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Synthesis of *N*-substituted-*N*-acylthioureas of 4-substituted piperazines endowed with local anaesthetic, antihyperlipidemic, antiproliferative activities and antiarrythmic, analgesic, antiaggregating actions

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Abstract

Three series of *N*-acyl and *N*-cyclohexyl- or *N*-methyl or *N*-phenyl-thioureas of 4-substituted (methyl, phenyl, 2-pyridyl)piperazines (**4**–**12**) were synthesised according to a highly convergent one-pot procedure and tested in vivo (local anaesthetic, anti-hyperlipoproteinemic, analgesic, anti-inflammatory, antiarrythmic activities) and in vitro (antiaggregating and, for some selected derivatives, antiproliferative activities) experiments. All the test compounds showed local anaesthesia in particular **4Ar$_4$**, **5Ar$_4$**, **12Ar$_3$** (after 5 min) and **5Ar$_2$**, **5Ar$_3$**, **9Ar$_4$** (after 30 min) were equipotent to lidocaine. In lowering triglyceride levels, compounds **6Ar$_4$** and **7Ar$_3$** were more active than nicotinic acid, whereas **7Ar$_4$** and **11Ar$_4$** were approximately equipotent. As concerns analgesic activity, **5Ar$_2$** and **5Ar$_4$** were as active as indomethacin. Appreciable anti-inflammatory activity was found in **8Ar$_1$**, **5Ar$_2$** and **11Ar$_2$**, but inferior to that of indomethacin. High levels of antiarrythmic activity, comparable with that of quinidine, were found in derivatives **4Ar$_2$** and **10Ar$_1$**. Compounds **4Ar$_2$** and **8Ar$_2$**, assayed in antitumor in vitro screening system at National Cancer Institute (NCI), showed significant antiproliferative activity against ACHN cell line (GI50: 0.13 μM) and NCI-H226 cell line (GI50: 1.03 μM), respectively.

Keywords: 3,3-Disustituted-1-acylthioureas; *N*-substituted-*N*-acyl 4-substituted piperazine-1-thiocarboxamides; Local anaesthetic, Antihyperlipidemic; Analgesic; Anti-inflammatory; Antiarrhythmic; Platelet antiaggregating and antiproliferative activities

1. Introduction

In previous papers [1,2], 3,3-disubstituted 1-phenyl(thio)ureas (**A**, **B**, Chart 1, embodying moieties of pharmacologically useful secondary amines, were reported to be endowed with various and interesting pharmacological properties. In order to expand the SAR studies on acylthioureas (ATUs) and confirm their pharmacological potential, we designed and synthesised *N*-substituted-*N*-acylthioureas of 4-methyl-, 4-phenyl- and 4-(2-pyridyl) piperazine **4**–**12**, as new analogues of **A** and **B**, incorporating three variable structural regions (R, R_{1-3}, Ar_{1-4}, Chart 2). These portions were designed with differing electronic, lipophilic and steric properties, so that the products are featured by a common scaffold with different shapes and different substitution patterns. The title compounds were assayed in some in vivo and in vitro tests. Additional goal of the present investigation is to validate ATUs as sources of new hit/lead compounds, exploiting an analogue design approach, even if there is no mechanistic understanding of the target(s) at all.

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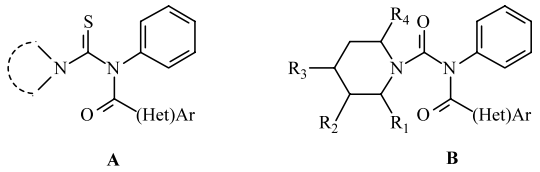


Chart 1. The shared structures of prieviously described acyl(thio)ureas [1,2].

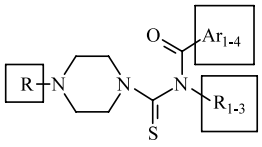


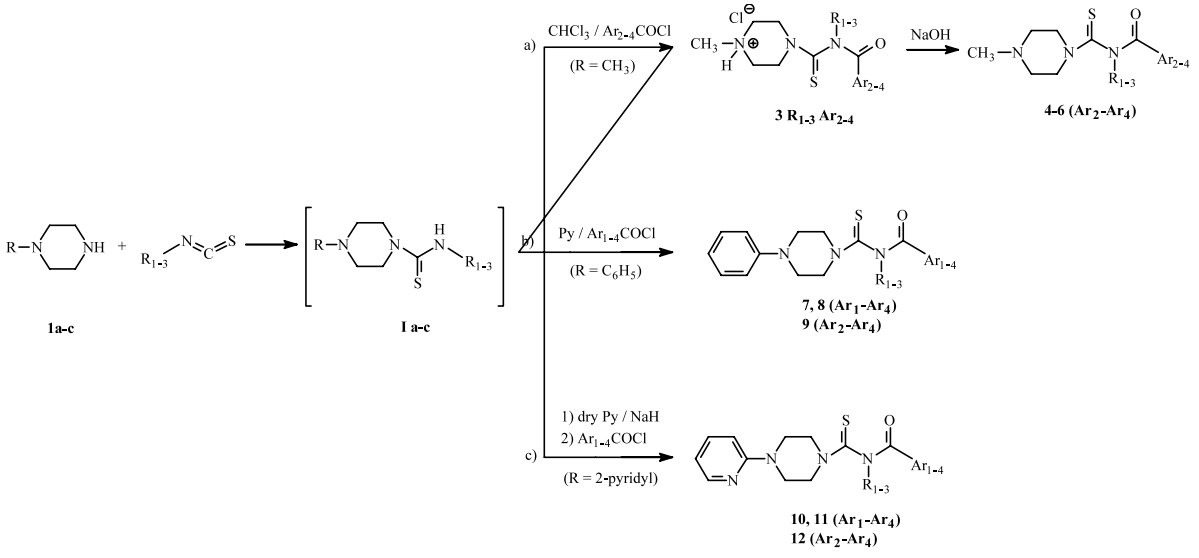
Chart 2. The variable structural regions of the title compounds for SAR studies.

2. Chemistry

The methodology employed for the preparation of the new ATUs is a highly convergent one-pot procedure achieved through three steps by sequentially combining three types of building blocks (amines, isothiocyanates, acyl chlorides, respectively).

The synthesis of **7**–**9** and **10**–**12** was accomplished according to the previously described synthetic variants (Scheme 1, paths b and c) [1], whereas **4**–**6** were obtained (path a) in the absence of base (pyridine, sodium hydride), and, owing to the presence of the 4-basic nitrogen, the primary reaction products were hydrochlorides $\mathbf{3R_{1-3}Ar_{2-4}}$. Indeed, the use of bases is not only unnecessary, but ineffective too, causing a decrease of yields and tedious and time-consuming work-up procedures. On the other hand, the isolation of the hydrochlorides indicates that the 4-nitrogen atom of the piperazine nucleus acts as an intramolecular scavenger of hydrogen chloride, thus playing the same role of tertiary base (pyridine). It is noteworthy that the three synthetic variants are featured by high atom economy, because either only one molecule of HCl is formally lost in the whole process (Scheme 1b and c) or all chemical features of the building blocks are incorporated into the products (Scheme 1a). Furthermore, no intermediate needs to be isolated and in general the overall yields are good to high. Owing the variety of the chemically accessible building blocks (the employed ones are commercially available), maximum need molecular diversity can be obtained in the smallest number of steps and under the mildest conditions possible. For these reasons, the above procedures could be easily adaptable to combinatorial synthesis of ATU libraries.

The reaction conditions of route (a) were somewhat similar to acylation (acetylation/benzoylation) of thiourea in aprotic medium, to afford *N*-acyl thiourea via isolable *S*-acylisothiouronium salt intermediate [3], which underwent sulphur-to-nitrogen acyl migration in consequence of basic treatment ($NaHCO_3$) or heating.



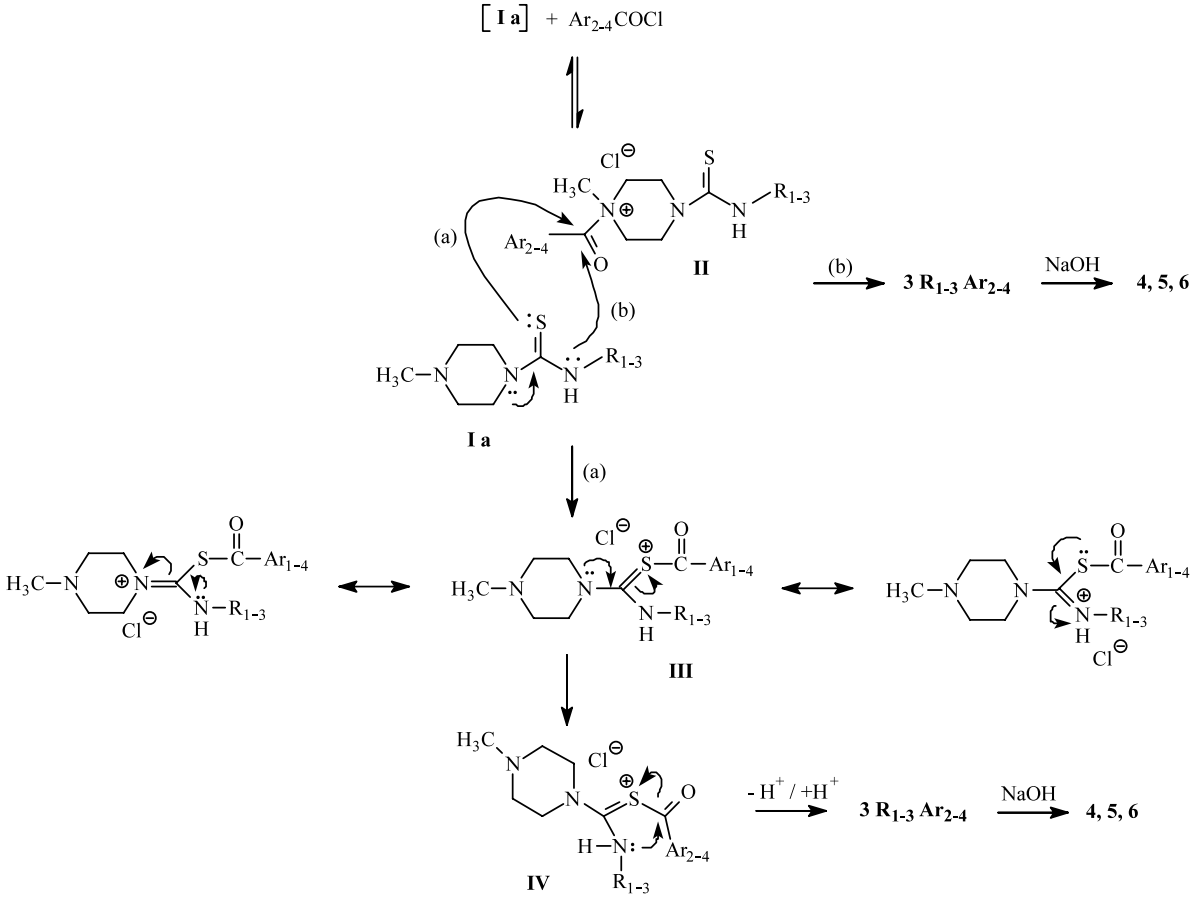
	R
1a, Ia	CH_3
1b, Ib	C_6H_5
1c, Ic	2-pyridyl

				R_{1-3}
Ia	**4**	**7**	**10**	CH_3
Ib	**5**	**8**	**11**	C_6H_{11}
Ic	**6**	**9**	**12**	C_6H_5

Ar_{1-4}
$\mathbf{Ar_1}$ = C_6H_5
$\mathbf{Ar_2}$ = 4-ClC_6H_4
$\mathbf{Ar_3}$ = 2-furyl
$\mathbf{Ar_4}$ = 2-thienyl

Scheme 1. One-pot synthesis of the title compounds.

Moreover, a similar intermediate was also proposed for acylation of carbothioamides, since benzoylation of thioformamide gives the corresponding isolable *S*-benzoyl derivative [4]. Starting from these observations, we hypothesised the following mechanism for formation of ATUs **4**–**6** (Scheme 2). Preliminarily, the formation of complexes **II** might occur between intermediate **Ia** and acyl chlorides, since it is well known that bases (as pyridine and triethylamine) having tertiary nitrogen form reversible complexes with acyl chlorides [5], at the same time enhancing reactivity of the latter. It is noteworthy that 3,3-disubstituted thioureas not having basic nitrogens cannot be acylated under base-free conditions and the slow release of hydrogen chloride from unreacted acyl reagents decomposes the key thiourea intermediate, yielding starting materials (amines and isothiocyanates) [6] due to reversibility of the reaction [7]. After this initial step, the sulphur atom would undergo a kinetically-controlled acyl attack, yielding resonance-stabilised isothiouronium salts **III**. Then, as reported above for simple thiourea [3], a subsequent in situ basic treatment should cause a thermodynamically-assisted S→N acyl transposition through a transient four-centre Chapman-type intermediate **IV**. This implies a *syn* configuration of **IV**, with the nitrogen lone pair in a proper steric alignment for attack upon the acyl carbonyl with a request of a major activation entropy. A further example of this sort of acyl cationotropy in thiourea derivatives, is given by nitrogen-to-nitrogen acyl migration, as proved by some of us [8]. Therefore, with the aim to verify existence of **III**, we isolated the primary reaction products as salts, before basic treatment to avoid any possible acyl transposition. Actually, all these products were soluble in water and two of them, being deliquescent, could not be characterised (i.e. $\mathbf{3R_2Ar_3}$ and $\mathbf{3R_2Ar_4}$, see Section 4). Their saline nature were also confirmed by the IR spectra, which exhibit typical absorption bands corresponding to the quaternary ammonium groups in the range of 2720–2100 cm^{-1}. Nevertheless, in order to make possible clear assignment of the acyl group, and in this way to confirm either the *S*-acyl or *N*-acyl structure (**III** or **3**, respectively), we achieved the ^{13}C NMR spectrum of one (R_{1-3}: C_6H_5, Ar_{1-4}: 2-thienyl) of these salts, assumed as a prototype. The presence of the thione group at δ 185.30 excludes the structure of type **III**, and therefore salts **3** possess the N-acyl structure. As a consequence, the subsequent treatment with sodium



Scheme 2. The proposed mechanism for formation of hydrochlorides **3**.

hydroxide simply converts salts **3** into free bases **4**, **5** and **6** (Scheme 2, paths a and b, and also see Scheme 1, route a).

Both mechanistic paths a and b of Scheme 2 can explain the outcome of the reaction. The intermediate **III** is likely unstable and, even though no base has been added, spontaneously undergoes sulphur-to-nitrogen rearrangement via **IV**, with concomitant internal proton transfer from the less basic nitrogen of the NHR_{1-3} group to the more basic 4-nitrogen. Alternatively (route b), the acyl attack would be directed towards the nitrogen atom and hydrogen chloride released was trapped by the 4-tertiary nitrogen. Unlike simple thiourea, intermediate **Ia** bears the groupings R_{1-3}, which might enhance the reactivity of the NH group, due to either an increase of nucleophilicity (Me, C_6H_{11}) or acid ionizability (C_6H_5). Moreover, this reaction pattern might be further justified by the HSAB principle [9]. From this point of view, **Ia** shows two basic reactive sites: one soft (S), the other hard (N). Since acyl chlorides are well-known 'hard acids', they would preferentially react with the hard site. It is noteworthy that of these two mechanisms the former, (exemplified by path a, Scheme 2), enjoyed the widest support [10].

3. Pharmacology

The title compounds were evaluated for infiltration anaesthesia, antihyperlipidemic, analgesic, anti-inflammatory, antiarrhythmic, platelet antiaggregating and antiproliferative activities.

3.1. Infiltration anaesthesia

All test compounds were active (Table 1). In particular, **$4Ar_4$**, **$5Ar_4$**, **$12Ar_3$** and **$5Ar_2$**, **$5Ar_3$**, **$9Ar_4$**, were about equipotent to lidocaine (after 5 and 30 min, respectively). Also **$8Ar_1$**, **$10Ar_2$**, **$10Ar_3$**, and **$12Ar_2$** showed good activity levels, whereas **$4Ar_3$**, **$6Ar_2$**, **$7Ar_4$**, were moderately active.

3.2. Antihyperlipidemic activity

Compounds **$4Ar_4$**, **$6Ar_2$**, **$6Ar_4$**, **$7Ar_3$**, **$8Ar_3$**, **$9Ar_3$**, **$11Ar_1$** and **$11Ar_2$** were capable of lowering the triglyceride levels rather than the cholesterol ones, and were more active than nicotinic acid (Table 2). Moreover, **$7Ar_4$** and **$11Ar_4$** were about equipotent to nicotinic acid and **$5Ar_4$**, **$8Ar_4$**, **10Ar**, and **$11Ar_3$** were still appreciably active, displaying the same activity trend. Conversely, **$7Ar_1$**, **$7Ar_2$**, **$8Ar_1$**, **$9Ar_2$** **$10Ar_3$**, and **$12Ar_4$** displayed an appreciable antihypercholesterolemic activity, causing smaller or no (only for **$12Ar_4$**) reduction of the triglyceride levels. In order to assess the antihyperlipidemic potency of **$7Ar_3$**, the following calculated ED_{50} values are reported (in parentheses: fiducial limits): 91.6 (30.7–277.2), 98.5 (44.6–208.9) and 63.6 mg/kg (33.4–121.3) for total, free and esterified hypercholesterolemia, respectively; 50.3 mg/kg (30.9–81.8) for hypertriglyceridemia.

3.3. Analgesic activity

In the hot plate test, **$5Ar_2$**, **$5Ar_4$**, and **$9Ar_2$** at the dose of 50 mg/kg were about equipotent to indometacin (5 mg/kg) (Table 3). Noteworthy activity was showed also by **$5Ar_3$**, **$10Ar_3$**, and **$12Ar_2$**. Conversely, **$4Ar_2$**, **$4Ar_4$**, **$6Ar_3$**, **$7Ar_2$**, **$8Ar_3$** and **$8Ar_4$** were moderately active, whereas **$9Ar_3$**, **$10Ar_4$**, **$12Ar_3$**, **$12Ar_4$** were poorly active or practically inactive.

3.4. Anti-inflammatory activity

In the carrageenan-induced paw oedema in rats, only **$8Ar_1$**, **$5Ar_3$**, **$11Ar_2$** turned out to be active, but to a lesser extent than indomethacin (Table 4). Some compounds exerted a better anti-inflammatory effect at the 2nd h (**$5Ar_2$**, **$10Ar_3$**) or the 3rd h (**$8Ar_2$**, **$8Ar_3$**, **$9Ar_3$**, **$9Ar_4$**, **$10Ar_2$**) after administration. Compounds **$4Ar_3$**, **$6Ar_2$**, **$6Ar_3$**, **$7Ar_2$**, **$7Ar_4$**, **$9Ar_2$**, **$12Ar_3$** were poorly inactive or practically inactive.

3.5. Antiarrhythmic activity

$4Ar_3$ and **$10Ar_1$** were as approximately active as quinidine (Table 5). Moreover, **$6Ar_4$** and **$7Ar_4$** exhibited good activity levels. Some compounds (**$5Ar_4$**, **$11Ar_2$**, **$11Ar_3$**) significantly protracted the appearance time of extrasystoles only. Compounds **$4Ar_4$**, **$5Ar_2$**, **$6Ar_2$**, **$6Ar_3$**, **$7Ar_2$**, **$8Ar_2$**, **$11Ar_1$**, **$12Ar_3$**, **$12Ar_4$** were poorly inactive or practically inactive.

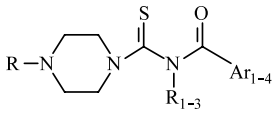
3.6. Platelet antiaggregation activity

In the inhibition test of aggregation induced by collagen, only **$5Ar_2$** was approximately equipotent to acetylsalicylic acid. Other prominent derivatives were **$4Ar_3$**, **$5Ar_3$**, **$6Ar_4$**, **$7Ar_3$**, **$9Ar_4$**, **$10Ar_3$**. The other reported compounds were poorly active (Table 6).

3.7. Antiproliferative activity

Some of compounds selected by NCI showed an interesting antiproliferative activity against subpanel cell lines. In particular, **$4Ar_2$** and **$8Ar_2$** were significantly active at very low concentrations against ACHN cell line (GI_{50}: 0.13 μM) and NCI-H226 cell line (GI_{50}: 1.03 μM), derived from renal cancer and non-small cell lung cancer, respectively. **$7Ar_2$**, **$11Ar_2$**, **$11Ar_4$**, **$12Ar_2$** and, to a lesser extent, **$5Ar_2$** exhibited an appreciable, broad

Table 1
Infiltration anaesthesia by pinch-tail test [a], induced by the compounds of series **4–12**



Comp.	R	R_{1-3}	Ar_{1-4}	Dose [b]	Activity [c]	
					5 min	30 min
4Ar$_2$	CH_3	CH_3	*p*-ClC_6H_5	0.2	20	40
4Ar$_3$	CH_3	CH_3	2-furyl	0.2	40	40
4Ar$_4$	CH_3	CH_3	2-thienyl	0.2	60	40
5Ar$_2$	CH_3	C_6H_{11}	*p*-ClC_6H_5	0.2	40	80
5Ar$_3$	CH_3	C_6H_{11}	2-furyl	0.2	30	80
5Ar$_4$	CH_3	C_6H_{11}	2-thienyl	0.2	60	60
6Ar$_2$	CH_3	C_6H_5	*p*-ClC_6H_5	0.2	40	40
6Ar$_3$	CH_3	C_6H_5	2-furyl	0.2	20	20
6Ar$_4$	CH_3	C_6H_5	2-thienyl	0.2	10	20
7Ar$_1$	C_6H_5	CH_3	C_6H_5	0.2	20	40
7Ar$_2$	C_6H_5	CH_3	*p*-ClC_6H_5	0.2	20	60
7Ar$_3$	C_6H_5	CH_3	2-furyl	0.2	10	30
7Ar$_4$	C_6H_5	CH_3	2-thienyl	0.2	40	40
8Ar$_1$	C_6H_5	C_6H_{11}	C_6H_5	0.2	40	60
8Ar$_2$	C_6H_5	C_6H_{11}	*p*-ClC_6H_5	0.2	20	40
8Ar$_3$	C_6H_5	C_6H_{11}	2-furyl	0.2	40	20
8Ar$_4$	C_6H_5	C_6H_{11}	2-thienyl	0.2	20	20
9Ar$_2$	C_6H_5	C_6H_5	*p*-ClC_6H_5	0.2	10	20
9Ar$_3$	C_6H_5	C_6H_5	2-furyl	0.2	20	40
9Ar$_4$	C_6H_5	C_6H_5	2-thienyl	0.2	40	80
10Ar$_1$	C_5H_4N	CH_3	C_6H_5	0.2	20	40
10Ar$_2$	C_5H_4N	CH_3	*p*-ClC_6H_5	0.2	40	60
10Ar$_3$	C_5H_4N	CH_3	2-furyl	0.2	40	60
10Ar$_4$	C_5H_4N	CH_3	2-thienyl	0.2	30	50
11Ar$_1$	C_5H_4N	C_6H_{11}	C_6H_5	0.2	20	40
11Ar$_2$	C_5H_4N	C_6H_{11}	*p*-ClC_6H_5	0.2	20	20
11Ar$_3$	C_5H_4N	C_6H_{11}	2-furyl	0.2	10	40
11Ar$_4$	C_5H_4N	C_6H_{11}	2-thienyl	0.2	20	60
12Ar$_2$	C_5H_4N	C_6H_5	*p*-ClC_6H_5	0.2	40	60
12Ar$_3$	C_5H_4N	C_6H_5	2-furyl	0.2	60	40
12Ar$_4$	C_5H_4N	C_6H_5	2-thienyl	0.2	20	20
Lidocaine				60	80	

[a] Ten mice (20–25 g)/group.
[b] Percent of glycofurol solution (0.2 ml).
[c] Percent of animals showing anaesthesia, 5 and 30 min after infiltration of test compounds into the tail root. Dieffenbach tweezers were applied for 10 s.

activity. The most sensitive cell lines are here reported, as follows (Table 7).

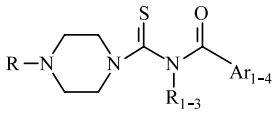
For **5Ar$_2$**: SNB-75 (GI_{50}: 20.1 μM) and HS 578T (GI_{50}: 27.4 μM); for **7Ar$_2$**: UO-31 (GI_{50}: 20.1 μM) and HS 578T (GI_{50}: 19.0 μM); for **11Ar$_2$**: HL-60(TB) (GI_{50}: 17.3 μM), MOLT-4 (GI_{50}: 24.3 μM), RPMI-8226 (GI_{50}: 25.5 μM), NCI-H226 (GI_{50}: 18.1 μM), and XF 498 (GI_{50}: 8.2 μM); for **11Ar$_4$**: NCI-H226 (GI_{50}: 19.5 μM), COLO 205 (GI_{50}: 22.4 μM) and RXF-393 (GI_{50}: 22.5 μM); finally, for **12Ar$_2$**: RXF-393 (GI_{50}: 15.6 μM).

4. Experimental

4.1. Chemistry

All the building blocks used are commercially available. Piperazines, isothiocyanates and acyl chlorides, 60% sodium hydride dispersion were purchased by Aldrich Chemical, Milan (Italy). Solvents (chloroform, pyridine) were reagent grade. Organic solutions were dried over anhydrous sodium sulphate and evaporated

Table 2
Effects of the compounds of series **4**–**12** on hypercholesterolemia and hypertriglyceridemia induced by Triton in rats



Comp.	R	R_{1-3}	Ar_{1-4}	Dose (mg/kg p.o.)	Serum cholesterol, mg%±SE [a]			Serum triglycerides, mg%±SE [a]
					Total	Free	Esterified	
4Ar$_2$	CH_3	CH_3	*p*-ClC_6H_5	50	302±79* (−33.2)	122±7* (−18.7)	180±63* (−40.4)	290±31* (−19.4)
4Ar$_3$	CH_3	CH_3	2-furyl	50	418±28 (−7.5)	178±12 (+18.7)	240±47 (−20.5)	310±20 (−13.9)
4Ar$_4$	CH_3	CH_3	2-thienyl	50	283±31** (−37.4)	118±15 (−21.3)	165±21** (−45.4)	212±44** (−41.1)
5Ar$_2$	CH_3	C_6H_{11}	*p*-ClC_6H_5	50	420±27 (−7.1)	168±18 (+12.0)	252±46 (−16.6)	357±21 (−0.8)
5Ar$_3$	CH_3	C_6H_{11}	2-furyl	50	387±55 (−14.4)	129±5* (−14.0)	258±48 (14.6)	278±25* (−22.8)
5Ar$_4$	CH_3	C_6H_{11}	2-thienyl	50	403±56 (−10.8)	163±12 (+8.7)	240±64 (−20.5)	268±24** (−25.6)
6Ar$_2$	CH_3	C_6H_5	*p*-ClC_6H_5	50	277±19** (−38.7)	112±6** (−25.3)	165±41** (−45.4)	201±19** (−44.2)
6Ar$_3$	CH_3	C_6H_5	2-furyl	50	383±27* (−15.3)	166±2 (+10.7)	217±35* (−28.1)	269±16** (−25.3)
6Ar$_4$	CH_3	C_6H_5	2-thienyl	50	241±19** (−46.7)	106±8** (−29.3)	135±17** (−55.3)	140±27** (−61.1)
7Ar$_1$	C_6H_5	CH_3	C_6H_5	50	301±42** (−33.4)	114±6** (−24.0)	187±61* (−38.1)	275±32* (−23.6)
7Ar$_2$	C_6H_5	CH_3	*p*-ClC_6H_5	50	315±36** (−30.3)	122±12 (−18.7)	193±43* (−36.1)	280±28* (−22.2)
7Ar$_3$	C_6H_5	CH_3	2-furyl	25	391±21 (−13.5)	153±12 (+2.0)	238±10* (−21.2)	279±22** (−22.5)
				50	255±19** (−43.6)	104±8** (−30.7)	151±19** (−50.0)	143±20** (−60.3)
				100	200±13** (−55.7)	97±9** (−35.3)	103±12** (−65.9)	112±14** (−68.9)
7Ar$_4$	C_6H_5	CH_3	2-thienyl	50	384±50 (−15.0)	156±8 (+4.0)	238±58 (−21.2)	251±19** (−30.3)
8Ar$_1$	C_6H_5	C_6H_{11}	C_6H_5	50	303±39** (−33.0)	122±1** (−18.7)	181±63* (−40.1)	271±32** (−24.7)
8Ar$_2$	C_6H_5	C_6H_{11}	*p*-ClC_6H_5	50	470±40 (+4.0)	142±7 (−5.3)	328±46 (+8.6)	358±25 (−0.6)
8Ar$_3$	C_6H_5	C_6H_{11}	2-furyl	50	413±28 (−8.6)	127±12 (−15.3)	286±18 (−5.3)	216±14** (−40.0)
8Ar$_4$	C_6H_5	C_6H_{11}	2-thienyl	50	368±30* (−18.6)	116±15* (−22.7)	252±55 (−16.6)	277±23* (−23.1)
9Ar$_2$	C_6H_5	C_6H_5	*p*-ClC_6H_5	50	256±38** (−43.4)	120±7* (−20.0)	136±39** (−55.0)	318±23 (−11.7)
9Ar$_3$	C_6H_5	C_6H_5	2-furyl	50	406±43 (−10.2)	193±12 (+28.7)	213±28 (−29.5)	222±18** (−38.3)
9Ar$_4$	C_6H_5	C_6H_5	2-thienyl	50	318±41** (−29.6)	137±16 (−8.7)	181±49* (−40.1)	287±19* (−20.3)
10Ar$_1$	C_5H_4N	CH_3	C_6H_5	50	408±46 (−9.7)	168±11 (+12.0)	240±51 (−20.5)	266±24** (−26.1)
10Ar$_2$	C_5H_4N	CH_3	*p*-ClC_6H_5	50	293±21** (−35.2)	133±6* (−11.3)	160±22** (−47.0)	323±18 (−10.3)
10Ar$_3$	C_5H_4N	CH_3	2-furyl	50	321±42** (−29.0)	124±12 (−17.3)	197±28** (−34.8)	274±30* (−23.9)
10Ar$_4$	C_5H_4N	CH_3	2-thienyl	50	411±28 (−9.1)	146±13 (−2.7)	265±32 (−12.2)	306±26 (−15.0)
11Ar$_1$	C_5H_4N	C_6H_{11}	C_6H_5	50	363±31** (−19.7)	173±16 (+15.3)	190±25** (−37.1)	213±18** (−40.8)
11Ar$_2$	C_5H_4N	C_6H_{11}	*p*-ClC_6H_5	50	323±14** (−28.5)	148±12 (−1.3)	175±13** (−42.0)	216±18* (−40.0)
11Ar$_3$	C_5H_4N	C_6H_{11}	2-furyl	50	407±55 (−10.0)	165±11 (+10.0)	242±60 (−19.9)	266±21 (−26.1)
11Ar$_4$	C_5H_4N	C_6H_{11}	2-thienyl	50	393±51 (−13.0)	158±10 (+5.3)	235±60 (−22.2)	248±19 (−31.1)
12Ar$_2$	C_5H_4N	C_6H_5	*p*-ClC_6H_5	50	458±32 (+1.3)	136±17 (−9.3)	322±15 (+6.6)	336±21 (−6.7)
12Ar$_3$	C_5H_4N	C_6H_5	2-furyl	50	328±41 (−27.4)	161±8 (+7.3)	167±46 (−44.7)	301±18 (−16.4)
12Ar$_4$	C_5H_4N	C_6H_5	2-thienyl	50	288±26** (−36.3)	113±16 (−24.7)	175±33* (−42.0)	386±15 (+7.2)
Controls					102±12	41±9	61±15	130±9
Triton				400	452±13	150±10	302±15	360±21
Triton+nicotinic acid				50	178±18** (−60.6)	79±10** (−47.3)	99±12** (−67.2)	243±18** (−32.5)

*, **Statistically significant value calculated in comparison with the test performed with Triton only ($P < 0.05$ and $P < 0.01$, respectively) [23].
[a] In parentheses, % variation compared with the group treated with Triton only.

using a rotatory evaporator operating at reduced pressure of about 10–20 Torr.

Thin layer chromatography 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}): chloroform was used as a developing solvent and detection of spots was made by UV light and/or by iodine vapours.

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.

^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 instrument, chemical shifts were reported in δ (ppm) units relative to the internal reference tetramethylsilane. Coupling constant values were given in Hertz. Elemental analyses for C, H, N were performed

by an EA1110 Analyser, Fison Instruments (Milan) and were within ±0.4% of theoretical values.

4.1.1. General procedure for preparation of hydrochlorides **3**

To a chloroform solution (15 ml) of 1-methylpiperazine (1.50 g, 15 mmol) and proper isothiocyanate (methyl-, cyclohexyl-, phenyl-isothiocyanate, 15 mmol), stirred for 10 min at room temperature (r.t.), neat acyl chloride (15 mmol) was added in a single portion. The resulting reaction mixture was kept under vigorously stirring at r.t. for 4 h. At different times, some of the hydrochlorides precipitated. Afterwards, small volumes of diethyl ether (0.5–1.0 ml) were added to the solution/suspension till no more turbidity formed. The solid was allowed to stand overnight, collected by filtration and recrystallized from proper solvent(s). The isolated hydrochlorides **3R$_2$Ar$_3$** and **3R$_2$Ar$_4$**, owing to their deliquescence, could not be characterised.

*4-{[4-Chlorobenzoyl(methyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_1$Ar$_2$**).* $C_{14}H_{19}Cl_2N_3OS$, MM 348.29, Yield 98%, m.p. 203–204 °C (MeOH/Et_2O). IR (KBr): 2700–2100, 1655 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.82 (s, 3H, CH_3), 3.10–4.10 (m, 4H, $2CH_2$/N-4), 3.44 (s, 3H, CH_3), 4.30–4.95 (m, 4H, $2CH_2$/N-1), 7.35–7.82 (m, 4H, arom. H), 12.55 (very bs, 1H, exchangeable, NH).

*4-{[2-Furoyl(methyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_1$Ar$_3$**).* $C_{12}H_{18}ClN_3O_2S$, MM 303.81, Yield 97%, m.p. 205–207 °C (MeOH/Et_2O). IR (KBr): 3100, 2700–2200, 1655 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.92 (s, 3H, CH_3), 3.11–3.73 (m, 4H, $2CH_2$/N-4), 3.34 (s, 3H, CH_3), 3.98–5.00 (m, 4H, $2CH_2$/N-1), 6.48–6.73 (m, 1H, H-4 fur.), 7.23 (d, $J = 4$ Hz, H-3 fur), 7.56–7.82 (m, 1H, H-5 fur.), 12.93 (very bs, 1H, exchangeable, NH).

*4-{[Methyl(thien-2-ylcarbonyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_1$Ar$_4$**).* $C_{12}H_{18}ClN_3OS_2 \cdot H_2O$, MM 337.88, Yield 97%, m.p. 188–189 °C (hot $CHCl_3$). IR (KBr): 3600–3300, 2720–2180, 1655 cm^{-1}; ^1H NMR (DMSO): δ 2.80 (s, 3H, CH_3), 3.01–3.80 (m, 4H, $2CH_2$/N-4), 3.25 (s, 3H, CH_3), 3.90–5.00 (m, 4H, $2CH_2$/N-1), 7.08–7.32 (m, 1H, H-3 thioph.), 7.48–7.58 (m, 1H, H-4 thioph.), 7.80–8.05 (m, 1H, H-5 thioph.), 12.15 (very bs, 1H, exchangeable, NH).

*4-{[4-Chlorobenzoyl(cyclohexyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_2$Ar$_2$**).* $C_{19}H_{27}Cl_2N_3OS$, MM 416.41, Yield 85%, m.p. 186–187 °C (MeOH/Et_2O). IR (KBr): 2700–2200, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.92–2.32 (m, 10H, $5CH_2$ cyclohexyl), 2.74 (s, 3H, CH_3), 3.20–5.02 (m, 9H, $4CH_2$/pip and CH), 7.23–7.94 (m, 4H, arom. H), 13.38 (very bs, 1H, exchangeable, NH).

*4-{[4-Chlorobenzoyl(phenyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_3$Ar$_2$**).* $C_{19}H_{21}Cl_2N_3OS$, MM 410.36, Yield 96%, m.p. 191–193 °C (MeOH/Et_2O). IR (KBr): 2600–2180, 1655 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.90 (s, 3H, CH_3), 2.95–3.30 (m, 4H, $2CH_2$/N-4), 3.95–5.00 (m, 4H, $2CH_2$/N-1), 7.05–7.80 (m, 9H, arom. H), 12.45 (very bs, 1H, exchangeable, NH). After change with D_2O: δ 2.88 (s, 3H, CH_3), 3.06–3.67 (m, 4H, $2CH_2$/N-4), 4.30–4.81 (m, 4H, $2CH_2$/N-1).

*4-{[2-Furoyl(phenyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_3$Ar$_3$**).* $C_{17}H_{20}ClN_3O_2S$, MM 365.88, Yield 96%, m.p. 182–183 °C (MeOH/Et_2O). IR (KBr): 2600–2180, 1640 cm^{-1}; ^1H NMR (DMSO): δ 2.82 (s, 3H, CH_3), 3.00–3.78 (m, 4H, $2CH_2$/N-4), 4.10–5.18 (m, 4H, $2CH_2$/N-1), 6.56–6.76 (m, 1H, H-4 fur.), 6.79–6.95 (d, $J = 4$ Hz, H-3 fur), 7.20–7.70 (m, 5H, arom H.), 7.98–8.14 (m, 1H, H-5 fur.), 12.50 (very bs, 1H, exchangeable, NH).

*4-{[Phenyl(thien-2-ylcarbonyl)amino]carbonothioyl}-1-methylpiperazin-1-ium chloride (**3R$_3$Ar$_4$**).* $C_{17}H_{20}ClN_3OS_2$, MM 381.94, Yield 85%, m.p. 196–199 °C ($CHCl_3$/Et_2O). IR (KBr): 2680–2200, 1655 cm^{-1}; ^1H NMR (DMSO): δ 2.90 (s, 3H, CH_3), 3.10–3.70 (m, 4H, $2CH_2$/N-4), 4.02–5.30 (m, 4H, $2CH_2$/N-1), 6.80–7.75 (m, 8H, arom H and H-3, H-4, H-5 thioph.), 12.88 (very bs, 1H, exchangeable, NH). ^{13}C NMR (DMSO): δ 85.30, 141.38, 137.33, 134,50, 134,29, 130,13, 129.27, 128.46, 52.01, 47.24, 42.28, 41.54.

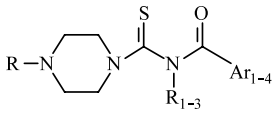
4.1.2. General procedure for the synthesis of ATU derivatives **4–6**

To a stirred chloroform solution (40 ml) of 1-methylpiperazine (1.00 g, 10 mmol) and proper isothiocyanate (methyl-, cyclohexyl-, phenyl-isothiocyanate, 10 mmol) neat acyl chloride (11 mmol) was added in a single portion after 10 min at r.t. The resulting mixture was allowed to react at r.t. for 4 h under stirring; then water (50 ml) was added and the chloroform layer was separated and washed two times with water (25 ml). The combined aqueous layers were made alkaline by treatment of 1 M NaOH (45 ml) and extracted with chloroform (3 × 15 ml). The organic phase was dried and evaporated under reduced pressure to dryness to afford an oily or solid residue, which was crystallized by proper solvents.

*4-Chloro-N-methyl-[(4-methylpiperazin-1-yl)carbonothioyl]benzamide (**4Ar$_2$**).* $C_{14}H_{18}ClN_3OS$, MM 311.84, Yield 71%, m.p. 111–112 °C (CH_2Cl_2/Et_2O). IR (KBr): 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.00–2.55 (m, 4H, $2CH_2$/pip), 2.25 (s, 3H, CH_3N/pip), 3.30–3.95 (m, 4H, $2CH_2N$/pip), 3.50 (s, 3H, CH_3N), 7.44–7.76 (m, 4H, arom. H).

*N-Methyl-[(4-methylpiperazin-1-yl)carbonothioyl]-2-furamide (**4Ar$_3$**).* $C_{12}H_{17}N_3O_2S$, MM 267.35, Yield 60%, m.p. 81–83 °C (CH_2Cl_2/Et_2O). IR (KBr): 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.22–2.65 (m, 4H, $2CH_2$/pip), 2.30 (s, 3H, CH_3N/pip), 3.32 (s, 3H, CH_3N), 3.80–

Table 3
Analgesic activity of the compounds of series **4**–**12**, evaluated by hot plate test [a]



Comp.	R	R_{1-3}	Ar_{1-4}	Dose (mg/kg p.o.)	Mean reaction time (s±SE) at the following times (hours) after treatment (in parentheses per cent change relative to 0 value) [b]				
					0	1	2	3	4
4Ar$_2$	CH_3	CH_3	*p*-ClC_6H_5	50	9±1.2	10±1.0 (11)	11±0.8* (22)	11±0.7* (22)	13±1.6* (44)
4Ar$_3$	CH_3	CH_3	2-furyl	50	8±2.3	8±1.9 (0)	9±2.6 (12)	9±1.4 (12)	11±3.8 (37)
4Ar$_4$	CH_3	CH_3	2-thienyl	50	9±1.2	9±2.1 (0)	10±1.8 (11)	12±1.1* (33)	13±1.6* (44)
5Ar$_2$	CH_3	C_6H_{11}	*p*-ClC_6H_5	50	7±1.8	9±1.6 (29)	12±2* (71)	14±1.2** (100)	16±2.1** (129)
5Ar$_3$	CH_3	C_6H_{11}	2-furyl	50	10±1.7	13±0.9 (30)	14±1.0* (40)	15±2.1* (50)	18±1.7** (80)
5Ar$_4$	CH_3	C_6H_{11}	2-thienyl	50	8±1.5	12±1.6* (50)	14±2.0* (75)	16±1.7** (100)	17±2.3** (112)
6Ar$_2$	CH_3	C_6H_5	*p*-ClC_6H_5	50	10±1.2	10±2.0 (0)	10±1.5 (0)	11±1.8 (10)	12±2.6 (20)
6Ar$_3$	CH_3	C_6H_5	2-furyl	50	7±1.9	8±1.4 (14)	9±1.6 (29)	10±3.1 (43)	10±2.6 (43)
6Ar$_4$	CH_3	C_6H_5	2-thienyl	50	9±2.1	9±1.7 (0)	10±1.8 (11)	12±3.2 (33)	13±1.7 (44)
7Ar$_1$	C_6H_5	CH_3	C_6H_5	50	9±1.4	9±1.5 (0)	10±1.2 (11)	11±1.9 (22)	12±2.3 (33)
7Ar$_2$	C_6H_5	CH_3	*p*-ClC_6H_5	50	8±1.3	9±1.2 (12)	10±1.9 (25)	12±1.2* (50)	13±2.0* (62)
7Ar$_3$	C_6H_5	CH_3	2-furyl	50	8±1.1	8±1.0 (0)	9±0.9 (12)	10±1.2 (25)	10±1.6 (25)
7Ar$_4$	C_6H_5	CH_3	2-thienyl	50	9±1.9	9±1.1 (0)	10±1.9 (11)	12±2.3 (33)	13±1.4 (44)
8Ar$_1$	C_6H_5	C_6H_{11}	C_6H_5	50	10±0.9	10±1.7 (0)	12±2.1* (20)	14±1.4* (40)	15±1.3** (50)
8Ar$_2$	C_6H_5	C_6H_{11}	*p*-ClC_6H_5	50	9±1.8	9±2.1 (0)	10±2.7 (11)	10±2.3 (11)	11±1.5 (22)
8Ar$_3$	C_6H_5	C_6H_{11}	2-furyl	50	8±0.7	10±0.9* (25)	11±1.1* (37)	11±1.2* (37)	12±1.6* (50)
8Ar$_4$	C_6H_5	C_6H_{11}	2-thienyl	50	7±1.9	9±2.1 (29)	9±1.8 (29)	10±1.9 (43)	11±2.4 (57)
9Ar$_2$	C_6H_5	C_6H_5	*p*-ClC_6H_5	50	7±2.1	9±1.7 (29)	10±2.0 (43)	12±1.8* (71)	14±1.9** (100)
9Ar$_3$	C_6H_5	C_6H_5	2-furyl	50	9±1.5	9±1.6 (0)	9±1.9 (0)	9±1.6 (0)	10±2.0 (11)
9Ar$_4$	C_6H_5	C_6H_5	2-thienyl	50	8±1.1	9±1.2 (12)	10±1.4 (25)	11±0.8* (37)	12±1.1* (50)
10Ar$_1$	C_5H_4N	CH_3	C_6H_5	50	9±2.1	10±1.9 (11)	11±2.1 (22)	11±1.8 (22)	12±1.7 (33)
10Ar$_2$	C_5H_4N	CH_3	*p*-ClC_6H_5	50	7±0.9	8±2.1 (14)	9±1.4 (29)	9±0.9 (29)	9±1.3 (29)
10Ar$_3$	C_5H_4N	CH_3	2-furyl	50	9±1.5	10±1.4 (11)	12±1.8 (33)	13±1.0* (44)	15±1.2** (67)
10Ar$_4$	C_5H_4N	CH_3	2-thienyl	50	10±1.4	10±1.5 (0)	11±1.7 (10)	12±2.5 (20)	12±1.9 (20)
11Ar$_1$	C_5H_4N	C_6H_{11}	C_6H_5	50	10±2.1	11±1.6 (10)	12±2.4 (20)	12±2.1 (20)	13±1.9 (30)
11Ar$_2$	C_5H_4N	C_6H_{11}	*p*-ClC_6H_5	50	8±1.9	8±2.0 (0)	9±2.3 (12)	10±2.5 (25)	11±3.1 (37)
11Ar$_3$	C_5H_4N	C_6H_{11}	2-furyl	50	9±2.7	9±2.3 (0)	10±1.8 (11)	12±3.1 (33)	13±2.7 (44)
11Ar$_4$	C_5H_4N	C_6H_{11}	2-thienyl	50	8±1.4	8±1.7 (0)	9±1.8 (12)	9±2.1 (12)	10±1.5 (25)
12Ar$_2$	C_5H_4N	C_6H_5	*p*-ClC_6H_5	50	10±1.7	12±1.4 (20)	15±1.0* (50)	17±2.1* (70)	17±1.8* (70)
12Ar$_3$	C_5H_4N	C_6H_5	2-furyl	50	7±1.2	7±2.5 (0)	8±1.8 (14)	9±2.3 (29)	9±1.9 (29)
12Ar$_4$	C_5H_4N	C_6H_5	2-thienyl	50	8±2.4	8±1.8 (0)	9±1.9 (12)	10±2.5 (25)	12±3.1 (50)
Control					7±0.2	7±1.0 (0)	7±0.8 (0)	7±0.9 (0)	7±1.1 (0)
Indomethacin				5	8±1.2	10±0.8* (25)	13±1.0** (62)	15±0.9** (87)	16±1.7** (100)

*, ** Statistically significant values calculated in comparison with the test performed with basis value ($P < 0.05$, $P < 0.01$, respectively) [23].
[a] Ten mice (20–25 g)/group.
[b] Mean value of five determinations.

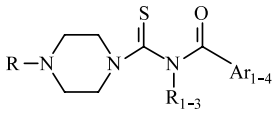
4.20 (m, 4H, $2CH_2N$/pip), 6.47–6.59 (m, 1H, H-4 fur.), 7.20 (d, 1H, $J = 4$ Hz, H-3 fur.), 7.59 (bs, 1H, H-5 fur.).

N-Methyl-[(4-methylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***4Ar$_4$***). $C_{12}H_{17}N_3OS_2$, MM 283.42, Yield 75%, m.p. 105–106 °C (CH_2Cl_2/Et_2O). IR (KBr): 1630 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.10–2.55 (m, 4H, $2CH_2$/pip), 2.24 (s, 3H, CH_3N/pip), 3.42 (s, 3H, CH_3N), 3.65–4.10 (m, 4H, $2CH_2N$/pip), 7.00–7.25 (m, 1H, H-4 thioph.), 7.50–7.90 (m, 2H, H-3 and H-5 thioph.)

4-Chloro-N-cyclohexyl-[(4-methylpiperazin-1-yl)carbonothioyl]benzamide (***5Ar$_2$***). $C_{19}H_{26}ClN_3OS$, MM 379.25, Yield 65%, m.p. 133–134 °C (CH_2Cl_2/Et_2O). IR (KBr): 1630 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.50 (m, 14H, $5CH_2$ and $2CH_2$/pip), 2.20 (s, 3H, CH_3N/pip), 3.05–4.00 (m, 4H, $2CH_2N$/pip), 4.05–4.80 (m, 1H, CHN), 7.37–7.82 (m, 4H, arom. H).

N-Cyclohexyl-[(4-methylpiperazin-1-yl)carbonothioyl]-2-furamide (***5Ar$_3$***). $C_{17}H_{25}N_3O_2S$, MM 335.47, Yield 82%, m.p. 104–105 °C (CH_2Cl_2/Et_2O). IR (KBr): 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.60 (m, 14H, $5CH_2$ and $2CH_2$/pip), 2.25 (s, 3H, CH_3N/pip), 3.65–4.15 (m, 4H, $2CH_2N$/pip), 4.10–4.60 (m, 1H, CHN), 6.44–6.54 (m, 1H, H-4 fur.), 7.25 (d, 1H, $J = 4$ Hz, H-3 fur.), 7.57 (bs, 1H, H-5 fur.).

Table 4
Anti-inflammatory activity of the compounds of series **4**–**12**, evaluated by carrageenan-induced rat paw oedema test [a]



Comp.	R	R_{1-3}	Ar_{1-4}	Dose (mg/kg p.o.)	% Oedema inhibition relative to control at the following times (h) after administration			
					1st h	2nd h	3rd h	4th h
4Ar$_2$	CH_3	CH_3	*p*-ClC_6H_5	50	−18	−18	−33	−39
4Ar$_3$	CH_3	CH_3	2-furyl	50	−36	−2	−15	−20
4Ar$_4$	CH_3	CH_3	2-thienyl	50	−9	−32	−25	−32
5Ar$_2$	CH_3	C_6H_{11}	*p*-ClC_6H_5	50	−31	−43	−44	−44
5Ar$_3$	CH_3	C_6H_{11}	2-furyl	50	−50	−50	−34	−50
5Ar$_4$	CH_3	C_6H_{11}	2-thienyl	50	0	−25	−34	−38
6Ar$_2$	CH_3	C_6H_5	*p*-ClC_6H_5	50	+14	−16	−25	−16
6Ar$_3$	CH_3	C_6H_5	2-furyl	50	+50	+61	+28	+12
6Ar$_4$	CH_3	C_6H_5	2-thienyl	50	+14	−16	−25	−30
7Ar$_1$	C_6H_5	CH_3	C_6H_5	50	−45	−16	−25	−30
7Ar$_2$	C_6H_5	CH_3	*p*-ClC_6H_5	50	0	0	−18	−25
7Ar$_3$	C_6H_5	CH_3	2-furyl	50	−18	−18	−19	−39
7Ar$_4$	C_6H_5	CH_3	2-thienyl	50	−36	−36	−36	−36
8Ar$_1$	C_6H_5	C_6H_{11}	C_6H_5	50	−50	−50	−67	−63
8Ar$_2$	C_6H_5	C_6H_{11}	*p*-ClC_6H_5	50	−18	−31	−61	−55
8Ar$_3$	C_6H_5	C_6H_{11}	2-furyl	50	−9	−31	−40	−55
8Ar$_4$	C_6H_5	C_6H_{11}	2-thienyl	50	−45	−16	−25	−30
9Ar$_2$	C_6H_5	C_6H_5	*p*-ClC_6H_5	50	+27	−2	−15	−5
9Ar$_3$	C_6H_5	C_6H_5	2-furyl	50	−9	−32	−40	−44
9Ar$_4$	C_6H_5	C_6H_5	2-thienyl	50	−45	−32	−45	−44
10Ar$_1$	C_5H_4N	CH_3	C_6H_5	50	−45	−16	−25	−15
10Ar$_2$	C_5H_4N	CH_3	*p*-ClC_6H_5	50	0	−25	−35	−50
10Ar$_3$	C_5H_4N	CH_3	2-furyl	50	−18	−39	−46	−49
10Ar$_4$	C_5H_4N	CH_3	2-thienyl	50	−23	−25	−37	−25
11Ar$_1$	C_5H_4N	C_6H_{11}	C_6H_5	50	+50	+14	−25	−25
11Ar$_2$	C_5H_4N	C_6H_{11}	*p*-ClC_6H_5	50	−45	−16	−45	−44
11Ar$_3$	C_5H_4N	C_6H_{11}	2-furyl	50	−9	−9	−25	−32
11Ar$_4$	C_5H_4N	C_6H_{11}	2-thienyl	50	−41	−36	−36	−36
12Ar$_2$	C_5H_4N	C_6H_5	*p*-ClC_6H_5	50	−18	−39	−33	−29
12Ar$_3$	C_5H_4N	C_6H_5	2-furyl	50	+27	−2	−10	−20
12Ar$_4$	C_5H_4N	C_6H_5	2-thienyl	50	0	−25	−34	−38
Control				Carr. 1% sol	+22	+44	+67	+89
Indomethacin				5	−45	−43	−63	−70

[a] Each compound was tested on a group of five albino rats (180–250 g) and given by gastric probe 30 min before carrageenan.

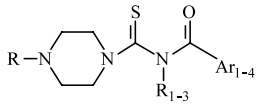
N-Cyclohexyl-[(4-methylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***5Ar$_4$***). $C_{17}H_{25}N_3OS_2$, MM 351.54, Yield 70%, m.p. 127–128 °C (CH_2Cl_2/Et_2O). IR (KBr): 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.65 (m, 14H, $5CH_2$ and $2CH_2$/pip), 2.25 (s, 3H, CH_3N/pip), 3.55–4.10 (m, 4H, $2CH_2N$/pip), 4.15–4.75 (m, 1H, CHN), 6.90–7.25 (m, 1H, H-4 thioph.), 7.40–7.60 (m, 1H, H-3 thioph.), 7.75–8.00 (m, 1H, H-5 thioph.).

4-Chloro-N-phenyl-[(4-methylpiperazin-1-yl)carbonothioyl]benzamide (***6Ar$_2$***). $C_{19}H_{20}ClN_3OS$, MM 373.91, Yield 65% (Py/TEA), 73% ($CHCl_3$), m.p. 191–193 °C (CH_2Cl_2/Et_2O). IR (KBr): 1675 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.25–2.75 (m, 4H, $2CH_2$/pip), 2.30 (s, 3H, CH_3N/pip), 3.80–4.40 (m, 4H, $2CH_2N$/pip), 7.05–7.80 and 8.00–8.30 (m, 9H, arom. H).

N-Phenyl-[(4-methylpiperazin-1-yl)carbonothioyl]-2-furamide (***6Ar$_3$***). $C_{17}H_{19}N_3O_2S$, MM 329.42, Yield 78%, m.p. 126–127 °C (CH_2Cl_2/Et_2O). IR (KBr): 1665 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.25 (s, 3H, CH_3N/pip), 2.25–2.70 (m, 4H, $2CH_2$/pip), 3.85–4.40 (m, 4H, $2CH_2N$/pip), 6.40–6.65 (m, 1H, H-4 fur.), 6.85 (d, 1H, $J = 4$ Hz, H-3 fur.), 7.25–7.78 (m, 9H, arom. H and H-5 fur.).

N-Phenyl-[(4-methylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***6Ar$_4$***). $C_{17}H_{19}N_3OS_2$, MM 345.49, Yield 95%, m.p. 148–149 °C (CH_2Cl_2/Et_2O). IR (KBr): 1615 cm^{-1}; IR

Table 5
Antiarrhythmic activity of the compounds of series **4**–**12**, evaluated as protection index against ecgraphic effects from aconitine in albino rats [a]



Comp.	R	R_{1-3}	Ar_{1-4}	Dose (mg/kg p.o.)	Appearance time (s ± SE) of extrasystoles [b]	Death time (sec ± SE) [b]
4Ar$_2$	CH_3	CH_3	*p*-ClC_6H_5	50	260 ± 24.1** (44)	760 ± 10.1** (31)
4Ar$_3$	CH_3	CH_3	2-furyl	50	345 ± 42.0** (92)	980 ± 17.2** (69)
4Ar$_4$	CH_3	CH_3	2-thienyl	50	210 ± 28.9 (17)	632 ± 30.3 (9)
5Ar$_2$	CH_3	C_6H_{11}	*p*-ClC_6H_5	50	220 ± 16.8 (22)	720 ± 44.3* (24)
5Ar$_3$	CH_3	C_6H_{11}	2-furyl	50	192 ± 14.5 (7)	740 ± 47.9** (28)
5Ar$_4$	CH_3	C_6H_{11}	2-thienyl	50	274 ± 40.4* (52)	682 ± 31.8 (18)
6Ar$_2$	CH_3	C_6H_5	*p*-ClC_6H_5	50	193 ± 9.8** (7)	641 ± 54.3 (10)
6Ar$_3$	CH_3	C_6H_5	2-furyl	50	185 ± 10.9 (3)	493 ± 9.4 (−15)
6Ar$_4$	CH_3	C_6H_5	2-thienyl	50	262 ± 30.3* (46)	945 ± 30** (63)
7Ar$_1$	C_6H_5	CH_3	C_6H_5	50	207 ± 45.2 (15)	692 ± 51* (19)
7Ar$_2$	C_6H_5	CH_3	*p*-ClC_6H_5	50	177 ± 8.6 (−2)	580 ± 18.3 (0)
7Ar$_3$	C_6H_5	CH_3	2-furyl	50	162 ± 16.4 (−10)	447 ± 10.4 (−23)
7Ar$_4$	C_6H_5	CH_3	2-thienyl	50	270 ± 24.3** (50)	892 ± 20.4** (54)
8Ar$_1$	C_6H_5	C_6H_{11}	C_6H_5	50	194 ± 8.3 (8)	586 ± 10.2 (1)
8Ar$_2$	C_6H_5	C_6H_{11}	*p*-ClC_6H_5	50	220 ± 19.2 (22)	964 ± 17.4** (66)
8Ar$_3$	C_6H_5	C_6H_{11}	2-furyl	50	194 ± 16.8 (8)	646 ± 20.7 (11)
8Ar$_4$	C_6H_5	C_6H_{11}	2-thienyl	50	236 ± 17.4* (31)	892 ± 44* (54)
9Ar$_2$	C_6H_5	C_6H_5	*p*-ClC_6H_5	50	210 ± 24.6 (17)	644 ± 22.3 (11)
9Ar$_3$	C_6H_5	C_6H_5	2-furyl	50	189 ± 12.3 (5)	704 ± 36.9* (21)
9Ar$_4$	C_6H_5	C_6H_5	2-thienyl	50	210 ± 7.9 (17)	904 ± 11.2 (56)
10Ar$_1$	C_5H_4N	CH_3	C_6H_5	50	325 ± 42.1** (81)	1058 ± 28.1** (82)
10Ar$_2$	C_5H_4N	CH_3	*p*-ClC_6H_5	50	207 ± 11.4 (15)	610 ± 17.4 (5)
10Ar$_3$	C_5H_4N	CH_3	2-furyl	50	227 ± 15.6* (26)	652 ± 18.2* (12)
10Ar$_4$	C_5H_4N	CH_3	2-thienyl	50	241 ± 33.4 (34)	692 ± 20.2** (19)
11Ar$_1$	C_5H_4N	C_6H_{11}	C_6H_5	50	192 ± 10.8 (7)	596 ± 12.8 (3)
11Ar$_2$	C_5H_4N	C_6H_{11}	*p*-ClC_6H_5	50	270 ± 36.4* (50)	606 ± 29.1 (4)
11Ar$_3$	C_5H_4N	C_6H_{11}	2-furyl	50	292 ± 45.6 (62)	674 ± 25.1 (16)
11Ar$_4$	C_5H_4N	C_6H_{11}	2-thienyl	50	240 ± 20.1* (33)	656 ± 15.4* (13)
12Ar$_2$	C_5H_4N	C_6H_5	*p*-ClC_6H_5	50	242 ± 49.6 (34)	746 ± 46.4 (29)
12Ar$_3$	C_5H_4N	C_6H_5	2-furyl	50	188 ± 20.5 (4)	532 ± 26.4 (−8)
12Ar$_4$	C_5H_4N	C_6H_5	2-thienyl	50	184 ± 12.1 (2)	606 ± 27.4 (4)
Controls (aconitine HCl)				[c]	180 ± 15.9	580 ± 27.2
Quinidine				25	360 ± 20.1** (100)	1020 ± 14.3* (76)

*, ** Statistically significant values calculated in comparison with the test performed with aconitine alone ($P < 0.05$, $P < 0.01$, respectively) [23].
[a] Five animals (200–250 g)/group.
[b] In parentheses % time increase as regard to the control.
[c] 15 mg/kg i.v. until death time.

(KBr): 1680 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.30 (s, 3H, CH_3N/pip), 2.20–2.70 (m, 4H, $2CH_2$/pip), 3.85–4.40 (m, 4H, $2CH_2N$/pip), 6.70–7.70 (m, 8H, arom. H and H-3, H-4, H-5 thioph.).

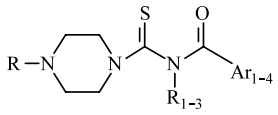
4.1.3. General procedure for preparation of ATU derivatives 7–9

In a non-anhydrous pyridine solution (20 ml) 1-phenylpiperazine (1.62 g, 10 mmol) and proper isothiocyanate (methyl-, cyclohexyl-, phenyl-isothiocyanate, 10 mmol) were allowed to react for 10 min at r.t. under stirring. After adding neat acyl chloride (11 mmol) in a single portion at r.t., the reaction mixture was stirred for 4 h at r.t. Evaporating pyridine in vacuo gave a crude residue, which was treated with water. The resulting suspension was extracted with dichloromethane (3 × 20 ml), washed three times with water (40 ml), dried, filtered through a plug of Florisil and finally evaporated under reduced pressure to yield a residue, which was crystallised from proper solvents.

*N-Methyl-[(4-phenylpiperazin-1-yl)carbonothioyl]benzamide (**7Ar$_1$**)*. $C_{19}H_{21}N_3OS$, MM 339.46, Yield 77%, m.p. 115–116 °C (CH_2Cl_2/MeOH). IR (KBr): 1665 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.90–3.35 (m, 4H, $2CH_2$/pip), 3.52 (s, 3H, CH_3N), 3.60–4.10 (m, 4H, $2CH_2N$/pip), 6.70–8.10 (m, 10H, arom. H).

*4-Chloro-N-methyl-[(4-phenylpiperazin-1-yl)carbonothioyl]benzamide (**7Ar$_2$**)*. $C_{19}H_{20}ClN_3OS$, MM

Table 6
Inhibition test of platelet aggregation induced by collagen in human plasma versus the compounds of series **4**–**12**



Comp.	R	R_{1-3}	Ar_{1-4}	Final concentration	% Aggregation ± SE	% Δ vs. collagen
4Ar$_2$	CH_3	CH_3	*p*-ClC_6H_5	10^{-6} g/ml	77.0 ± 2.5	−5.5
				10^{-5}	74.5 ± 3.8	−8.6
4Ar$_3$	CH_3	CH_3	2-furyl	10^{-6} g/ml	75.0 ± 5.6	−8.0
				10^{-5}	73.2 ± 4.1	−10.2
5Ar$_2$	CH_3	C_6H_{11}	*p*-ClC_6H_5	10^{-6} g/ml	74.5 ± 5.5	−8.6
				10^{-5}	71.0 ± 7.5	−12.9
5Ar$_3$	CH_3	C_6H_{11}	2-furyl	10^{-6} g/ml	76.5 ± 2.6	−6.1
				10^{-5}	70.5 ± 4.3	−13.5
5Ar$_4$	CH_3	C_6H_{11}	2-thienyl	10^{-6} g/ml	78.4 ± 5.9	−3.8
				10^{-5}	76.5 ± 2.4	−6.1
6Ar$_3$	CH_3	C_6H_5	2-furyl	10^{-6} g/ml	79.2 ± 3.5	−2.8
				10^{-5}	74.5 ± 4.3	−8.6
6Ar$_4$	CH_3	C_6H_5	2-thienyl	10^{-6} g/ml	77.0 ± 2.7	−5.5
				10^{-5}	69.5 ± 5.7	−14.7
7Ar$_3$	C_6H_5	CH_3	2-furyl	10^{-6} g/ml	76.0 ± 5.0	−6.7
				10^{-5}	72.4 ± 4.9	−11.2
8Ar$_2$	C_6H_5	C_6H_{11}	*p*-ClC_6H_5	10^{-6} g/ml	79.2 ± 2.4	−2.8
				10^{-5}	78.6 ± 5.1	−3.6
8Ar$_4$	C_6H_5	C_6H_{11}	2-thienyl	10^{-6} g/ml	79.6 ± 3.7	−2.3
				10^{-5}	79.0 ± 4.1	3.1
9Ar$_2$	C_6H_5	C_6H_5	*p*-ClC_6H_5	10^{-6} g/ml	79.5 ± 3.7	−2.4
				10^{-5}	70.1 ± 4.8	−14.0
9Ar$_4$	C_6H_5	C_6H_5	2-thienyl	10^{-6} g/ml	76.9 ± 5.3	−5.6
				10^{-5}	72.4 ± 3.3	−11.2
10Ar$_3$	C_5H_4N	CH_3	2-furyl	10^{-6} g/ml	76.4 ± 7.6	−6.3
				10^{-5}	70.5 ± 8.9	−13.5
11Ar$_2$	C_5H_4N	C_6H_{11}	*p*-ClC_6H_5	10^{-6} g/ml	79.4 ± 4.8	−2.6
				10^{-5}	73.9 ± 3.7	−9.3
11Ar$_3$	C_5H_4N	C_6H_{11}	2-furyl	10^{-6} g/ml	77.9 ± 5.1	−4.4
				10^{-5}	71.3 ± 3.9	−12.5
12Ar$_4$	C_5H_4N	C_6H_5	2-thienyl	10^{-6} g/ml	79.9 ± 3.7	−2.0
				10^{-5}	74.6 ± 4.9	−8.5
Collagen				10^{-5} g/ml	81.5 ± 2.3	
Acetylsalicylic Acid + Collagen				10^{-6} g/ml	74.5 ± 1.0	−8.6
				10^{-5}	68.9 ± 1.1	−15.5

373.91, Yield 78%, m.p. 161–163 °C (CH_2Cl_2/MeOH). IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.90–3.30 (m, 4H, $2CH_2$/pip), 3.50 (s, 3H, CH_3N), 3.65–4.10 (m, 4H, $2CH_2N$/pip), 6.70–7.95 (m, 9H, arom. H).

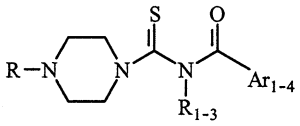
*N-Methyl-[(4-phenylpiperazin-1-yl)carbonothioyl]-2-furamide (**7Ar$_3$**)*. $C_{17}H_{19}N_3O_2S$, MM 329.42, Yield 64%, m.p. 159–160 °C (CH_2Cl_2/MeOH). IR (KBr): 1665 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.10–3.30 (m, 4H, $2CH_2$/pip), 3.40 (s, 3H, CH_3N), 3.82–4.62 (m, 4H, $2CH_2N$/pip), 6.40–6.65 (m, 1H, H-4 fur.), 6.80–7.70 (m, 7H, arom. H and H-3, H-5 fur.).

*N-Methyl-[(4-phenylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (**7Ar$_4$**)*. $C_{17}H_{19}N_3OS_2$, MM 345.49, Yield 58%, m.p. 123–124 °C (CH_2Cl_2/MeOH). IR (KBr): 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.90–3.35 (m, 4H, $2CH_2$/pip), 3.50 (s, 3H, CH_3N), 3.75–4.20 (m, 4H, $2CH_2N$/pip), 6.70–7.95 (m, 8H, arom. H and H-3, H-4, H-5 thioph.).

*N-Cyclohexyl-[(4-phenylpiperazin-1-yl)carbonothioyl]benzamide (**8Ar$_1$**)*. $C_{24}H_{29}N_3OS$, MM 407.58, Yield 53%, m.p. 164–166 °C (CH_2Cl_2/MeOH). IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.70 (m, 10H, $5CH_2$), 2.80–3.25 (m, 4H, $2CH_2$/pip), 3.40–4.20 (m, 4H, $2CH_2N$/pip), 4.25–4.90 (m, 1H, CHN), 6.60–8.15 (m, 10H, arom. H).

*4-Chloro-N-cyclohexyl-[(4-phenylpiperazin-1-yl)carbonothioyl]benzamide (**8Ar$_2$**)*. $C_{24}H_{28}N_3ClOS$, MM 442.02, Yield 66%, m.p. 119–120 °C (CH_2Cl_2/MeOH). IR (KBr): 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.65 (m, 10H, $5CH_2$), 2.75–3.30 (m, 4H, $2CH_2$/pip), 3.35–4.85 (m, 5H, $2CH_2N$/pip and CHN), 6.65–7.65 and 7.75–8.15 (m, 9H, arom. H).

Table 7
Antiproliferative activity of selected compounds



	R	R_{1-3}	Ar_{1-4}		R	R_{1-3}	Ar_{1-4}
$4Ar_2$	CH_3	CH_3	p-ClC_6H_5	**$11Ar_2$**	C_5H_4N	C_6H_{11}	p-ClC_6H_5
$5Ar_2$	CH_3	C_6H_{11}	p-ClC_6H_5	**$11Ar_4$**	C_5H_4N	C_6H_{11}	2-thienyl
$7Ar_2$	C_6H_5	CH_3	p-ClC_6H_5	**$12Ar_2$**	C_5H_4N	C_6H_5	2-thienyl
$8Ar_2$	C_6H_5	C_6H_{11}	p-ClC_6H_5				

	GI_{50} (μM) [a]						
Panel/cell line	**$4Ar_2$**	**$5Ar_2$**	**$7Ar_2$**	**$8Ar_2$**	**$11Ar_2$**	**$11Ar_4$**	**$12Ar_2$**
Leukemia							
CCRF-CEM		72.5	56.6		48.5	81.1	
HL-60(TB)		61.6	35.5	94.6	17.3		32.6
K-562		51.3	42.1	58.2	37.6	47.0	41.7
MOLT-4		63.1	58.4	54.2	24.3	33.1	43.6
RPMI-8226			64.3	63.5	25.5	43.1	82.5
SR		58.8	35.2	67.6	41.1	36.6	39.3
Non-small cell lung cancer							
A549/ATCC			89.1		45.1	43.7	94.2
HOP-62					71.8		
HOP-92			89.6			66.2	
NCI-H226			47.6	1.03	18.1	19.5	24.9
NCI-H23					96.8		
NCI-H322M					45.3		
NCI-H460			37.8		30.0	54.6	
NCI-H522			51.5		38.7	66.2	
LXFL 529					75.6	58.6	55.2
Small cell lung cancer							
DMS 114				70.0	34.9	36.9	42.4
DMS 273				46.9	34.3	43.1	78.4
Colon cancer							
COLO 205			51.0		44.4	22.4	
DLD-1					81.6	63.8	
HCT-116				45.7	36.6	49.3	97.5
HCT-15			87.5		43.8	78.0	
HT29			77.6		55.3	52.0	
KM12			53.2		68.0	60.6	
KM20L2					74.2	70.7	
SW-620					83.6		
CNS cancer							
SF-268					27.3		
SF-295					74.1		99.3
SF-539					61.6		
SNB-19					96.5		
SNB-75		20.1	46.7	85.2			
U251					37.1	79.1	
XF 498					8.2		
Melanoma							
LOX IMVI				79.4	56.1	77.4	
MALME-3M						36.3	
M14						76.4	
M19-MEL						87.2	
SK-MEL-2						58.6	
SK-MEL-28							
SK-MEL-5			35.5		38.7	31.3	39.7
UACC-257			71.5	91.1	30.8	51.0	
UACC-62			92.4				
Ovarian cancer							
OVCAR-3					45.9	57.2	66.3
Renal cancer							
786-0					36.4		
ACHN	0.13				43.6	69.2	
CAKI-1						76.3	
RXF-393					35.7	22.5	15.6
UO-31			20.1		48.3	44.4	68.8
Breast cancer							
MCF7			79.0				
HS 578T		27.4	19.0				

[a] The notation GI_{50} refers to the tested compound concentration that produced 50% growth inhibition. Only the values < 100 μM are reported.

N-Cyclohexyl-[(4-phenylpiperazin-1-yl)carbonothioyl]-2-furamide (***8Ar₃***). $C_{22}H_{27}N_3O_2S$, MM 397.54, Yield 43%, m.p. 132–133 °C (CH_2Cl_2/MeOH). IR (KBr): 1635 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.55 (m, 10H, $5CH_2$), 2.95–3.55 (m, 4H, $2CH_2$/pip), 3.80–4.70 (m, 5H, $2CH_2N$/pip and CHN), 6.40–6.65 (m, 1H, H-4 fur.), 6.70–7.70 (m, 7H, arom. H and H-3, H-5 fur.).

N-Cyclohexyl-[(4-phenylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***8Ar₄***). $C_{22}H_{27}N_3OS_2$, MM 413.61, Yield 68%, m.p. 135–136 °C (CH_2Cl_2/MeOH). IR (KBr): 1615 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.90–2.70 (m, 10H, $5CH_2$), 2.80–3.50 (m, 4H, $2CH_2$/pip), 3.65–4.80 (m, 5H, $2CH_2N$/pip and CHN), 6.65–7.650 and 7.80–8.05 (m, 8H, arom H. and H-3, H-4, H-5 thioph.).

4-Chloro-N-phenyl-[(4-phenylpiperazin-1-yl)carbonothioyl]benzamide (***9Ar₂***). $C_{24}H_{22}ClN_3OS$, MM 435.98, Yield 71%, m.p. 145–147 °C (CH_2Cl_2/MeOH). IR (KBr): 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.00–3.50 (m, 4H, $2CH_2$/pip), 3.90–4.50 (m, 4H, $2CH_2N$/pip), 6.65–7.80 (m, 14H, arom. H).

N-Phenyl-[(4-phenylpiperazin-1-yl)carbonothioyl]-2-furamide (***9Ar₃***). $C_{22}H_{21}N_3O_2S$, MM 391.49, Yield 67%, m.p. 141–142 °C (CH_2Cl_2/MeOH). IR (KBr): 1675 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.05–3.50 (m, 4H, $2CH_2$/pip), 4.00–4.45 (m, 4H, $2CH_2N$/pip), 6.40–6.60 (m, 1H, H-4 fur.), 6.70–7.75 (m, 12H, arom. H and H-3, H-5 fur.).

N-Phenyl-[(4-phenylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***9Ar₄***). $C_{22}H_{21}N_3OS_2$, MM 407.56, Yield 92%, m.p. 165–167 °C (CH_2Cl_2/MeOH). IR (KBr): 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.15–3.55 (m, 4H, $2CH_2$/pip), 4.10–4.50 (m, 4H, $2CH_2N$/pip), 6.80–7.75 (m, 13H, arom. H and H-3, H-4, H-5 thioph.).

4.1.4. General procedure for preparation of ATU derivatives 10–12

In an anhydrous pyridine solution (20 ml) 1-(2-pyridyl)piperazine (1.63 g, 10 mmol) and proper isothiocyanate (methyl-, cyclohexyl-, phenyl-isothiocyanate, 10 mmol) were allowed to react for 10 min at r.t. under stirring. After adding a 60% sodium hydride dispersion in mineral oil (0.4 g, $\sim$10 mmol) portion wise at r.t., the reaction mixture was kept under stirring, until hydrogen evolution subsided. Then, neat acyl chloride (10 mmol) was added in a single portion at r.t. The reaction mixture was heated at 55–60 °C for 4 h. For work-up procedure see Section 4.1.3.*N*-Methyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]benzamide (**10Ar₁**)

$C_{18}H_{20}N_4OS$, MM 340.45, Yield 63%, m.p. 101–103 °C (CH_2Cl_2/MeOH). IR (KBr): 1655 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.80–4.10 (m, 8H, $4CH_2$/pip), 3.52 (s, 3H, CH_3N), 6.40–6.85 and 7.25–8.00 and 8.10–8.40 (m, 9H, arom. H and H-3, H-4, H-5 pyr.).

4-Chloro-N-methyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]benzamide (***10Ar₂***). $C_{18}H_{19}ClN_4OS$, MM 374.89, Yield 74%, m.p. 153–154 °C (CH_2Cl_2/MeOH). IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.35–4.00 (m, 8H, $4CH_2$/pip), 3.50 (s, 3H, CH_3N), 6.48–6.90 and 7.25–7.95 and 8.10–8.40 (m, 8H, arom. H and H-3, H-4, H-5 pyr.).

N-Methyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]-2-furamide (***10Ar₃***). $C_{16}H_{18}N_4O_2S$, MM 330.41, Yield 67%, m.p. 104–105 °C (CH_2Cl_2/MeOH). IR (KBr): 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.38 (s, 3H, CH_3N), 3.50–3.85 (m, 4H, $2CH_2$/pip), 3.90–4.30 (m, 4H, $2CH_2N$/pip), 6.40–6.95 (m, 3H, H-4 fur. and 2H pyr.), 7.25 (d, $J = 4$ Hz, 1H, H-3 fur.), 7.45–7.85 (m, 2H, H-5 fur; and 1H pyr.), 8.15–8.45 (m, 1H, H-6 pyr.).

N-Methyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***10Ar₄***). $C_{16}H_{18}N_4OS_2$, MM 346.48, Yield 82%, m.p. 149–150 °C (CH_2Cl_2/MeOH). IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.38–3.78 (m, 4H, $2CH_2$/pip), 3.48 (s, 3H, CH_3N), 3.80–4.25 (m, 4H, $2CH_2N$/pip), 6.50–6.90 and 7.00–7.25 and 7.35–7.90 and 8.15–8.45 (m, 7H, pyr. and thioph. arom. H.).

N-Cyclohexyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]benzamide (***11Ar₁***). $C_{23}H_{28}N_4OS$, MM 408.57, Yield 59%, m.p. 136–137 °C (CH_2Cl_2/MeOH). IR (KBr): 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.80 (m, 10H, $5CH_2$), 3.15–4.05 (m, 8H, $4CH_2$/pip), 4.20–4.90 (m, 1H, CHN), 6.35–6.95 and 7.10–8.05 (m, 8H, arom. H and H-3, H-4, H-5 pyr.), 8.10–8.35 (m, 1H, H-6 pyr.).

4-Chloro-N-cyclohexyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]benzamide (***11Ar₂***). $C_{23}H_{27}ClN_4OS$, MM 443.01, Yield 59%, m.p. 117–119 °C (CH_2Cl_2/MeOH). IR (KBr): 1660 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.80 (m, 10H, $5CH_2$), 3.10–4.90 (m, 8H, $4CH_2$/pip and CHN), 6.35–7.00 and 7.20–8.40 (m, 8H, arom. H and H-3, H-4, H-5, H-6 pyr.).

N-Cyclohexyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]-2-furamide (***11Ar₃***). $C_{21}H_{26}N_4O_2S$, MM 398.53, Yield 65%, m.p. 139–140 °C (CH_2Cl_2/MeOH). IR (KBr): 1640 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.60 (m, 10H, $5CH_2$), 3.35–3.80 (m, 4H, $2CH_2$/pip), 3.85–4.80 (m, 5H, $2CH_2N$/pip and CHN), 6.35–6.95 and 7.10–7.80 (m, 6H, H-3, H-4, H-5 fur. and H-3, H-4, H-5 pyr.), 8.05–8.35 (m, 1H, H-6 pyr.).

N-Cyclohexyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (***11Ar₄***). $C_{21}H_{26}N_4OS_2$, MM 414.50, Yield 60%, m.p. 118–119 °C (CH_2Cl_2/MeOH). IR (KBr): 1620 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.00–2.65 (m, 10H, $5CH_2$, 3.20–3.68 (m, 4H, $2CH_2$/pip), 3.72–4.80 (m, 5H, $2CH_2N$/pip and CHN), 6.45–7.20 and 7.30–8.00 (m, 6H, arom H.

and H-3, H-4, H-5 thioph. and H-3, H-4, H-5 pyr.), 8.10–8.40 (m, 1H, H-6 pyr.).

*4-Chloro-N-phenyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]benzamide (**12Ar$_2$**)*. $C_{23}H_{21}ClN_4OS$, MM 436.96, Yield 57%, m.p. 125–126 °C (CH_2Cl_2/MeOH). IR (KBr): 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.50–3.85 (m, 4H, $2CH_2$/pip), 3.90–4.50 (m, 4H, $2CH_2N$/pip), 6.50–6.95 and 7.05–7.80 (m, 12H, arom. H and H-3, H-4, H-5 pyr.), 8.10–8.40 (m, 1H, H-6 pyr.).

*N-Phenyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]-2-furamide (**12Ar$_3$**)*. $C_{21}H_{20}N_4O_2S$, MM 392.48, Yield 76%, m.p. 127–128 °C (CH_2Cl_2/MeOH). IR (KBr): 1670 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.50–3.90 (m, 4H, $2CH_2$/pip), 3.95–4.40 (m, 4H, $2CH_2N$/pip), 6.40–7.00 and 7.30–7.80 (m, 11H, arom H. and H-3, H-4, H-5 fur. and H-3, H-4, H-5 pyr.), 8.10–8.40 (m, 1H, H-6 pyr.).

*N-Phenyl-[(4-pyridin-2-ylpiperazin-1-yl)carbonothioyl]thiophene-2-carboxamide (**12Ar$_4$**)*. $C_{21}H_{20}N_4OS_2$, MM 408.55, Yield 94%, m.p. 159–160 °C (CH_2Cl_2/MeOH). IR (KBr): 1650 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.50–3.90 (m, 4H, $2CH_2$/pip), 4.00–4.40 (m, 4H, $2CH_2N$/pip), 6.50–7.10 and 7.20–7.80 and 8.10–8.40 (m, 11H, arom H. and H-3, H-4, H-5 thoph. and H-3, H-4, H-5 pyr.), 8.10–8.40 (m, 1H, H-6 pyr.).

4.2. Pharmacology

All the compounds were assayed for local anaesthetic [11], anti-iperlipidemic [12], antiarrythmic [13], analgesic [14], anti-inflammatory [15], platelet antiaggregating [16] and antiproliferative [17–19] activities, evaluated according to previously described standard procedures.

5. Discussion and conclusion

The SARs will be discussed taking into account the principal structural parameters, relevant to each of the tested activities.

Widespread local anaesthetic activity, reported in Table 1, seems to be due to the features of the whole molecule rather than of one variable region. Nevertheless, it emerges that the benzoyl group does not favour activity. When compared to derivatives **B** (Chart 1), the tested compounds are in general more active, indicating that their substitution patterns elicit better anaesthetic effects.

In evaluating their antihyperlipidemic properties (Table 2), several compounds (**4Ar$_4$**, **6Ar$_2$**, **6Ar$_4$**, **7Ar$_3$**, **8Ar$_3$**, **9Ar$_3$**, **11Ar$_1$** and **11Ar$_2$**) belonging to the three series were more active than nicotinic acid in lowering trygliceride levels, but they showed lower efficacy in decreasing serum hypercholesterolemia. Notably, **6Ar$_4$** and **7Ar$_3$**, the most active compounds, reduced the almost tripled triglyceride levels to near normality, at a time showing an outstanding reduction of hypercholesterolemia. Interestingly, also some aryloxyalkylthioimidazoles, inhibitors of acyl-CoA: cholesterol-*O*-acyltransferase, gave similar effects [20]. The activity trend in the 4-methylpiperazine derivatives is in the order: **6Ar$_2$** > **4Ar$_4$** > **5Ar$_4$** ≈ **6Ar$_3$** > **5Ar$_3$**, indicating that the best results are obtained, when R_{1-3} and Ar_{1-4} are aliphatic and prevalently heteroaromatic, respectively. Also in the 4-(hetero)arylpiperazines, when R_{1-3}, is an aliphatic function (compounds **11Ar$_1$**, **8Ar$_3$**, **11Ar$_2$**, **9Ar$_3$**), an enhanced activity was observed, whereas a major tolerability to the nature of the acyl substituent is allowed. In some cases, it is worthwhile noting how activity of the close analogues could be deeply affected by alteration of only one of the variable groups, in particular R_{1-3} or Ar_{1-4}, (**6Ar$_4$** ≫ **4Ar$_4$**; **7Ar$_3$** > **8Ar$_3$** > **9Ar$_3$**; **6Ar$_4$** ≫ **6Ar$_2$**). Interestingly, some compounds showed a certain degree of selectivity. Thus, **8Ar$_3$** caused a 40% reduction of triglycerides, scarcely influencing the total, free and esterified cholesterol levels. On the contrary, **9Ar$_2$** and **12Ar$_4$** significantly decreased hypercholesterolemia, causing about 12% (**9Ar$_2$**) or no (**12Ar$_4$**) reduction of hypertriglyceridemia. Interestingly, R and R_{1-3} of these ATUs are (hetero)aromatic (phenyl for **8Ar$_3$** and **9Ar$_2$**; 2-pyridyl for **12Ar$_4$**) and cyclic (ciclohexyl for **8Ar$_3$**, phenyl for **9Ar$_2$** and **12Ar$_4$**), respectively. Therefore, selectivity might depend on fine-tuning these substituents. Because in some forms of dysliproteinemia, only one of the above serum lipid parameters is altered, it is important to find new agents, which reduce either hypercholesterolemia or hypertriglyceridemia. Moreover, the pharmacological protocol available for the treatment of hypertriglyceridemia is quite small [21].

As concerns analgesic activity (Table 3), the best results were obtained (**5Ar$_2$**, **5Ar$_4$**, **9Ar$_2$** and **5Ar$_3$**, **10Ar$_3$** **12Ar$_2$**), when R_{1-3} is cyclic (C_6H_{11} > C_6H_5), and Ar_{1-4} is heteroaryl or 4-chlorophenyl. Taken together, the data indicate that electronic factors are not critical for activity.

Only few compounds, **5Ar$_3$**, **8Ar$_1$** and **11Ar$_2$**, were endowed with significant anti-inflammatory activity (Table 4), that seems to depend on the presence of the cyclohexyl substituent. When compared to piperidinoacylureas **B**, the title compounds turn out to be less active as anti-inflammatory agents. This indicates that isosteric replacements of the urea carbonyl group and the piperidine nucleus of **B** with the thione group and the 4-substituted piperazine ring, respectively, are not beneficial to anti-inflammatory activity.

The SARs concerning antirrhythmic activity (Table 5) display that the most active compounds **4Ar$_2$**, **10Ar$_1$** are featured by the same group $R_{1-3}(CH_3)$, and by the benzoyl and 4-chlorobenzoyl moieties as substituents of the thiourea nitrogen, respectively. In these two series, replacement of the methyl group with the cyclohexyl

ring gives either derivatives still capable of protracting the appearance time of extrasystoles (**11Ar$_2$**) or poorly active (**5Ar$_2$**).

In the inhibition test of aggregation (Table 6), a number of the most active compounds belongs to the 4-methylpiperazine series (**4Ar$_3$**, **5Ar$_2$**, **6Ar$_4$**). More in general, the best combination of the variable substituents is R_{1-3}: CH_3, C_6H_{11} and Ar_{1-4}: 4-chlorophenyl or heteroaryl, respectively.

Finally, it is apparent that the 4-chlorophenyl and, in a lesser degree, 2-thienyl substituents are molecular determinants for antiproliferative activity (**4Ar$_2$**, **8Ar$_2$**, **5Ar$_2$**, **7Ar$_2$**, **11Ar$_2$**, **11Ar$_2$**) (Table 7), the other variable groups playing a secondary role. The cytotoxic effects of these ATUs were approximately similar to that of some isosteres **B**. This can be reasonably related to the presence of the acyl(thio)urea moiety in both the series. The NCI anticancer drug discovery screen has been designed to distinguish between broad spectrum antitumor compounds and subpanel-selective antiproliferative agents. In the present study, **4Ar$_2$** and, to a lesser extent, **8Ar$_2$** showed a distinctive pattern of selectivity (ACHN and NCI-H226 cell lines, respectively), whereas **5Ar$_2$**, **7Ar$_2$**, **11Ar$_2$**, **11Ar$_2$** and **12Ar$_2$** exhibited a broad spectrum antitumor activity, even if at higher concentrations. Provided that only mechanistic investigations can explain this differential response, selective cytotoxicity of **4Ar$_2$** and **8Ar$_2$** might be mediated, at least in part, by their preferential transport and accumulation in the above sensitive cell lines. Effectively, these phenomena can influence cytotoxicity, as proved for some selective cytotoxic agents [22].

In conclusion, we have synthesised and evaluated three series of ATUs sharing the 4-substituted piperazine scaffold. The results emerged from in vivo and in vitro tests show that several compounds are endowed with various interesting pharmacological properties. In particular, local anaesthetic, antihyperlipemic and antiproliferarive activities turn out to be prominent. Therefore, further SAR studies of ATU derivatives are in progress.

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