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N-Acyl-*N*-phenyl ureas of piperidine and substituted piperidines endowed with anti-inflammatory and anti-proliferative activities

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Abstract

Six series of *N*-acyl-*N*-phenyl ureas **1**–**6** of piperidine (**1**), and 2-ethyl- (**2**), 3-methyl- (**3**), 4-methyl- (**4**), 4-phenyl- (**5**), *cis*-2,6-dimethyl- (**6**) piperidine were synthesised and evaluated for their anti-inflammatory, anaesthetic, anti-pyretic properties. Some derivatives of series **1** and **5** were also assayed for anti-proliferative activity. Several compounds showed an anti-inflammatory activity comparable or slighty inferior to that of indomethacin in rats (**1c**,**d**, **2a**,**b**,**g**,**h**, **3b**, **4h**, **5d**,**e**). Moreover, an appreciable anti-inflammatory activity was also found in **2c**,**e**, **3e**,**f**,**g**, **4g**, **5a**,**b**,**c**,**f**,**h**, and **6a**,**b**,**d**. All the compounds were devoid of anti-pyretic activity and only a few of them exhibited a low level of infiltration anaesthesia in mice. Compound **5a** showed a broad spectrum anti-cancer activity (at low micromolar concentrations), particulary significant against leukemia subpanel.

Keywords: 3,3-Disubstituted-1-acyl-1-phenyl ureas; *N*-Acyl-*N*-phenyl-piperidine-1-carboxamides; Anti-inflammatory activity; Anti-proliferative activity

1. Introduction

In previous papers we reported a facile and efficient one-pot acylation strategy of 3-monosubstituted- and 3,3-disubstituted-1-phenylthioureas, prepared starting from proper amines and phenylisothiocyanate. These thioureas reacted in situ regiospecifically at nitrogen(s) of the thiourea triad with (hetero)aromatic acyl chloride to give the corresponding 1-acyl-, 3-acyl-, and 1,3-diacyl-derivatives, respectively [1,2].

Our current interest in acylthio- and acyl-urea derivatives comes from their scarse consideration from a biological point of view, although simple acylthioureas and acylureas have been described for almost a century [3–6]. Nevertheless, a literature survey indicates that open-chain acylureas possess a number of activities, i.e. they can act as hypnotic [7], anti-epilectic [8], anti-mycotic [9] and platelet anti-aggregating agents [10]. Among cyclic ureas, sedative–hypnotic and anti-convulsant properties of barbiturates are well established and therapeutically useful [11]. On this basis, in an attempt to discover novel lead compounds endowed with anti-inflammatory activity, we designed and synthesized a series of *N*-acyl-*N*-phenyl ureas of piperidine and substituted piperidines **1**–**6**, because no other study has hitherto been reported on anti-inflammatory activity of acylurea derivatives. The choice of the piperidine ring was made on account of an interesting anti-inflammatory activity found by us in some *N*-acyl-*N*-phenyl-3-piperidinobornene-2-thiocarboxamides [12] (which can be considered as vinylogous thioderivatives of the title compounds) and in 1-methyl-4-(*N*-aroyl)-piperidinamides [13]. On the other hand, some selective 1-acyl-4-phenylpiperidine-based non-peptide NK_1 antagonists [14] have been developed to be used, together with the

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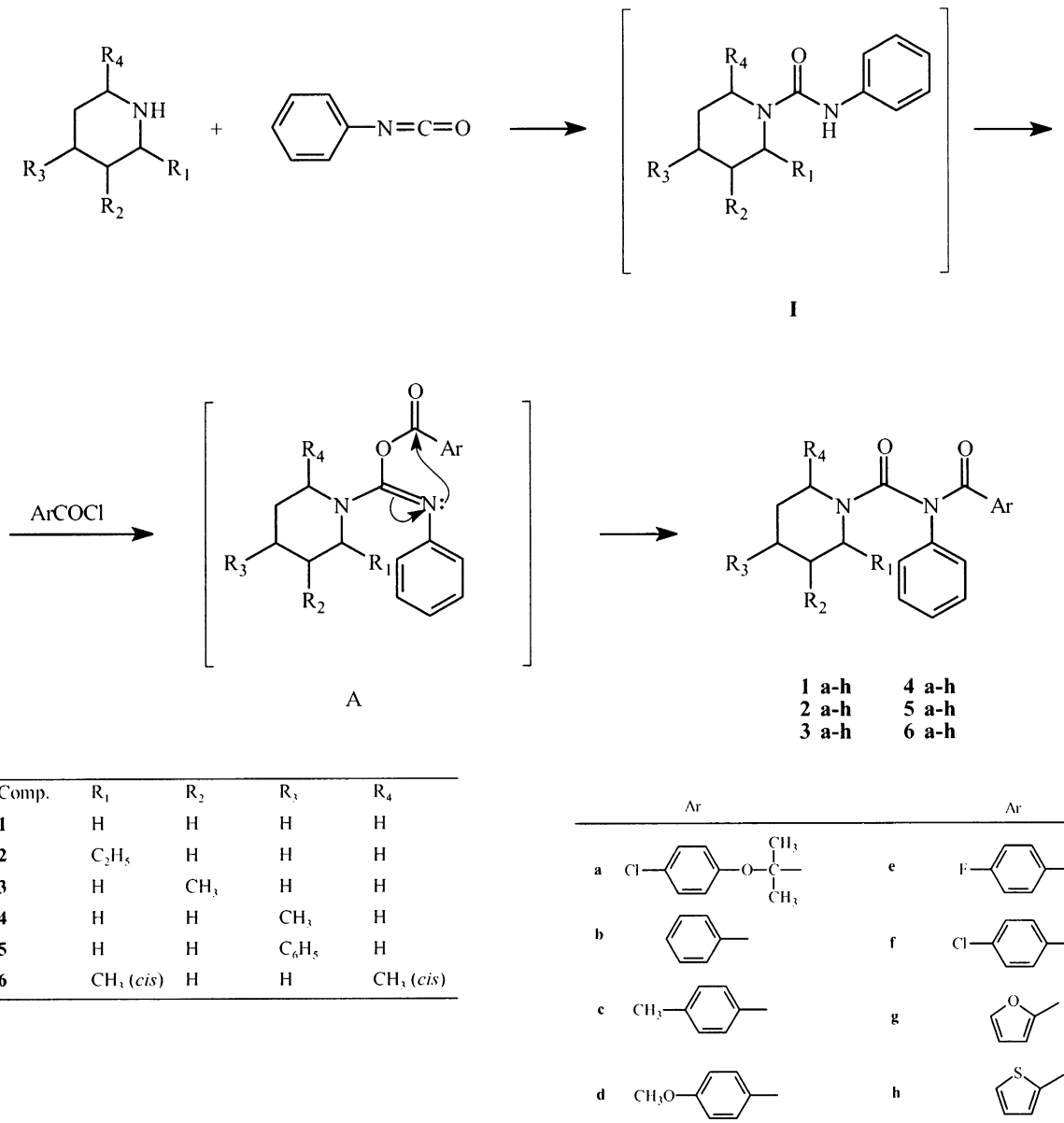
availability of the cloned human NK_1 receptor, to define a putative pharmacophore for these latter compounds. NK_1 receptor and substance P have implicated, inter alia, in neurogenic inflammation, pain transmission and dural inflammation in migraine. Finally, the –NCON–CO– sub-structure characterizing the title compounds, is also present in some arylbiurets, which were found to be more potent than phenylbutazone and aminopyrine as regard to anti-inflammatory and analgesic activities, respectively [15].

2. Chemistry

Six structural types of piperidines were chosen for synthesis, having either no substituent or one (2-ethyl-, 3-methyl-, 4-methyl-, 4-phenyl-) or two (*cis*-2,6-dimethyl-) hydrophobic substituents with different size and in the different ring positions. The synthesis of the title compounds was carried out according to a previously described method for 3,3-disubstituted 1-acyl-1-phenylthioureas [1] and is depicted in Fig. 1. Briefly, the reaction of the above piperidines afforded *N*-phenylurea intermediate **I** with phenylisocyanate in anhydrous pyridine medium. **I** was subsequently reacted in situ with the proper acyl chloride at room temperature to give in good to excellent yields *N*-acylureas **1**–**6**. The synthesis deserves some comments. In the acylation of thiourea with acetyl/benzoyl chloride, Dixon and Hawthorne isolated the intermediates resulting from acetyl/benzoyl attack on the sulphur atom, which either for treatment with sodium bicarbonate or by heating,



Comp.	R_1	R_2	R_3	R_4
1	H	H	H	H
2	C_2H_5	H	H	H
3	H	CH_3	H	H
4	H	H	CH_3	H
5	H	H	C_6H_5	H
6	CH_3 (*cis*)	H	H	CH_3 (*cis*)

Fig. 1. Synthetic pathway (and suggested mechanism) for preparation of the title compounds.

through hydrogen chloride loss, rearranged to *N*-acetyl/benzoyl thiourea [6]. Also in the case of urea and alkylureas, it can be possible that acylation occurs first on oxygen [16], then followed by a sort of Chapman rearrangement. This would involve a four-centre rearrangement with 1,3 oxygen-to-nitrogen acyl transfer, probably due to the *syn* configuration of the isourea transient intermediate **A** with the nitrogen lone pair in the proper steric alignment for attack upon the acyl group (see Fig. 1). We are unable to detect any intermediate **A** resulting from attack on oxygen, probably as a consequence of the reaction conditions (basic medium), which could greatly favour the above rearrangement, as in the case of thiourea where the basic treatment caused S→N acyl transposition. Even if ^{15}N NMR spectroscopy is the best technique for a unambigous differentiation between the individual acylation positions in ureas and thioureas, in this simpler case N-acylation has been proved by ^{13}C NMR spectrum of **1b**, selected as a representative compound of the series. In this spectrum the two diagnostic values at δ 170.63 and 155.95 were assigned, on the basis of literature data [17], to CO carbon signals of the benzoyl and urea moieties, respectively. It is indicative to note the downfield effect on the urea carbonyl signal in comparison with that of the thione carbon of the corresponding thiourea analogue (δ 155.95 vs. 184.50) [1]. Conversely, as expected, the two signals of the carbonyl carbons relative to the benzoyl substituents had similar δ values (169.46 and 170.63 [1]) and were not affected by the sulphur/oxygen change.

3. Pharmacology

All derivatives were evaluated for anti-inflammatory activity (Table 1) and randomly for anaesthetic (Table 2) and anti-pyretic activities (data not shown). Derivatives **1c**,**d**, **2a**,**b**,**g**,**h**, **3b**,**c**, **4h**, **5d**,**e**, and **2c**,**e**,**f**, **3e**–**g**, **4g**, **5a**–**c**,**f**,**h**, were endowed with anti-inflammatory activity comparable or slightly inferior to that of indomethacin. Moreover, **2c**,**e**,**f**, **3e**–**g**, **4g**, **5a**–**c**,**f**,**h** **6a**,**b**,**d** showed a moderate activity, whereas **1a**,**b**,**e**–**h**, **2d**, **3a**,**d**,**h**, **5g**, **6e** were scarcely active. In addition, derivatives **3c**, **6c**, and **2d** showed transient anti-inflammatory effects. In order to assess their relative potency, ED_{50} values (mg/kg/os) of compounds **2a**, **4h**, and **5d** were reported at 3 and 4 h after the treatment (Table 1). As regards infiltration anaesthesia, only the active compounds have been reported (Table 2). Among them, **5h** showed 67 and 50% effect of lidocaine after 5 and 30 min, respectively, from administration, whereas compounds **1b**,**d**,**h**, **2c**, **3c**–**g** exhibited only 33 and/or 25%. All tested compounds were devoid of anti-pyretic activity (data not shown). Furthermore, eight compounds belonging to the series **1** and **5** (**1a**,**f**–**h**; **5a**,**f**–**h**) were selected by the National Cancer Institute (NCI) for in vitro human tumor cell screen in order to evaluate their antiproliferative activity. Only the activity of the most active agents, **5a**,**f**,**h** and **1a**, have been reported in Table 3. Compound **5a** exhibited not only a significant antiproliferative activity ($GI_{50} \leq 3$–4 μM) against leukemia cell line subpanel and breast cancer cell line T-47D, but also cytotoxic effects at relatively low micromolar concentrations (GI_{50} values in the range 3–10 μM) against colon cancer cell line sub-panel and some cell lines derived from non-small cell lung cancer (EKVX), CNS cancer (SNB-75, U251), melanoma (LOX IMVI, SK-MEL-5, UACC-257), ovarian cancer (OVCAR-8), renal cancer (TK-10, UO-31), prostate cancer (PC-3), breast cancer (MCF7, MCF7/ADR-RES). By virtue of its broad activity, **5a** was selected by the NCI to undergo the in vivo hollow fiber assay. Also **5f**–**h** and **1a** showed an approximatively comparable anti-cancer activity, but only against a limited number of cell lines. In particular, for **1a**: HL-60(TB) (GI_{50}: 2.16 μM), K-562, MOLT-4, RPMI-8226 (leukemia), HTC-116, HCT-l5, HT29, KM12 (colon c.), SF-539 (CNS c.), IGROV1 (ovarian c.), PC-3 (prostate c., GI_{50}: 3.40 μM), T-47D (breast c., GI_{50}: 0.89 μM); for **5f**: HT29 (GI_{50}: 3.13 μM), SW-620 (colon c.), SF-268, SNB-75 (CNS c., GI_{50}: 1.83 μM), OVCAR-5 (ovarian c., GI_{50}: 0.55 μM), PC-3; for **5g**: EKVX (non-small cell lung c., GI_{50}: 2.02 μM), SNB-75 (CNS c., GI_{50}: 1.95 μM); for **5h**: EKVX, PC-3 (GI_{50}: 0.87 μM).

4. Experimental

4.1. Chemistry

Chemicals were purchased from Aldrich Chimica. Thin layer chromatography for routine monitoring of the course of reactions and confirming the homogeneity of analytical samples was performed on 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 disc. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 200 instrument. Chemical shifts are reported in δ units (ppm) relative to the internal reference tetramethylsilane. Elemental analyses were within $\pm 0.4\%$ of the theoretical values.

4.1.1. General procedure for the one-pot synthesis of compounds *1*, *2*, *3*, *4*, *5*, and *6 (a–h)*

A stirred anhydrous pyridine (25 ml) solution of piperidine or substituted piperidines (15 mmol), was

Table 1
Anti-inflammatory activity of compounds **1**, **2**, **3**, **4**, **5**, **6 (a–h)**

Comp.	Dose (mg/kg p.o.)	% Oedema inhibition relative to control at the				ED_{50} (mg/kg) (fiducial limits)	
		1st hour [a]	2nd hour [a]	3rd hour [a]	4th hour [a]	3rd hour [a]	4th hour [a]
Control	carr. 1% sol	+31	+44	+50	+62		
Indomethacin	5	−77	−84	−86	−89		
1a	50	−60	−42	−50	−50		
1b	50	−60	−57	−38	−60		
1c	50	−80	−86	−75	−80		
1d	50	−80	−86	−88	−90		
1e	50	−40	−57	−50	−50		
1f	50	−60	−57	−50	−60		
1g	50	−40	−43	−63	−60		
1h	50	−40	−43	−50	−60		
2a	25	−60	−70	−60	−68	16.03	10.54
	50	−68	−77	−80	−84	(11.96–21.49)	(7.56–14.68)
	100	−80	−86	−88	−90		
2b	50	−68	−77	−70	−84		
2c	50	−61	−73	−64	−71		
2d	50	−65	−61	−66	−38		
2e	50	−40	−70	−63	−60		
2f	50	−60	−70	−50	−60		
2g	50	−80	−70	−75	−70		
2h	50	−80	−70	−63	−80		
3a	50	−40	−43	−63	−80		
3b	50	−80	−71	−63	−70		
3c	50	−80	−71	−63	−50		
3d	50	−60	−57	−63	−60		
3e	50	−80	−71	−63	−60		
3f	50	−60	−71	−63	−60		
3g	50	−60	−71	−75	−70		
3h	50	−60	−57	−38	−50		
4a	50	−3	−9	0	−5		
4b	50	−10	−11	0	−3		
4c	50	−6	−20	−6	−6		
4d	50	−6	−34	−30	−16		
4e	50	−6	−14	−24	−32		
4f	50	−14	−24	−30	−50		
4g	50	−65	−75	−68	−80		
4h	25	−64	−75	−66	−73	6.00	6.91
	50	−65	−75	−68	−83	(3.25–11.07)	(4.20–11.38)
	100	−84	−75	−78	−83		
5a	50	−68	−77	−68	−75		
5b	50	−58	−70	−74	−60		
5c	50	−60	−68	−63	−60		
5d	25	−45	−71	−75	−80	10.44	6.11
	50	−65	−75	−78	−83	(6.79–15.64)	(3.93–9.48)
	100	−80	−84	−86	−90		
5e	50	−68	−68	−80	−84		
5f	50	−60	−70	−74	−70		
5g	50	−42	−57	−52	−35		
5h	50	−60	−57	−75	−70		
6a	50	−58	−70	−62	−70		
6b	50	−61	−73	−64	−63		
6c	50	−58	−70	−34	−25		
6d	50	−58	−70	−74	−70		
6e	50	−60	−43	−50	−50		
6f	50	−42	−7	−6	−6		
6g	50	−45	−36	0	−3		
6h	50	−65	−61	−20	−11		

[a] Times from administration.

treated with phenylisocyanate (1.79 g, 15 mmol) at room temperature. After 10 min, the proper acyl chloride (18 mmol) was added and the reaction mixture was allowed to stir at room temperature for 6 h. After water (200 ml) addition, a solid or oily phase was separated. The precipitate was allowed to stand for 1 h, filtered and dissolved in dichloromethane. The resulting solution was washed with brine (30 ml), dried over anhydrous sodium sulphate and evaporated in vacuo: the residue was crystallized from suitable solvents. The separated oily phase was extracted directly with diethyl ether or dichloromethane. The extract was washed with 1 M HCl (3 × 25 ml) and dried as above. Evaporating in vacuo afforded a residue that was crystallised from suitable solvents. Melting points, yields, ^{1}H NMR (^{13}C NMR of **1b**) data and crystallisation solvents of the compounds are reported below. In the IR spectra (KBr), the acyl and urea carbonyl groups showed both a single band (range: 1675–1665 cm^{-1}; **1c**, **2a**–**c**,**f**, **3a**,**b**,**d**,**g**, **4a**,**b**,**d**–**f**, **5a**,**b**,**d**, **6b**,**c**,**e**–**g**) and two well-split bands, which could be clearly identified (**1a**–**d**,**f**–**h**, **2d**,**e**,**g**,**h**, **3c**,**e**,**f**,**h**, **4c**,**g**,**h**, **5c**,**e**–**h**, **6a**,**d**,**h**). The band at higher frequencies (range: 1690–1670 cm^{-1}) was assigned to the acyl carbonyl, whereas the one at lower frequencies was referred to the urea carbonyl group (range: 1670–1630 cm^{-1}).

1a: *N*-[2-(4-Chlorophenoxy)-2-methyl]propionyl-*N*-phenylpiperidine-1-carboxamide. Formula $C_{22}H_{25}N_2O_3C1$, MM 400.91, yield 82%, m.p. 150–151 °C (dichloromethane–diethyl ether 1:7). ^{1}H NMR ($CDCl_3$): δ 0.85–1.50 (m, 6H, 3CH_2/pip), 1.65 (s, 6H, 2CH_3), 3.00–3.65 (m, 4H, 2CH_2N), 6.95–7.55 (m, 9H, arom. H).

Table 2
Infiltration anaesthesia by pinch-tail test

Comp.	Activity [a]	
	5 min	30 min
Lidocaine	60	80
1b	0	20
1d	20	0
1h	0	20
2c	20	20
3c	20	0
3d	20	20
3e	20	0
3f	0	20
3g	0	20
4a	20	0
4d	20	20
4f	20	0
4h	0	20
5f	20	0
5g	20	0
5h	40	40
6e	0	20
6f	20	0

Dose: 0.2 ml of 0.1% glycofurol solution except for lidocaine.
[a] Percent of animals showing anaesthesia, 5 and 30 min after infiltration of test compounds into the tail root.

1b: *N*-Benzoyl-*N*-phenyl piperidine-l-carboxamide. Formula $C_{19}H_{20}N_2O_2$, MM 308.38, yield 81%, m.p. 144–145 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 1.25–1.75 (m, 6H, 3CH_2/pip), 3.30–3.72 (m, 4H, 2CH_2N/pip), 7.07–7.92 (m, 10H, arom. H). ^{13}C NMR ($CDCl_3$): δ 24.31, 25.72, 126.78, 127.53, 128.74, 128.84, 129.83, 131.97, 135.47, 139.98, 155.95 (CO, ureido), 170.63 (CO, benzoyl).

1c: *N*-(4-Methyl)benzoyl-*N*-phenyl piperidine-1-carboxamide. Formula $C_{20}H_{22}N_2O_2$, MM 322.41, yield 70%, m.p. 109–111 °C (diethyl ether). ^{1}H NMR ($CDC1_3$): δ 1.20–1.75 (m, 6H, 3CH_2/pip), 3.25–3.75 (m, 4H, 2CH_2N/pip), 6.98–7.80 (m, 9H, arom. H).

1d: *N*-(4-Methoxy)benzoyl-*N*-phenyl piperidine-1-carboxamide. Formula $C_{20}H_{22}N_2O_3$, MM 338.41, yield 63%, m.p. 89–91 °C (dichloromethane–diethyl ether 1:7). ^{1}H NMR ($CDCl_3$): δ 1.21–1.87 (m, 6H, 3CH_2/pip), 3.27–3.70 (m, 4H, 2CH_2N/pip), 6.72–7.87 (m, 9H, arom. H).

1e: *N*-(4-Fluoro)benzoyl-*N*-phenyl piperidine-1-carboxamide. Formula $C_{19}H_{19}N_2O_2F$, MM 326.37, yield 83%, m.p. 121–122 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.07–1.92 (m, 6H, 3CH_2/pip), 3.22–3.27 (m, 4H, 2CH_2N/pip), 6.82–7.92 (m, 9H, arom. H).

1f: *N*-(4-Chloro)benzoyl-*N*-phenyl piperidine-1-carboxamide. Formula $C_{19}H_{19}N_2O_2Cl$, MM 342.83, yield 61%, m.p. 119–120 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.2–1.8 (m, 6H, 3CH_2/pip), 3.28–3.75 (m, 4H, 2CH_2N/pip), 7.10–7.80 (m, 9H, arom. H).

1g: *N*-(2-Furane)carbonyl-*N*-phenyl piperidine-1-carboxamide. Formula $C_{17}H_{18}N_2O_3$, MM 298.34, yield 96%, m.p. 152–153 °C (dichloromethane–diethyl ether 1:7). ^{1}H NMR ($CDCl_3$): δ 1.30–1.8 (m, 6H, 3CH_2/pip), 3.30–3.70 (m, 4H, 2CH_2N/pip), 6.38–6.60 (m, 1H, H-4 fur.), 6.93 (d, 1H, $J = 4$ Hz, H-3 fur), 7.20-7.60 (m, 6H, 5 arom. H and H-5 fur).

1h: *N*-(2-Thiophene)carbonyl-*N*-phenyl piperidine-1-carboxamide. Formula $C_{17}H_{18}N_2O_2S$, MM 314.40, yield 77%, m.p. 149–150 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.45–1.85 (m, 6H, 3CH_2/pip), 3.35–3.85 (m, 4H, 2CH_2N/pip), 6.85–7.15 and 7.30–7.65 (m, 8H, 5 arom. H and 3H thioph.).

2a: *N*-[2-(4-Chlorophenoxy)-2-methyl]propionyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{24}H_{29}N_2O_3C1$, MM 428.96, yield 90%, m.p. 134–135 °C (diethyl ether–petroleum ether 60–80 °C 1:1). ^{1}H NMR ($CDCI_3$): δ 0.30–1.80 (m, 11H, 3CH_2/pip + 5H/ethyl), 1.60 (s, 6H, 2CH_3–C), 2.30–2.85 (m, 1H, CH), 3.05–4.50 (m, 2H, CH_2N), 6.80–7.50 (m, 9H, arom. H).

2b: *N*-Benzoyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{21}H_{24}N_2O_2$, MM 336.43, yield 72%, m.p. 100–101 °C (diethyl ether–petroleum ether 60–

Table 3
Anti-proliferative activities of selected compounds **5a**, **f**–**h**, **1a**

Panel/cell line	GI_{50} (μM)				
	5a	**5f**	**5g**	**5h**	**1a**
Leukemia					
CCRF-CEM	3.0	18.9	26.5	95.6	16.7
HL-60(TB)	0.85	17.1	19.3	28.9	2.16
K-562	3.67	32.1	29.1	22.7	8.17
MOLT-4	2.74	21.81	17.7	34.7	3.41
RPMI-8226	2.54	13.7	17.4	12.6	7.55
Non-small cell lung cancer					
EKVX	6.03	10.3	2.02	7.67	24.6
NCI-H226	37.1	17.8	31.7	33.9	17.6
NCI-H23	8.80	23.9	45.7	40.6	14.2
NCI-H322M	>100	>100	>100	>100	16.0
Colon cancer					
COLO 205	7.89	33.0	39.7	27.2	17.4
HCT-116	4.33	17.5	42.7	42.4	5.32
HCT-15	7.20	15.0	42.0	28.0	6.29
HT29	7.52	3.13	47.7	25.4	8.34
KM12	6.68	11.4	27.2	14.4	5.88
SW-620	6.30	9.20	>100	51.6	13.0
CNS cancer					
SF-268	>100	8.21	99	73.7	20.1
SF-295	17.5	31.0	n.d.	42.7	
SF-539	>100	65.0	74.5	>100	8.34
SNB-19	>100	72.0	>100	>100	16.8
SNB-75	6.78	1.83	1.95		
U251	6.34	39.6	76.3	46.1	14.2
Melanoma					
LOX IMVI	5.92	28.1	59.8	41.5	11.4
MALME-3M	25.4	10.4	>100	15.8	12.0
M14	93.7	18.4	41.4	40.5	17.5
SK-MEL-2	35.7	37.3	42.5	13.8	13.1
SK-MEL-28	>100	45.2	62.7	43.9	15.3
SK-MEL-5	4.34	15.4	21.6	16.8	10.4
UACC-257	5.88	25.4	38.9	40.0	
UACC-62	>100	31.7	52.1	50.6	13.2
Ovarian cancer					
IGROV1	28.4	17.9	25.9	14.7	5.09
OVCAR-3	54.1	44.7	47.0	53.6	23.9
OVCAR-5	n.d.	0.55	>100	>100	12.0
OVCAR-8	8.48	33.8	40.8	39.4	14.1
SK-OV-3	99.5	>100	81.5	88.4	19.7
Renal cancer					
786-0	-	70.2	93.0	>100	13.4
A498	68.4	20.4	94.9	51.4	17.0
ACHN	19.2	57.8	>100	63.1	14.9
CAKI-1	30.7	54.0	>100	>100	14.4
RXF 393	13.6	11.9	34.0	16.8	18.0
SN12C	11.6	99.6	>100	62.3	17.0
TK-10	9.07	30.7	55.3	67.0	
UO-31	5.81	12.1	24.4	18.0	10.8
Prostate cancer					
PC-3	4.43	6.39	22.3	0.87	3.40
DU-145	>100	59.0	82.9	>100	14.7
Breast cancer					
MCF7	7.37	39.4	66.5	35.5	19.6
MCF7/ADR-RES	8.73	16.6	23.2	24.8	14.5
MDA-MB-231/ATCC	>100	26.1	>100	>100	10.6

Table 3 (*Continued*)

Panel/cell line	GI_{50} (μM)				
	5a	**5f**	**5g**	**5h**	**1a**
HS 578T	>100	24.2	60.3	27.5	16.9
MDA-MB-435	>100	25.7	35.0	29.4	16.0
MDA-N	18.0	39.3	42.5	43.7	24.5
BT-549	34.1	19.5	33.7	27.0	15.4
T-47D	2.39	11.1	21.4	26.1	0.89

80 °C 1:1). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, $J = 7$ Hz, 3H, CH_3/ethyl), 1.10–1.98 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.74–3.32 (m, 1H, CH), 3.80–4.60 (m, 2H, CH_2N), 6.90–7.90 (m, 10H, arom. H).

2c: *N*-(4-Methyl)benzoyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{22}H_{26}N_2O_2$, MM 350.46, yield 68%, m.p. 127–128 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, $J = 7$ Hz, 3H, CH_3/ethyl), 1.09–2.05 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.35 (s, 3H, CH_3/tolyl), 2.60–3.33 (m, 1H, CH), 3.73–4.64 (m, 2H, CH_2N/pip), 6.83–7.78 (m, 9H, arom. H).

2d: *N*-(4-Methoxy)benzoyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{22}H_{26}N_2O_3$, MM 366.46, yield 65%, m.p. 140–141 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.86 (t, $J = 7$ Hz, 3H, CH_3), 1.26–1.90 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.76–3.30 (m, 1H, CH), 3.84 (s, 3H, CH_3O), 4.04–4.61 (m, 2H, CH_2N/pip), 6.69–7.60 (m, 9H, arom. H).

2e: *N*-(4-Fluoro)benzoyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{21}H_{23}N_2O_2F$, MM 354.42, yield 68%, m.p. 115–116 °C (diethyl ether–petroleum ether 60–80 °C 1:1). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, $J = 7$ Hz, 3H, CH_3), 1.22–1.92 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.72–3.32 (m, 1H, CH), 3.74–4.60 (m, 2H, CH_2N/pip), 6.72–7.97 (m, 9H, arom. H).

2f: *N*-(4-Chloro)benzoyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{21}H_{23}N_2O_2C1$, MM 370.88, yield 79%, m.p. 120–121 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.83 (t, $J = 7$ Hz, 3H, CH_3), 1.07–2.00 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.70–3.30 (m, 1H, CH), 3.65–4.65 (m, 2H, CH_2N/pip), 6.85–7.80 (m, 9H arom. H).

2g: *N*-(2-Furane)carbony1-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{19}H_{22}N_2O_3$, MM 326.40, yield 61%, m.p. 98–99 °C (diethyl ether–petroleum ether 60–80 °C 1:1). ^{1}H NMR ($CDCl_3$): δ 0.86 (t, $J = 7$ Hz, 3H, CH_3), 1.28–1.88 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.68–3.28 (m, 1H, CH), 3.76–4.59 (m, 2H, CH_2N/pip), 6.38 (m, 1H, H-4 fur.), 6.96 (d, 1H, $J = 4$ Hz, H-3 fur.), 7.22–7.62 (m, 6H, 5 arom. H and H-5 fur.).

2h: *N*-(2-Thiophene)carbonyl-*N*-phenyl-2-ethylpiperidine-1-carboxamide. Formula $C_{19}H_{22}N_2O_2S$, MM 342.46, yield 63%, m.p. 113–115 °C (diethyl ether–petroleum ether 60–80 °C 1:1). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, $J = 7$ Hz, 3H, CH_3), 1.33–2.00 (m, 8H, $3CH_2$/pip and CH_2/ethyl), 2.73–3.38 (m, 1H, CH), 3.79–4.73 (m, 2H, CH_2N/pip), 6.83–7.13 and 7.20–7.69 (m, 8H, 5 arom. H and 3H thioph.).

3a: *N*-[2-(4-Chlorophenoxy)-2-methyl]propionyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{23}H_{27}N_2O_3C1$, MM 414.93, yield 79%, m.p. 115–116 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.5–1.5 (m, 8H, $2CH_2$, CH and CH_3/pip), 1.65 (s, 6H, $2CH_3C$), 2.00–2.60 (m, 2H, CH_2N/pip), 3.45–4.30 (m, 2H, CH_2N/pip), 6.90–7.55 (m, 9H, arom. H).

3b: *N*-Benzoyl-*N*-phenyl-3-methyl piperidine-1-carboxamide. Formula $C_{20}H_{22}N_2O_2$, MM 322.41, yield 72%, m.p. 95–97 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.88 (d, $J = 5$ Hz, 3H, CH_3/pip), 0.90–2.00 (m, 5H, $2CH_2 + CH$/pip), 1.72–3.17 (m, 2H, CH_2N/pip), 3.80–4.37 (m, 2H, CH_2N/pip), 6.92–7.90 (m, 10H, arom. H).

3c: *N*-(4-Methyl)benzoyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{21}H_{24}N_2O_2$, MM 336.43, yield 88%, m.p. 98–100 °C (diethyl ether–petroleum ether 60–80 °C 7:1). ^{1}H NMR ($CDCl_3$): δ 0.88 (d, $J = 5$ Hz, 3H, CH_3/pip) 0.90–2.03 (m, 5H $2CH_2 + CH$/pip), 2.27 (s, 3H, CH_3/tolyl), 2.33–3.13 (m, 2H, CH_2N/pip), 3.83–4.49 (m, 2H, CH_2N/pip), 6.99–7.18 (m, 9H, arom. H).

3d: *N*-(4-Methoxy)benzoyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{21}H_{24}N_2O_3$, MM 352.43, yield 85%, m.p. 83–85 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.86 (d, $J = 5$ Hz, 3H, CH_3/pip) 0.90–1.98 (m, 5H, $2CH_2 + CH$/pip), 2.14–3.20 (m, 2H, CH_2N/pip), 3.80–4.34 (m, 2H, CH_2N/pip), 3.84 (s, 3H, CH_3O), 6.74–7.87 (m, 9H, arom. H).

3e: *N*-(4-Fluoro)benzoyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{20}H_{21}N_2O_2F$, MM 340.40, yield 80%, m.p. 74–76 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.83 (d, $J = 5$ Hz, 3H, CH_3/pip) 0.90–1.98 (m, 5H, $2CH_2 + CH$/pip), 2.13–3.13 (m, 2H, CH_2N/pip), 4.00–4.38 (m, 2H, CH_2N/pip), 6.83–7.99 (m, 9H, arom. H).

3f: *N*-(4-Chloro)benzoyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{20}H_{21}N_2O_2Cl$, MM

356.85, yield 77%, m.p. 107–109 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.88 (d, $J = 5$ Hz, 3H, CH_3/pip) 0.90–1.98 (m, 5H, $2CH_2 + CH$/pip), 2.23–3.08 (m, 2H, CH_2N/pip), 3.68–4.38 (m, 2H, CH_2N/pip), 7.08–7.80 (m, 9H, arom. H).

3g: *N*-(2-Furane)carbonyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{18}H_{20}N_2O_3$, MM 312.37, yield 79%, m.p.132–133 °C (dichloromethane–diethyl ether 1:8). ^{1}H NMR ($CDCl_3$): δ 0.88 (d, $J = 5$ Hz, 3H, CH_3/pip) 0.90–2.00 (m, 5H, $2CH_2 + CH$/pip), 2.17–3.16 (m, 2H, CH_2N/pip), 3.84–4.44 (m, 2H, CH_2N/pip), 6.44–6.62 (m, 1H, H-4 fur.), 6.95 (d, $J = 4$ Hz, 1H, H-3 fur.), 7.13–7.69 (m, 6H, 5 arom. H and H-5 fur.).

3h: *N*-(2-Thiophene)carbonyl-*N*-phenyl-3-methylpiperidine-1-carboxamide. Formula $C_{18}H_{20}N_2O_2S$, MM 328.43, yield 83%, m.p. 113–114 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.88 (d, $J = 5$ Hz, 3H, CH_3/pip) 0.90–2.05 (m, 5H, $2CH_2 + CH$/pip), 2.33–3.20 (m, 2H, CH_2N/pip), 3.87–4.45 (m, 2H, CH_2N/pip), 6.85–7.15 and 7.25–7.68 (m, 8H, 5 arom. H and 3H thioph.).

4a: *N*-[2-(4-Chlorophenoxy)-2-methyl]propionyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{23}H_{27}N_2O_3C1$, MM 414.93, yield 72%, m.p. 120–121 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.77 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.09–1.50 (m, 5H, $2CH_2 + CH$/pip), 1.64 (s, 6H, $2CH_3C$), 2.27–2.98 (m, 2H, CH_2N/pip), 3.10–4.40 (m, 2H, CH_2N/pip), 6.87–7.64 (m, 9H, arom. H).

4b: *N*-Benzoyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{20}H_{22}N_2O_2$, MM 322.41, yield 81%, m.p. 90–92 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.87 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.09–1.87 (m, 5H, $2CH_2 + CH$/pip), 2.52–3.14 (m, 2H, CH_2N/pip), 3.90–4.15 (m, 2H, CH_2N/pip), 6.80–7.92 (m, 10H, arom. H).

4c: *N*-(4-Methyl)benzoyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{21}H_{24}N_2O_2$, MM 336.43, yield 92%, m.p. 122–123 °C (diethyl ether–petroleum ether 60–80 °C 2:1). ^{1}H NMR ($CDCl_3$): δ 0.87 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.1–1.85 (m, 5H, $2CH_2 + CH$/pip), 2.35 (s, 3H, CH_3/tolyl), 2.50–3.10 (m, 2H, CH_2N/pip), 3.90–4.45 (m, 2H, CH_2N/pip), 6.75–7.27 (m, 9H, arom. H).

4d: *N*-(4-Methoxy)benzoyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{21}H_{24}N_2O_3$, MM 352.43, yield 70%, m.p. 102–103 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.89 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.07–1.93 (m, 5H, $2CH_2 + CH$/pip), 2.50–3.15 (m, 2H, CH_2N/pip), 3.80 (s, 3H, CH_3O), 3.90–4.25 (m, 2H, CH_2N/pip), 6.70–7.90 (m, 9H, arom. H).

4e: *N*-(4-Fluoro)benzoyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{20}H_{21}N_2O_2F$, MM 340.40, yield 76%, m.p. 130–131 °C (dichloromethane–diethyl ether 1:9). ^{1}H NMR ($CDCl_3$): δ 0.87 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.05–1.92 (m, 5H, $2CH_2 + CH$/pip), 2.50–3.13 (m, 2H, CH_2N/pip), 3.38–4.48 (m, 2H, CH_2N/pip), 6.67–7.90 (m, 9H, arom. H).

4f: *N*-(4-Chloro)benzoyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{20}H_{21}N_2O_2Cl$, MM 356.85, yield 75%, m.p. 113–115 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.88 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.05–1.88 (m, 5H, $2CH_2 + CH$/pip), 2.50–3.15 (m, 2H, CH_2N/pip), 3.80–4.15 (m, 2H, CH_2N/pip), 7.05–7.80 (m, 9H, arom. H).**4g**: *N*-(2-Furane)carbonyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{18}H_{20}N_2O_3$, MM 312.37, yield 90%, m.p. 118–119 °C (dichloromethane–diethyl ether 1:9). ^{1}H NMR ($CDC1_3$): δ 0.88 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.03–1.90 (m, 5H, $2CH_2 + CH$/pip), 2.27–3.20 (m, 2H, CH_2N/pip), 3.95–4.50 (m, 2H, CH_2N/pip), 6.40–6.60 (m, 1H, H-4 fur.), 6.68–7.19 (d, $J = 4$ Hz, 1H, H-3 fur.) 7.03–7.70 (m, 6H, 5 arom. H and H-5 fur.).

4h: *N*-(2-Thiophene)carbonyl-*N*-phenyl-4-methylpiperidine-1-carboxamide. Formula $C_{18}H_{20}N_2O_2S$, MM 328.43, yield 85%, m.p. 109–111 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.85 (d, $J = 6$ Hz, 3H, CH_3/pip), 1.05–1.95 (m, 5H, $2CH_2 + CH$/pip), 2.60–3.20 (m, 2H, CH_2N/pip), 3.98–4.50 (m, 2H, CH_2N/pip), 6.88–7.03 and 7.21–7.64 (m, 8H, 5 arom. H and 3H tioph.).

5a: *N*-[2-(4-Chlorophenoxy)-2-methyl]propionyl-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{28}H_{29}N_2O_3C1$, MM 477.00, yield 65%, m.p. 159–160 °C (dichloromethane–diethyl ether 1:7). ^{1}H NMR ($CDCl_3$): δ 1.01–1.90 (m, 4H, $2CH_2$/pip), 1.66 (s, 6H, $2CH_3C$), 2.03–2.96 (m, 3H, CH_2N and CH/pip), 3.71–4.70 (m, 2H, CH_2N/pip), 6.50–7.93 (m, 14H, arom. H).

5b: *N*-Benzoyl-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{25}H_{24}N_2O_2$, MM 384.48, yield 76%, m.p. 166–167 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.00–2.07 (m, 4H, $2CH_2$/pip), 2.40–3.27 (m, 3H, CH_2N and CH/pip), 4.13–4.65 (m, 2H, CH_2N/pip), 6.80–7.98 (m, 15H, arom. H).

5c: *N*-(4-Methyl)benzoyl-*N*-pheny1-4-phenylpiperidine-1-carboxamide. Formula $C_{26}H_{26}N_2O_2$, MM 398.50, yield 75%, m.p. 139–140 °C (diethyl ether). ^{1}H NMR ($CDC1_3$): δ 1.00–2.08 (m, 4H, $2CH_2$/pip), 2.45–3.26 (m, 3H, CH_2N and CH/pip), 4.08–4.66 (m, 2H, CH_2N/pip), 6.68–7.88 (m, 14H, arom. H).

5d: *N*-(4-Methoxy)benzoyl-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{26}H_{26}N_2O_3$, MM 414.50, yield 71%, m.p. 114–116 °C (diethyl ether–petroleum ether 60–80 °C 9:1). ^{1}H NMR ($CDCl_3$): δ 1.04–2.03 (m, 4H, $2CH_2$/pip), 2.38–3.34 (m, 3H, CH_2N and CH/pip), 3.85 (s, 3H, CH_3O), 4.13–4.68 (m, 2H, CH_2N/pip), 6.70–7.97 (m, 14H, arom. H).

5e: *N*-(4-Fluoro)benzoyl-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{25}H_{23}N_2O_2F$, MM 402.47, yield 75%, m.p. 163–164 °C (dichloromethane–diethyl ether 1:7). ^{1}H NMR ($CDCl_3$): δ 1.00–2.07 (m, 4H, $2CH_2$/pip), 2.35–3.27 (m, 3H, CH_2N and

CH/pip), 3.96–4.62 (m, 2H, CH_2N/pip), 6.57–7.99 (m, 14H, arom. H).

5f: *N*-(4-Chloro)benzoy1-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{25}H_{23}N_2O_2C1$, MM 418.92 yield 77%, m.p. 107–109 °C (diethyl ether–petroleum ether 60–80 °C 9:1). ^{1}H NMR ($CDCl_3$): δ 1.00–2.10 (m, 4H, $2CH_2$/pip), 2.40–3.29 (m, 3H, CH_2N and CH/pip), 3.56–4.65 (m, 2H, CH_2N/pip), 6.70–7.85 (m, 14H, arom. H).

5g: *N*-(2-Furane)carbonyl-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{23}H_{22}N_2O_3$, MM 374.44, yield 70%, m.p.157–158 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.08–2.07 (m, 4H, $2CH_2$/pip), 2.40–3.31 (m, 3H, CH_2N and CH/pip), 4.10–4.66 (m, 2H, CH_2N/pip), 6.40–6.60 (m, 1H, H-4 fur.), 6.93 (d, $J = 4$ Hz, 1H, H-3 fur.), 7.05-7.70 (m, 11H, 10 arom. H and H-5 fur.).

5h: *N*-(2-Thiophene)carbonyl-*N*-phenyl-4-phenylpiperidine-1-carboxamide. Formula $C_{23}H_{22}N_2O_2S$, MM 390.50, yield 72%, m.p. 171–173 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.13–2.12 (m, 4H, $2CH_2$/pip), 2.43–3.38 (m, 3H, CH_2N and CH/pip), 4.21–4.73 (m, 2H, CH_2N/pip), 6.85–7.70 (m, 13H, 10 arom. H and 3H thioph.).

6a: *N*-[2-(4-Chlorophenoxy)-2-methyl]propionyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{24}H_{29}N_2O_3C1$, MM 428.96, yield 58%, m.p. 165–166 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 0.50–1.5 (m, 12H, $3CH_2 + 2CH_3$/pip), 1.61 (s, 6H, $2CH_3C$), 4.10–4.90 (m, 2H, 2CH/pip), 7.05–7.65 (m, 9H, arom. H).

6b: *N*-Benzoyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{21}H_{24}N_2O_2$, MM 336.43, yield 82%, m.p. 137–139 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, CH_3/pip), 1.20 (s, 3H, CH_3/pip), 1.25–1.90 (m, 6H, $3CH_2$/pip), 4.25–4.80 (m, 2H, 2CH/ pip), 7.10–7.90 (m, 10H, arom. H).

6c: *N*-(4-Methyl)benzoyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{22}H_{26}N_2O_2$, MM 350.46, yield 75%, m.p. 139–141 °C (diethyl ether–petroleum ether 60–80 °C 3:1). ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, CH_3/pip), 1.20 (s, 3H, CH_3/pip), 1.30–1.90 (m, 6H, $3CH_2$/pip), 2.35 (s, 3H, CH_3/tolyl), 4.15–4.80 (m, 2H, 2CH/pip), 6.58–7.75 (m, 9H, arom. H).

6d: *N*-(4-Methoxy)benzoyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{22}H_{26}N_2O_3$, MM 366.46, yield 70%, m.p. 124–126 °C (diethyl ether–petroleum ether 60–80 °C 3:1). ^{1}H NMR ($CDCl_3$): δ 1.11 (s, 3H, CH_3/pip), 1.22 (s, 3H, CH_3/pip), 1.30–1.95 (m, 6H, $3CH_2$/pip), 3.83 (s, 3H, CH_3O), 4.20–4.80 (m, 2H, 2CH/pip), 6.60–8.35 (m, 9H, arom. H).

6e: *N*-(4-Fluoro)benzoyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{21}H_{23}N_2O_2F$, MM 354.42, yield 78%, m.p. 154–155 °C (dichloromethane–diethyl ether 1:7). ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, CH_3/pip), 1.21 (s, 3H, CH_3/pip), 1.33–1.93 (m, 6H, $3CH_2$/pip), 4.20–4.83 (m, 2H, 2CH/pip), 6.63–8.03 (m, 9H, arom. H).

6f: *N*-(4-Chloro)benzoyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{21}H_{23}N_2O_2C1$, MM 370.88, yield 74%, m.p. 131–132 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.10 (s, 3H, CH_3/pip), 1.21 (s, 3H, CH_3/pip), 1.20–2.0 (m, 6H, $3CH_2$/pip), 4.08–4.80 (m, 2H, 2CH/pip), 6.60–7.82 (m, 9H, arom. H).

6g: *N*-(2-Furane)carbonyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{19}H_{22}N_2O_3$, MM 326.40, yield 70%, m.p.133–135 °C (dichloromethane–petroleum ether 1:7). ^{1}H NMR ($CDCl_3$): δ 1.14 (s, 3H, CH_3/pip), 1.26 (s, 3H, CH_3/pip), 1.34–1.98 (m, 6H, $3CH_2$/pip), 4.24–4.84 (m, 2H, 2CH/pip), 6.40–6.66 (m, 1H, H-4 fur.), 7.08 (d, $J = 4$ Hz, 1H, H-3 fur.), 7.24–7.69 (m, 6H, 5 arom. H and H-5 fur.).

6h: *N*-(2-Thiophene)carbonyl-*N*-phenyl-*cis*-2,6-dimethylpiperidine-1-carboxamide. Formula $C_{19}H_{22}N_2O_2S$, MM 342.46, yield 85%, m.p. 144–145 °C (diethyl ether). ^{1}H NMR ($CDCl_3$): δ 1.15 (s, 3H, CH_3/pip), 1.28 (s, 3H, CH_3/pip), 1.35–1.99 (m, 6H, $3CH_2$/pip), 4.24–4.89 (m, 2H, 2CH/pip), 6.81–7.68 (m, 8H, 5 arom. H and 3H thioph.).

4.2. Pharmacology

All the compounds were assayed, according to standard procedures, in a series of in vivo tests in order to evaluate specifically anti-inflammatory activity and randomly local anaesthetic and antipyretic activities. Some of them, selected by the NCI, were also tested in vitro for antiproliferative activity.

4.2.1. Anti-inflammatory activity

Anti-inflammatoriy activity was evaluated by carrageenan-induced paw edema in rats [18]. Each compound was assayed on a groups of five albino animals of both sexes (body weight: 180–250 g), pregnant females excluded, and administered by gastric probe 30 min before subcutaneous injection of 0.2 ml of 1% carrageenan suspension in 0.9% NaCl solution into the plantar aponeurosis of the hind paw at ten-fold dosage (50 mg/kg) than that of indomethacin (5 mg/kg), used as reference drug. A control group of five rats was treated only with carrageenan. The paw volume was measured by a water plethysmometer Socrel at 0, 1, 2, 3, 4 h after administration of the agents. The data reported in Table 1 are the percentage inhibition values, calculated from the mean increase of paw volume at each time interval in comparison with that of the control group at the same time intervals. The lower (25 mg/kg) and higher dosage (100 mg/kg) of derivatives **2a**, **4h**, and **5d** were administered, in order to evaluate dose-dependent activity and to calculate ED_{50} values (Table 1) at 3 and 4 h after compound administration.

4.2.2. Local anaesthetic activity

Local anaesthetic activity was evaluated, according to standard procedure [19], on a group of ten mice (body weight: 20–25 g) after 5 and 30 min following infiltration of 0.2 ml of 0.1% glycofurol (Sigma–Aldrich, Milan) solution of each compound and lidocaine (reference drug) into the tail root. Dieffenbach tweezers were applied for 10 s. At the above times, percent of mice showing anaesthesia was monitored (Table 2).

4.2.3. Anti-proliferative activity

The NCI developed a high-flux anti-cancer drug screen utilizing a panel of about 60 human tumour cell lines in culture, derived from nine clinically isolated neoplastic diseases [20–22]. The NCI provided a detailed, computer-generated set of meangraphs that facilitate rapid perusal of the screening data to uncover possible indication of broad spectrum anti-tumour compounds and tumour sub-panel selectivity. Cell lines were incubated in 96-well plates for 48 h with five concentrations (0.01–100 μmol/l) of each test compound and were stained for total protein with sulphorodamine B according to the standard NCI protocol, in order to estimate cell viability or growth. Dose–responce curves (% growth inhibition vs. $\log_{10}$ concentration) were calculated. The cytotoxicity of each test compound is evaluated through the growth inhibition parameters, for example GI_{50} which represents the compound molar concentration required to produce 50% growth inhibition at the end of incubation time (Table 3).

5. Discussion and conclusions

Data reported in Table 1 show evidence that many of the most active compounds have been found in the series **2** and **5**, characterised by ethyl and phenyl substituents at the 2- and the 4-position of the piperidine ring, respectively. Provided also that the two unsubstituted piperidine derivatives **1c**,**d** showed anti-inflammatory activity comparable to that of indomethacin, it is probable that the ethyl and the phenyl groups can play an important but not essential role for activity, being reasonably involved in prominent, but secondary interactions. The activity of **3b** and, to a lesser degree, **3e**–**g**, indicates that the introduction of a smaller alkyl, as the methyl group, in the 3-position of the piperidine ring also gave good results; but moving the methyl group from the 3- to the 4- position caused, in several compounds **4**, decrease in activity. In spite of this, it is noteworthy that **4h** and **4g**, carrying the 2-furoyl and 2-thenoyl moieties, showed anti-inflammatory activity comparable with that of indomethacin. In particular **4h** was approximatively equipotent to **5d** and **2a**, as can be seen on the basis of their reported ED_{50} values (Table 1). Finally, *cis*-2,6-dimetyl substitution led to some decline in activity, given that only **6a**,**b**,**d** were moderately active. Probably, the steric crowd around the piperidine nitrogen, due to the *cis*-2-6-dimethyl substitution pattern, is detrimental for activity. As a consequence, only one of the two positions around the piperidine nitrogen atom can be occupied. Interestingly, the aroyl groups of the most active compounds were characterized either by the electron-rich rings (**2g**,**h**, **4h**), or by the benzene nucleus without substituents (**2b**, **3b**) or with electron-donating (CH_3O/CH_3) **(1d**,**c**, **5d)** and electron-withdrawing (F/Cl) *p*-substituents (**2a**, **5e**), so being apparent that electronic factors do not play a critical role. These results, taken together, would indicate that activity parameters include size and position of the piperidine substituent(s) and, reasonably, lipophilic character of the acyl moieties. Structure–activity correlations concerning antitumor screening data (Table 3) revealed that the 2-(4-chlorophenyl)-2-methyl propionyl group is the preferred substituent as demonstrated by activity of agents **5a** and **1a**. Nevertheless, the 4-phenyl-substitution seems to confer a wider spectrum of activity. Thus, **5a** showed a spectrum of activity particulary significant against leukemia cell line subpanel and, in a lesser degree, colon cancer cell line subpanel, whereas compound **1a** was significantly active only against cell lines HL-60(TB), PC-3, and T-47D.

In summary, this preliminary study demonstrates that the title compounds exhibit interesting anti-inflammatory and antiproliferative properties, depending on a delicate balance between the piperidine substitution pattern and nature of the acyl moities. Work is in progress to optimize anti-inflammatory activity, to establish the mechanism of action and to evaluate gastric ulcerogenic action. Positive results in all these parameters will be reported in a subsequent manuscript.

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