.9 | 23.2 | 17.8 | 17.1 | 17.4 | 16.9 | 21.9 | 48.5 | 18.0 |
| OVCAR-8 | 22.1 | 19.0 | 15.3 | 21.9 | 8.8 | 5.3 | 15.6 | 15.0 | 19.0 | 24.0 | 16.8 |
| SK-OV-3 | 22.8 | 18.2 | 15.3 | 20.2 | 16.0 | 15.9 | 25.0 | 18.9 | 17.5 | 21.9 | 26.6 |
| Renal cancer | | | | | | | | | | | |
| 786-0 | 18.6 | 7.2 | 2.9 | 11.4 | 3.3 | 2.4 | 6.8 | 4.5 | 4.7 | 12.7 | 3.3 |
| A498 | 16.1 | 20.2 | 15.5 | 17.7 | 16.6 | 15.1 | 14.8 | 15.8 | 15.3 | 20.5 | 16.5 |
| ACHN | 21.3 | 19.2 | 17.4 | 17.8 | 4.7 | 15.0 | 17.3 | 17.3 | 16.9 | 20.1 | 6.5 |
| CAKI-1 | 22.1 | 14.0 | 9.7 | 12.3 | 5.1 | 3.4 | 14.0 | 14.9 | 13.3 | 13.6 | 5.1 |
| RXF 393 | 18.5 | 17.8 | 12.7 | 14.3 | 3.6 | 2.9 | 16.8 | 17.5 | 16.0 | 16.1 | 3.3 |
| SN12C | 18.1 | 18.3 | 9.0 | 17.1 | 3.5 | 2.6 | 14.7 | 13.6 | 15.5 | 20.2 | 5.3 |
| TK-10 | 36.3 | 18.5 | 10.7 | 17.0 | 6.3 | 5.4 | 15.0 | 16.5 | 15.3 | 17.1 | 14.3 |
| UO-31 | n.t.[c] | n.t.[c] | 2.3 | 0.2 | 3.7 | 2.3 | n.t.[c] | n.t.[c] | n.t.[c] | 0.07 | 4.5 |

Table 6 (*continued*)

| Panel | Compound $GI_{50}$ (μM)[b] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell line | **1m** | **1n** | **1o** | **1q** | **1s** | **1t** | **3j** | **3n** | **3o** | **3q** | **3s** |
| Prostate cancer | | | | | | | | | | | |
| PC-3 | 22.7 | 16.7 | 16.9 | 15.0 | 3.9 | 6.1 | 15.8 | 14.7 | 16.6 | 22.2 | 5.0 |
| DU-145 | 30.6 | 14.7 | 10.5 | 13.6 | 2.7 | 3.3 | 15.7 | 15.7 | 16.8 | 19.6 | 2.5 |
| Breast cancer | | | | | | | | | | | |
| MCF7 | 18.3 | 5.6 | 3.1 | 5.2 | 3.0 | 3.2 | 2.8 | 4.6 | 3.5 | 5.5 | 2.7 |
| NCI/ADR-RES | 24.8 | 34.4 | 31.7 | 26.8 | 15.6 | 19.1 | 36.5 | 32.9 | 28.9 | 36.1 | 24.5 |
| MDA-MB-231/ATCC | 19.1 | 18.8 | 16.2 | 16.7 | 12.2 | 9.4 | 16.4 | 14.6 | 15.6 | 21.5 | 15.9 |
| HS 578T | 41.0 | 29.1 | 21.4 | 24.7 | 6.8 | 16.6 | 49.6 | 39.6 | 21.0 | 27.6 | 11.0 |
| MDA-MB-435 | 16.4 | 15.4 | 9.0 | 13.1 | 4.0 | 2.8 | 14.8 | 16.6 | 14.2 | 15.5 | 6.4 |
| MDA-N | 20.0 | 18.3 | 13.0 | 15.0 | 5.0 | 2.6 | 16.6 | 15.6 | 18.8 | 17.9 | 9.1 |
| BT-549 | 21.1 | 23.6 | 22.4 | 17.4 | 4.6 | 10.0 | 25.4 | 18.0 | 17.3 | 25.0 | 2.3 |
| T-47D | 19.5 | 17.5 | 6.5 | 13.1 | 4.9 | 4.6 | 12.8 | 15.3 | 10.7 | 20.7 | 4.0 |

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell lines screen. Only the values <100 μM are reported.
[b] Compound concentration that produces 50% growth inhibition.
[c] Not tested.
[d] Not determined.

2-thenoyl led to an activity increase (compare **1r–t** with **1d**, **2s** with **2d** and **3s** with **3d**), while the replacement with an alkylic acyl moiety (small acetyl or sterically demanding pivaloyl) caused a drop or loss in activity (**1a** vs **1d**; **3a** and **3b** vs **3d**). ATU **3d**, vinylogue of **3c**, turned out to be inactive. Regarding the substitution on the the benzoyl moiety phenyl ring, a chlorine atom at position *para* led to a remarkable potency increase in series **1** and **2** (compare **1j** with **1d** and **2j** with **2d**) and to the activity halving in series **3** (**3j** vs **3d**). The chlorine-atom shift to the *meta* and *ortho* position led to less active (*para* **1j** > *meta* **1i** > *ortho* **1h**) or inactive (*ortho* **2h**) compounds. The phenyl substitution with a more electron-withdrawing group (*nitro*) or an electron-donating function (for inductive effect: methyl, *t*-butyl; for mesomeric effect: methoxy) led to a decrease (4-chloro **1j** vs 4-methyl **1f**; 4-chloro **3j** vs 4-methyl **3f**) or loss in activity (4-*t*-butyl **1g**, 2-methyl **1e**, 3-nitro **1k**, 4-methoxy **1l**, 4-methyl **2f**). At position *para*, the chlorine atom and the methyl group were tolerated better than the more encoumbering *t*-butyl (compare **1j** and **1f** with **1g**). Regarding the dichlorosubstitution, patterns 3,4 (**1n–3n**) and 3,5 (**1o–3o**) were particularly effective and in some cases more beneficial than the 4-chlorosubstitution (compare **1n** with **1j**, **2o** with **2j** and **3o** with **3j**). On the contrary, pattern 2,4 was unfavourable (**1n**, **1o** vs **1m**; **2n**, **2o** vs **2m**; **3n**, **3o** vs **3m**), thus confirming that the presence of a substituent at *ortho* position is detrimental (compare also **1e** with **1f**; **1h** with **1i** and **1j**; **2h** with **2j**). The 3,4-dichlorobenzoyl ATU **1n** turned out to be the most active compound of the three series with an $IC_{50}$ of 4.6 μM. The good results obtained in series **1** by the 4-chloro-3-nitro derivative (**1p**, $IC_{50}$: 11 μM) and the 3,4,5-trimethoxy congener (**1q**, $IC_{50}$: 7.0 μM) suggest that the activity would not be affected by the electronic properties of the substituents. The 3,4,5-trimethoxybenzoyl acyl moiety was favourable also in series **3** (**3q**: $IC_{50} = 13$ μM), but led to the inactive six-membered ring ATU **2q**. The acyl portions

Table 7
$GI_{50}$ values of **4d**, **4s**–**6s** and **8s**–**14s** against different cancer cell lines in culture[a]

| Panel | Compound $GI_{50}$ (μM)[b] | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell line | **4d** | **4s** | **5s** | **6s** | **8s** | **9s** | **10s** | **11s** | **12s** | **13s** | **14s** |
| Leukemia | | | | | | | | | | | |
| CCRF-CEM | | 79.7 | | 41.9 | | 22.5 | | n.t.[c] | n.t.[c] | n.t.[c] | 15.6 |
| HL-60 (TB) | 33.5 | 19.8 | 6.9 | 31.5 | 22.0 | 10.5 | 5.8 | 8.2 | 7.5 | 9.9 | 14.9 |
| K-562 | 79.5 | 6.6 | 3.7 | 5.5 | 9.1 | 16.1 | 38.4 | 6.7 | 10.2 | 3.2 | 17.7 |
| MOLT-4 | 39.2 | 5.2 | 7.4 | 43.7 | 39.8 | 17.7 | 8.2 | 15.9 | 20.8 | 15.2 | 19.6 |
| RPMI-8226 | 35.3 | 4.5 | 3.3 | 5.6 | 12.4 | 10.5 | 2.7 | 3.8 | 5.5 | 8.4 | 12.4 |
| SR | 18.8 | 2.0 | 2.1 | 2.8 | 3.0 | 1.6 | 2.3 | 2.7 | 2.1 | 1.3 | 2.4 |
| Non-small cell lung cancer | | | | | | | | | | | |
| A549/ATCC | 37.1 | 13.5 | 14.6 | 22.0 | 34.5 | 25.8 | 11.2 | 10.8 | 19.3 | 11.3 | 11.6 |
| EKVX | 3.5 | 11.9 | 10.2 | 17.8 | 0.3 | 0.01 | 11.2 | 7.9 | 2.6 | 8.1 | 9.5 |
| HOP-62 | | n.t.[c] | 22.3 | 22.0 | 25.6 | 25.7 | 22.0 | 11.7 | 20.6 | 15.9 | 17.3 |
| HOP-92 | 56.8 | 22.4 | 14.0 | 26.9 | 25.5 | 21.5 | 11.7 | 11.1 | 20.8 | 27.0 | 23.0 |
| NCI-H23 | 26.1 | 5.8 | 13.2 | 15.2 | 21.4 | 12.3 | 16.4 | n.t.[c] | 12.2 | 7.0 | 7.7 |
| NCI-H322M | 33.4 | 15.5 | 14.0 | 23.4 | 17.6 | 28.6 | 4.7 | 27.9 | 12.4 | 2.2 | 14.2 |
| NCI-H460 | 20.0 | 3.3 | 2.5 | 9.5 | 4.0 | 19.9 | 1.5 | 7.0 | 2.3 | 2.4 | 4.9 |
| NCI-H522 | 15.9 | n.t.[c] | n.t.[c] | 3.7 | 18.7 | 16.7 | n.t.[c] | n.t.[c] | n.t.[c] | n.t.[c] | n.t.[c] |
| Colon cancer | | | | | | | | | | | |
| COLO 205 | n.d.[d] | 16.5 | 31.7 | 23.2 | 24.9 | 25.6 | 23.4 | 16.5 | 12.6 | 20.0 | 19.2 |
| HCT-116 | 34.0 | n.t.[c] | 4.0 | 9.1 | 21.2 | 15.2 | 4.1 | 6.4 | 7.4 | 7.9 | 8.9 |
| HCT-15 | 36.4 | 5.2 | 3.0 | 5.7 | 3.8 | 21.3 | 2.2 | 4.7 | 2.2 | 2.6 | 6.3 |
| HT29 | 42.0 | 27.7 | 12.2 | 30.5 | 24.1 | 21.4 | 17.1 | 16.9 | 15.8 | 16.9 | 19.2 |
| KM12 | 32.3 | 4.1 | 3.3 | 6.5 | 7.5 | 16.3 | 2.8 | 8.4 | 3.0 | 2.3 | 10.1 |
| SW-620 | 33.7 | 5.6 | 5.2 | 5.9 | 22.1 | 17.3 | 14.6 | 6.1 | 3.9 | 8.7 | 7.9 |
| CNS cancer | | | | | | | | | | | |
| SF-268 | 21.3 | 31.4 | 4.8 | 31.2 | 11.7 | 17.8 | 2.6 | 10.7 | 5.8 | n.d.[d] | 7.2 |
| SF-295 | 46.2 | 31.3 | 26.0 | 43.6 | 32.1 | 20.2 | 21.7 | 19.3 | 18.6 | 11.1 | 16.4 |
| SF-539 | n.t.[c] | 2.5 | n.t.[c] | 2.2 | n.t.[c] | n.t.[c] | n.t.[c] | 1.1 | n.t.[c] | n.t.[c] | n.t.[c] |
| SNB-19 | | 38.7 | 54.9 | 39.7 | 36.4 | 27.6 | 30.1 | 16.3 | 41.7 | 29.2 | 35.6 |
| SNB-75 | | 40.6 | 15.5 | 68.2 | | | 9.0 | 10.9 | 21.0 | 27.4 | 46.0 |
| U251 | 38.2 | 6.6 | 6.8 | 17.0 | 11.5 | 17.3 | 4.8 | 13.4 | 5.5 | 2.7 | 11.5 |
| Melanoma | | | | | | | | | | | |
| LOX IMVI | | 3.3 | 2.4 | 17.9 | 2.5 | 16.7 | 2.1 | 8.9 | 1.6 | 1.2 | 6.5 |
| MALME-3M | 10.2 | 47.8 | 32.7 | 30.7 | 12.5 | 21.4 | 20.7 | 9.3 | 5.6 | 3.1 | 13.3 |
| M14 | 37.3 | n.t.[c] | 6.4 | 14.9 | 16.5 | 14.2 | 4.0 | 6.3 | 10.7 | 7.0 | 8.9 |
| SK-MEL-2 | 18.4 | 4.3 | 2.9 | 3.7 | 24.4 | 16.7 | 3.3 | n.t.[c] | 15.2 | 12.1 | n.t.[c] |
| SK-MEL-28 | 45.5 | 12.5 | 13.0 | 28.0 | 15.8 | 18.7 | 3.9 | 28.0 | 9.1 | 17.8 | 22.2 |
| SK-MEL-5 | 23.3 | 2.9 | 14.8 | 8.3 | 16.5 | 11.2 | 16.6 | 8.1 | 5.3 | 10.9 | <0.005 |
| UACC-257 | 25.3 | n.t.[c] | 5.9 | n.t.[c] | 23.3 | 16.5 | 2.5 | n.t.[c] | 17.2 | 11.7 | n.t.[c] |
| UACC-62 | 50.4 | 8.8 | 10.8 | 12.9 | 14.0 | 16.9 | 5.5 | 7.3 | 11.5 | 6.9 | 7.6 |
| Ovarian cancer | | | | | | | | | | | |
| IGROV1 | 28.2 | 6.6 | 6.2 | 19.4 | 24.0 | 25.3 | 3.6 | 10.9 | 10.4 | 5.6 | |
| OVCAR-3 | 36.4 | 11.2 | 4.9 | 18.2 | 19.0 | 15.5 | 11.4 | 17.2 | 2.7 | 7.3 | 20.2 |
| OVCAR-4 | 59.3 | 20.1 | 23.3 | 29.8 | 18.5 | 25.2 | 10.6 | 20.1 | 17.4 | 16.1 | 31.0 |
| OVCAR-8 | 77.0 | 14.6 | 15.5 | 25.4 | 43.6 | 20.6 | 8.2 | 8.8 | 27.8 | 16.2 | 11.7 |
| SK-OV-3 | 66.7 | 33.1 | 28.2 | 37.4 | 42.6 | 41.2 | 20.0 | 17.5 | 31.3 | 41.8 | 20.0 |
| Renal cancer | | | | | | | | | | | |
| 786-0 | 38.9 | n.t.[c] | 6.3 | 16.1 | 18.0 | 17.4 | 5.3 | 11.2 | 11.2 | 8.2 | 12.9 |
| ACHN | 18.8 | 9.4 | 4.8 | 16.6 | 11.0 | 15.3 | 2.9 | 6.9 | 4.1 | 5.8 | 8.5 |
| CAKI-1 | 28.7 | 4.8 | 3.4 | 12.5 | 11.8 | 13.2 | 5.2 | 9.3 | 2.8 | 7.0 | 15.9 |
| RXF 393 | 22.7 | 2.6 | 3.1 | 4.1 | 19.2 | 13.1 | 1.8 | n.t.[c] | 4.4 | 5.8 | n.t.[c] |
| SN12C | 24.6 | 3.1 | 3.7 | 8.6 | 12.0 | 11.8 | 2.6 | 7.7 | 5.9 | 6.1 | 8.1 |
| TK-10 | 23.3 | 4.6 | 3.6 | 10.5 | 12.1 | 19.4 | 4.2 | 10.6 | 3.5 | 6.9 | 12.4 |
| UO-31 | 20.4 | 10.5 | 5.2 | 10.2 | 23.5 | 12.0 | 2.9 | 8.8 | 13.5 | 8.7 | 10.0 |
| Prostate cancer | | | | | | | | | | | |
| PC-3 | 60.0 | 17.2 | 6.8 | 31.7 | 35.6 | 18.9 | 4.8 | 17.1 | 18.1 | 23.6 | 19.4 |
| DU-145 | 24.4 | 5.5 | 3.5 | 5.0 | 29.8 | 15.9 | n.t.[c] | 11.0 | n.t.[c] | 12.4 | 8.0 |
| Breast cancer | | | | | | | | | | | |
| MCF7 | 16.7 | 3.5 | n.t.[c] | 4.5 | 1.7 | 21.0 | n.t.[c] | 1.3 | n.t.[c] | n.t.[c] | n.t.[c] |
| NCI/ADR-RES | | n.t.[c] | 24.0 | n.t.[c] | 25.4 | 28.1 | 23.2 | n.t.[c] | 22.2 | 9.2 | n.t.[c] |
| MDA-MB-231/ATCC | 36.1 | 2.9 | 4.2 | 13.7 | 13.1 | 18.4 | 2.6 | 9.5 | 9.0 | 7.4 | 10.8 |
| MDA-MB-435 | 38.8 | 5.3 | 6.8 | 11.0 | 14.6 | 15.2 | 12.6 | 10.7 | 4.5 | 7.2 | 14.4 |
| BT-549 | 22.8 | 6.9 | 4.2 | 7.8 | 22.5 | 8.6 | 14.0 | 5.6 | 3.5 | 10.0 | 9.0 |
| T-47D | 89.2 | 21.2 | 4.6 | 7.2 | 9.3 | | 3.7 | 4.3 | 6.0 | | 4.1 |

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell lines screen. Only the values <100 μM are reported.
[b] Compound concentration that produces 50% growth inhibition.
[c] Not tested.
[d] Not determined.

that gave the best results in all the three series **1**–**3** were the 2-furoyl (**1s**: 7.4 μM; **2s**: 36 μM; **3s**: 9.0 μM) and, to a lesser extent, the 3,5-dichlorobenzoyl (**1o**: 13 μM; **2o**: 30 μM; **3o**: 15 μM).

The replacement of one of the two benzoyl moieties in ATUs **1d** and **1s** with the electron-poor α,β-unsaturated system *N*-methylene(malononitrile) (**4d**, **4s**: Y = W = cyano, Table 4) enhanced the antiproliferative activity 8.5 and 1.7 times, respectively (**4d** vs **1d**, **4s** vs **1s**). The benzoyl/furoyl interchange confirmed to be effective, with a 2.5-fold increase in potency (**4s** vs **4d**). Data of Table 4 display that the replacement of one or both cyano groups with an acetyl (**10s**–**12s**), pivaloyl (**7s**), benzoyl (**6s**, **11s**, **13s**, **14s**), methoxycarbonyl (**5s**, **12s**), ethoxycarbonyl (**14s**), aminocarbonyl (**8s**) or *p*-chlorophenylaminocarbonyl (**9s**) did not affect significantly the activity (compare **5s**–**14s** with **4s**), as the $IC_{50}$ values remained in the 3.0–7.0 μM range, except for the encumbering pivaloyl derivative **7s**. Since the isosteric oxygen/sulphur exchange produced an 11-fold drop (**17s** vs **14s**) or loss (**15s** vs **4s** and **16s** vs **11s**) in activity and thioureas **V**–**XV** resulted to be inactive (data not shown), the importance of the presence of the sulphur atom and of the acyl moiety for the activity is highlighted.

The data reported in Table 5 show that, among the ATUs tested at NCI, compounds of series **1** and **3** (with the exception of **3b** and **3m**) showed a $GI_{50}$ value inferior to 50 μM against all the cell lines investigated (57–60), a TGI (measure of the cytostatic activity) lower than 100 μM against most of the cell lines (50–58) and an $LC_{50}$ (measure of the cytocidal effect) inferior to 100 μM against still a high number of cell lines (26–52). In particular, ATUs **1o**, **1s**, **1t** and **3o** displayed a significant TGI value against 57–58 out of 57–60 cell lines investigated and a significant $LC_{50}$ against 46–52 cell lines. Compounds **2s**, **2t**, **3b** and **3m** resulted as the least effective. Most of the remaining ATUs (**4d**, **4s**–**6s**, **8s**–**14s**) showed antiproliferative activity against all or nearly all the cell lines investigated (46–52 out of 47–53), but only compounds **5s**, **10s**, **12s** and **13s** demonstrated to be cytostatic at micromolar concentrations in a good number of cell lines (37–44) and cytocidal in 19–25 cell lines.

Analysis of data of Tables 6 and 7 indicates that the ATUs tested at NCI showed in general a wide spectrum of activity in the micromolar concentration range (in most cases $GI_{50} < 25$ μM) with a slight higher sensitivity towards leukemia and renal cancer.

Among all the cell lines investigated, compounds **1** and **3** (Table 6) exhibited particular sensitivity for leukemia CCRF–CEM, RPMI-8226 and SR, melanoma LOX IMVI and non-small cell lung HOP-92 and NCI-H522, colon KM12, renal 786-0 and UO-31, breast MCF7, MDA-MB-435 and T-47D cancer cell lines. ATUs **1o**, **1s**, **1t** and **3s** resulted effective against all leukemia cell lines in the 0.3–4.8 μM concentration range. The most prominent compounds **1s**, **1t** and **3s** turned out to be highly active also against melanoma, colon, renal and prostate cancer cell lines and many lines of the other subpanels. Some compounds exhibited high sensitivity for a particular tumor cell line: **1s** for leukemia MOLT-4 ($GI_{50}$: 300 nM), **1q**, **3b** and **3q** for renal cancer UO-31 [$GI_{50} = 200$, 70 and 70 nM, respectively ($GI_{50}$ values of **3b** not reported in Table 6)]; **3j** for non-small cell lung cancer HOP-92 ($GI_{50}$: 700 nM).

The data reported in Table 7 show that compounds **4s–14s** exhibited particular sensitivity for renal cancer, leukemia RPMI-8226 and SR, melanoma LOX IMVI and SK-MEL-5, and non-small cell lung EKVX and NCI-H460, colon HCT-116, HCT-15, KM-12 and SW-620, breast MCF7, MDA-MB-231/ATCC, BT-549 and T-47D cancer cell lines. The 2-furoyl derivative **4s** was more active than the corresponding benzoyl analogue **4d** against all cell lines (except for EKVX). The nature of the electron-withdrawing groups Y and W of the α,β-unsaturated system seems to slightly influence the antiproliferative activity: the ATUs which turned out to be in general the most effective (**4s**, **5s** and **10s–13s**) carried the cyano, acetyl, benzoyl, methoxy- and ethoxycarbonyl groups, whereas ATUs **8s** and **9s**, bearing an amide group, turned out to be in general the least active, but selective for non-small cell lung cancer EKVX (**8s**, $GI_{50}$: 300 nM; **9s**, $GI_{50}$: 10 nM). Among the most prominent compounds, **5s** was particularly effective against leukemia ($GI_{50}$: 2.1–7.4 μM), renal ($GI_{50}$: 3.1–6.3 μM) and prostate ($GI_{50}$: 3.5, 6.8 μM) cancer cell lines, most of the lines of colon and breast subpanels and various lines of the other panels. ATU **14s** exhibited high selectivity for melanoma SK-MEL-5 cell line ($GI_{50} < 5$ nM).

At the moment, studies are in progress in the attempt to understand the target and the mechanism of action of the title compounds. From a mechanistic point of view, it could be hypothesized that ATUs act as acyl group vectors, able to inactivate enzymes essential for cell proliferation through the acylation of particular aminoacids (e.g. lysine amino group or serine hydroxy function).

All acyl(thio)ureas and starting thioureas **V–XV** were also assayed against HIV-1 infected MT-4 cells for their ability to inhibit the virus-induced cytopathogenicity. However, none of them was able to prevent the HIV-induced cytopathogenicity at non cytotoxic concentrations (data not shown).

## 4. Conclusion

The ATUs synthesized exhibited interesting antiproliferative properties with a wide spectrum of activity at micromolar, low micromolar and, in some cases, nanomolar concentrations. The antitumor effect was higher in imidazolidine-2-thione ATUs than in tetrahydropyrimidine-2(1*H*)-thione ATUs and appeared strongly dependent on the nature of the acyl moiety. The furoyl group led to the most cytotoxic compounds in all the series. The understanding of the target and mechanism of action of the title compounds will provide useful information to modify the ATU structures in order to improve their anticancer properties.

## 5. Experimental protocols

### 5.1. Chemistry

All chemicals were purchased by Sigma–Aldrich Chemical Co. and used without further purification, unless otherwise stated. Solvents were of reagent grade. DMF was dried on molecular sieves (5 Å 1/16″ inch pellets). Organic solutions were dried over anhydrous sodium sulphate. Thin layer chromatography (TLC) system for routine monitoring the course of reactions and confirming the purity of analytical samples employed aluminium-backed silica gel plates (Merck DC-Alufolien Kieselgel 60 $F_{254}$): $CHCl_3$ or $CHCl_3$/methanol were used as developing solvents and detection of spots was made by UV light and/or by iodine vapours. The reactions of acylation were performed in parallel (except for **3a** and **4d**) by using Carousel-6 and Carousel-12 Reaction Stations™ (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna). The evaporation of solutions was performed in parallel with an Evaposel™ apparatus (Radleys Discovery Technologies, Italian distributor: StepBio, Bologna) operating at reduced pressure of about 15–20 Torr. For the reactions not performed in parallel fashion, the organic solutions were evaporated using a rotatory evaporator operating at reduced pressure of about 10–20 Torr. Yields were not optimized. Melting points were determined on a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 398 spectrometer as KBr discs or solutions in $CHCl_3$. $^1H$ and $^{13}C$ NMR spectra were recorded in DMSO-$d_6$, $CDCl_3$ or $CF_3COOD$ on a Varian Gemini 200 instrument. Chemical shifts were reported in ppm units relative to the internal reference tetramethylsilane, and the splitting patterns were described as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), quintet and m (multiplet). The first order values reported for coupling constants *J* were given in hertz. Elemental analyses were performed by an EA1110 Elemental Analyser (Fison-Instruments, Milan) and were within ±0.4% of the theoretical values. The synthesis of compounds **IV–XVIII** was accomplished according to the published procedure [21].

#### 5.1.1. Parallel procedure for the preparation of 1,3-diacylimidazolidine-2-thiones *Ia* and *Id–t* and 1,3-diacyl-

*tetrahydropirimidin-2(1H)-thiones* ***2d***, ***2f***, ***2h***, ***2j***, ***2m–o***, ***2q***, ***2s*** *and* ***2t***

The suitable acyl chloride (22 mmol) (for **1a**: acetic anhydride, 22 mmol, 2.08 mL) was added in one portion to each numbered round-bottomed flask of a Carousel-6 Reaction Station™, containing a stirred solution of thiourea **I** (10 mmol, 1.022 g) or **II** (10 mmol, 1.162 g) in pyridine (20 mL). The resulting mixtures were stirred for 30 min at 90 °C. After cooling to rt, 150 mL of water were added into each round-bottomed flask. The precipitates obtained were filtered off in parallel by an in-house device, washed with water and a mixture of diethyl ether and petroleum ether (1:1), and purified by crystallization from the suitable solvents or solvent mixtures.

*5.1.1.1. 1,3-Diacetylimidazolidine-2-thione (**1a**).* Yield: 76%; m.p.: 93–95 °C (DCM/diethyl ether); IR ($CHCl_3$): 1687 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.81 (s, 6H, $2CH_3$), 4.01 (s, 4H, $2CH_2$). Anal. Calcd for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N, 15.04; S, 17.22. Found: C, 45.25; H, 5.42; N, 15.11; S, 16.89.

*5.1.1.2. 1,3-Dibenzoylimidazolidine-2-thione (**1d**).* Yield: 90%; m.p.: 243–244 °C (acetone/$CHCl_3$) (1it.: m.p. 228–231 °C, 232–233 °C (methanol) [22]); IR (KBr): 1681 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.22 (s, 4H, $2CH_2$), 7.28–7.64 (m, 10H, Ar-H) (consistent with the data reported in lit. [22]). $^{13}C$ NMR ($CDCl_3$) $\delta$: 44.79 ($2CH_2$), 127.31 (4CH), 128.21 (4CH), 131.39 (2CH), 133.80 (2C), 171.40 (2CO), 178.00 (CS). Anal. Calcd for $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03; S, 10.33. Found: C, 65.85; H, 4.52; N, 9.09; S, 10.28.

*5.1.1.3. 1,3-Bis(2-methylbenzoyl)imidazolidine-2-thione (**1e**).* Yield: 97%; m.p.: 154–156 °C (diethyl ether); IR (KBr): 1683 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.15 (s, 6H, $2CH_3$), 4.12 (s, 4H, $2CH_2$), 7.04–7.27 (m, 8H, Ar-H). Anal. Calcd for $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28; S, 9.47. Found: C, 67.45; H, 5.35; N, 8.32; S, 9.20.

*5.1.1.4. 1,3-Bis(4-methylbenzoyl)imidazolidine-2-thione (**1f**).* Yield: 92%; m.p.: 174–176 °C (DCM/diethyl ether); IR (KBr): 1679 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.34 (s, 6H, $2CH_3$), 4.18 (s, 4H, $2CH_2$), 7.13–7.37 and 7.52–7.77 (m, 8H, Ar-H). Anal. Calcd for $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28; S, 9.47. Found: C, 67.22; H, 5.30; N, 8.19; S, 9.28.

*5.1.1.5. 1,3-Bis(4-tert-butylbenzoyl)imidazolidine-2-thione (**1g**).* Yield: 80%; m.p.: 218–219 °C (DCM/petroleum ether); IR (KBr): 1678 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 1.28 (s, 9H, $3CH_3$), 4.20 (s, 4H, $2CH_2$), 7.31–7.79 (m, 8H, Ar-H). Anal. Calcd for $C_{25}H_{30}N_2O_2S$: C, 71.05; H, 7.15; N, 6.63; S, 7.59. Found: C, 71.31; H, 7.18; N, 6.72; S, 7.71.

*5.1.1.6. 1,3-Bis(2-chlorobenzoyl)imidazolidine-2-thione (**1h**).* Yield: 87%; m.p.: 156–158 °C (diethyl ether); IR (KBr): 1663 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.29 (s, 4H, $2CH_2$), 7.18–7.47 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_2S$: C, 53.84; H, 3.19; N, 7.39; S, 8.45. Found: C, 53.53; H, 3.18; N, 7.29; S, 8.41.

*5.1.1.7. 1,3-Bis(3-chlorobenzoyl)imidazolidine-2-thione (**1i**).* Yield: 82%; m.p.: 198–200 °C (DCM/diethyl ether); IR (KBr): 1672 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.22 (s, 4H, $2CH_2$), 7.25–7.57 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_2S$: C, 53.84; H, 3.19; N, 7.39; S, 8.45. Found: C, 54.08; H, 3.33; N, 7.52; S, 8.30.

*5.1.1.8. 1,3-Bis(4-chlorobenzoyl)imidazolidine-2-thione (**1j**).* Yield: 93%; m.p.: 168–170 °C (DCM/diethyl ether); IR (KBr): 1671 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.22 (s, 4H, $2CH_2$), 7.38–7.87 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{12}Cl_2N_2O_2S$: C, 53.84; H, 3.19; N, 7.39; S, 8.45. Found: C, 54.06; H, 3.24; N, 7.48; S, 8.39.

*5.1.1.9. 1,3-Bis(3-nitrobenzoyl)imidazolidine-2-thione (**1k**).* Yield: 90%; m.p.: 258–260 °C (DCM/diethyl ether); IR (KBr): 1683, 1526, 1349 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.33 (s, 4H, $2CH_2$), 7.60–8.65 (m, 8H, Ar-H). Anal. Calcd for $C_{17}H_{12}N_4O_6S$: C, 51.00; H, 3.02; N, 13.99; S, 8.01. Found: C, 50.92; H, 3.08; N, 14.11; S, 8.01.

*5.1.1.10. 1,3-Bis(4-methoxybenzoyl)imidazolidine-2-thione (**1l**).* Yield: 97%; m.p.: 205–207 °C (DCM/diethyl ether); IR (KBr): 1670 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 3.84 (s, 6H, $2CH_3$), 4.17 (s, 4H, $2CH_2$), 6.87–7.16 and 7.65–7.88 (m, 8H, Ar-H). Anal. Calcd for $C_{19}H_{18}N_2O_4S$: C, 61.61; H, 4.90; N, 7.56; S, 8.66. Found: C, 61.86; H, 4.93; N, 7.65; S, 8.78.

*5.1.1.11. 1,3-Bis(2,4-dichlorobenzoyl)imidazolidine-2-thione (**1m**).* Yield: 88%; m.p.: 151–153 °C (DCM/diethyl ether); IR (KBr): 1677 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.30 (s, 4H, $2CH_2$), 7.20–7.42 (m, 6H, Ar-H). Anal. Calcd for $C_{17}H_{10}Cl_4N_2O_2S$: C, 45.56; H, 2.25; N, 6.25; S, 7.15. Found: C, 45.36; H, 2.32; N, 6.23; S, 6.95.

*5.1.1.12. 1,3-Bis(3,4-dichlorobenzoyl)imidazolidine-2-thione (**1n**).* Yield: 78%; m.p.: 178–180 °C (DCM/diethyl ether); IR (KBr): 1689 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.21 (s, 4H, $2CH_2$), 7.36–7.42 (m, 4H, Ar-H), 7.63–7.67 (m, 2H, Ar-H). $^{13}C$ NMR ($CDCl_3$) $\delta$: 44.56 ($2CH_2N$), 127.23 (2CH), 129.45 (2CH), 130.05 (2CH), 131.85 (2C), 133.18 (2C), 135.86 (2C), 168.77 (2CO), 177.66 (CS). Anal. Calcd for $C_{17}H_{10}Cl_4N_2O_2S$: C, 45.56; H, 2.25; N, 6.25; S, 7.15. Found: C, 45.68; H, 2.29; N, 6.28; S, 6.96.

*5.1.1.13. 1,3-Bis(3,5-dichlorobenzoyl)imidazolidine-2-thione (**1o**).* Yield: 81%; m.p.: 231–233 °C (DCM/diethyl ether); IR (KBr): 1675 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.25 (s, 4H, $2CH_2$), 7.25–7.57 (m, 6H, Ar-H). Anal. Calcd for $C_{17}H_{10}Cl_4N_2O_2S$: C, 45.56; H, 2.25; N, 6.25; S, 7.15. Found: C, 45.20; H, 2.32; N, 6.26; S, 7.05.

*5.1.1.14. 1,3-Bis(4-chloro-3-nitrobenzoyl)imidazolidine-2-thione (**1p**).* Yield: 84%; m.p.: 198–200 °C (DCM/diethyl

ether); IR (KBr): 1674 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.25 (s, 4H, $2CH_2$), 7.45–7.74 (m, 6H, Ar-H). Anal. Calcd for $C_{17}H_{10}Cl_2N_4O_6S$: C, 43.51; H, 2.15; N, 11.94; S, 6.83. Found: C, 43.41; H, 2.27; N, 11.92; S, 6.95.

*5.1.1.15. 1,3-Bis(3,4,5-trimethoxybenzoyl)imidazolidine-2-thione (**1q**).* Yield: 80%; m.p.: 189–191 °C (DCM/diethyl ether); IR (KBr): 1671 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 3.88 (s, 12H, $4CH_3$), 3.92 (s, 6H, $2CH_3$), 4.23 (s, 4H, $2CH_2$), 6.97 (s, 4H, Ar-H). Anal. Calcd for $C_{23}H_{26}N_2O_8S$: C, 56.32; H, 5.34; N, 5.71; S, 6.54. Found: C, 56.60; H, 5.33; N, 5.74; S, 6.39.

*5.1.1.16. 1,3-Di-1-naphthoylimidazolidine-2-thione (**1r**).* Yield: 68%; m.p.: 189–191 °C (DCM/diethyl ether); IR (KBr): 1678 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.39 (s, 4H, $2CH_2$), 7.25–8.02 (m, 14H, Ar-H). Anal. Calcd for $C_{25}H_{18}N_2O_2S$: C, 73.15; H, 4.42; N, 6.82; S, 7.81. Found: C, 73.09; H, 4.39; N, 6.82; S, 7.52.

*5.1.1.17. 1,3-Di-2-furoylimidazolidine-2-thione (**1s**).* Yield: 86%; m.p.: 145–147 °C (DCM/diethyl ether); IR (KBr): 1675 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.23 (s, 4H, $2CH_2$), 6.78 (dd, $J = 3.7$ Hz, $J = 1.8$ Hz, 2H, 2 fur H-4), 7.45 (d, $J = 3.7$ Hz, 2H, 2 fur H-3), 8.06 (d, $J = 1.8$ Hz, 2H, 2 fur H-5). Anal. Calcd for $C_{13}H_{10}N_2O_4S$: C, 53.79; H, 3.47; N, 9.65; S, 11.05. Found: C, 53.70; H, 3.49; N, 9.57; S, 11.06.

*5.1.1.18. 1,3-Bis(thien-2-ylcarbonyl)imidazolidine-2-thione (**1t**).* Yield: 84%; m.p.: 131–132 °C (DCM/diethyl ether); IR (KBr): 1675 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.24 (s, 4H, $2CH_2$), 7.16–7.38 (m, 2H, 2 thioph H-4), 7.793–8.18 (m, 4H, 2 thioph H-3 and 2 thioph H-5). Anal. Calcd for $C_{13}H_{10}N_2O_2S_3$: C, 48.43; H, 3.13; N, 8.69; S, 29.83. Found: C, 48.46; H, 3.05; N, 8.60; S, 29.91.

*5.1.1.19. 1,3-Dibenzoyltetrahydropyrimidine-2(1H)-thione (**2d**).* Yield: 91%; m.p.: 228–230 °C (DCM/diethyl ether); IR (KBr): 1701 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.44 (quintet, $J = 6.6$ Hz, 2H, $CCH_2C$), 3.98 (t, $J = 6.6$ Hz, 4H, $2CH_2N$), 7.40–8.05 (m, 10H, Ar-H). Anal. Calcd for $C_{18}H_{16}N_2O_2S$: C, 66.65; H, 4.97; N, 8.64; S, 9.88. Found: C, 66.54; H, 4.96; N, 8.63; S, 9.92.

*5.1.1.20. 1,3-Bis(4-methylbenzoyl)tetrahydropyrimidine-2(1H)-thione (**2f**).* Yield: 98%; m.p.: 209–211 °C (DCM/diethyl ether); IR (KBr): 1699 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.32 (s, 6H, $2CH_3$), 2.35 (quintet, $J = 6.6$ Hz, 2H, $CCH_2C$), 3.85 (t, $J = 6.6$ Hz, 4H, $2CH_2N$), 7.18–7.28 and 7.59–7.69 (m, 8H, Ar-H); $^{13}C$ NMR (DMSO-$d_6$) $\delta$: 21.07 ($2CH_3$), 22.09 ($CH_2$), 44.88 ($2CH_2N$), 129.09 (4CH), 129.23 (4CH), 131.40 (2C), 142.97 (2C), 173.22 (2CO), 181.10 (CS). Anal. Calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95; S, 9.10. Found: C, 68.24; H, 5.78; N, 8.29; S, 9.20.

*5.1.1.21. 1,3-Bis(2-chlorobenzoyl)tetrahydropyrimidine-2(1H)-thione (**2h**).* Yield: 68%; m.p.: 196–198 °C (DCM/diethyl ether); IR (KBr): 1700 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.47 (quintet, $J = 6.8$ Hz, 2H, $CCH_2C$), 4.14 (t, $J = 6.8$ Hz, 4H, $2CH_2N$), 7.16–7.45 (m, 8H, Ar-H). Anal. Calcd for $C_{18}H_{14}Cl_2N_2O_2S$: C, 54.97; H, 3.59; N, 7.12; S, 8.15. Found: C, 55.16; H, 3.60; N, 7.26; S, 7.90.

*5.1.1.22. 1,3-Bis(4-chlorobenzoyl)tetrahydropyrimidine-2(1H)-thione (**2j**).* Yield: 98%; m.p.: 207–209 °C (DCM/diethyl ether); IR (KBr): 1705 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.37 (quintet, $J = 6.6$ Hz, 2H, $CCH_2C$), 3.90 (t, $J = 6.6$ Hz, 4H, $2CH_2N$), 7.44–7.54 and 7.67–7.77 (m, 8H, Ar-H). Anal. Calcd for $C_{18}H_{14}Cl_2N_2O_2S$: C, 54.97; H, 3.59; N, 7.12; S, 8.15. Found: C, 55.17; H, 3.81; N, 6.80; S, 7.98.

*5.1.1.23. 1,3-Bis(2,4-dichlorobenzoyl)tetrahydropyrimidine-2(1H)-thione (**2m**).* Yield: 65%; m.p.: 201–203 °C (DCM/diethyl ether); IR (KBr): 1683 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.46 (quintet, $J = 7.0$ Hz, 2H, $CCH_2C$), 4.12 (t, $J = 7.0$ Hz, 4H, $2CH_2N$), 7.22–7.43 (m, 6H, Ar-H). Anal. Calcd for $C_{18}H_{12}Cl_4N_2O_2S$: C, 46.78; H, 2.62; N, 6.06; S, 6.94. Found: C, 47.08; H, 2.72; N, 6.25; S, 6.96.

*5.1.1.24. 1,3-Bis(3,4-dichlorobenzoyl)tetrahydropyrimidine-2(1H)-thione (**2n**).* Yield: 73%; m.p.: 158–160 °C (DCM/diethyl ether); IR (KBr): 1674 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.46 (quintet, $J = 6.6$ Hz, 2H, $CCH_2C$), 4.00 (t, $J = 6.6$ Hz, 4H, $2CH_2N$), 7.40–7.63 and 7.70–7.85 (m, 2H, Ar-H). Anal. Calcd for $C_{18}H_{12}Cl_4N_2O_2S$: C, 46.78; H, 2.62; N, 6.06; S, 6.94. Found: C, 46.73; H, 2.76; N, 6.24; S, 7.10.

*5.1.1.25. 1,3-Bis(3,5-dichlorobenzoyl)tetrahydropyrimidine-2(1H)-thione (**2o**).* Yield: 98%; m.p.: 201–203 °C (DCM/diethyl ether); IR (KBr): 1719 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.37 (quintet, $J = 6.4$ Hz, 2H, $CCH_2C$), 3.94 (t, $J = 6.4$ Hz, 4H, $2CH_2N$), 7.68–7.77 (m, 6H, Ar-H). Anal. Calcd for $C_{18}H_{12}Cl_4N_2O_2S$: C, 46.78; H, 2.62; N, 6.06; S, 6.94. Found: C, 46.71; H, 3.00; N, 6.28; S, 6.75.

*5.1.1.26. 1,3-Bis(3,4,5-trimethoxybenzoyl)tetrahydropyrimidine-2(1H)-thione (**2q**).* Yield: 80%; m.p.: 132–134 °C (DCM/diethyl ether); IR (KBr): 1683 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.48 (quintet, $J = 6.6$ Hz, 2H, $CCH_2C$), 3.77–4.15 (m, 22H, $2CH_2N + 6CH_3$), 7.06 (s, 4H, Ar-H). $^{13}C$ NMR ($CDCl_3$) $\delta$: 22.66 ($CH_2$), 43.87 ($2CH_2N$), 55.63 ($4CH_3$), 60.26 ($2CH_3$), 106.43 (4CH), 128.17 (2C), 141.81 (2C), 152.29 (4C), 172.33 (2CO), 182.20 (CS). Anal. Calcd for $C_{24}H_{28}N_2O_8S$: C, 57.13; H, 5.59; N, 5.55; S, 6.35. Found: C, 56.74; H, 5.50; N, 5.41; S, 6.29.

*5.1.1.27. 1,3-Di-2-furoyltetrahydropyrimidine-2(1H)-thione (**2s**).* Yield: 97%; m.p.: 199–201 °C (acetone/chloroform); IR (KBr): 1694 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.26 (quintet, $J = 6.4$ Hz, 2H, $CCH_2C$), 3.76 (t, $J = 6.4$ Hz, 4H, $2CH_2N$), 6.68 (dd, $J = 3.6$ Hz, $J = 1.6$ Hz, 2H, 2 fur H-4), 7.36 (d, $J = 3.6$ Hz, 2H, 2 fur H-3), 7.96 (d, $J = 1.6$ Hz, 2H, 2 fur H-5); $^{13}C$ NMR (DMSO-$d_6$) $\delta$: 22.17 ($CH_2$), 44.84 ($2CH_2N$), 112.96 (2CH), 119.78 (2CH), 147.35 (2C), 147.67 (2CH),

162.04 (2CO), 181.10 (CS). Anal. Calcd for $C_{14}H_{12}N_2O_4S$: C, 55.26; H, 3.97; N, 9.21; S, 10.54. Found: C, 55.32; H, 4.04; N, 9.48; S, 10.54.

*5.1.1.28. 1,3-Bis(thien-2-ylcarbonyl)tetrahydropyrimidine-2(1H)-thione (**2t**).* Yield: 97%; m.p.: 223–225 °C (acetone/DCM); IR (KBr): 1681 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.30 (quintet, $J = 6.4$ Hz, 2H, $CCH_2C$), 3.79 (t, $J = 6.4$ Hz, 4H, $2CH_2N$), 7.18 (dd, $J = 5.0$ Hz, $J = 3.8$ Hz, 2H, 2 thioph H-4), 7.83 (dd, $J = 3.8$ Hz, $J = 1.2$ Hz, 2H, 2 thioph H-3), 8.01 (dd, $J = 5.0$ Hz, $J = 1.2$ Hz, 2H, 2 thioph H-5); $^{13}C$ NMR (DMSO-$d_6$) $\delta$: 21.55 ($CH_2$), 45.38 ($2CH_2N$), 128.50 (2CH), 134.96 (2CH), 135.70 (2CH), 138.00 (2C), 166.80 (2CO), 179.01 (CS). Anal. Calcd for $C_{14}H_{12}N_2O_2S_3$: C, 49.98; H, 3.60; N, 8.33; S, 28.59. Found: C, 50.33; H, 3.62; N, 8.60; S, 28.58.

### *5.1.2. Preparation of 3-acetyl-2-thioxoimidazolidine-1-carbaldehyde (**3a**)*

Acetic anhydride (large excess, 8 mL) was added in one portion to a stirred suspension of 2-thioxo-imidazolidine-1-carbaldehyde (10 mmol, 1.301 g) in pyridine (8 mL). The resulting mixture was stirred for 3 h at 100 °C. After cooling to rt, 30 mL of water were added. The precipitate obtained was filtered off, dried and crystallized from DCM/petroleum ether. Yield: 55%; m.p.: 99–101 °C; IR ($CHCl_3$): 1708 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.85 (s, 3H, $CH_3$), 3.84–4.24 (m, 4H, $2CH_2$), 9.58 (s, 1H, HCO). Anal. Calcd for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27; S, 18.62. Found: C, 41.86; H, 4.70; N, 16.40; S, 18.83.

### *5.1.3. Parallel procedure for the preparation of 3-acyl-2-thioxoimidazolidine-1-carbaldehydes **3b–d**, **3f**, **3j**, **3m–o**, **3q** and **3s***

The suitable acyl chloride (11 mmol) was added in one portion to each numbered round-bottomed flask of a Carousel-6 Reaction Station™, containing a stirred solution of 2-thioxo-imidazolidine-1-carbaldehyde (10 mmol, 1.301 g) in pyridine (20 mL). The resulting mixtures were stirred for 12 h at rt. Then, 100 mL of water were added into each round-bottomed flask. The contents of the flasks were transferred into a set of separating funnels. After parallel extraction with $CH_2Cl_2$, the combined extracts of each reaction were washed with a saturated $NaHCO_3$ solution, a 2 N HCl solution and brine, and dried over anhydrous $Na_2SO_4$. Evaporation in parallel under reduced pressure using an Evaposel™ apparatus gave residues which were purified by crystallization from the suitable solvents or solvent mixtures.

*5.1.3.1. 3-(2,2-Dimethylpropanoyl)-2-thioxoimidazolidine-1-carbaldehyde (**3b**).* Yield: 49%; m.p.: 116–118 °C (DCM/petroleum ether); IR ($CHCl_3$): 1691 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 1.43 (s, 9H, $3CH_3$), 4.01 (s, 4H, $2CH_2$), 9.46 (s, 1H, HCO). Anal. Calcd for $C_9H_{14}N_2O_2S$: C, 50.45; H, 6.59; N, 13.07; S, 14.96. Found: C, 50.26; H, 6.60; N, 13.08; S, 14.88.

*5.1.3.2. 3-[(2E)-3-Phenylprop-2-enoyl]-2-thioxoimidazolidine-1-carbaldehyde (**3c**).* Yield: 96%; m.p.: 158–160 °C (DCM/petroleum ether); IR ($CHCl_3$): 1680 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 3.89–4.24 (m, 4H, $2CH_2$), 7.28–7.73 (m, 5H, Ar-H), 7.78 (d, $J = 15.6$ Hz, 1H, CH=CPh), 8.36 (d, $J = 15.6$ Hz, 1H, C = CHPh), 9.62 (s, 1H, HCO). Anal. Calcd for $C_{13}H_{12}N_2O_2S$: C, 59.98; H, 4.65; N, 10.76; S, 12.32. Found: C, 60.16; H, 4.84; N, 10.61; S, 12.00.

*5.1.3.3. 3-Benzoyl-2-thioxoimidazolidine-1-carbaldehyde (**3d**).* Yield: 65%; m.p.: 125–127 °C (DCM/petroleum ether); IR (KBr): 1679 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 3.92–4.28 (m, 4H, $2CH_2$), 7.44–7.93 (m, 5H, Ar-H), 9.51 (s, 1H, HCO). Anal. Calcd for $C_{11}H_{10}N_2O_2S$: C, 56.40; H, 4.30; N, 11.96; S, 13.69. Found: C, 56.13; H, 4.30; N, 12.01; S, 13.52.

*5.1.3.4. 3-(4-Methylbenzoyl)-2-thioxoimidazolidine-1-carbaldehyde (**3f**).* Yield: 88%; m.p.: 178–180 °C (DCM/petroleum ether); IR ($CHCl_3$): 1685 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.43 (s, 3H, $CH_3$), 3.99–4.28 (m, 4H, $2CH_2$), 7.15–7.39 and 7.54–7.81 (m, 4H, Ar-H), 9.50 (s, 1H, HCO). Anal. Calcd for $C_{12}H_{12}N_2O_2S$: C, 58.05; H, 4.87; N, 11.28; S, 12.91. Found: C, 58.13; H, 4.93; N, 11.01; S, 12.73.

*5.1.3.5. 3-(4-Chlorophenyl)-2-thioxoimidazolidine-1-carbaldehyde (**3j**).* Yield: 73%; m.p.: 158–160 °C (DCM/petroleum ether); IR ($CHCl_3$): 1695 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.00–4.31 (m, 4H, $2CH_2$), 7.30–7.85 (m, 4H, Ar-H), 9.50 (s, 1H, HCO). Anal. Calcd for $C_{11}H_9ClN_2O_2S$: C, 49.17; H, 3.38; N, 10.42; S, 11.93. Found: C, 49.27; H, 3.39; N, 10.30; S, 11.68.

*5.1.3.6. 3-(2,4-Dichlorophenyl)-2-thioxoimidazolidine-1-carbaldehyde (**3m**).* Yield: 58%; m.p.: 103–105 °C (DCM/petroleum ether); IR ($CHCl_3$): 1688 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 3.84–4.47 (m, 4H, $2CH_2$), 7.24–7.53 (m, 3H, Ar-H), 9.50 (s, 1H, HCO). Anal. Calcd for $C_{11}H_8Cl_2N_2O_2S$: C, 43.58; H, 2.66; N, 9.24; S, 10.58. Found: C, 43.52; H, 2.89; N, 9.21; S, 10.32.

*5.1.3.7. 3-(3,4-Dichlorophenyl)-2-thioxoimidazolidine-1-carbaldehyde (**3n**).* Yield: 64%; m.p.: 164–166 °C (DCM/petroleum ether); IR ($CHCl_3$): 1689 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.00–4.30 (m, 4H, $2CH_2$), 7.49–7.60 (m, 2H, Ar-H), 7.73–7.85 (m, 1H, Ar-H), 9.50 (s, 1H, HCO). Anal. Calcd for $C_{11}H_8Cl_2N_2O_2S$: C, 43.58; H, 2.66; N, 9.24; S, 10.58. Found: C, 43.72; H, 2.81; N, 9.16; S, 10.27.

*5.1.3.8. 3-(3,5-Dichlorophenyl)-2-thioxoimidazolidine-1-carbaldehyde (**3o**).* Yield: 78%; m.p.: 164–166 °C (DCM/petroleum ether); IR ($CHCl_3$): 1695 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.00–4.32 (m, 4H, $2CH_2$), 7.48–7.66 (m, 3H, Ar-H), 9.50 (s, 1H, HCO). $^{13}C$ NMR (DMSO-$d_6$) $\delta$: 41.17 ($CH_2N$), 46.17 ($CH_2N$), 127.07 (2CH), 130.90 (2C), 133.78 (CH),

137.93 (C), 161.93 (CHO), 167.95 (CO), 179.95 (CS). Anal. Calcd for $C_{11}H_8Cl_2N_2O_2S$: C, 43.58; H, 2.66; N, 9.24; S, 10.58. Found: C, 43.33; H, 2.79; N, 9.00; S, 10.54.

*5.1.3.9. 3-(3,4,5-Trimethoxybenzoyl)-2-thioxoimidazolidine-1-carbaldehyde (**3q**).* Yield: 78%; m.p.: 229–231 °C (diethyl ether); IR ($CHCl_3$): 1695 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 3.90 (s, 6H, $2CH_3$), 3.95 (s, 3H, $CH_3$), 3.83–4.32 (m, 4H, $2CH_2$), 7.02 (s, 2H, Ar-H), 9.53 (s, 1H, HCO). Anal. Calcd for $C_{14}H_{16}N_2O_5S$: C, 51.84; H, 4.97; N, 8.64; S, 9.89. Found: C, 51.97; H, 5.01; N, 8.66; S, 9.70.

*5.1.3.10. 3-(2-Furoyl)-2-thioxoimidazolidine-1-carbaldehyde (**3s**).* Yield: 75%; m.p.: 119–121 °C (diethyl ether); IR ($CHCl_3$): 1693 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 4.00–4.30 (m, 4H, $2CH_2$), 6.63 (dd, $J = 3.8$ Hz, $J = 1.6$ Hz, 1H, fur H-4), 7.35 (d, $J = 3.8$ Hz, 1H, fur H-3), 7.65 (d, $J = 1.6$ Hz, 1H, fur H-5), 9.54 (s, 1H, HCO). Anal. Calcd for $C_9H_8N_2O_3S$: C, 48.21; H, 3.60; N, 12.49; S, 14.30. Found: C, 48.30; H, 3.69; N, 12.19; S, 14.23.

### *5.1.4. Preparation of [(3-benzoyl-2-thioxoimidazolidin-1-yl)methylene]malononitrile (**4d**)*

Benzoyl chloride (5.5 mmol, 0.638 mL) was added dropwise to a stirred solution of **V** (5 mmol, 0.891 g) and TMEDA (5.5 mmol, 0.825 mL) in pyridine (10 mL). The resulting mixture was stirred at rt for 2 h. After dilution with 120 mL of a 2 N HCl solution, the solid precipitated was filtered off, dried and crystallized from acetone. Yield: 52%; m.p.: 238–240 °C; IR (KBr): 2223, 1697, 1596 $cm^{-1}$; $^1H$ NMR ($CF_3COOD$) $\delta$: 4.48–4.86 (m, 4H, $2CH_2$), 7.40–7.72 (m, 3H, Ar-H), 8.01–8.29 (m, 2H, Ar-H), 8.67 (s, 1H, CH=C). Anal. Calcd for $C_{14}H_{10}N_4OS$: C, 59.56; H, 3.57; N, 19.85; S, 11.36. Found: C, 59.46; H, 3.57; N, 20.15; S, 11.16.

### *5.1.5. Parallel procedure for the preparation of N-acyl(thio)ureas **4s–17s***

2-Furoyl chloride (5.5 mmol, 0.542 mL) was added dropwise to each numbered reaction tube of a Carousel-12 Reaction Station™, containing a stirred solution of the suitable (thio)urea (**V–XVIII**) (5 mmol) and TMEDA (5.5 mmol, 0.825 mL) in pyridine (20 mL). The resulting mixtures were stirred for 4 h at 65 °C. After cooling to rt, a 2 N HCl solution (25 mL) was added into each tube. The contents of the tubes were then transferred into a set of beakers. More 2 N HCl solution (125 mL) was added into each beaker. Three different types of work-up were carried out. Work-up (*i*) (**4s**, **6s–9s**, **12s** and **13s**): the precipitates obtained were filtered off in parallel by an in-house device, dried and crystallized from the suitable solvent or solvent mixture. Work-up (*ii*) (**5s**, **10s**, **11s** and 1**5s–17s**): the amorphous precipitates obtained were filtered off in parallel by an in-house device and dissolved in $CH_2Cl_2$. The solutions were washed with brine, dried over anhydrous $Na_2SO_4$ and filtered in parallel through pads of Florisil (diameter 5 × 2 cm) by an in-house device. Parallel evaporating *in vacuo* using an Evaposel™ apparatus gave residues which were purified by crystallization from the suitable solvent mixtures. Work-up (*iii*) (**14s**): the mixture was extracted with $CH_2Cl_2$. The organic layer was washed with brine, filtered through a plug of Florisil (diameter 5 × 2 cm) and evaporated *in vacuo* using an Evaposel™ apparatus, to give a residue which was purified by crystallization from DCM/ethanol.

*5.1.5.1. {[3-(2-Furoyl)-2-thioxoimidazolidin-1-yl]methylene}-malononitrile (**4s**).* Yield: 44%; m.p.: 205–207 °C (acetone); IR (KBr): 2231, 1695, 1599 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.23–4.60 (m, 4H, $2CH_2$), 6.81 (dd, $J = 4.2$ Hz, $J = 1.8$ Hz, 1H, fur H-4), 7.56 (d, $J = 4.2$ Hz, 1H, fur H-3), 8.10 (d, $J = 1.8$ Hz, 1H, fur H-5), 8.61 (s, 1H, CH=C). Anal. Calcd for $C_{12}H_8N_4O_2S$: C, 52.93; H, 2.96; N, 20.58; S, 11.77. Found: C, 53.20; H, 3.10; N, 20.62; S, 11.54.

*5.1.5.2. Methyl (2E)-2-cyano-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylate (**5s**).* Yield: 79%; m.p.: 185–187 °C (acetone); IR (KBr): 2216, 1727, 1678, 1604 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 3.83 (s, 3H, $CH_3$), 4.30–4.60 (m, 4H, $2CH_2$), 6.73–6.89 (m, 1H, fur H-4), 7.54 (d, $J = 4.2$ Hz, 1H, fur H-3), 8.01–8.12 (m, 1H, fur H-5), 8.98 (s, 1H, CH=C). Anal. Calcd for $C_{13}H_{11}N_3O_4S$: C, 51.14; H, 3.63; N, 13.76; S, 10.50. Found: C, 51.05; H, 3.70; N, 13.95; S, 10.28.

*5.1.5.3. (2E)-2-Benzoyl-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylonitrile (**6s**).* Yield: 57%; m.p.: 218–220 °C (acetone/DCM); IR (KBr): 2214, 1682, 1640, 1596 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.34–4.72 (m, 4H, $2CH_2$), 6.73–6.91 (m, 1H, fur H-4), 7.49–7.90 (m, 6H, 5Ar-H and fur H-3), 8.01–8.17 (m, 1H, fur H-5), 8.90 (s, 1H, CH=C). Anal. Calcd for $C_{18}H_{13}N_3O_3S$: C, 61.53; H, 3.73; N, 11.96; S, 9.12. Found: C, 61.44; H, 3.83; N, 11.89; S, 9.02.

*5.1.5.4. (2E)-2-(2,2-Dimethylpropanoyl)-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylonitrile (**7s**).* Yield: 63%; m.p.: 160–162 °C (DCM/ethanol); IR (KBr): 2206, 1685, 1565 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 1.37 (s, 9H, $C(CH_3)_3$), 4.25–4.71 (m, 4H, $2CH_2$), 6.60–6.71 (m, 1H, fur H-4), 7.37 (d, $J = 4.2$ Hz, 1H, fur H-3), 7.60–7.70 (m, 1H, fur H-5), 9.23 (s, 1H, CH=C). Anal. Calcd for $C_{16}H_{17}N_3O_3S$: C, 57.99; H, 5.17; N, 12.68; S, 9.67. Found: C, 58.28; H, 5.32; N, 12.76; S, 9.89.

*5.1.5.5. (2E)-2-Cyano-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylamide (**8s**).* Yield: 75%; m.p.: 246–248 °C (acetone/methanol); IR (KBr): 3436, 3187, 2210, 1693, 1649, 1590 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.25–4.61 (m, 4H, $2CH_2$), 6.73–6.91 (m, 1H, fur H-4), 7.46–7.76 (m, 3H, $NH_2$ and fur H-3), 8.00–8.16 (m, 1H, fur H-5), 8.96 (s, 1H, CH=C). Anal. Calcd for $C_{12}H_{10}N_4O_3S$: C, 49.65; H, 3.47; N, 19.30; S, 11.04. Found: C, 49.61; H, 3.67; N, 19.14; S, 10.96.

*5.1.5.6. (2E)-N-(4-Chlorophenyl)-2-cyano-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylamide (**9s**).* Yield: 90%; m.p.: 236–238 °C (acetone/DCM); IR (KBr): 3338, 2213, 1683,

1591 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.23–4.67 (m, 4H, $2CH_2$), 6.73–6.94 (m, 1H, fur H-4), 7.36–7.89 (m, 5H, 4Ar-H and fur H-3), 8.03–8.18 (m, 1H, fur H-5), 9.00 (s, 1H, CH=C). Anal. Calcd for $C_{18}H_{13}ClN_4O_3S$: C, 53.94; H, 3.27; N, 13.98; S, 8.00. Found: C, 53.80; H, 3.38; N, 13.83; S, 7.92.

*5.1.5.7. 3-{[3-(2-Furoyl)-2-thioxoimidazolidin-1-yl]methylene}pentane-2,4-dione (**10s**).* Yield: 64%; m.p.: 164–166 °C (DCM); IR (KBr): 1696, 1677, 1648, 1601 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.38 (s, 3H, $CH_3$), 2.45 (s, 3H, $CH_3$), 3.75–4.01 (m, 2H, $CH_2$), 4.05–4.34 (m, 2H, $CH_2$), 6.72–6.90 (m, 1H, fur H-4), 7.49 (d, $J = 4.2$ Hz, 1H, fur H-3), 7.99–8.12 (m, 1H, fur H-5), 8.52 (s, 1H, CH=C). $^{13}C$ NMR (DMSO-$d_6$) $\delta$: 26.18 ($CH_3$), 32.41 ($CH_3$), 46.61 ($2CH_2N$), 112.73 (CH), 120.28 (CH), 126.05 (C), 137.21 (CH), 146.09 (C), 147.41 (=CH), 159.39 (NCO), 179.17 (CS), 196.41 (CO), 202.82 (CO). Anal. Calcd for $C_{14}H_{14}N_2O_4S$: C, 54.89; H, 4.61; N, 9.14; S, 10.47. Found: C, 54.92; H, 4.65; N, 9.07; S, 10.34.

*5.1.5.8. (2Z)-1-Phenyl-2-{[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]methylene}butane-1,3-dione (**11s**).* Yield: 71%; m.p.: 179–181 °C (acetone/petroleum ether); IR (KBr): 1671, 1618, 1575 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.37 (s, 3H, $CH_3$), 3.40–3.77 (m, 2H, $CH_2$), 3.85–4.30 (m, 2H, $CH_2$), 6.66–6.88 (m, 1H, fur H-4), 7.35–8.12 (m, 7H, 5Ar-H, fur H-3 and fur H-5), 8.78 (s, 1H, CH=C). Anal. Calcd for $C_{19}H_{16}N_2O_4S$: C, 61.94; H, 4.38; N, 7.60; S, 8.70. Found: C, 62.11; H, 4.47; N, 7.56; S, 8.37.

*5.1.5.9. Methyl (2Z)-2-acetyl-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylate (**12s**).* Yield: 44%; m.p.: 159–160 °C (acetone); IR (KBr): 1716, 1680, 1607 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 2.38 (s, 3H, $CH_3$); 3.68–4.25 (m, 7H, $2CH_2$ and $OCH_3$), 6.52–6.72 (m, 1H, fur H-4), 7.24–7.41 (m, 1H, fur H-3), 7.57–7.69 (m, 1H, fur H-5), 8.68 (s, 1H, CH=C). Anal. Calcd for $C_{14}H_{14}N_2O_5S$: C, 52.17; H, 4.38; N, 8.69; S, 9.95. Found: C, 52.14; H, 4.52; N, 8.75; S, 10.00.

*5.1.5.10. 1,3-Diphenyl-2-{[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]methylene}propane-1,3-dione (**13s**).* Yield: 80%; m.p.: 199–201 °C (DCM/ethanol); IR (KBr): 1675, 1603, 1571 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$: 3.67–3.84 (m, 2H, $CH_2$), 3.99–4.15 (m, 2H, $CH_2$), 6.50–6.60 (m, 1H, fur H-4), 7.25–7.95 (m, 12H, 10Ar-H, fur H-3 and fur H-5), 8.70 (s, 1H, CH=C). Anal. Calcd for $C_{24}H_{18}N_2O_4S$: C, 66.96; H, 4.21; N, 6.51; S, 7.45. Found: C, 67.08; H, 4.23; N, 6.26; S, 7.49.

*5.1.5.11. Ethyl (2E)-2-benzoyl-3-[3-(2-furoyl)-2-thioxoimidazolidin-1-yl]acrylate (**14s**).* Yield: 97%; m.p.: 142–144 °C (DCM/ethanol); IR (KBr): 1698, 1684, 1653, 1607 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 1.02 (t, $J = 6.6$ Hz, 3H, $CH_3$), 3.46–3.78 (m, 2H, $NCH_2$), 3.90–4.25 (m, 4H, $NCH_2$ and $OCH_2$), 6.64–6.85 (m, 1H, fur H-4), 7.39–8.12 (m, 7H, 5Ar-H, fur H-3 and fur H-5), 8.81 (s, 1H, CH=C). Anal. Calcd for $C_{20}H_{18}N_2O_5S$: C, 60.29; H, 4.55; N, 7.03; S, 8.05. Found: C, 60.20; H, 4.54; N, 6.94; S, 7.97.

*5.1.5.12. {[3-(2-Furoyl)-2-oxoimidazolidin-1-yl]methylene}malononitrile (**15s**).* Yield: 87%; m.p.: 233–234 °C (acetone); IR (KBr): 2223, 1776, 1664, 1594 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 4.29–4.38 (m, 4H, $2CH_2$), 6.76 (dd, $J = 4.8$ Hz, $J = 1.2$ Hz, 1H, fur H-4), 7.50 (d, $J = 4.8$ Hz, 1H, fur H-3), 8.0–8.11 (m, 1H, fur H-5), 8.28 (s, 1H, CH=C). Anal. Calcd for $C_{12}H_8N_4O_3$: C, 56.25; H, 3.15; N, 21.87. Found: C, 55.26; H, 3.19; N, 22.09.

*5.1.5.13. (2Z)-1-Phenyl-2-{[3-(2-furoy)-2-oxoimidazolidin-1-yl]methylene}butane-1,3-dione (**16s**).* Yield: 67%; m.p.: 205–207 °C (DCM/ethanol); IR (KBr): 1767, 1746, 1675, 1620, 1577 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 2.31 (s, 3H, $CH_3$), 3.25–3.60 (m, 2H, $CH_2$), 3.69–4.01 (m, 2H, $CH_2$), 6.58–6.76 (m, 1H, fur H-4), 7.35–7.97 (m, 7H, 5Ar-H, fur H-3 and fur H-5), 8.10 (s, 1H, CH=C). Anal. Calcd for $C_{19}H_{16}N_2O_5$: C, 64.77; H, 4.58; N, 7.95. Found: C, 65.01; H, 4.69; N, 7.96.

*5.1.5.14. Ethyl (2E)-2-benzoyl-3-[3-(2-furoy)-2-oxoimidazolidin-1-yl]acrylate (**17s**).* Yield: 48%; m.p.: 155–157 °C (ethanol); IR (KBr): 1757, 1694, 1668, 1611, 1575 $cm^{-1}$; $^1H$ NMR (DMSO-$d_6$) $\delta$: 1.03 (t, $J = 6.6$ Hz, 3H, $CH_3$), 3.18–3.52 (m, 2H, $NCH_2$), 3.72–4.19 (m, 4H, $NCH_2$ and $OCH_2$), 6.64–6.84 (m, 1H, fur H-4), 7.40–8.06 (m, 7H, 5Ar-H, fur H-3 and fur H-5), 8.15 (s, 1H, CH=C). Anal. Calcd for $C_{20}H_{18}N_2O_6$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.97; H, 4.71; N, 7.27.

### 5.2. Pharmacology

#### 5.2.1. Evaluation of antiproliferative and antiviral activity in MT-4 cells (MTT method)

Antiproliferative activity of compounds, based on the viability of mock-infected cells as monitored by the MTT method, was evaluated in parallel with their antiviral activity. Activity against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 lymphoblastoid T cells [23] [cells from American Type Culture Collection (ATCC, USA) grown in RPMI 1640 containing 10% foetal calf serum (FCS), 100 UI/mL penicillin G and 100 μg/mL streptomycin; cell culture checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco)]. Briefly, 50 μL of RPMI 10% FCS containing $1 \times 10^4$ cells were added to each well of flat-bottomed microtiter trays containing 50 μL of medium and serial dilutions of test compounds (test compound initially dissolved in DMSO at the concentration of 100 μM and then serially diluted in culture medium). Twenty microlitres of an HIV-1 suspension containing 100 $CCID_{50}$ were then added. After a four-day incubation at 37 °C the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [24]. The compound concentrations resulting in 50% cell growth inhibition in comparison with untreated controls and expressed as $IC_{50}$ values were determined by linear regression analysis.

*5.2.2. Evaluation of anticancer activity at NCI*

The NCI high-flux anticancer drug screen [25–27] utilized a panel of 60 human tumor cell lines in culture derived from nine cancer types (lung, colon, CNS, ovarian, renal, prostate and breast cancer, leukemia and melanoma). The compound were tested at 10-fold dilutions of five concentrations ranging from $10^{-4}$ to $10^{-8}$ M. According to the NCI protocol, cell lines were exposed to test agents in 96-well plates for the last 48 of a 72 h incubation and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. For each compound, the drug concentration required to produce 50% ($GI_{50}$) and total (TGI) growth inhibition, and 50% cytocidal effect ($LC_{50}$) were obtained for 47–60 cell lines. Values were calculated for each of these parameters if the level activity was reached; if the effect was not reached or was exceeded, the value is expressed as greater or lesser than the maximum or minimum concentration tested.

## Acknowledgements

The authors are very grateful to Dr. V.L. Narayanan, Chief of Drug Synthesis and Chemistry Branch, National Cancer Institute (Bethesda, MD, USA) for the in vitro evaluation of anticancer activity. This work was supported by MURST (Cofinanziamento Nazionale), CNR (Rome) and Ministero Italiano della Salute – Istituto Superiore di Sanità (grant no. 40D.46). Fondazione Carige is gratefully acknowledged for financially supporting S.C. The authors thank Mr. O. Gagliardo for the microanalyses, Mr. F. Tuberoni, Dr. C. Rossi and Dr. R. Raggio for the IR and NMR spectra.

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