

IN VITRO AND IN SILICO BIOLOGICAL ACTIVITY OF NEW IBUPROFEN HYBRIDS

Stanimir P. Manolov, Iliyan I. Ivanov*, Dimitar G. Bojilov

University of Plovdiv, Faculty of Chemistry, 24 Tsar Assen Str., Plovdiv 4000, Bulgaria

Abstract

Herein we report the biological evaluation of new hybrids of 1,2,3,4-tetrahydroisoquinoline with ibuprofen. The hybrids are screened for their *in vitro* inhibition of albumin denaturation, antioxidant, and antitryptic activity. The tested compounds have shown significant results. The lipophilicity was established using both reversed-phase thin layer chromatography and *in silico* calculations.

Keywords: *in vitro*, *in silico*, ibuprofen, NSAIDs, anti-inflammatory, antitryptic, antioxidant activity

1. INTRODUCTION

Drug discovery is very challenging for scientific and economic reasons. The scientists involved in the process face a lot of difficulties in the way of the creation of new drugs. It's very important to know how to limit investments and health costs at a reasonable level in this process. Analysis of the drugs market shows that the market is dominated by small molecules, given that over 80% of new drug developments are currently based on small and hybrid molecules [1]. The efficacy of a medicine is controlled by different parameters including distribution, absorption, mechanism of interaction between the drug and its cellular target, metabolism, excretion, and toxicity. The tailor-made design of the molecules is essential for those parameters to be optimized. Hybrid molecules, obtained by the combination of structural features of two fragments with a different activity, are probably the most useful chemical entities to work upon for developing modified structures with much improved properties in the area of pharmaceutical science [2].

In this article, we report *in vitro* and *in silico* biological assessment of newly synthesized by us hybrids [3]. The main structure of the obtained hybrids is presented in Figure 1. They all have ibuprofen fragment attached to 1,2,3,4-tetrahydroisoquinoline skeleton (Fig.1).

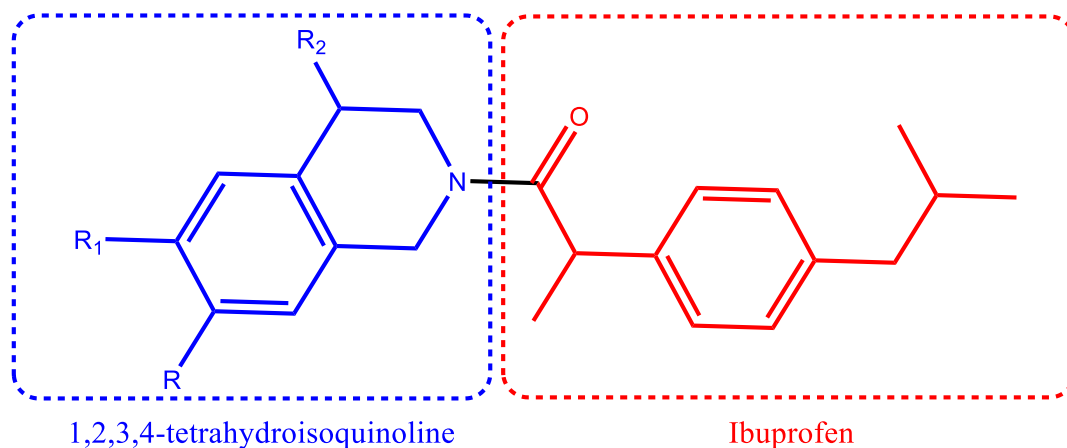


Fig. 1. Novel hybrids of 1,2,3,4-tetrahydroisoquinoline and ibuprofen.

The isoquinoline structural motifs are widely distributed in nature. These alkaloids and their synthetic analogs have been studied since the end of the 19th century, and have been reported to display a wide spectrum of biological and pharmacological activities [4].

2- (*p*-Isobutylphenyl) propionic acid or ibuprofen is a non-steroidal anti-inflammatory drug (NSAIDs) with a strong antipyretic, anti-inflammatory, and analgesic effect. Ibuprofen is a drug used to treat inflammatory and painful diseases, such as rheumatoid arthritis, soft tissue damage, osteoarthritis, etc. [5].

In this regard, we are interested in assessing the *in vitro* and *in silico* antioxidant, anti-inflammatory, and anti-arthritic activity of the newly obtained hybrid molecules (**H1-H5**). Lipophilicity as the most regularly applied parameter used in structure-activity relationship (SAR) drug discovery studies have also been evaluated.

2. RESULTS AND DISCUSSION

Biological evaluation

All synthesized ibuprofen derivatives were tested for their *in vitro* inhibition of albumin denaturation (IAD), antioxidant and antitryptic activity (ATA). The obtained *in vitro* results were compared to the predicted *in silico* ones. The results are presented in Table 1.

Compounds	$IC_{50} \pm SD, \mu\text{g/mL}$			$R_M \pm SD$	Pa	
	HPSA	IAD	ATA		cAnti-I	cAnti-A
AA	24,84 $\pm$ 0,35	-	-	-	-	-
Qrc	69,25 $\pm$ 1,82	-	-	-	-	-
Ibu	-	81,50 $\pm$ 4,95	259,82 $\pm$ 9,14	1,11 $\pm$ 0,010	0,903	0,573
H1	93,18 $\pm$ 1,69	106,22 $\pm$ 0,27	389,91 $\pm$ 5,33	1,19 $\pm$ 0,008	0,505	0,054
H2	76,00 $\pm$ 1,36	84,09 $\pm$ 1,09	199,90 $\pm$ 2,16	1,31 $\pm$ 0,009	0,504	0,039
H3	252,91 $\pm$ 3,96	187,20 $\pm$ 3,38	661,02 $\pm$ 8,41	1,33 $\pm$ 0,006	0,439	-
H4	103,53 $\pm$ 2,55	166,37 $\pm$ 9,50	512,93 $\pm$ 9,90	1,46 $\pm$ 0,006	0,438	-
H5	156,37 $\pm$ 1,28	67,09 $\pm$ 1,25	309,20 $\pm$ 6,85	1,29 $\pm$ 0,012	0,444	-

Table 1. *In vitro* and *in silico* biological activity results. Hydrogen peroxide scavenging activity (HPSA), inhibition albumin denaturation (IAD), and antitryptic activity (ATA) were expressed as IC_{50} ($\mu\text{g/mL}$). Ascorbic acid (AA), quercetin (Qrc) and ibuprofen (Ibu) were used as standards.

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

Hydrogen peroxide scavenging activity

It has been demonstrated that free radicals play an important role in the pathogenesis of specific diseases and aging [6, 7]. To compare the antioxidant activity of ibuprofen derivatives, we used ascorbic acid and quercetin as standards that have proven antioxidant properties. The obtained ibuprofen hybrids were screened *in vitro* for scavenging activity utilizing hydrogen peroxide. The antioxidant activity values of the ibuprofen hybrids vary from 76 to 252.91 $\mu\text{g/mL}$. Hydrogen peroxide is an oxidant and is formed continuously in living tissues as a result of a number of metabolic processes. The scavenging of hydrogen peroxide is a very important step that prevents the reaction between iron ions and H_2O_2 , which generate extremely reactive oxygen species $\cdot\text{OH}$ radicals [8]. Compared to ascorbic acid (24.84 $\mu\text{g/mL}$), the obtained ibuprofen derivatives demonstrated lower *in vitro* antioxidant activity. But compared with quercetin, compound **H2** shows significant antioxidant activity close to it. Compound **H1** (93.18 $\mu\text{g/mL}$) and **H2** (76.00 $\mu\text{g/mL}$) demonstrate higher antioxidant activity compared to the rest of ibuprofen derivatives (Table 1, Figure 2).

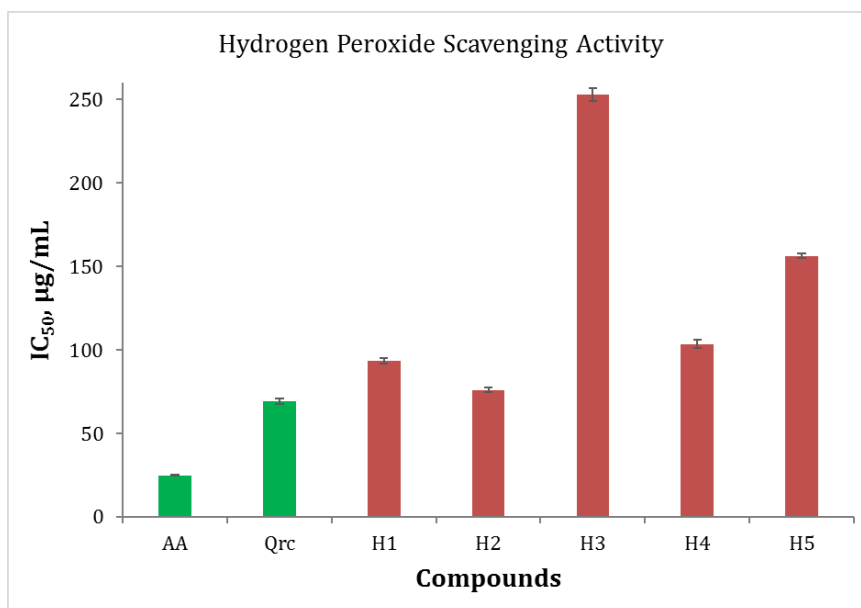


Fig. 2. HPSA of ibuprofen hybrids. Ascorbic acid (AA) and quercetin (Qrc) were used as standards. The results of antioxidant activity were expressed as IC₅₀ (µg/mL).

Although hydrogen peroxide is not very reactive, it can cause cytotoxicity by generating hydroxyl radicals in the cell [9]. Hydroxy 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 [10]. Therefore, the removal of H_2O_2 is very important in the prevention of the generation of $\cdot OH$.

Inhibition of albumin denaturation

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, fluid extravasation, cell migration, tissue breakdown, and repair [11]. 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 [12]. The obtained ibuprofen derivatives were screened for inhibition of albumin denaturation. This method provides information on the extent to which albumin is protected from denaturation when heated [8]. For this purpose, we have used human albumin. The percentage of inhibition of ibuprofen hybrids is presented in Figure 4. The results of the study are presented as IC₅₀. These newly obtained ibuprofen derivatives are considered hybrid molecules as they are composed of two structural domains owning different biological functions. They contain two pharmacophores - pharmacophore I - 1,2,3,4-tetrahydroisoquinoline and ibuprofen is considered as pharmacophores II (Figure 3).

In this study, we have investigated the effect of substituents in the structure of pharmacophore I, while pharmacophore II did not change structurally. As ibuprofen has proven properties and presence in the structure of new molecules, we have decided to use it as a benchmark to compare the activities of new 1,2,3,4-tetrahydroisoquinoline-ibuprofen hybrid molecules. The IC₅₀ values of ibuprofen, estimated as IAD is 81.50 µg/mL (Table 1, Figure 4). The obtained results show the IC₅₀ values of ibuprofen hybrid molecules are in the range between 67.09 µg/mL and 187.20 µg/mL (Table 1, Figure 4). Hybrid molecule **H5** is characterized by the highest degree of albumin protection from denaturation and compound **H3** is the least active.

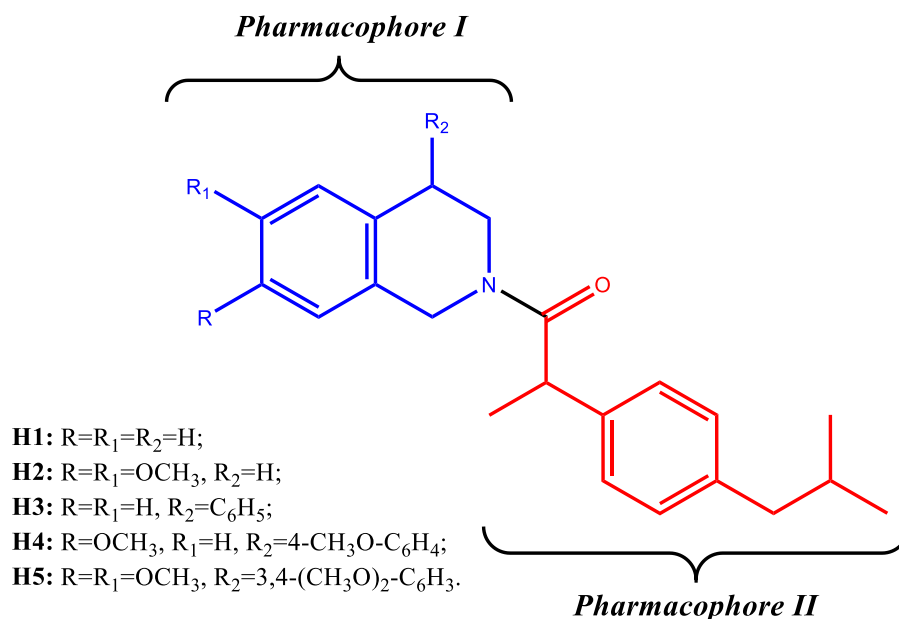


Fig. 3. Structure of ibuprofen hybrids formed by two pharmacophores. Pharmacophore I (blue) - has the structure of 1,2,3,4-tetrahydroisoquinoline, Pharmacophore II (red) - the structure of ibuprofen.

Analyzing the obtained results, it is seen that the presence of methoxy groups in pharmacophore I lead to an increase in the degree of protection against albumin denaturation compared to ibuprofen (Figure 4). We have also noted that the activity of hybrid molecules depends on the number of methoxy groups. As the number of methoxy groups in the structure of 1,2,3,4-tetrahydroisoquinoline-ibuprofen hybrid molecules increases, so does the stabilization of the albumin molecule. We found out this effect in our previous studies [8, 13]. The activity of hybrid molecule **H2** and ibuprofen molecule did not differ statistically due to the replacement of hydrogen atoms with methoxy groups at positions 6 and 7. Therefore **H2** is more active than **H1**. In addition, at position 4 in pharmacophore I, the hydrogen atom was replaced by aryl substituents (R_2). In R_2 we varied the number of methoxy groups - with one, two, and without methoxy groups. Analyzing the results of IAD experiments shows that the activity depends on the number of methoxy groups. As the number of methoxy groups increases, the IC_{50} values for IAD decrease, and the degree of albumin protection from denaturation increases. Therefore, the hybrid molecule **H5** shows higher activity than **H3** and **H4**. Assessing the results, we can conclude that hybrid molecules **H2** and **H5** will have a beneficial effect on human health, which makes them reliable and effective anti-inflammatory agents.

Furthermore, IAD analysis reveals that lipophilicity is a major physicochemical parameter. The study of hybrid molecules shows average lipophilicity (R_M) from 1.19 to 1.46, which to some extent affects the albumin protection. The R_M values depend on the retention factor parameter (R_f). R_M is a function of $1/R_f$, which means that R_M is inversely proportional to R_f . The hybrid molecules with low values of R_f (**H3**, **H4**) are characterized by a low degree of albumin protection from denaturation. While the R_M values of the hybrid molecules **H2** and **H5** did not differ statistically, they showed the highest activity. (Table 1, Figure 4). The *in vitro* analysis of new 1,2,3,4-tetrahydroisoquinoline-ibuprofen hybrid molecules by IAD is essential for the study of new potential anti-inflammatory agents.

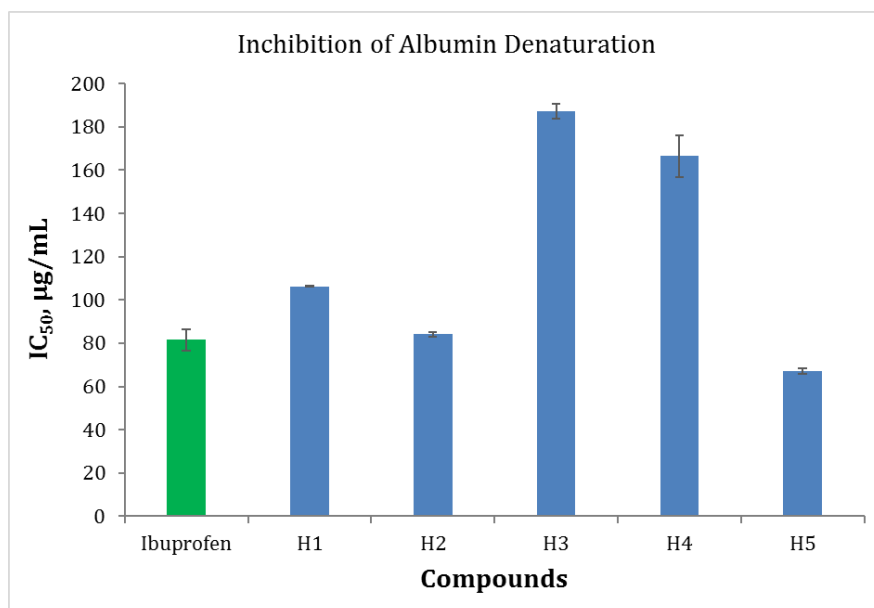


Fig. 4. The inhibition of albumin denaturation activity of ibuprofen derivatives. Ibuprofen is used as standard. The results were expressed as IC₅₀ (µg/mL).

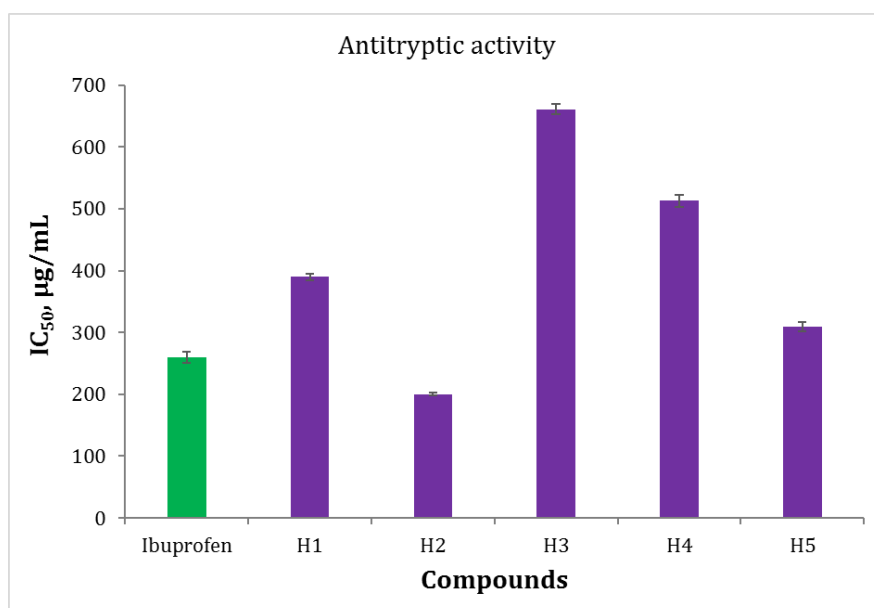


Fig. 5. The antitryptic activity of ibuprofen derivatives. Ibuprofen is used as standard. The results are expressed as IC₅₀ (µg/mL).

Antitryptic 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 a significant level of protection was provided by proteinase inhibitors [12, 14]. *In vitro* anti-arthritic activity was assessed as antitryptic activity [14]. The obtained hybrid molecules were screened for ATA (Table 1, Figure 5). The IC₅₀ results for the ATA range from 199.90 µg/mL to 661.02 µg/mL. The highest activity was demonstrated by compound **H2** (199.90 µg/mL). Hence this compound turns out to be the most promising.

ATA analysis results show the same dependence as for the IAD. Inhibition of trypsin is influenced by the position of both the methoxy groups and the aryl substituents at positions 6, 7 and 4, respectively. The presence of methoxy groups in the structures of the ibuprofen hybrid molecules leads to an increase in their activity. The low activity of **H3** (661.02 µg/mL) is due to the lack of methoxy groups in the structure. The decreasing order of their antitryptic activity is as follows **H2** > **H5** > **H1** > **H4** > **H3** (Table 1, Figure 5).

Lipophilicity

Lipophilicity is the most regularly applied parameter used in SAR drug discovery studies. It can be experimentally determined or calculated. Lipophilicity has been correlated to permeability, solubility, and increases in target potency and toxicity. We determined the lipophilicity by reverse phase thin layer chromatography (RPTLC) method as R_M values. This is considered to be a reliable, fast, and convenient method for expressing lipophilicity [15]. 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 [10].

In the present work, we have investigated the antioxidant and *in vitro* biological activity of new 1,2,3,4-tetrahydroisoquinoline-ibuprofen hybrid molecules. Lipophilicity proved to be an important factor in their activity. Although hybrid molecules are lipophilic, some of them call for significant antioxidant activity (**H1**, **H2**, and **H4**) and others low (**H3** and **H5**). It should be noted that antioxidant activity is not a criterion for the biological activity of the studied hybrid molecules. However, the use of lipophilic antioxidants is necessary to neutralize harmful radicals in cell membranes [10].

	HPSA	IAD	ATA	R_M	cAnti-I	cAnti-A
HPSA	1	0,5346	0,7285	0,0651	-0,6578	-0,6284
IAD		1	0,9286	0,5330	-0,5231	-0,4319
ATA			1	0,3453	-0,6427	-0,5210
R_M				1	-0,6481	-0,6896
cAnti-I					1	0,9790
cAnti-A						1

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

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 ATA and IAD with ATA ($r > 0.7$) shows that the hybrid molecules 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).

In general, the *in vitro* studies results show that the 1,2,3,4-tetrahydroisoquinoline-ibuprofen hybrid molecules exhibit IAD and ATA. From both experiments, we derive important information about the properties of potential new drugs, and both experiments are related to preserving the integrity of the albumin molecule. HSA is known to have two major binding sites for drugs: Sudlow sites I and II. According to Sudlow's nomenclature, bulky heterocyclic molecules bind to Sudlow's site I (located in subdomain IIA), whereas Sudlow's site II (located in subdomain IIIA) is preferred by aromatic carboxylates with an extended conformation (Fig.6) [16].

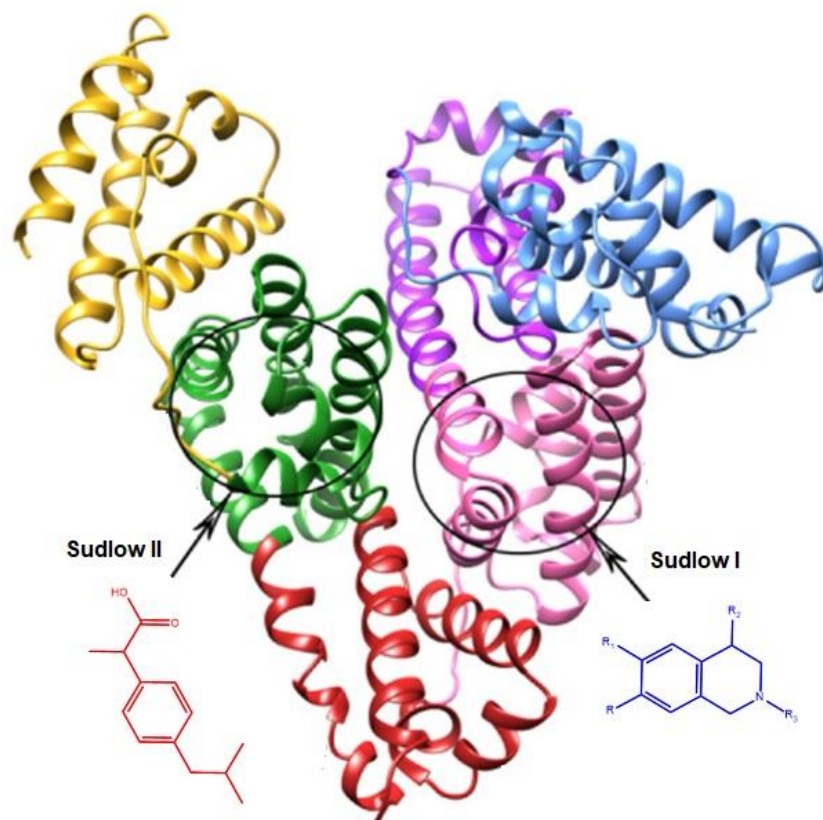


Fig. 6. Human serum albumin binding sites Sudlow I and Sudlow II.

Ibuprofen has been shown to have good anti-inflammatory activity. But it is also known that 1,2,3,4-tetrahydroisoquinoline alkaloids have biological effects such as anti-HIV and anti-tumor [17-19]. Therefore, we combined the two biologically active compounds in a new hybrid molecule. From all designed and carried experiments, we found out that the new hybrid molecules exhibit IAD and ATA, and two of them (**H2** and **H5**) show significantly higher activity.

3. EXPERIMENTAL SECTION

Chemicals and Reagents

Chromatographic grade methanol for HPLC analyses was used (VWR, Austria). Water for HPLC was prepared with a Millipore purifier (Millipore, USA). Ibuprofen, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium chloride, potassium chloride, hydrogen peroxide, ascorbic acid, trypsin, Tris-HCl buffer, and perchloric acid were purchased from Sigma-Aldrich. Human albumin 20 % - BB, 200g/l was purchased from BB-NCIPD Ltd., Bulgaria. Chromatographic plates Kieselgel 60 F₂₅₄ were purchased from Merck (Germany).

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 [20] with minor modification as described by Manolov et al. [8]. 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 hydrogen peroxide (43 mM), 1 ml sample/standard with different concentrations (20-1000 µg/ml) and 2.4 ml potassium phosphate buffer solution. The mixture was stirred and incubated in dark for 10 minutes 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 and quercetin were used as standards. The percentage HPSA of the samples was evaluated by comparing with a blank sample and calculated using the following formula:

$$I, \%(HPSA) = \left[\frac{A_{blank} - (A_{TS} - A_{CS})}{A_{blank}} \right] * 100$$

Where A_{blank} is the absorbance of the blank sample (phosphate buffer and hydrogen peroxide), A_{CS} is the absorbance of the control sample (test sample + phosphate buffer) and A_{TS} is the absorbance of the test sample (test sample + phosphate buffer + hydrogen peroxide). The mean IC_{50} value was estimated based on three replicates by means of interpolating the graphical dependence of scavenging hydrogen peroxide on concentration.

Inhibition of albumin denaturation (IAD)

In vitro analysis of anti-inflammatory activity was assessed as inhibition of albumin denaturation (IAD). The analysis was performed according to the Sakat method [21] and Manolov et al. [8] with minor modifications. The experiment was performed with human albumin. The solution of albumin (1 %) was prepared in distilled water. The tested samples/standards were dissolved firstly in methanol so the final concentration of the stock solution is 1000 µg/ml. Then a series of working solutions with different concentrations (20-500 µg/ml) in PBS were prepared. The reaction mixture was containing a 2 ml test sample/standard of different concentrations and 1 ml albumin (1 %). The mixture was incubated at 37°C for 15 min and then heated at 70°C for 15 min in a water bath. After cooling the turbidity was measured at 660 nm with a spectrophotometer (Camspec M508, England). The experiment was performed three times. Percentage inhibition of albumin denaturation (IAD) was calculated against control. The control sample is albumin with the same concentration dissolved in distilled water.

$$\%IAD = \left[\frac{A_{control} - A_{sample}}{A_{control}} \right] * 100$$

Antitryptic activity (ATA)

The analysis was performed according to the method of Oyedapo and Femurewa [14] with minor modification as described by Manolov et al. [8]. The reaction mixture was containing 2 ml 0.06 mg/ml trypsin, 1 ml Tris-HCl buffer (20 mM, pH 7.4) and 1 ml test sample/standard (in methanol) of different concentrations (20-1000 µg/ml). The mixture was incubated at 37°C 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 minutes. The absorbance of the supernatant was measured at 280 nm with a spectrophotometer (Camspec M508, England) against control solution. The control solution was sample/standard in methanol with different concentrations. Ibuprofen was used as standard. The analysis was performed three times. The percentage of antitryptic activity (ATA) of the samples was evaluated by comparing with a blank sample. The blank sample is prepared as the test sample but with a small exception - perchloric acid is added before albumin.

$$\%ATA = \left[\frac{A_{blank} - (A_{TS} - A_{CS})}{A_{blank}} \right] * 100$$

Where A_{blank} is the absorbance of the blank sample, A_{CS} is the absorbance of the control solution (test sample in different concentrations) and A_{TS} is the absorbance of the test samples. The mean IC_{50} values were estimated by means of interpolating the graphical dependence of ATA on concentration.

Physicochemical characterisation

Determination of lipophilicity as R_M values.

Determination of lipophilicity of ibuprofen derivatives was estimated according to the method reported by Pontiki and Hadjipavlou-Litina [10]

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 [22, 23].

Statistical Analysis

All the analyses were made in triplicates. Data were expressed as mean $\pm$ SD. The level of significance was set at $p < 0.05$.

4. CONCLUSIONS

In conclusion, the newly obtained 1,2,3,4-tetrahydroisoquinoline-ibuprofen hybrid molecules were biologically evaluated *in vitro* and *in silico* for their antioxidant, ATA, and IAD activities. Lipophilicity as R_M values as a fundamental property has also been evaluated. Hybrids **H2** and **H5** show significantly high results for all activities.

ACKNOWLEDGMENTS

We acknowledge financial support from the National Science Fund of the Bulgarian Ministry of Education and Science (Grant number KII-06-M29/1 – 2018). Dimitar G. Bojilov is thankful to the National Program of Ministry of Education and Science “Young Scientists and Postdoctoral Students” (PMC 577/2018) for financial support.

REFERENCES

1. Meunier, B, 2008, ‘Hybrid molecules with a dual mode of action: dream or reality’, *Accounts of chemical research*, vol. 41, pp. 69-77.
2. Shaveta, Mishra, S, Singh, P, 2016, ‘Hybrid molecules: The privileged scaffolds for various pharmaceuticals’, *European Journal of Medicinal Chemistry*, vol. 124, pp. 500-536.
3. Manolov, S, Ivanov, I, Bojilov, D, 2021, ‘Green synthesis of new hybrid molecules of ibuprofen and 1,2,3,4-tetrahydroisoquinoline’, *Materials, Methods and Technologies*, vol. 15, in press.
4. Huang, Zh, Meng, Y, Wu, Y, Song, Ch, Chang, J, 2021, ‘Synthesis of isoindolo [1,2-a]isoquinoline and isoindolo [2,1-a]quinoline derivatives via trifluoroacetic acid-mediated cascade reactions’, *Tetrahedron*, pp. 132280, In press.
5. Uzunov, P 1994, "Handbook of Medicines", *Medicine and Physical Education*, Sofia.
6. Kadhum, A, Al-Amiery, A, Musa, Y, Mohamad, B, 2011, ‘The antioxidant activity of new coumarin derivatives’, *International Journal of Molecular Sciences*, vol. 12, pp. 5747–5761.
7. Naama, J, Alwan, G, Obayes, H, Al-Amiery, A, Al-Temimi, A, Kadhum, A, Mohamad, A, 2013, ‘Curcuminoids as antioxidants and theoretical study of stability of curcumin isomers in gaseous state’, *Research on Chemical Intermediates*, vol. 39, pp. 4047–4059.
8. Manolov, S, Ivanov, I, Bojilov, D, 2021, ‘Microwave-assisted synthesis of 1,2,3,4-tetrahydroisoquinoline sulfonamide derivatives and their biological evaluation’, *Journal of the Serbian Chemical Society*, vol. 86, pp. 139–151.

9. Ebrahimzadeh, M, Nabavi, S, Nabavi, S, Bahramian, F, Bekhradnia, A, 2010, 'Antioxidant and free radical scavenging activity of *H. officinalis* L. Var. *angustifolius*, *V. odorata*, *B. hyrcana* and *C. speciosum*', *Pakistan Journal of Pharmaceutical Sciences*, vol. 23, pp. 29–34.
10. Pontiki, E, Hadjipavlou-Litina, D, 2007, 'Synthesis and pharmacochemical evaluation of novel aryl-acetic acid inhibitors of lipoxygenase, antioxidants, and anti-inflammatory agents', *Bioorganic & Medicinal Chemistry*, vol. 15, pp. 5819–5827.
11. Vane, J, Botting, R, 1995, 'New insights into the mode of action of anti-inflammatory drugs', *Inflammation Research*, vol. 44, pp. 1–10.
12. Jayashree, V, Bagyalakshmi, S, Manjula Devi, K, Richard Daniel, D, 2016, 'In-vitro anti-inflammatory activity of 4-benzylpiperidine', *Asian Journal of Pharmaceutical and Clinical Research*, vol. 9, pp. 108–110.
13. Manolov, S, Ivanov, I, Bojilov, D, Yuliyana Voinikov, 2021, 'Evaluation of antioxidant, anti-inflammatory and anti-arthritis activity of new ibuprofen derivatives', *Bulgarian Chemical Communications*, vol. 53, pp. 66–71.
14. Oyedapo, O, Famurewa, A, 1995, 'Antiprotease and membrane stabilizing activities of extracts of *fagara zanthoxyloides*, *olax subscorpioides* and *tetrapleura tetraptera*', *International Journal of Pharmacognosy*, vol. 33, pp. 65–69.
15. Hansch, C, Leo, D, 1995, Hoekman, *Exploring QSAR: hydrophobic, electronic, and steric constants*, American Chemical Society, Washington, DC.
16. Fasano, M, Curry, S, Terreno, E, Galliano, M, Fanali, G, Narciso, P, Ascenzi, P, 2005, 'The extraordinary ligand binding properties of human serum albumin', *IUBMB Life*, vol. 57, pp. 787–796.
17. Zhang, H, Zembower, D, Chen, Z, 1997, 'Structural analogues of the michellamine anti-HIV agents. Importance of the tetrahydroisoquinoline rings for biological activity', *Bioorganic & Medicinal Chemistry Letters*, vol. 7, pp. 2687–2690.
18. Scott, J, Williams, R, 2002, 'Chemistry and Biology of the Tetrahydroisoquinoline Antitumor Antibiotics', *Chemical Reviews*, vol. 102, pp. 1669–1730.
19. Lane, J, Estevez, A, Mortara, K, Callan, O, Spencer, J, Williams, R, 2006, 'Antitumor activity of tetrahydroisoquinoline analogues 3-epi-jorumycin and 3-epi-renieramycin G', *Bioorganic & Medicinal Chemistry Letters*, vol.16, pp. 3180–3183.
20. Ruch, R, Cheng, S, Klaunig, J, 1989, 'Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea', *Carcinogenesis*, vol. 10, pp. 1003–1008.
21. Sakat, S, Juvekar, A, Gambhire, M, 2010, 'In-vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* linn', *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 2, pp. 146–155.
22. Sadym, A, Lagunin, A, Filimonov, D, Poroikov, V, 2003, 'Prediction of biological activity spectra via the Internet', *SAR and QSAR in Environmental Research*, vol. 14, pp. 339–347.
23. Filimonov, D, Lagunin, A, Glorizova, T, Rudik, A, Druzhilovskii, D, Pogodin, P, Poroikov, V, 2014, 'Prediction of the Biological Activity Spectra of Organic Compounds Using the Pass Online Web Resource', *Chemistry of Heterocyclic Compounds*, vol. 50, pp. 444–457.