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Original article

Adamantane derivatives of thiazolyl-*N*-substituted amide, as possible non-steroidal anti-inflammatory agents

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Abstract

A series of adamantane derivatives of thiazolyl-*N*-substituted amides were synthesized in a three-step reaction and tested for anti-inflammatory activity as well as lipoxygenase and cycloxygenase inhibitory actions. Theoretical calculation of their lipophilicity, as *C* log *P* was performed. The effect of the synthesized compounds on inflammation, using the carrageenin-induced mouse paw oedema model was studied and compared to indomethacin. In general, the studied compounds were found to be potent anti-inflammatory agents (29.6–81.5%). Anti-inflammatory activity was influenced by some structural characteristics of the synthesized compounds. The lipoxygenase inhibitory activity was tested by the conversion of sodium linoleate to 13-hydroperoxylinoleic acid. Low inhibitory activity was observed. Evaluation of COX-1 and COX-2 inhibitory activities of the compounds revealed a COX-1 inhibitory potential, comparable to that of naproxen for some of the compounds and a low to moderate COX-2 inhibitory potential. Comparison of the in vivo and in vitro results leads to the conclusion that most compounds of this series may be involved in other mechanisms of inflammation, too.

Keywords: Adamantane; COX; LOX; Anti-inflammatory; C log P; Thiazole derivatives

1. Introduction

Inflammation is one of the most important natural defence mechanisms against internal and external threats. However, the inflammatory elements produced can also be deadly to the body when ''switched on'' for too long, a condition known as chronic inflammation. Research has indicated that chronic inflammation is common in the nerve cells of patients with neurodegenerative diseases.

Eicosanoids are a family of lipid mediators derived from the metabolism of arachidonic acid. Eicosanoids such as prostanoids and leucotrienes have a wide range of biological action, including potent effects on inflammation and immunity. Once liberated from cell membranes by the action of phospholipase A2, arachidonic acid may become substrate for various metabolic pathways that produce biological mediators [1]. The most important of these are the cycloxygenase and lipoxygenase pathways.

Non-steroidal anti-inflammatory drugs (NSAIDs), alleviate inflammation by counteracting the cycloxygenase isoforms (COX-1, COX-2) [2] preventing the synthesis of prostaglandins and eliminating inflammation and pain. Although COX-2 is concerned to be the main isoenzyme related to inflammation, most NSAIDs in the market today block both forms of COX isoenzymes. Side effects such as gastrointestinal pain have been associated with NSAID use due to the inhibition of COX-1. Beside, COX-2 specific inhibition was associated with cardiovascular problems. Balanced inhibition of COX-1/2 isoenzymes combined to LOX inhibition seems to be the choice of preference for some scientists in an effort to achieve the goal of effective anti-inflammatory agents with low side effects.

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Thiazole and benzothiazole derivatives have been found to possess analgesic and anti-inflammatory actions also exhibiting lipoxygenase inhibitory activity [3–11]. Adamantane derivatives have been found to interfere with various enzymes and possess a variety of therapeutic activities, such as anti-inflammatory [12], anti-viral [13], anti-Parkinson [14] and antimicrobial–anticancer activities as well as dihydrofolate reductase (DHFR) inhibitory activity [15–17].

Moreover, adamantane has also been used as a brain-directed drug carrier for poorly absorbed drugs. Tsuzuki et al. proved in 1994, that the introduction of the 1-adamantane moiety to azidothymidine (AZT) resulted in the enhancement of the BBB penetration [18].

Several 2-[3-(1-adamantyl)-4-substituted-5-thioxo-1,2,4-triazolin-1-yl]acetic acids, 2-[3-(1-adamantyl)-4-substituted-5-thioxo-1,2,4-triazolin-1-yl] propionic acids and related derivatives, 2-(1-adamantyl)-5-substituted-1,3,4-oxadiazoles and 2-(1-adamantylamino)-5-substituted-1,3,4-thiadiazoles, produced good in vivo dose-dependent anti-inflammatory activities using the carrageenin-induced paw oedema method in rats [19,20]. Since the combination of two pharmacophores on the same scaffold is a well established approach to the synthesis of more potent drugs [21,22] we decided to incorporate an adamantane moiety to the thiazolyl ring in an effort to synthesize new compounds with anti-inflammatory activity.

The synthesized compounds contain a thiazolyl ring linked to different amino amides and to the highly lipophilic adamantane group. These structural characteristics of the compounds account for a high possibility to be potential inhibitors of the enzymes involved in inflammation [23].

The anti-inflammatory properties of the adamantane derivatives were evaluated by their ability to inhibit carrageenin-induced mouse paw oedema. Moreover, lipoxygenase and cycloxygenase, COX-1 and COX–2, inhibitory action of the compounds were tested in an attempt to elucidate the mechanism of anti-inflammatory action.

2. Results and discussion

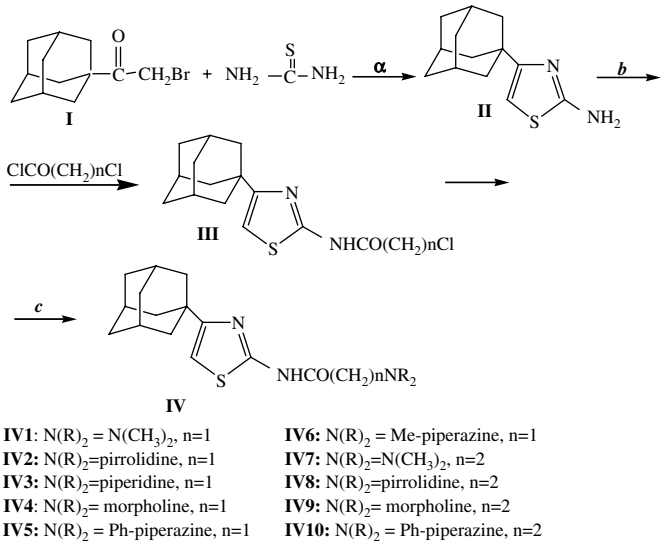
2.1. Chemistry

The general method employed to prepare the final compounds is shown in Scheme 1.

The compounds of the title were prepared in two steps. Initial 4-(1-adamantanyl)-2-aminothiazole (**II**) was treated with chloracetyl/propionyl chloride in benzene to give the corresponding chloroacetamides/propionamides (**III**) which in the second step undergo condensation with a series of secondary amines in ethanol to give the title compounds (**IV 1–10**). Overall the reactions proceeded smoothly in good yields. Yields, physical properties and molecular formulae are given in Section 4.

Structures of all synthesized compounds were established on the basis of elemental and spectral analyses (IR, ^{1}H NMR, MS).

The IR spectra were in agreement with the proposed structures. IR spectra showed a sharp band in the region 1680–1700 cm^{-1} (NHC=O) [24]. As an example, spectral data of compound **2** are presented here while spectral features of other



IV1: $N(R)_2 = N(CH_3)_2$, n=1
IV2: $N(R)_2$=pirrolidine, n=1
IV3: $N(R)_2$=piperidine, n=1
IV4: $N(R)_2$= morpholine, n=1
IV5: $N(R)_2$ = Ph-piperazine, n=1
IV6: $N(R)_2$ = Me-piperazine, n=1
IV7: $N(R)_2=N(CH_3)_2$, n=2
IV8: $N(R)_2$=pirrolidine, n=2
IV9: $N(R)_2$= morpholine, n=2
IV10: $N(R)_2$ = Ph-piperazine, n=2

Scheme 1. Synthetic procedure for the preparation of title compounds. Reagents and conditions. (a) Isopropanol, 0.5 h, r.t, yield 94%; (b) C_6H_6, reflux, yield 99% ($n = 1$) and 74% ($n = 2$); (c) RNH_2, EtOH, reflux, yield 43–87%.

derivatives are given in Section 4. IR (KBr) spectrum of this compound showed bands at $\nu = 3030$ cm^{-1} (N–H) and 1652 cm^{-1} (C=O). In the ^{1}H NMR spectrum, the two singlets observed at 3.32 ppm (methylen protons, $COCH_2$) and 6.74 ppm (thiazolyl proton) are in agreement with the values expected for these protons and with findings from studies of similar compounds [11]. The protons of pyrrolidine and adamantane revealed multiple signals at 2.48–2.53 ppm and 1.69–2.00 ppm. The results are consistent with the proposed structures.

In the EI-mass spectra, the existence of daughter ions was assigned from the suggested fragmentation pattern, which is in agreement with the findings from pertinent studies of simpler compounds [24,25]. Almost all substances gave stable molecular ions. Initial loss of pyrrolidine (*m/z* 71) led to ion with *m/z* 276 which in turn gave ion with *m/z* 261 by loosing methyl. The latter ions with *m/z* 234, *m/z* 134 and *m/z* 85 and *m/z* 84, which are the base peaks (thiazole) exhibit the expected fragmentation pattern of this kind of structure [24,25].

2.2. Physicochemical studies

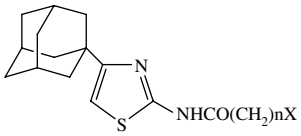
Since lipophilicity is a significant physicochemical property that determines distribution, bioavailability, metabolic activity and elimination we tried to theoretically calculate the corresponding *C* log *P* values in *n*-octanol buffer [26].

2.3. Biological evaluation

2.3.1. In vivo inhibition of the carrageenin-induced oedema

Compounds **IV1–10** were examined in vivo for their anti-inflammatory activity using the carrageenin mouse paw oedema as a model of inflammation and they are given in Table 1, as percentage inhibition of weight increase at the right hind paw in comparison to the uninjected left hind paw. Carrageenin-induced

Table 1
Inhibition % of induced carrageenin rat paw oedema (CPE %); in vitro % inhibition of soybean lipoxygenase (LOX); in vitro % inhibition of cycloxygenase-1 (COX-1); *C* log *P* values



Compound	X	*n*	CPE %[a] (0.2 mmol/kg bw)	% LOX[b] inhibition	% COX-1[b] inhibition	% COX-2[b] inhibition	*C* log *P*
IV1	$N(CH_3)_2$	1	48*	26.4%	25%	3.6%	3.562
IV2	Pyrrolidinyl	1	69.7**	5%	33.6%	12.2%	4.360
IV3	Piperidinyl	1	26.6*	0%	nt	nt	4.995
IV4	Morpholinyl	1	57.8*	0%	27.9%	35.3%	3.726
IV5	Ph-piperazinyl	1	47.6*	33%	43.6%	15.8%	6.264
IV6	Me-piperazinyl	1	30*	nt	nt	nt	4.350
IV7	$N(CH_3)_2$	2	81.5**	1%	25.0%	8.6%	3.790
IV8	Pyrrolidinyl	2	36.8**	nt	nt	nt	4.633
IV9	Morpholinyl	2	49.2**	8.4%	nt	nt	3.923
IV10	Ph-piperazinyl	2	55.9**	39%	28.6%	17.3%	6.461

nt: Not tested.
[a] Each value represents the mean of two independent experiments with six animals in each group, statistical studies were done with student's *T*-test, $^{**}p < 0.01$, $^{*}p < 0.05$.
[b] Values are means of tree determinations and deviation from the mean is <10% of the mean value. Compound concentration for LOX-inhibition assay was 100 μM. Compound concentration in COX-1 and COX-2 inhibition assays was 200 μM with the exception of compound **10** which was tested at a concentration of 20 μM because of solubility problems.

oedema is a non-specific inflammation resulting from a complex of diverse mediators. Since oedema of this type is highly sensitive to NSAIDs, carrageenin has been accepted as a useful agent for studying new anti-inflammatory drugs. As shown in Table 1, all the investigated compounds provided protection against carrageenin-induced paw oedema. Compound **IV7** is the most potent followed by compounds **IV2**, **IV4** and **IV10**. Compound **IV3** is the less active. All compounds contain the adamantane substituent. The structural modifications involve the nature of the substituent X (alicyclic ring or chain) and the length of the intermediate aliphatic chain ($n = 1$ or 2). It was observed that among the compounds with one-carbon bridge the most active compound is the pyrrolidinyl derivative, **IV2**, (69.7%) followed by morpholinyl, **IV4**, (57.8%) and dimethylamino, **IV1**, (48%) derivatives. The less active in this series is piperidinyl derivative, **IV3**, (26.6%). On the contrary, among compounds with two-carbon atom bridge the dimethylamino derivative, **IV7**, had the best anti-inflammatory action (81.5%), followed by phenyl-piperazinyl, **IV10**, (55.9%) and morpholinyl derivatives, **IV9**, (49.2%). The less active compound was pyrrolidinyl derivative, **IV8**, (36.8%). As gathered by the comparison between the one-carbon and the two-carbon atom series, the effect of the length of intermediate chain is ambiguous. Compound **IV1**, with one-carbon intermediate chain has about half of the activity (48%) of the two-carbon chain analogue, compound **IV7** (81.5%). A decrease in activity due to diminished length of the intermediate chain is also observed in the phenyl-piperazinyl derivatives (compounds **IV10** and **IV5**). However, the opposite is observed when the activities of the pyrrolidinyl- (**IV8** and **IV2**) and morpholinyl- (**IV9** and **IV4**) derivatives are taken into account. It is of interest to note that in the one-carbon chain series the five-atom heterocyclic substituent in the pyrrolidilyl derivative, **IV2**, (69.7%), strongly favours anti-inflammatory activity compared to the six-atom piperidinyl substituent in compound **IV3** (26.6%). In the series of piperazinyl derivatives ($n = 1$), the presence of a C_6H_5 group seems to be related with more active compounds (compounds **IV5**, **IV6**). Besides, as lipophilicity increases, the anti-inflammatory action increases too, but not continuously.

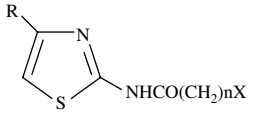
Comparison of the anti-inflammatory activity of the adamantane derivatives with the activity of relevant compounds lacking the adamantane group [11] may lead to interesting conclusions (Table 2).

(a) For the dimethylamino amides, having one-carbon intermediate chain ($n = 1$), the presence of the adamantane group, **IV1**, led to an increase of the activity (**1** > **1c** >**1b** > **1d** > **1a**). The anti-inflammatory activity of compound **IV1** is similar to the phenyl-substituted derivative **1c**. A positive effect of adamantane is also observed in case of dimethylamino amides **IV7** and **7a**, having two-carbon intermediate chain ($n = 2$). Compound **IV7**, having the adamantane group, in the position 4 of thiazole ring exhibited much higher activity (81.5%) than derivative, **7a**, (53.4%), with *p*-OCH_3-phenyl group in the same position of the ring.
(b) The pyrrolidinyl derivatives with R = adamantane and $n = 1$, are also more potent anti-inflammatory agents (**IV2** > **2b** > **2a**) whereas the presence of adamantane in the piperidinyl- and piperazinyl-analogues is not correlated with higher biological response.

2.3.2. In vitro soybean lipoxygenase inhibition

The synthesized compounds were further evaluated for inhibition of soybean lipoxygenase LOX by the UV absorbance based enzyme assay (Table 1). The conversion of sodium linolate to 13-hydroxy-peroxylinoleic acid measured at 234 nm was

Table 2
Comparison of the in vivo data (new and previous reported results) [11]



Compound	R	X	*n*	CPE %[a] (0.2 mmol/kg bw)
IV1	Adamantanyl	$N(CH_3)_2$	1	48
1a[a]	H	$N(CH_3)_2$	1	28.4
1b[a]	CH_3	$N(CH_3)_2$	1	37.6
1c[a]	C_6H_5	$N(CH_3)_2$	1	52
1d[a]	*p*-OCH_3–C_6H_4	$N(CH_3)_2$	1	35
IV2	Adamantanyl	Pyrrolidinyl	1	69.7
2a[a]	C_6H_5	Pyrrolidinyl	1	49.2
2b[a]	*p*-OCH_3-C_6H_4	Pyrrolidinyl	1	55
IV3	Adamantanyl	Piperidinyl	1	26.6
3a[a]	*p*-OCH_3-C_6H_4	Piperidinyl	1	53.3
IV5	Adamantanyl	Ph-piperazinyl	1	47.6
5a[a]	H	Ph-piperazinyl	1	72.1
IV7	Adamantanyl	$N(CH_3)_2$	2	81.5
7a[a]	*p*-OCH_3–C_6H_4	$N(CH_3)_2$	2	53.4
IV8	Adamantanyl	Pyrrolidinyl	2	36.8
8a[a]	H	Pyrrolidinyl	2	64.1
8b[a]	C_6H_5	Pyrrolidinyl	2	68.4
8c[a]	*p*-OCH_3–C_6H_4	Pyrrolidinyl	2	56.2
IV9	Adamantanyl	Morpholinyl	2	49.2
9a[a]	*p*-OCH_3–C_6H_4	Morpholinyl	2	55.4

[a] As reported in reference [11].

recorded and compared with appropriate standard (NDGA). The studied compounds were added in the reaction mixture at a final concentration of 0.1 mM. While one may not extrapolate the quantitative results of this assay to the inhibition of mammalian 5-LOX, it has been shown that inhibition of plant LOX activity by NSAIDs is qualitatively similar to their inhibition of the rat mast cell LOX and may be used as a simple qualitative screen for such activity. Phenyl-piperazinyl derivatives, **IV10** ($n = 2$) and **IV5** ($n = 1$), exhibited the best inhibitory action (39% and 33% respectively). The dimethylamino derivative with one-carbon intermediate chain, **IV1**, also showed considerable inhibitory effect (26.4%). On the contrary, the two-carbon intermediate chain dimethylamino derivative, **IV7**, had no inhibitory activity (1%). All other compounds were practically inactive.

2.3.3. In vitro cycloxygenase inhibition

Cycloxygenase inhibitory action was measured using human recombinant COX-1 and ovine COX-2 isoenzymes included in ''COX inhibitor screening assay'' kit purchased by Cayman. It was found that Ph-piperazinyl derivatives of both series **IV5** ($n = 1$) and **IV10** ($n = 2$) showed the highest COX-1 inhibitory activity (43.6% and 28.6% respectively) while the dimethylamino derivatives **IV1** and **IV7** exhibited the lowest activity (25%). It should be mentioned that the activity of compound **IV5** was better than that of naproxen under the same conditions (40%). Although the results are preliminary, it was observed that the COX-1 inhibitory activity of compounds from the same series ($n = 1$ or $n = 2$) is in correlation with lipophilicity theoretically calculated *C* log *P* values. The higher activity corresponds to higher *C* log *P* value.

In case of COX-2 inhibition, the most active compound was morpholinyl derivative **IV4** (35.3%), followed by phenylpiperazine derivatives **IV10** and **IV5** (17.3% and 15.8%, respectively) while the less active compounds of both series were dimethylamino derivatives **IV1** and **IV7** (3.6% and 8.6%, respectively). These are in accordance to the COX-1 inhibitory results. No correlation with *C* log *P* values was observed for derivatives with $n = 1$.

3. Conclusions

The results prove that the presence of adamantane moiety generally leads to thiazolyl-*N*-substituted amides with improved anti-inflammatory properties compared to the thiazolyl-*N*-substituted amides previously studied by our team [11].

Some of the compounds may act as COX/LOX inhibitors. Ph-piperazinyl derivatives **IV10** and **IV5** exhibited the best LOX and COX-1/2 inhibitory actions. Interestingly, derivative **IV10** exhibited considerable inhibitory action although added in the reaction mixture at a much lower concentration (20 μM instead of 200 μM) compared to other compounds and naproxen, due to solubility problems. These compounds may represent a good starting point for discovery of new COX/LOX inhibitors of the *N*-[4-(1-adamantyl)-thiazol-2-yl]-2/3-substituted aminoacetamide/propionamide series.

However, Ph-piperazinyl derivative ($n = 2$), **IV10**, exhibited moderate anti-inflammatory action in the in vivo experiment. On the contrary, the dimethylamino derivative ($n = 1$), **IV7**, with the best anti-inflammatory action had very low LOX and COX-1/2 inhibitory activities. Since, other mechanisms are involved at the first step of oedema formation a straight correlation between in vitro results and LOX and COX inhibitory activities cannot be obtained. As a matter of fact this contradiction is commonly observed at the results of many researchers [27] and further investigation is needed for the elucidation of the mechanism of action of many potent anti-inflammatory agents. In a future study, further investigation on the mechanism of action of some of our compounds may reveal new series of non-COX/LOX inhibitors with potent anti-inflammatory action.

4. Experimental

4.1. Materials

All the chemicals used were of analytical grade and commercially available by Merck, nordihydroguaretic acid (NDGA) is purchased from the Aldrich Chemical Co. (Milwaukee, WI, USA). Soybean lipoxygenase, linoleic acid sodium salt Arachidonic Acid (AA), and indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA) and carrageenin, type K, was commercially available. COX Inhibitor Screening Assay kit was provided by Cayman (Cayman Chemical Co., Ann Arbor, MI, USA, Catalog No. 560131). For the in vivo experiments, male and female AKR mice were used.

^{1}H NMR spectra were recorded with a Mercury 200 (Varian) spectrometer using $CDCl_3$ as solvent and hexamethyldisiloxane (HMDSO) as internal standard (for unsilylated compounds). Melting points were determined on a Boetius table and were uncorrected. The results of elemental analysis for C, H, N, S were in agreement with the calculated.

4.2. Chemistry

4.2.1. Synthesis N-[4-(1-adamantyl)]-2-aminothiazole (II)

To a solution of 1-adamantyl bromomethyl ketone, **I**, (257 mg, 1 mmole) in 5 ml of isopropanol, a suspension of thiourea (152 mg, 2 mmole) in 10 ml of isopropanol was added. The mixture was stirred for half an hour. After this time the resulting solution was poured into a solution of sodium carbonate, and the precipitate formed was filtered and dried to give, after recrystallization from ethyl acetate, 220 mg (94%) of pure product, **II**. M.p. 215–215.5 °C. IR(KBr): $\nu = 3050\ cm^{-1}$ (N–H), $1650\ cm^{-1}$ (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.65$–2.00 (m, 15H adamantane), 6.00 (s, 1H, thiaz.), 6.75 (s, 2H, NH_2).

4.2.2. Synthesis of N-[(4-(1-adamantyl)-thiazol-2-yl)] 2/3-chloroacetamide/propionamide (III)

The synthesis was performed according to the procedure described in our previous paper [24,28]. To a solution of 4-adamantyl-2-aminothiazole (0.02 mol) in dry benzene a cooled solution of chloroacetyl or 3-chloropropionyl chloride (0.033 mol) in dry benzene (7.5 ml) was added drop wise. The reaction mixture was refluxed in a water bath at 80 °C for 3 h. Benzene and excess 2-chloracetyl/3-chloropropionyl chlorides were removed by distillation. The residue was washed with aqueous sodium bicarbonate (5% w/v) followed by cold water. The crude product was dried and crystallized from ethanol.

Yield: 99% and 74% for 2- and 3-chloroacetamides respectively. M.p.185–187 °C and 190–192 °C, respectively.

4.2.3. Synthesis of N-[(4-(1-adamantyl) thiazol-2-yl)] 2/3-substituted acetamides/propionamides (IV 1–10)

The synthesis was performed according to the procedure described in our previous paper [24,28]. A mixture of 2-chloroacetamido or 3-chloropropionamido thiazole (0.006 mol), amines (0.7 mol), absolute ethanol (15 ml) and anhydrous sodium carbonate (1.48 g) was heated under reflux in a water bath for 12 h. The excess of amine and ethanol was removed by distillation and the residue was treated with 5% sodium bicarbonate solution to remove acid impurities, filtered, washed with water and dried. It was crystallized from ethanol (95%) to give white crystals.

4.2.4. N-[4-(1-adamantyl)-thiazol-2-yl]-2 dimethylamino acetamide (IV1)

Yield: 85%; mp 139–139.5 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.74$.

IR(KBr): $\nu = 3050\ cm^{-1}$ (N–H), 1680 nm (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.69$–2.01 (m, 15H, adamantane), 2.25 (s, 6H, $N(CH_3)_2$), 3.18 (s, 2H, $COCH_2$), 6.67 (s, 1H, thiazole). MS for $C_{17}H_{25}N_3OS$, *m/z*: M^+ 319.

4.2.5. N-[4-(1-adamantyl)-thiazol-2-yl]-2-pyrrolidino acetamide (IV2)

Yield: 74%; mp 190–191 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.83$. IR(KBr): $\nu = 3030\ cm^{-1}$ (N–H), $1652\ cm^{-1}$ (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.69$–2.00 (m, 15H, adamantane and 4H pyrrolidine), 2.48–2.53 (m, 4H, pyrrolidine), 3.32 (s, 2H, $COCH_2$), 6.74 (s, 1H, thiazolyl). MS for $C_{19}H_{27}N_3OS$, *m/z*: M^+ 345.

4.2.6. N-[4-(1-adamantyl)-thiazol-2-yl]-2-piperidino acetamide (IV3)

Yield: 78%; mp 144–145 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.78$. IR(KBr): $\nu = 3055\ cm^{-1}$ (N–H), $1680\ cm^{-1}$ (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.42$–1.56 (m, 6H, piperidine), 1.75–1.91 (m, 15H, adamantane), 2.06 (m, 4H piperidine), 3.24 (s, 2H, $COCH_2$), 6.71 (s, 1H, thiazole). MS for $C_{20}H_{29}N_3OS$, *m/z*: M^+ 359.

4.2.7. N-[4-(1-adamantyl)-thiazol-2-yl]-2-morpholino acetamide (IV4)

Yield: 81%; mp 125–126 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.81$. IR(KBr): $\nu = 3050\ cm^{-1}$ (N–H), $1680\ cm^{-1}$ (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.82$–2.14 (m, 15H, adamantane), 2.63–2.73 (m, 4H, morpholine CH_2NCH_2), 3.51 (br s, 2H, $COCH_2$), 3.67 (t, 4H, morpholine), 6.74 (s, 1H, thiazole). MS for $C_{19}H_{27}N_3O_2S$, *m/z*: M^+ 361.

4.2.8. N-[4-(1-adamantyl)-thiazol-2-yl]-2-(4′-phenylpiperazino) acetamide (IV5)

Yield: 95%; mp 134–135 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.80$. IR(KBr): $\nu = 3120\ cm^{-1}$ (N-H), $1685\ cm^{-1}$ (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.70$–2.01 (m, 15H adamantane), 2.64–2.66 (m, 4H piperazine), 3.14 (m, 4H, piperazine), 3.30 (s, 2H, $COCH_2$), 6.66 (s, 1H, thiazole), 6.73–6.78 (q, 1H, ArH), 6.90–6.93 (d, 2H ArH, $J = 8.13$ Hz), 7.17–7.22 (q, 2H, ArH). MS for $C_{25}H_{32}N_4OS$, *m/z*: M^+ 436.

4.2.9. N-[4-(1-adamantyl)-thiazol-2-yl]-2-methylpiperazino acetamide (IV6)

Yield: 87%; mp 184–186 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.62$.

IR(KBr): $\nu = 3150\ cm^{-1}$ (N–H), $1680\ cm^{-1}$ (C=O). ^{1}H NMR (DMSO-d_6): $\delta = 1.69$–2.01 (m, 15H, adamantane), 2.80 (s, 3H, NCH_3), 3.48–3.68 (m, 8H, piperazine), 4.21 (s, 2H, $COCH_2$), 6.78 (s, 1H, thiazole). MS for $C_{20}H_{30}N_4OS$, *m/z*: M^+ 374.

4.2.10. N-[4-(1-adamantyl)-thiazol-2-yl]-3-dimethylaminopropionamide (IV7)

Yield: 77%; mp 133–135 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.71$.

IR(KBr): $\nu = 3080\ cm^{-1}$ (N–H), $1685\ cm^{-1}$ (C=O). ^1H NMR (DMSO-d_6): $\delta = 1.75$–2.04 (m, 15H, adamantane), 2.39 (s, 6H, $N(CH_3)_2$), 2.64–2.67 (m, 4H, $COCH_2CH_2N$) 3.2 (s, 2H, $COCH_2$), 6.4 (s, 1H, thiazole), 7.27 (s, 1H, NH), MS for $C_{18}H_{27}N_3OS$, *m/z*: M^+ 333.

4.2.11. N-[4-(1-adamantyl)-thiazol-2-yl]-3-pyrrolidino propionamide (*IV8*)

Yield: 43%; mp 149–150 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.60$.

IR(KBr): $\nu = 3120\ cm^{-1}$ (N–H), $1680\ cm^{-1}$(C=O). ^1H NMR (DMSO-d_6): $\delta = 1.68$–1.97 (m, 15H, adamantane; 4H, pyrrolidine), 2.42–2.68 (m, 8H pyrrolidine, $COCH_2CH_2$), 6.76 (s, 1H, thiazole), MS for $C_{20}H_{29}N_3OS$, *m/z*: M^+ 359.

4.2.12. N-[4-(1-adamantyl)-thiazol-2-yl]-3-morpholino propionamide (*IV9*)

Yield: 77%; mp 185–187 °C (ethanol). TLC: eluent = benzene–ethanol (8:2), $R_f = 0.69$.

IR(KBr): $\nu = 3100\ cm^{-1}$ (N–H), $1680\ cm^{-1}$ (C=O). ^1H NMR (DMSO-d_6): $\delta = 1.67$–1.95 (m, 15H, adamantane), 2.45–2.82 (m, 4H, morpholine and 4H, $COCH_2CH_2$), 3.75–3.92 (m, 4H, morpholine), 6.78 (s, 1H, thiazole). MS for $C_{20}H_{29}N_3O_2S$, *m/z*: M^+ 375.

4.2.13. N-[4-(1-adamantyl)-thiazol-2-yl]-3-(4′-phenylpiperazine)-propionamide (*IV10*)

Yield: 75%; mp 230–232 °C (ethanol). TLC: eluent = benzol–ethanol (8:2), $R_f = 0.64$.

IR(KBr): $\nu = 3080\ cm^{-1}$ (N–H), $1680\ cm^{-1}$ (C=O). ^1H NMR (DMSO-d_6): $\delta = 1.65$–2.00 (m, 15H, adamantane), 2.65 (br s, 4H, piperazine), 3.13 (br s, 4H, piperazine), 3.25–3.44 (m, 4H, $COCH_2CH_2$), 6.67 (9s, 1H, thiazole), 6.73–6.78 (t, 1H, ArH), 6.90–6.93 (d, 2H, ArH. $J = 8.43$ Hz), 7.17–7.22 (t, 2H ArH). MS for $C_{26}H_{34}N_4OS$, *m/z*: M^+ 450.

4.3. Physicochemical studies

4.3.1. Determination of lipophilicity as C log P

Lipophilicity was theoretically calculated as *C* log P values in octanol–water-buffer by ClogP program of Biobyte. [26].

4.4. Biological evaluation

4.4.1. In vivo inhibition of the carrageenin-induced oedema

Oedema was induced in the right hind paw of AKR mice (20–/30 g, 2–/3 months old) by the intradermal injection of 0.05 ml 2% w/v carrageenin in water [11]. These studies were in accordance with recognized care and use of laboratory animals published by the Greek Government 160/1991, based on EU regulations 86/609. All the tested compounds 0.2 mmol/kg bw were suspended in water, with few drops of Tween 80 and ground in a mortar before use and were given intraperitoneally (i.p) at the same time as the carrageenin. The animals were euthanized 3.5 h after carrageenin injection. The experiment was repeated twice for each compound (two groups of six animals). The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight was compared with that in control animals (injected with water) and expressed as a percent inhibition of the oedema (CPE % values Table 1).

Indomethacin in 0.2 mmol/kg (44%) was administered as a standard comparative drug.

4.4.2. In vitro soybean lipoxygenase inhibition

The tested compounds dissolved in DMSO were incubated in different concentrations at room temperature with sodium linoleate (0.1 mM) and 0.2 ml of enzyme solution ($1/3 \times 10^4$ w/v in saline) added in a reaction mixture of 3 ml [7]. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with nordihydroguairetic acid ((NDGA) = 94.4% at 0.1 mM), an appropriate standard inhibitor. The results are summarized in Table 1.

4.4.3. In vitro cycloxygenase inhibition

The COX-1 and COX-2 activities of the compounds were measured using ovine COX-1 and human recombinant COX-2 enzymes included in the ‘‘COX Inhibitor Screening Assay’’ kit provided by Cayman (Cayman Chemical Co., Ann Arbor, MI, USA) [29]. The assay directly measures PGF_{2a} produced by $SnCl_2$ reduction of COX-derived PGH_2. The prostanoids production was quantified via enzyme immunoassay using a broadly specific antibody that binds to all the major prostaglandin compounds.

Estimation of % inhibition (Table 1) was performed at a substrate concentration of 0.1 μM. The compounds were added at the reaction mixture at a final concentration of 200 μM with the exception of compound **IV10** which was tested at a concentration of 20 μM because of solubility problems. Naproxen, used as positive control, was added to the reaction mixture at the same concentration, 200 μM, as the tested compounds (COX-1 inhibition: 40%, COX-2 inhibition 51%).

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