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Evaluation of antioxidant, anti-inflammatory and anti-arthritic activity of new ibuprofen derivatives

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Herein, we present the synthesis and *in-vitro* anti-inflammatory, antioxidant, and anti-arthritic activities of new ibuprofen derivatives. All structures were confirmed by spectral analysis (^{1}H NMR, ^{13}C NMR, UV, IR and HRMS). The lipophilicity was established using reversed-phase thin layer chromatography and *in silico* calculations. The anti-inflammatory and anti-arthritic activities correlated with the lipophilicity of the compounds.

Keywords: Ibuprofen derivatives, anti-inflammatory, H_2O_2 scavenging activity, anti-arthritic, lipophilicity, *in-vitro*.

INTRODUCTION

2-Arylpropanoic acids are an important class of non-steroidal anti-inflammatory drugs (NSAIDs) used for the treatment of pain and inflammation in various diseases [1]. One of the most widely used NSAIDs is ibuprofen or 2- (4-isobutylphenyl) propionic acid, well known for its analgesic, antipyretic and anti-inflammatory properties [2]. However, prolonged use of NSAIDs is known to cause gastrointestinal ulceration and bleeding, as well as nephrotoxicity [3].

Numerous ibuprofen amides and other derivatives have been examined for different biological activities [4]. There are data in the literature on isoquinoline derivatives containing electron-donating substituents such as methoxy groups exhibiting diverse biological activity. It is the presence of methoxy groups that enhances the activity of the commented compounds [5]. That is why we have selected these examples, varying the substituents and all with Ibuprofen residue so that we could assess their impact.

In recent years, many newly synthesized organic compounds show significant anti-inflammatory activity [6, 7]. The last few years, despite the hard research efforts in searching of efficient anti-inflammatory drugs, a set of greatly important fundamental questions remains unresolved [8]. Much research has been focused on studying compounds which are capable of decreasing the inflammation while conserving structural silhouettes [9]. In this regard, attempts to create new non-harmful molecules with anti-inflammatory properties continue.

NSAIDs are the most important therapeutic agents used for the treatment of inflammation. Among them, ibuprofen has been widely used, due to its inhibitory activity against cyclooxygenase (COX) enzymes that catalyze the formation of prostaglandin precursors from arachidonic acid. Inflammatory processes increase the concentration of ROS (reactive oxygen species) in the human body. H_2O_2 is transformed into $^{\cdot}$OH radicals that damage the cell membrane. Thus, one of the aims of the present study was to evaluate the scavenging activity for H_2O_2 of the newly obtained ibuprofen derivatives [10, 11].

MATERIALS AND METHODS

General

All the reagents and chemicals for the synthesis and analysis were purchased from commercial sources (Sigma-Aldrich, S.A., Germany) and used as received. Melting points were determined on a Boetius hot stage apparatus and are non-corrected. The spectral data were recorded on a Bruker Avance II + 600 spectrometer (BAS-IOCCP—Sofia, Sofia, Bulgaria). The ^{1}H-NMR and ^{13}C-NMR spectra were taken in $CDCl_3$ or DMSO at 600 MHz and 150.9 MHz, respectively. Chemical shifts are given in ppm relative and were referenced to TMS (δ = 0.00 ppm) as an internal standard with the coupling constants indicated in Hz. The NMR spectra were taken at room temperature (ac. 295 K). Mass analyses were carried out on a Q Exactive Plus mass spectrometer (ThermoFisher Scientific) equipped with a heated electrospray ionization (HESI-II) probe (ThermoScientific) (Medical University of Sofia). TLC was carried out on precoated 0.2 mm Fluka silica gel 60 plates and Kieselgel 60 F_{254}.

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Synthesis

Synthesis of amides 3. To the solution of ibuprofen (1 mmol) in 25 ml of CH_2Cl_2, DCC (1 mmol) was added. The reaction mixture was stirred at room temperature for 10 min. After the addition of the corresponding amine 1 (1 mmol) the reaction mixture was stirred for 50 min and formation of white crystals was observed. The side product dicyclohexylurea (white crystals) was separated *via* sintered glass filter. The filtrate was washed with diluted hydrochloric acid, saturated solution of Na_2CO_3 and brine. The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure.

2-(4-isobutylphenyl)-N-phenethylpropanamide ***(3a).*** Yield = 97%, MP = 100-104°C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.16 – 7.09 (m, 3H), 7.06 – 7.00 (m, 4H), 6.94 – 6.91 (m, 2H), 5.29 (s, 1H), 3.46 – 3.37 (m, 1H), 3.31 (dt, J = 20.1, 6.9 Hz, 1H), 2.67 – 2.58 (m, 1H), 2.39 (d, J = 7.2 Hz, 2H), 1.86 (dd, J = 12.7, 3.4 Hz, 1H), 1.78 (td, J = 13.6, 6.8 Hz, 1H), 1.65 – 1.61 (m, 1H), 1.41 (d, J = 7.2 Hz, 3H), 0.84 (s, 3H), 0.83 (s, 3H). ^{13}C NMR (151 MHz, $CDCl^3$) δ 174.41, 140.72, 138.84, 138.38, 129.62, 128.75, 128.53, 127.39, 126.37, 46.79, 45.04, 40.69, 35.56, 30.22, 25.58, 22.42, 18.34. UV (Methanol for HPLC), λ_{max} = 232 (ε 1173); λ_{max} = 264 (ε 350). IR(KBr): 701, 748, 760, 779, 856 γ(C_{sp}^2-H); 1382 ($\delta_s CH_3$); 1467, 1549, 1633, 1654 (νC=C); 1672, 1700 ν(C=O); 2854 $\nu_s(CH_2)$; 2932 $\nu_{as}(CH_2)$; 2954, 2968 $\nu_{as}(CH_3)$; 3026, 3069 ν(C_{sp}^2-H); 3258, 3292 ν(N-H). HRMS found for $C_{21}H_{27}NO$: m/z 310.2157 $[M+H]^+$ calcd. m/z 310.2165.

N-(3,4-dimethoxyphenethyl)-2-(4-isobutylphenyl)propanamide ***(3b).*** Yield = 98%, MP = 62-64°C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.06 – 7.01 (m, 4H), 6.64 (dd, J = 8.1, 3.9 Hz, 1H), 6.55 (t, J = 3.6 Hz, 1H), 6.46 (dd, J = 8.1, 2.0 Hz, 1H), 5.32 (t, J = 5.3 Hz, 1H), 3.78 (s, 3H), 3.74 (s, 3H), 3.43 – 3.36 (m, 2H), 3.31 (qd, J = 7.0, 5.9 Hz, 1H), 2.59 (td, J = 6.9, 3.6 Hz, 2H), 1.81 – 1.73 (m, 2H), 1.41 (d, J = 7.2 Hz, 3H), 1.37 (d, J = 6.8 Hz, 1H), 0.83 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.44, 148.94, 147.55, 140.69, 138.40, 131.32, 129.58, 127.37, 120.60, 111.73, 111.15, 55.88, 55.79, 46.78, 45.01, 40.80, 35.16, 30.21, 22.40, 18.48. UV (Methanol for HPLC), λ_{max} = 234 (ε 3707); λ_{max} = 280 (ε 1807). IR(KBr): 698, 724, 766, 785, 805, 851 γ(C_{sp}^2-H); 1381 ($\delta_s CH_3$); 1465, 1559, 1633, 1654 (νC=C); 1672, 1701 ν(C=O); 2859 ν(OCH_2-H); 2931 $\nu_{as}(CH_2)$; 2952, $\nu_{as}(CH_3)$; 2999, 3064 ν(C_{sp}^2-H); 3239, 3294 ν(N-H). HRMS found for $C_{23}H_{31}NO_3$: m/z 370.2370 $[M+H]^+$ calcd. m/z 370.2377.

N-(2,2-diphenylethyl)-2-(4-isobutylphenyl) propanamide ***(3c).*** Yield = 99%, MP = 74-76°C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.19 – 7.15 (m, 4H), 7.11-7.09 (m, 2H), 7.07 – 7.05 (m, 2H), 7.02 – 7.01 (m, 2H), 6.93 – 6.87 (m, 4H), 5.20 (s, 1H), 4.00 (t, J = 8.0 Hz, 1H), 3.84 – 3.78 (m, 1H), 3.65 (ddd, J = 10.4, 8.2, 5.5 Hz, 1H), 3.33 (q, J = 7.2 Hz, 1H), 2.36 (d, J = 7.2 Hz, 2H), 1.80 – 1.72 (m, 1H), 1.36 (d, J = 7.2 Hz, 3H), 0.83 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.42, 141.72, 140.60, 138.06, 129.55, 128.64, 128.03, 127.30, 126.72, 50.37, 46.71, 45.03, 43.83, 30.21, 22.44, 18.20. UV (Methanol for HPLC), λ_{max} = 229 (ε 3834); λ_{max} = 262 (ε 533). IR(KBr): 699, 750, 764, 850, 885 γ(C_{sp}^2-H); 1364, 1384 $\nu_s(CH_3)$; 1457 $\nu_{as}(CH_3)$; 1497, 1559 ν(C=C); 1653 ν(C=O); 2867 $\nu_s(CH_2)$; 2930 $\nu_{as}(CH_2)$; 3087 ν(C_{sp}^2-H); 3337, 3447 ν_{as} (N-H). HRMS found for $C_{27}H_{31}NO$: m/z 386.2468 $[M+H]^+$ calcd. m/z 386.2478.

N-(2,2-bis(4-methoxyphenyl)ethyl)-2-(4-isobutylphenyl)propanamide ***(3d).*** Yield = 94%, Oil, ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.94 (t, J = 2.0 Hz, 1H), 6.93 – 6.92 (m, 1H), 6.91 (d, J = 3.0 Hz, 1H), 6.90 (d, J = 2.7 Hz, 1H), 6.70 (ddd, J = 6.5, 4.8, 3.3 Hz, 1H), 5.20 (t, J = 5.5 Hz, 1H), 3.88 (t, J = 8.0 Hz, 1H), 3.76 – 3.71 (m, 1H), 3.69 (s, 1H), 3.69 (s, 1H), 3.61 – 3.56 (m, 1H), 3.34 (q, J = 7.3 Hz, 1H), 2.36 (d, J = 7.2 Hz, 1H), 1.79 – 1.73 (m, 1H), 1.36 (d, J = 7.4 Hz, 1H), 0.83 (s, 1H), 0.82 (s, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.42, 158.22, 140.58, 138.12, 134.12, 129.50, 128.84, 127.32, 113.96, 113.74, 55.22, 46.71, 45.01, 44.09, 30.21, 22.41, 18.22. UV (Methanol for HPLC), λ_{max} = 234 (ε 4126); λ_{max} = 276 (ε 1800). IR(KBr): 639, 750, 771, 807, 826, 891 γ(C_{sp}^2-H); 1367, 1383 $\nu_s(CH_3)$; 1465 $\nu_{as}(CH_3)$; 1510, 1559 ν(C=C); 1636 ν(C=O); 2851 $\nu_s(CH_2)$; 2929, 2953 $\nu_{as}(CH_2)$; 3318, 3436 ν_{as}(N-H). HRMS found for $C_{29}H_{35}NO_3$: m/z 446.2681 $[M+H]^+$ calcd. m/z 446.2690.

N-(2,2-bis(3,4-dimethoxyphenyl)ethyl)-2-(4-isobutylphenyl)propanamide ***(3e).*** Yield = 96%, MP = 86-88°C, ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.91 (dd, J = 19.6, 8.1 Hz, 4H), 6.68 (dd, J = 9.5, 8.0 Hz, 2H), 6.61 – 6.55 (m, 4H), 5.26 (t, J = 5.6 Hz, 1H), 3.92 (t, J = 8.0 Hz, 1H), 3.78 (s, 6H), 3.76 – 3.73 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.66 – 3.59 (m, 1H), 3.34 (q, J = 7.2 Hz, 1H), 2.35 (d, J = 7.2 Hz, 2H), 1.79 – 1.70 (m, 1H), 1.36 (d, J = 7.2 Hz, 2H), 0.82 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.47, 148.98, 148.96, 147.73, 140.62, 138.06, 134.36, 129.47, 127.26, 119.73, 119.64, 111.07, 118.18, 111.22, 55.84, 55.81, 49.54, 46.70, 44.97, 44.05, 30.20, 25.53, 24.88, 22.38, 18.29. UV (Methanol for HPLC), λ_{max} = 234 (ε 7000); λ_{max} = 264 (ε 4158). IR(KBr): 647, 765, 806, 852 γ(Csp2-

H); 1365 $\nu_s(CH_3)$; 1464, 1541, 1606 ν(C=C); 1649, 1654 ν(C=O); 2835 $\nu(OCH_2\text{-H})$; 2869 $\nu_s(CH_2)$; 2932 $\nu_{as}(CH_2)$; 2964 $\nu_{as}(CH_3)$; 3323, 3370 ν(N-H). HRMS found for $C_{31}H_{39}NO_5$: m/z 506.2891 $[M+H]^+$ calcd. m/z 506.2901.

Biological experiments

Hydrogen peroxide scavenging activity (HPSA). The ability of ibuprofen derivatives to scavenge hydrogen peroxide was assessed according to the method reported by Ruch *et al*. [12] with minor modification. The solution of hydrogen peroxide (43 mM) was prepared in potassium phosphate buffer solution (0.2 M, pH 7.4). Sample analysis was performed as follows: in test tubes were mixed 0.6 ml of hydrogen peroxide (43 mM), 0.1 ml of sample/standard with different concentration (15-1000 μg/ml) and 2.4 ml of potassium phosphate buffer solution. The mixture was stirred and incubated in dark for 10 min at 37 °C. Absorbance was measured at 230 nm with a spectrophotometer (Camspec M508, England) against a blank solution containing phosphate buffer and hydrogen peroxide without the sample. Ascorbic acid (AA) was used as a standard.

$$I, \%\ (\text{HPSA}) = [(A_{blank} - A_{sample}) / A_{blank}] * 100$$

In-vitro anti-inflammatory activity. *In-vitro* analysis of anti-inflammatory activity was carried out by inhibition of albumin denaturation. The analysis was performed according to Sakat's method [13] with minor modification. The solution of 1 % albumin (egg and human) was prepared in distilled water. The tested compounds were dissolved firstly in 1.2 ml of DMF and PBS up to 25 ml, so the final concentration of the stock solution was 1000 μg/ml. Then, a series of working solutions with different concentrations in PBS (20-500 μg/ml) was prepared. The reaction mixture contained 2 ml of test sample/standard of different concentrations and 1 ml of albumin (1%). The mixture was incubated at 37ºC for 15 min and then heated at 70ºC for 15 min in a water bath. After cooling the turbidity was measured at 660 nm with a spectrophotometer (Camspec M508, England). Ibuprofen was used as a standard. Percentage inhibition of albumin denaturation (IAD) was calculated against control. The control sample was albumin with the same concentration dissolved in distilled water.

$$\%\ \text{IAD} = [(A_{control} - A_{sample}) / A_{control}] * 100$$

In-vitro anti-arthritic activity. The analysis was performed according to the method of Sakat [13] with minor modification. The reaction mixture contained 2 ml of 0.06 mg/ml trypsin, 1 ml of Tris–HCl buffer (20 mM, pH 7.4) and 1 ml of test sample/standard (in methanol) of different concentrations (20-500 μg/ml). The mixture was incubated at 37ºC for 5 min. Then, 1 ml of human albumin (4% v/v) was added. The mixture was incubated for an additional 20 min. To the mixture, 2 ml of 70% perchloric acid was added for termination of the reaction. The cloudy suspension was cooled and centrifuged at 5000 rpm for 20 min. The absorbance of the supernatant was measured at 280 nm with a spectrophotometer (Camspec M508, England) against a control solution.

$$\%\ \text{PIA} = [(A_{control} - A_{sample}) / A_{control}] * 100$$

Prediction of anti-inflammatory and anti-arthritic activity. A computerized prediction of biological activity (anti-inflammatory and anti-arthritic) for the obtained compounds was performed using PASS Online program [14].

Physicochemical characterisation

Determination of lipophilicity as R_M values. Determination of lipophilicity of ibuprofen derivatives was performed according to the method reported by Pontiki and Hadjipavlou-Litina [16].

Determination of lipophilicity as clogP. The lipophilicity was evaluated by calculating LogP of the obtained compounds *via* ACD/ChemSketch/ LogP Predictor v.14.08.

Statistical analysis

The presented experimental data were generated in triplicate. Data were expressed as mean ± SD. The level of significance was set at $p<0.05$. The correlation between HPSA, IAD, PIA and lipophilicity was analysed using Pearson's test.

RESULTS AND DISCUSSION

Chemistry

The amides synthesis is very important in the pharmaceutical industry. Different amides are present in around 25% of the top-selling pharmaceutical products and in many other medicinally important compounds [17]. The desired amides can be obtained from wide variety of different precursors by a range of reaction pathways. Three of the most effective methods for synthesis of amides are nucleophilic acyl substitution, partial hydrolysis of nitriles and combination of amines with carboxylic acids in the presence of DCC, EDC or other "dehydrating" reagents. Using *N*,*N*'-dicyclohexylcarbodiimide

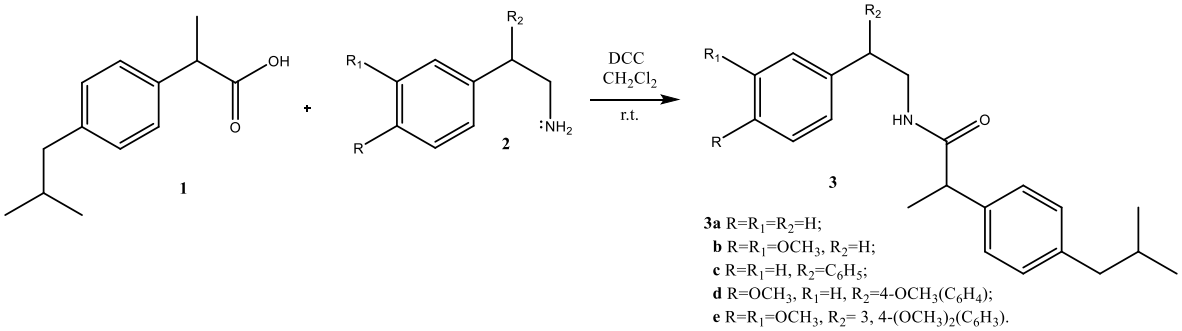
different amides can be formed under very mild conditions (Scheme 1). A convenient method for the preparation of the amides of amines and carboxylic acids is the method described in the literature using *N*,*N*-dicyclohexylcarbodiimide. DCC reacts with the carboxyl group of ibuprofen to produce an activated acylation agent that reacts with the amino group of the other molecule to form an amide bond (Scheme 1). All compounds are characterized for their melting points (MP), ^{1}H and ^{13}C NMR, UV, IR and HRMS spectra.

Biological evaluation

All synthesized ibuprofen derivatives were tested for their *in-vitro* antioxidant, anti-inflammatory and anti-arthritic activity, assessed by HPSA, IAD and PIA. The data from these analyses were compared with the *in-silico* obtained data (Table 1).

H_2O_2 scavenging activity

It has been demonstrated that free radicals play an important role in the pathogenesis of specific diseases [16]. Comparing with ascorbic acid (87.60 μg/ml), the newly obtained ibuprofen derivatives demonstrated lower *in-vitro* antioxidant activity. Despite their lipophilic nature, the synthetic analogue **3c** (195.24 μg/ml) showed the highest antioxidant activity compared to ibuprofen, while the rest of the compounds exhibited weaker antioxidant activity (Table 1, Figure 1). We assume that the high activity of **3c** is due to the obtained reactive OH radicals bound to the benzene nuclei at the free *para*-positions. From the literature is known that OH radicals hydroxylate the most electron-rich phenolic moiety over the benzoic ring, generating mainly ortho and para phenols [15]. Although hydrogen peroxide is not very reactive, it can cause cytotoxicity by generating hydroxyl radicals in the cell. Hydroxyl radicals are the most reactive and are thought to be responsible for some tissue damage caused by inflammation. In living organisms, the superoxide anion radical ($O_2^{-\cdot}$) and H_2O_2 are transformed into $^{\cdot}OH$ radicals and $^{\cdot}O_2$, which are responsible for cell damage. The inflammatory process causes the generation of a superoxide anionic radical at the inflammation site and this is associated with the formation of other oxidizing species such as $^{\cdot}OH$. Scavengers of hydroxyl radicals can increase the synthesis of prostaglandins [16]. Therefore, the removal of H_2O_2 is very important in the prevention of the generation of $^{\cdot}OH$.



Scheme 1. Synthesis of ibuprofen amide derivatives.

Table 1. *In-vitro* and *in-silico* results. The *in-vitro* results are expressed as IC_{50}.

Compound	HPSA μg/ml	$IAD_{Egg\ Alb}$ μg/ml	$IAD_{Human\ Alb}$ μg/ml	PIA μg/ml	R_M	*clogP*	*cAnti-I*	*cAnti-A*
AA	87.60 ± 7.48	–	–	–	–	–	–	–
Ibuprofen	382.62 ± 12.40	69.34 ± 5.59	81.50 ± 4.95	259.82 ± 9.14	1.02 ± 0.024	3.72	0.903	0.573
3a	930.41 ± 79.74	243.52 ± 17.15	200.33 ± 5.59	79.51 ± 3.98	1.46 ± 0.029	4.93	0.402	0.311
3b	542.26 ± 52.03	133.98 ± 0.59	137.61 ± 3.54	85.45 ± 4.68	1.37 ± 0.041	4.67	0.408	0.34
3c	195.24 ± 15.89	133.97 ± 0.69	130.34 ± 0.65	22.85 ± 3.04	1.30 ± 0.040	6.52	0.330	0.264
3d	263.22 ± 17.15	152.47 ± 0.56	157.52 ± 3.77	61.04 ± 4.52	1.44 ± 0.045	6.35	0.342	0.282
3e	603.88 ± 63.48	178.83 ± 13.43	146.41 ± 6.11	49.15 ± 4.14	1.35 ± 0.043	6.00	0.350	0.298

R_M – lipophilicity; cAnti-I – calculated anti-inflammatory activity; cAnti-A – calculated anti-arthritic activity.

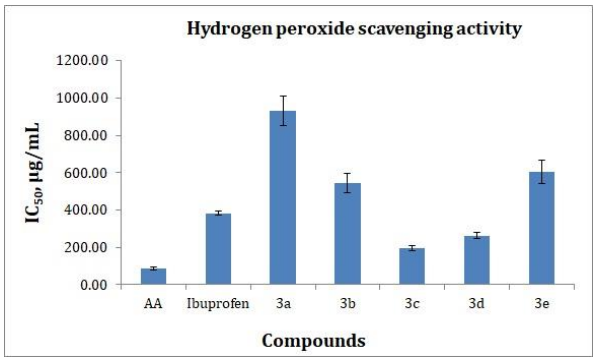


Figure 1. HPSA of ibuprofen derivatives. Ascorbic acid (AA) used as a standard.

Anti-inflammatory activity

Denaturation of proteins is a well-documented cause of inflammation in rheumatoid arthritis. Several anti-inflammatory drugs have shown dose-dependent ability to inhibit thermally induced protein denaturation [13]. The newly obtained ibuprofen amides were screened for anti-inflammatory activity *via* inhibition of albumin denaturation method. For this purpose, we used egg and human albumin.

Anti-arthritic activity

Proteinases have been implicated in arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases. It was previously reported that leukocytes' proteinase plays an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors [13].

Anti-arthritic activity was assessed by PIA. The obtained results present that ibuprofen derivatives show higher anti-arthritic activity compared to ibuprofen (Table 1, Figure 2B). The highest activity was demonstrated by compound **3c** (22.85 µg/ml), which correlated well with the results of the HPSA and IAD tests. Despite the low anti-arthritic activity shown by compound **3c** in the *in-silico* analysis, the experimental data showed the opposite results. The percentages of inhibition of synthesized ibuprofen derivatives are presented in Figure 2A. The IC_{50} values of ibuprofen, estimated as IAD against egg and human albumin, are 69.34 µg/ml and 81.50 µg/ml, respectively (Table 1, Figure 2A). It is therefore used as a standard to compare the anti-inflammatory activity of synthetic ibuprofen analogues. Compounds **3b** and **3c** showed the highest anti-inflammatory activity, followed by **3d** and **3e**. In vitro analysis of ibuprofen derivatives by IAD is essential for the study of new potential anti-inflammatory agents. Obtaining these data may allow creating a model that can reliably predict the anti-inflammatory efficacy of new synthetic analogues of ibuprofen. The studied synthetic analogues showed high lipophilicity, which to some extent affects the anti-inflammatory activity.

Lipophilicity

Lipophilicity is the most regularly appliedparameter used in SAR drug discovery studies. It can be experimentally determined or calculated. Lipophilicity has been correlated to permeability, solubility, increases in target potency and toxicity. We determined the lipophilicity by reverse-phase thin layer chromatography (RPTLC) method as R_M values and compared them with the corresponding theoretically calculated c*LogP* values in *n*-octanol-buffer using ACD/ChemSketch/ LogP Predictor v.14.08. This is considered to be a reliable, fast, and convenient method for expressing lipophilicity. Aside from the essential role of lipophilicity for the kinetics of biologically active compounds, antioxidants of hydrophilic or lipophilic character are both needed to act as radical scavengers in the aqueous phase or as chain-breaking antioxidants in biological membranes [16].

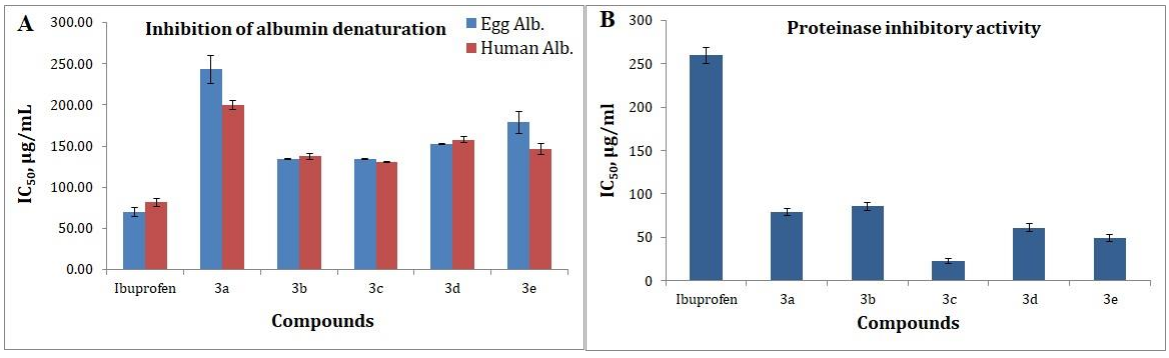


Figure 2. (**A**) *In-vitro* anti-inflammatory activity of ibuprofen derivatives. (**B**) *In-vitro* anti-arthritic activity of ibuprofen derivatives. Ibuprofen is used as a standard.

Table 2. Correlation between antioxidant, anti-inflammatory and antiarthritic activities with lipophilicity, expressed as r.

	HPSA	$IAD_{Egg\ Alb}$	$IAD_{Human\ Alb}$	*PIA*	R_M
HPSA	1	0.8579	0.7643	0.6568	0.5030
$IAD_{Egg\ Alb}$		1	0.9355	0.3781	0.6326
$IAD_{Human\ Alb}$			1	0.5238	0.8360
PIA				1	0.6928
R_M					1

The obtained results allowed us to establish a correlation between *in-vitro* biological activity and lipophilicity, expressed as r (Table 2). Good correlation dependence of HPSA with IAD and PIA ($r > 0.6$) shows that the synthetic analogues of ibuprofen possess complex activity, i.e. antioxidant, anti-inflammatory and anti-arthritic. In addition, we can assume that the results of anti-inflammatory and anti-arthritic activities show that they are influenced by lipophilicity (Table 2).

CONCLUSION

In conclusion, we have successfully synthesized a sequence of amides containing ibuprofen fragment in their scaffold. The compounds were biologically evaluated *in-vitro* and *in-silico* for their anti-inflammatory, anti-arthritic and antioxidant activities. Lipophilicity as R_M values and Log*P* as a fundamental property were also evaluated. Good correlation dependence of HPSA with IAD and PIA ($r > 0.6$) was observed. The newly synthesized compounds showed low antioxidant and anti-inflammatory and high anti-arthritic activity.

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