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Aminothiazole Derivatives with Antidegenerative Activity on Cartilage

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Abstract—A series of 2-dialkylamino-*N*-(4-substituted thiazolyl-2)acetamides and 3-dialkylamino-*N*-(4-substituted thiazolyl-2)propionamides were synthesized and evaluated for their anti-inflammatory activity. Encouraging results led us to investigate the effect of these compounds on NO production and GAGs release. Their effects were evaluated in vitro on the metabolism of pig cartilage, treated with IL-1β. The amount of glycosaminoglycans (GAGs) and the production of nitric oxide (NO) in the culture medium were determined. The results, obtained, showed that all compounds, in the presence of IL-1β, blocked the cartilage breakdown, with different behavior. A quantitative structure–activity relationship (QSAR) study was performed.

Introduction

The thiazolyl group is of great importance in treating of biological systems. Anti-inflammatory, analgesic and antipyretic activities are known for some thiazolyl and benzothiazolyl derivatives.[1–5] Meloxicam, for example, is a new NSAID with the thiazolyl group.

Nonsteroidal anti-inflammatory drugs (NSAIDs) form a basis for the treatment of inflammatory disease. They play a major role in the management of acute and chronic rheumatic diseases, but their use needs to be tempered with the realization that they can cause potentially serious adverse reactions, and in fact may themselves compromise the tissue metabolism of the cartilage.[6–9] The development of selective NSAIDs, which might reduce inflammation without side effects, would therefore be an important advancement. Nitric oxide (NO) and glycosaminoglycans (GAGs) can be considered key molecules in cartilage destruction. In the case of inflammatory disease, GAGs release is the consequence of increased matrix protease activity leading to the cleavage of collagen and proteoglycans, fundamental constituents of the cartilage. Moreover, the catabolic effect of NO determines the inhibition of proteoglycan (GAGs) synthesis and stimulates the condrocytes production of proenzymes that converted into active enzyme (metalloproteinases), causes cartilage breakdown.[10–12] Nitric oxide attracts considerable interest because it mediates many functions. It is produced in the animal species by three enzymes, called NO synthases. Two NO synthases are constructively expressed, one in the nervous system and the other one in the blood vessels. The third NO synthase is expressed in response to cytokines or lipopolysaccarides in smooth muscle cells, macrophages and hepatocytes as well as in chondrocytes and it could play a role in inflammatory and immunological host defence reactions. The effect of iNO, undoubtedly involved in most inflammatory disease, determines the formation of free radicals responsible for tissue damage and cartilage matrix degradation. Interleukin-1β is a cytokine, which is released, in inflammatory disease. This cytokine induces connective tissue cells, including chondrocytes and fibroblast to produce inflammatory mediators (NO) and matrix metalloproteinases, which are capable of degrading the components of the cartilage extra cellular matrix.[13–15]

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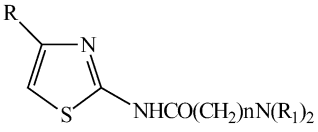
Based on the above considerations, in the present work we decided to investigate the effect on NO production and GAGs release of some thiazolyl-*N*-substituted amides, which were synthesized by us (Fig. 1),[17] previously demonstrated to possess anti-inflammatory activity.[16] Their effects on the metabolism of pig cartilage, treated with IL-1β, a cytokine released during inflammatory diseases, were evaluated in vitro. Parallel experiments were also carried out using of indomethacin as the reference drug.

In order to define some relationships between the chemical structure of the compounds and their activities, a QSAR analysis was performed. Although the small number of the compounds under study (only nine) a trend between the basic properties and the activities was found.

Results and Discussion

The thiazole derivatives of the title were prepared according to the procedure shown in Scheme 1.



Compound	R	n	$N(R_1)_2$
1	C_6H_5	1	$N(CH_3)_2$
2	C_6H_4-OCH_3	1	pyrrolidin
3	C_6H_4-OCH_3	1	piperidin
4	$CH_2COOC_2H_5$	1	piperidin
5	H	2	pyrrolidin
6	C_6H_5	2	pyrrolidin
7	C_6H_4-pOCH_3	2	$N(CH_3)_2$
8	C_6H_4-pOCH_3	2	pyrrolidin
9	$CH_2COOC_2H_5$	2	morpholin

Figure. 1. Aminothiazole derivatives under study.

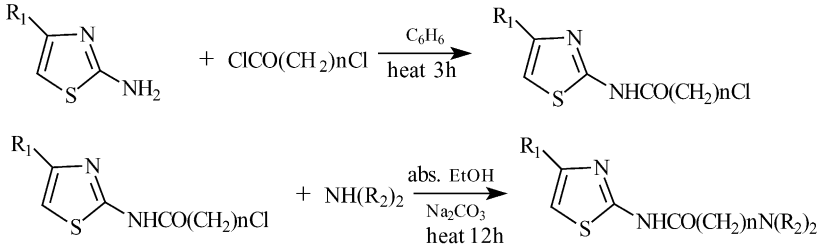
The 2-dialkylamino-*N*-(4-substituted-thiazolyl-2)-acetamides and propionamides (Fig. 1) **1**–**9** were tested for their antidegenerative effect.

Table 1 shows the results obtained at different concentrations (1–10–100 μg/mL) after 120 h in pig cartilage on NO and GAGs release, respectively (Figs. 2 and 3). These activities are dose dependent.

Tissue samples of pig articular cartilage were treated, in the present study, with compounds **1**–**9** as well as with the same compounds combined with IL-1β. This cytokine was used for stimulation of the inflammation process. It is known that during the inflammatory process, accompanying inflammatory disease, monocytes and macrophages release interleukin IL-1β, which was shown to reduce proteoglycan synthesis of these macromolecules.[16] This effect was also confirmed by our experiments as observed in Figures 4 and 5 by comparing with control data.

Compared with control data, a remarkable increase (28–35% and 300–357, respectively) in release of NO and GAGs (Figs. 4 and 5) was found after treatment with IL-1β at concentrations of 100 μg/mL. The controls after 24 h produced a very low amount (12 μM) of NO, mainly due to the constitutive NOS and consequently a low amount (150 μg/mL) of GAGs, but this level increased a little (22 μM and 270 μg/mL for NO and GAGs respectively) after 120 h. Indomethacin was used as the reference compound and samples treated with indomethacin combined with IL-1β showed a significant decrease ($p<0.01$) compared with the IL-1β treated samples, both on NO and GAGs release. As shown in Figure 2, all tested compounds when combined with IL-1β exhibited a significant reduction in NO release compared with the samples treated with IL-1β. The effect of all compounds, especially compound **N6** (20 ± 1.4) and **N4** (30 ± 0.5) was higher than that observed with indomethacin and IL-1β (40 ± 0.5), while compounds **N8** and **N1** had almost similar behaviour with indomethacin. Concerning the activity on GAGs release (Fig. 5) all compounds tested showed an inhibitory effect compared with the samples treated with IL-1β. Only the compound **N6** exhibited a better effect (283 ± 10) than that observed when the samples were treated with indomethacin (400 ± 15).

As shown in Figure 4 the NO release inhibition % compared with samples treated with IL-1β are 60% for compound **N6** and 44% for the two compounds: **N5** and **N4**; much higher than that observed in the samples



Scheme 1. Synthetic pathway of the title compounds.

Table 1. NO production and GAGs release (means ± SEM) from the pig nasal cartilage into the cultural medium 120 h after addition of aminothiazole derivatives in different concentrations (1–10–100 μg/mL): (a) compounds **N1**–**4**, (b) compounds **5**–**9**

Treatments	Concentrations (μg/mL)	GAGs release, 120 h (μg/mL)	NO production, 120 h (μM/mL)
(a)			
Control		270 ± 8	22 ± 1
IL-1β	10 ng/mL	450 ± 12	50 ± 1.8
Indomethacin	10^{-3} M	300 ± 13	25 ± 1.2
Indomethacin + IL-1β		400 ± 15	40 ± 0.5
Compound **N1**	1	231 ± 18	23 ± 0.9
	10	295 ± 13	26 ± 0.5
	100	301 ± 10	27 ± 0.2
Compound **N2**	1	255 ± 15	23 ± 0.4
	10	263 ± 12	24 ± 0.8
	100	302 ± 8	29 ± 0.3
Compound **N3**	1	271 ± 11	22 ± 0.5
	10	263 ± 7	25 ± 0.1
	100	321 ± 13	24 ± 0.7
Compound **N4**	1	267 ± 15	19 ± 1.3
	10	273 ± 16	20 ± 1.5
	100	329 ± 19	22 ± 1.2
Compound **N1** + IL-1β	1	392 ± 11	41 ± 1.7
	10	363 ± 7	38 ± 1.4
	100	357 ± 16	35 ± 1.1
Compound **N2** + IL-1β	1	386 ± 22	38 ± 1.1
	10	354 ± 18	36 ± 0.9
	100	316 ± 19	30 ± 0.5
Compound **N3** + IL-1β	1	382 ± 17	40 ± 1.8
	10	360 ± 9	35 ± 1.5
	100	327 ± 14	32 ± 1.2
Compound **N4** + IL-1β	1	375 ± 10	36 ± 0.7
	10	355 ± 18	30 ± 0.5
	100	320 ± 20	28 ± 0.1
(b)			
Control		270 ± 8	22 ± 1
IL-1β	10 ng/mL	450 ± 12	50 ± 1.8
Indomethacin	10^{-3} M	300 ± 13	25 ± 1.2
Indomethacin + IL-1β		400 ± 15	40 ± 0.5
Compound **N5**	1	254 ± 18	20 ± 0.8
	10	263 ± 16	21 ± 1.2
	100	292 ± 13	23 ± 0.7
Compound **N6**	1	231 ± 21	18 ± 1.2
	10	238 ± 19	20 ± 1.4
	100	264 ± 15	20 ± 0.7
Compound **N7**	1	262 ± 16	23 ± 1.1
	10	281 ± 14	23 ± 0.5
	100	315 ± 12	27 ± 1.7
Compound **N8**	1	258 ± 17	17 ± 1.3
	10	270 ± 8	18 ± 0.6
	100	278 ± 9	20 ± 0.7
Compound **N9**	1	290 ± 17	19 ± 1
	10	296 ± 13	22 ± 1.2
	100	311 ± 11	24 ± 0.8
Compound **N5** + IL-1β	1	380 ± 16	36 ± 1.4
	10	305 ± 14	30 ± 1.2
	100	301 ± 7	28 ± 1.3
Compound **N6** + IL-1β	1	364 ± 18	25 ± 0.5
	10	290 ± 15	22 ± 0.9
	100	283 ± 10	20 ± 0.3
Compound **N7** + IL-1β	1	391 ± 11	41 ± 1.1
	10	364 ± 9	37 ± 0.7
	100	332 ± 6	34 ± 0.9
Compound **N8** + IL-1β	1	385 ± 19	38 ± 0.6
	10	325 ± 16	35 ± 0.4
	100	305 ± 13	30 ± 0.3
Compound **N9** + IL-1β	1	380 ± 18	37 ± 1.5
	10	343 ± 14	33 ± 0.9
	100	326 ± 10	32 ± 0.8

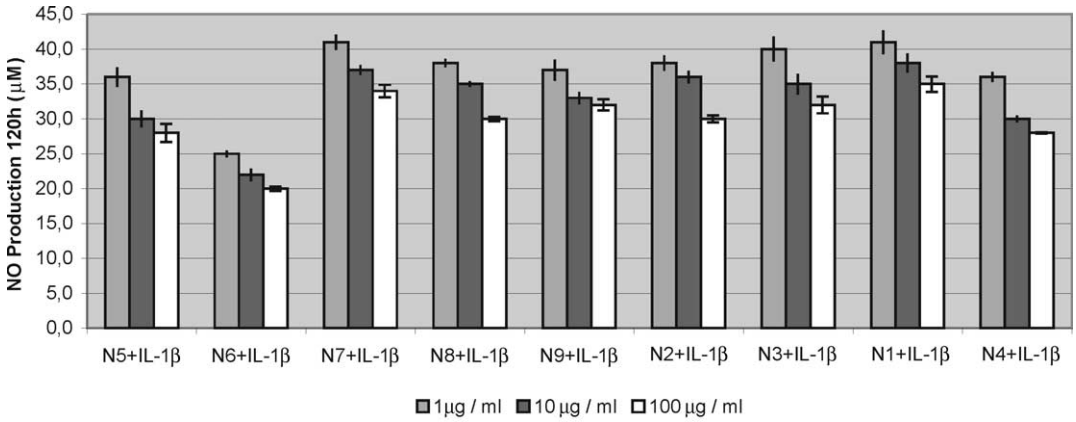


Figure 2. NO production (means ± SEM) from pig nasal cartilage into the culture medium 120 h after addition of aminothiazole derivatives **1**–**9** in different concentrations (1–10–100 μg/mL). Values are expressed as μM.

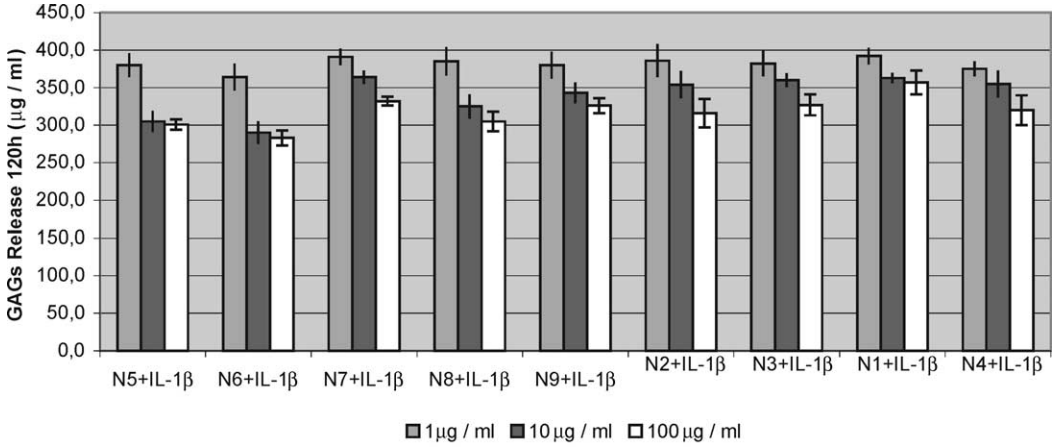


Figure 3. GAGs release (means ± SEM) from pig nasal cartilage into the culture medium 120 h after addition of aminothiazole derivatives **1**–**9** in different concentrations (1–10–100 μg/mL). Values are expressed as μg/mL.

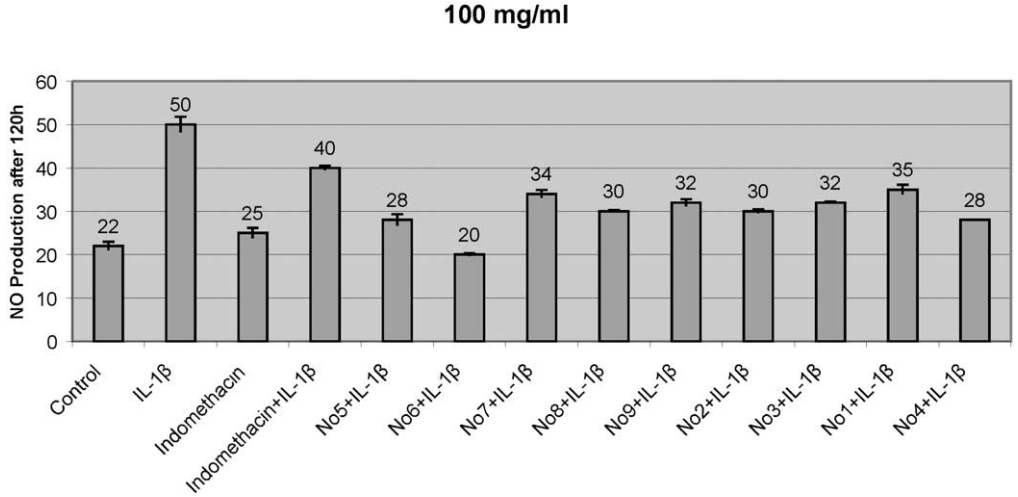


Figure 4. NO production (means ± SEM) from pig nasal cartilage into the culture medium 120 h after addition of aminothiazole derivatives **1**–**9** (100 μg/mL) or indomethacine with IL-1β. Values are expressed as μM.

treated with indomethacin and IL-1β (20%), while other compounds have better effects too (% inhibition, 30–40%). As regard the inhibitory effect of these compounds on GAGs release (Fig. 5), again the highest inhibitory effect was observed in compound **N6** (42%).

When the inhibitory activity of tested compounds is compared with their lipophilicity, it is obvious that the former does not depend on the latter, but that the structure of the compounds plays an important role.

From a structural point of view, compound **N6** the most effective in preventing IL-1β harmful effects on the cartilage is characterized by the presence of a phenyl group as R and pyrrolidine, while dimethylamino, piperidine and morpholine are allowed as R_1. The substitution on position 4 of phenyl as well as using hydrogen as R, leads to a decrease in activity. The presence of pyrrolidine substituent as R_1 seems necessary for this type of pharmacological activity, as piperidine, dimethyl and morpholine derivatives (**N9**, **N3**, **N8**, **N1**) showed lower effect on NO and GAGs release. It is obvious that the presence of two methylene group in combination with phenyl in position 4 of thiazolyl ring and pyrrolidine as R_1 is necessary too.

It should be mentioned that the compound **N6** exhibited the most potent action on the pig cartilage concerning NO and GAGs release compared with the other compounds, but even the influence on GAGs release in general is slight (283 μg/mL (compared with 450 for IL-1β, Fig. 5).

Pearson correlation matrix of the parameters involved in the study and the NO and GAG activities of the thiazoles is shown in Table 2. The plots experimental/predicted GAGs activities of the thiazoles are shown in Figure 6. A good correlation exists between both activities. pK_a values show high correlation between both of them as well ($R > 0.7$). The stepwise multivariable regression analysis led to the following models:

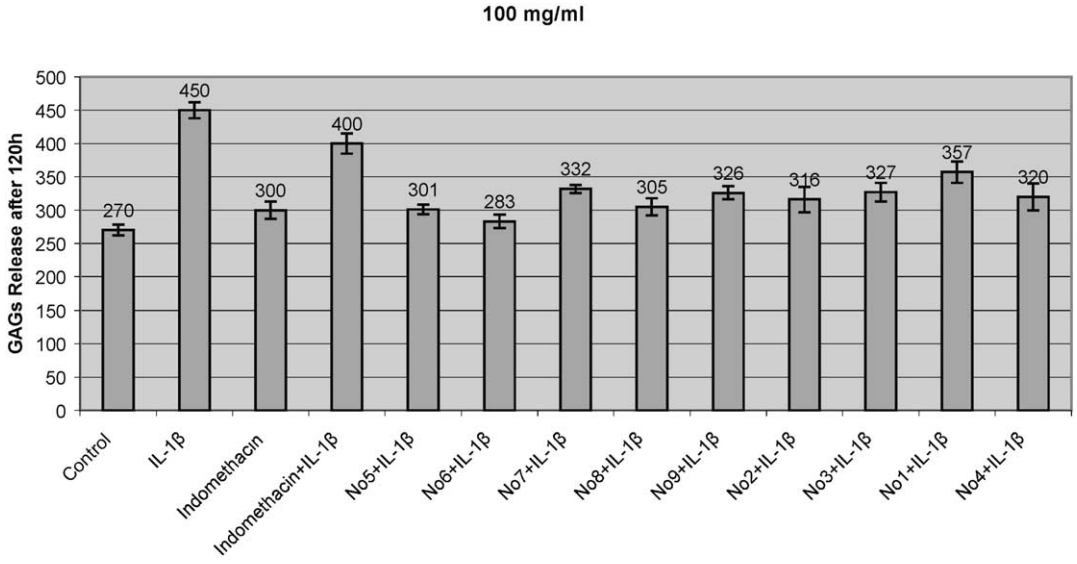


Figure 5. GAGs release (means ± SEM) from porcine nasal cartilage into the culture medium 120 h after addition of aminothiazole derivatives **1**–**9** (100 μg/mL) or indomethacine with IL-1β. Values are expressed as μg/mL.

Table 2. Pearson correlation matrix of the parameters involved in the study

	NOinh.	GAG	MV	MR	q_{Nring}	q_S	q_{Nco}	q_{NR1}	Pol	P	f_R	f_{R1}	CLOGP	$CLOGD_{7.4}$	R_M	pK_a
GAG	**0.892**	1.000														
MV	−0.156	−0.132	1.000													
MR	−0.008	0.055	**0.966**	1.000												
q_{Nring}	−0.053	−0.166	0.234	0.036	1.000											
q_S	−0.116	−0.116	0.416	0.235	**0.942**	1.000										
q_{Nco}	−0.213	−0.434	0.273	0.162	0.416	0.243	1.000									
q_{NR1}	−0.419	−0.414	−0.155	−0.220	−0.100	−0.053	−0.522	1.000								
Pol	−0.008	0.056	**0.966**	**1.000**	0.036	0.235	0.162	−0.220	1.000							
P	0.008	0.079	**0.967**	**0.963**	0.275	0.462	0.240	−0.303	**0.963**	1.000						
f_R	−0.107	−0.171	0.594	0.670	−0.504	−0.386	−0.051	0.224	0.670	0.458	1.000					
f_{R1}	0.434	0.413	0.032	0.164	−0.282	−0.343	0.428	**−0.876**	0.164	0.133	0.035	1.000				
CLOGP	0.056	0.094	0.565	0.565	−0.594	−0.479	0.125	−0.296	**0.709**	0.527	**0.824**	0.542	1.000			
$CLOGD_{7.4}$	−0.437	−0.403	**0.833**	**0.833**	−0.313	−0.204	0.406	−0.126	**0.712**	0.579	**0.760**	0.293	**0.833**	1.000		
R_M	−0.122	0.203	0.440	0.023	**−0.808**	−0.612	−0.565	0.059	0.023	−0.134	0.260	0.193	0.440	0.243	1.000	
pK_a	**0.723**	**0.730**	−0.231	−0.461	−0.273	−0.299	−0.654	0.038	−0.462	−0.488	−0.260	0.093	−0.231	−0.691	0.254	1.000
$I_{CH_2CH_2}$	0.292	0.514	−0.333	−0.128	−0.055	0.132	**−0.896**	0.315	−0.128	−0.098	−0.218	−0.417	−0.333	−0.581	0.358	0.636

Correlation coefficients above 0.7 are given in bold.

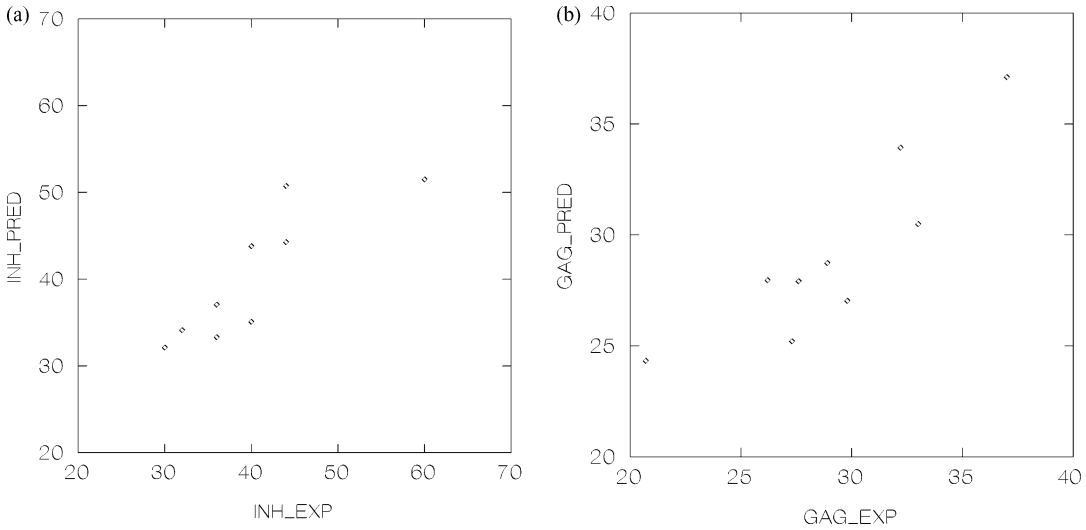


Figure 6. Experimental versus predicted activities: (a) inhibition of NO release; (b) inhibition of GAGs release.

$$\mathrm{NOinh} = -196.156(94.346)q_{\mathrm{NR1}} + 4.742(1.377)\mathrm{p}K_{\mathrm{a}} - 57.001(31.635) \quad (1)$$

$$n = 9,\ \ r^2 = 0.723\ \ \mathrm{SEE} = 5.383\ \ F_{2,6} = 7.829\ \ p = 0.021$$

$$\mathrm{GAG} = 0.035(0.013)P + 3.381(0.734)\mathrm{p}K_{\mathrm{a}} - 19.241 \times (12.455) \quad (2)$$

$$n = 9,\ \ r^2 = 0.781\ \ \mathrm{SEE} = 2.508\ \ F_{2,6} = 10.681\ \ p = 0.011$$

Aminothiazoles with higher pK_a values exhibit higher NO and GAG activities. The basic properties of the investigated compounds depends mainly on the substituent R_1 and they decrease in the order pyrrolidine > piperidine > $N(CH_3)_2$ > morpholine. Thus, pyrrolidine is the preferred substituent for the R_1 position. Additionally, the charge at N_{R1} and the parachor of the molecules influence the activities. Because of the limited number of compounds, the results from QSAR study should be considered only as initial information of the relationship between structure and activity.

Conclusion

The insertion of selected substituent in the thiazolyl ring can supply compounds able to block cartilage destruction during the inflammatory process, as simulated in our experimental model, and further investigations are warranted in order to reach a further understanding of structure–activity relationships in this very promising class of anti-inflammatory compounds.

Our preliminary QSAR study indicates that the basic properties of the aminothiazoles play an important role in their ability to block cartilage destruction during the inflammatory process.

Experimental

Chemistry

The thiazolyl-*N*-substituted amides **1**–**9** (Fig. 1) selected for this study were prepared according to the method previously described in our paper.[17]

The compounds were dissolved in dimethylsulphoxide 10%, diluted in culture medium and each one used at a concentration of 1, 10 and 100 μg/mL,

Biological evaluation

Preparation of cartilage.[18] Nasal pig cartilage was obtained from a local abattoir and washed in Hank's balance salt solution containing antibiotics (50 U/mL penicillin and 50 μg/mL streptomycin). Cartilage was sliced in disk (3–4 mm in diameter) and weight. The samples were placed into 24-well plates containing 1 mL of Dulbecco's modified Eagle's medium (Sigma, Italy), phenol red free, glutamine (10 mM), penicillin/streptomycin (50 U/mL and 50 μg/mL, respectively) and 10% heat inactivated fetal calf serum. Cartilage pieces were incubated at 37 °C in humidified 5% CO_2/95% air incubator. After 24 h, the media was removed and cartilage samples were treated for 120 h as follows: (a) control medium; (b) IL-1β 10 ng/mL; indomethacin 10^{-5} M and IL-1β 10 ng/mL; (e) compounds (**N1**–**9**) in dose of 1, 10 and 100 μg/mL; compounds (**N1**–**9**) and IL-1β.

Determination of NO levels

NO levels were determined in the tissue culture media using the Griess reaction.[19] 100 μL of sulphanilamide

(1% w/v) were added to single samples (100 μL) placed into 96-well microplate. The plate was wrapped in aluminium foil and shaken briefly. The absorbance was measured at 570 nm and finally the nitrite concentration determined from a sodium nitrite standard curve (0–120 μM). The values were expressed as μM nitrite released into the culture medium.

Determination of GAGs

GAGs were tested in the culture medium by utilizing a spectrophotometric assay using 1.9-dimethyl methylene blue (DMB) at a wavelength of 535 nm.[20,21] A standard curve for determination of GAGs concentration (100–500 μg/mL) was used. As a standard, GAG chondroitin sulphate derived from shark cartilage, was used.

Statistical analysis

Each experiment was repeated at least three times in quadruplicate samples. All statistical analyses were performed using statistical software package SYSTAT. Difference were considered significant at $p < 0.05$.

QSAR

The chemical structure of the compounds was described by three groups of parameters: steric, electrostatic and hydrophobic. Some of the parameters described local properties, others referred to global ones. Molecular volume (MV) and molecular refractivity (MR) were used in the QSAR as global steric properties. The partial charges at some of the main atoms (q_{Nring}, q_S, q_{Nco} and q_{NR1}) were applied to describe the local electron density. Polarizability (Pol) and parachor (P) were used as global electrostatic descriptors. The local hydrophobicity was presented by the parameters f_R and f_{R1}, and the global one by CLOGP. $CLOGD_{7.4}$ and the TLC-derived parameter R_M presented the distribution of the compounds in the octanol/buffer $pH_{7.4}$ system. pK_a values were also included in the study. An indicator variable $I_{CH_2CH_2}$ was included in the study to distinguish between CH_2 and CH_2CH_2 bridges. MV, MR, Pol, P, f_R and f_{R1} were calculated by ACD/ChemSketch.[22] The partial atomic charges were computed by AM1 semi-empirical method implemented in SYBYL6.7.[23] after MM optimisation of the molecules. CLOGP, $CLOGD_{7.4}$, pK_a and R_M were taken from our previous study.[17] Stepwise multivariable regression analysis was applied to develop QSAR models using SYSTAT. The models were presented in normalised form and assessed by the explained variance r^2, standard error of estimate (SEE) and F ratio.

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