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SYNTHESIS AND EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY PROPERTIES OF NOVEL IBUPROFEN ANALOGS

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Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to ameliorate the symptoms of inflammation and pain, particularly those associated with rheumatoid arthritis. Chronic use of these drugs including ibuprofen may elicit appreciable gastrointestinal (GI) toxicity. In order to synthesize novel analgesic and anti-inflammatory agents with reduced ulcerogenic effects, carboxylic acid of ibuprofen has been modified with respect to various heterocyclic amide groups, which is the most active area of research in this family. In this article, synthesis of a series of hybrid molecules containing important pharmacophore of ibuprofen and substituted benzothiazoles are described. All the synthesized compounds (**I – V**) were tested for their analgesic and anti-inflammatory properties on mice, in comparison to standard (ibuprofen) and control (saline) groups. All the synthesized compounds exhibited significant analgesic and anti-inflammatory activities when compared to both standard drug and control. Findings indicated that addition of substituted amino benzothiazoles (especially methyl, bromine, and nitro groups) to ibuprofen moiety as the main pharmacophore, is a desirable strategy for reduction of pain and inflammation which may lead to the production of new drugs with higher activities compared to ibuprofen.

Keywords: non-steroidal anti-inflammatory drugs, ibuprofen, anti-inflammatory, analgesic, substituted benzothiazoles.

INTRODUCTION

Ibuprofen (2-*p*-isobutylphenylpropionic acid) belongs to a class of non-steroidal anti-inflammatory drugs (NSAIDs) which are used to reduce the pain and for the treatment of degenerative inflammatory joint diseases and rheumatic disorders[1].

However, their therapeutic use is often limited by common side effects, such as gastrointestinal (GI) hemorrhage, ulceration, bleeding, and nephrotoxicity. In spite of abundance of NSAIDs in the market, the search continues to develop new drugs that have potent analgesic and anti-inflammatory activities with minimum side effects [2, 3].

The GI damage from NSAIDs is generally attributed to two factors, local irritation by the carboxylic acid moiety, common to most NSAIDs (topical effect), and decreased tissue prostaglandin production, which undermines the physiological role of cytoprotective prostaglandins in maintaining the GI health and homeostasis [4].

The pharmacological activity of NSAIDs is related to the suppression of prostaglandin (PG) biosynthesis from arachidonic acid by inhibiting cyclooxygenase (COX) enzymes [3].

PGs are well known to be the mediators of inflammation, pain, and swelling. They are produced by the action of COX enzymes on arachidonic acid. In fact, NSAIDs block the formation of PGs and produce analgesic, antipyretic and anti-inflammatory effects.COX enzyme exists in two isoforms: COX-1 (constitutive) and COX-2 (inducible). COX-1 is constitutively expressed and provides cytoprotection in the GI tract while COX-2 is inducible and mediates inflammation. The traditional NSAIDs (containing free carboxylic acid group) such as ibuprofen show greater selectivity for COX-1 than COX-2, therefore produce more GI toxicity. Thus, the development of new NSAIDs without these side effects has long been awaited [5].

Previous studies described that converting carboxylic acid containing NSAIDs into gastroprotective amide groups

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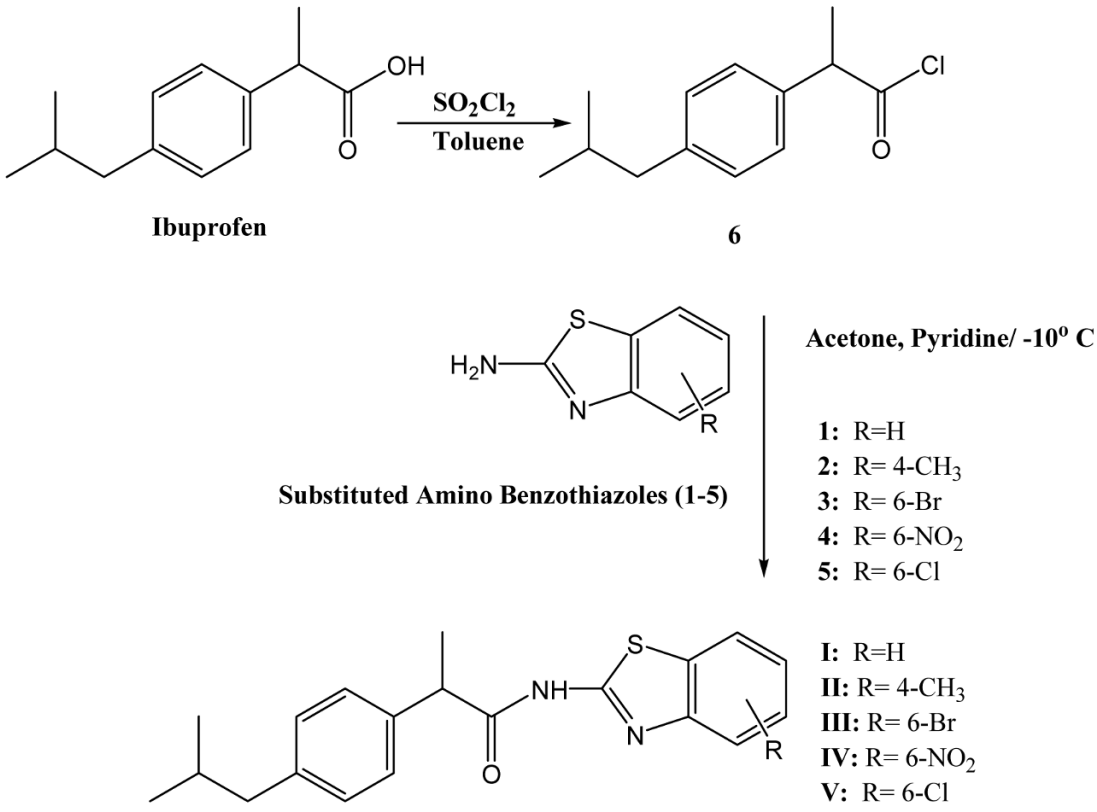

Scheme 1. Preparation route of new substituted benzothiazoles **I – V.**

could cause masking the acidic moiety of these drugs and may shift the enzyme selectivity from COX-1 to COX-2 and also increased anti-inflammatory activity with reduced ulcerogenic and GI effects [1, 4, 6].

Selective COX-2 inhibitors with better safety profile have been marketed as a new generation of NSAIDs. They elicit less or no GI damage and bleeding compared with conventional NSAIDs. As widely reported in literature, the selective COX-2 inhibitors also cause significant adverse effects in the renal and cardiovascular systems, possibly more serious than those caused by conventional NSAIDs. Their side effects as well as therapeutic actions are related to their ability to inhibit cyclooxygenase enzymes involved in the first step of the arachidonic acid cascade [7].

In the present work, new analogs (**I – V**) of ibuprofen with substituted benzothiazoles (**1 – 5**) possessing significant analgesic and anti-inflammatory activities [8 – 11] were synthesized**.** The analgesic and anti-inflammatory effects of new compounds **I – V** were evaluated in tail immersion (as a model of acute thermal pain) [12], formalin (as a model of acute chemical and chronic pain) [13], and paw edema [14] tests on mice and the results were compared to those in ibuprofen (standard) and control (saline) groups.

EXPERIMENTAL

General

All chemicals, including ibuprofen and substituted 2-aminobenzothiazoles1 – 5were purchased from Merck Chemical Company. Melting points (uncorrected) were determined with a digital Electro Thermal Melting Point apparatus (model 9100, Electrothermal Engineering Ltd., Essex, UK). The ^{1}H and^{13}C NMR spectra were recorded with a Bruker 400MHz (AMX model, Karlsruhe, Germany) spectrometer. IR spectra were recorded with a Thermo Nicolet FT-IR (Nexus-870 model, Nicolet Instrument Corp, Madison, Wisconsin, USA.) spectrophotometer. Mass spectra were recorded with an Agilent Technologies 5973, Mass Selective Detector (MSD) spectrometer (Wilmington, USA). Elemental analyses were carried out with a Perkin-Elmer, CHN elemental analyzer Model 2400.The elemental analysis data were approximately equal to calculated values.

Syntheses

Preparations were made according to Scheme 1.

Synthesis of 2-(4-isobutylphenyl)propanoyl chloride(6). This compound was prepared as light yellow liquid

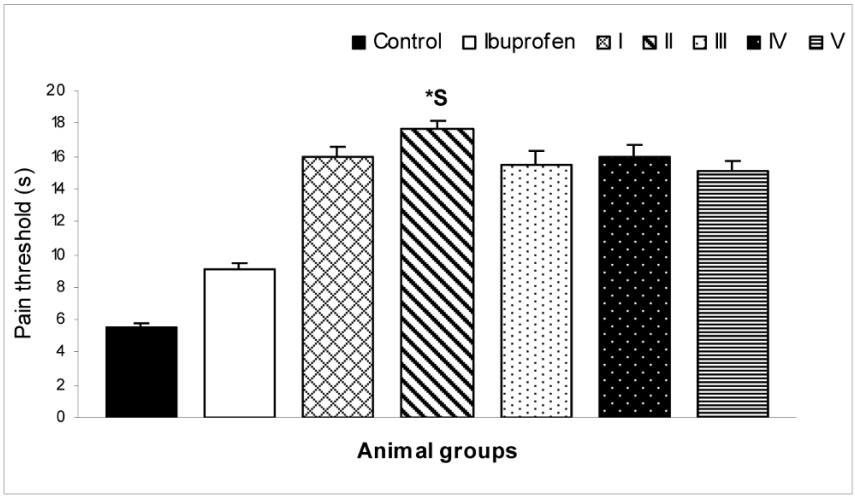

Fig. 1. Comparison of tail immersion pain threshold (s) between control, ibuprofen and other treatment groups (**I – V**). Symbols * and $ indicate the difference ($p < 0.05$) with control and ibuprofen groups, respectively. Bars show mean ± SEM of pain thresholds ($n = 12$ in each group).

from ibuprofen and thionyl chloride in dry toluene at 80°C, following a published method [15].

*New Substituted Benzothiazoles of Ibuprofen***(I – V).**

To a mixture of substituted 2-aminobenzothiazole (**1 – 5**) (0.01 mol) and pyridine (2 mL) in acetone (25 mL) maintained at –10°C was added with stirring a solution of compound **6** (2.25 g, 0.01 mol) in acetone (25 mL) over a period of 1 h. The reaction mixture was stirred for one week at 40°C and poured into crushed ice. The residue obtained was filtered, dissolved in chloroform (100 mL), washed with 5% hydrochloric acid (3 × 50 mL), 5% sodium bicarbonate (3 × 50 mL), and finally with brine solution (2 × 25 mL). The organic layer was filtered and dried to obtain substituted benzothiazoles **I – V**.

2-(4-Isobutylphenyl)-*N*-(3-mercapto-3*H*-indol-2-yl)propanamide(I): Brown viscous liquid; IR (KBr, ν_{max}, cm^{-1}): 3373, 3199, 2954, 1695, 1599, 1537, 1442, 1268, 1164, 755;^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.85 – 2.59 (m, 12H), 3.78-3.93 (m, 1H), 7.05-7.83 (m, 8H); $^{13}C\{^1H\}$NMR spectrum in $CDCl_3$ (δ, ppm): 18.5, 22.4, 30.3, 44.8, 46.6, 121.5, 124.3, 125.6, 127.9, 129.3, 136.5, 141.2, 147.7, 173.2, 180.2; Mass spectrum, *m/z* (I_{rel}, %): 338 (38), 293 (12), 282 (16), 206 (28), 195 (66), 188 (85), 161 (100), 153 (19), 145 (38), 117 (66), 107 (28), 91 (42); $C_{20}H_{22}N_2OS$.

2-(4-Isobutylphenyl)-*N*-(3-mercapto-4-methyl-3*H*-indol-2-yl)propanamide(II): Brown viscous liquid;IR (KBr, ν_{max}, cm^{-1}): 3370, 3199, 2954, 1699, 1592, 1537, 1454, 1408, 1248, 1261, 764; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.91 – 2.6 (m, 15H), 3.85-3.87 (m,1H,), 7.07-7.63 (m,7H,); $^{13}C\{^1H\}$NMR spectrum in $CDCl_3$ (δ, ppm): 18.1, 18.9, 22.4, 30, 44.8, 46.6, 118.8, 124.3, 125.6, 126.5, 129.1, 130.4, 131.5, 141, 146.7, 169.4, 173; Mass spectrum, *m/z* (I_{rel}, %): 352 (38), 321 (13), 195 (43), 188 (75), 161 (100), 145 (56), 117 (88), 91 (50); $C_{21}H_{24}N_2OS$.

***N*-(6-Bromo-3-mercapto-3*H*-indol-2-yl)-2-(4-isobutylphenyl)propanamide(III)**: Brown viscous liquid;IR (KBr, ν_{max}, cm^{-1}): 3390, 3181, 2953, 1693, 1591, 1536, 1454, 1408, 1283, 1262, 763; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.86 – 2.6 (m,12 H,), 3.67 – 3.79 (m,1H), 6.88 – 7.97 (m,7H); $^{13}C\{^1H\}$NMR spectrum in $CDCl_3$ (δ, ppm): 18.5, 22.4, 29.7, 45, 46.3, 116.7, 124.3, 126.1, 128.5, 133.6, 141.1, 142.9, 173.3, 174.5; Mass spectrum, *m/z* (I_{rel}, %): 418 (14), 416 (28), 387 (10), 295 (10), 255 (14), 228 (42), 188 (93), 161 (100), 145 (43), 117 (70), 91 (50); $C_{20}H_{21}BrN_2OS$.

2-(4-Iisobutylphenyl)-*N*-(3-mercapto-6-nitro-3*H*-indol-2-yl)propanamide(IV): Brown viscous liquid**;** IR (KBr, ν_{max}, cm^{-1}): 3338, 2954, 1679, 1613, 1596, 1507, 1407, 1338, 1253, 751;^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.84-2.5 (m,12H), 3.64 – 3.79 (m,1H), 7.11 – 8.25 (m,7H); $^{13}C\{^1H\}$NMR spectrum in $CDCl_3$ (δ, ppm): 18.6, 22.3, 30.1, 44.9, 47.4, 119.2, 119.7, 121.6, 124.8, 129.1, 133, 140.9, 144.8, 163.3, 173.7, 173.9; Mass spectrum, *m/z* (I_{rel}, %): 383 (24), 381 (25), 327 (63), 307 (13), 249 (7), 188 (65), 161 (100), 145 (19), 117 (56), 91 (37); $C_{20}H_{21}N_3O_3S$.

***N*-(6-Chloro-3-mercapto-3*H*-indol-2-yl)-2-(4-isobutylphenyl)propanamide(V)**: Brown viscous liquid; IR (KBr, ν_{max}, cm^{-1}): 3296, 2954, 1734, 1608, 1586, 1490, 1403, 1350, 1242, 825; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 0.68 – 2.53 (m, 12H), 3.64 – 4.04 (m, 1H), 6.71 – 7.82 (m, 7H); $^{13}C\{^1H\}$NMR spectrum in $CDCl_3$ (δ, ppm): 18.3, 22.7, 30.2, 45, 45.3, 120.7, 126.7, 127.3, 128.9, 129.9, 136.4, 136.6, 147.9, 171.3, 176.9; Mass spectrum, *m/z* (I_{rel}, %): 372 (40), 348 (35), 279 (40), 211 (60), 184 (35), 169 (33), 161 (100), 153 (42), 145 (33), 127 (85), 117 (84), 91 (42); $C_{20}H_{21}ClN_2OS$.

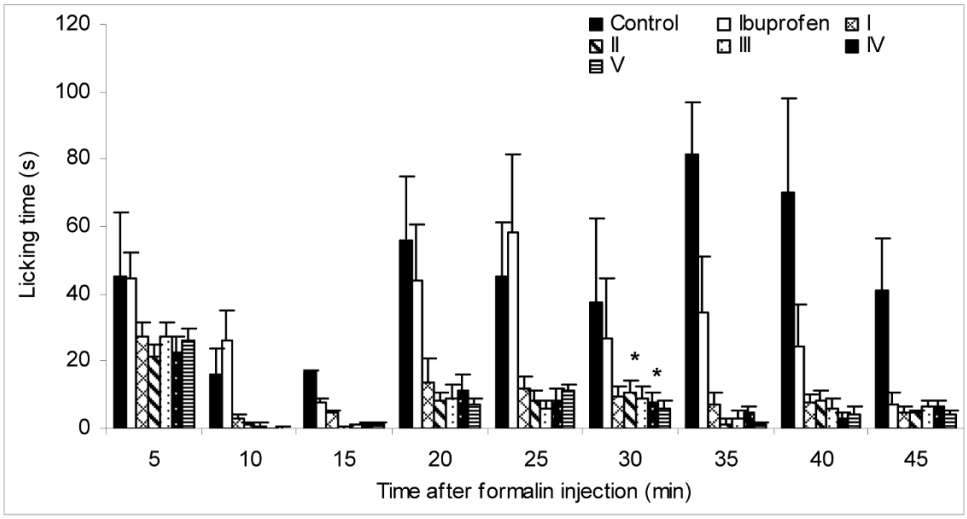

Fig. 2. Licking time for control, ibuprofen, and new drugs (**I – V**) in various groups upon formalin injection. Symbol *shows the difference ($p < 0.05$) from control group. Bars show the licking time mean±SEM for $n = 12$ in each animal group.

Animals

Male NMRI mice weighing 25 – 30 g were prepared (Pasteur's Institute, Tehran) at the beginning of the experiment and randomly housed, four per cage in a temperature-controlled colony room under 12 h light/dark cycle. Animals were given free access to water and standard laboratory rat chow (Pars Company, Tehran, Iran). All behavioral experiments were carried out between 9 am and 4 pm under normal room light and at 25 ^{o}C. Animals were divided into 7 groups:

- Group 1: control received saline;
- Group 2: standard received Ibuprofen (100 mg/kg)[16];
- Groups 3, 4, 5, 6, and 7 received new synthesized drugs (**I – V**, 100 mg/kg).

This study was carried out in accordance with the guidelines set forth in the Guide for the Care and Use of Laboratory Animals (NIH) and those of the Research Council of Shahed University of Medical Sciences (Tehran, Iran).

Pain Assessment

Tail immersion Test. The acute thermal pain was normally modeled by the tail immersion test. Thirty minutes after injection of drugs ibuprofen and newly derived **I – V** (100 mg/kg, i.p.) or an equivalent volume of saline (control), mice ($n = 8$ in each group) were housed in an animal restrainer. Then the terminal 5cm of their tails was first submerged into room temperature water (22 – 24°C) to check their aversion to water and then immersed in 52°C water. The reaction time between immersing the tail and its removal from heated water was measured and recorded as pain threshold. The record of pain threshold was repeated 5 times with 2 min interval for each animal group test. The cut-off time latency in 15s was employed to avoid animal tail damaging [12].

Formalin test. In this test, formaldehyde solution (50 μL, 2.5%) was injected subcutaneously into the plantar surface of control mice hind paw. Then the animal was placed in a Plexiglas chamber (30×30×30 cm^3), with a mirror at 45^{o} angle underneath for accurate observation. In the treatment groups, the drugs ibuprofen and its new derivatives (**I – V**) each one at a dose of 100 mg/kg (i.p.) was administered 30 min before formaldehyde injection. Prior to the experiments, all animals were brought to the test chamber 5 times at 5 minutes intervals to adapt them with the environment. The behavioral pain reactions, i.e., the licking time and frequency were detected and recorded in 5 min intervals during 45 min period after formalin injection. The first 10 min after formalin injection is known as the acute neurogenic pain and the period between 15 – 45 min is known as chronic inflammatory pain[13].

Formalin-induced paw edema. Formalin (50 μL, 3%) was injected to the right hind paw sub-plantar surface of each animal groups test ($n = 12$) including, control (normal saline), ibuprofen and new synthetic compounds (**I – V**). Except control animals, treatment mice received the drug (100 mg/kg, i.p.) 30 min prior to formalin injection. The paw volume was immediately measured before (zero time) and then at 60, 90, 120, 150 and 180 min after the formalin injection by using caliper[14]. The difference in paw diameter between control and treatment groups was considered as data for statistical analysis.

Statistical Analysis

Sigma stat 3.5 soft was used for statistical analysis. The measured data were presented as means ± S.E.M. Compari-

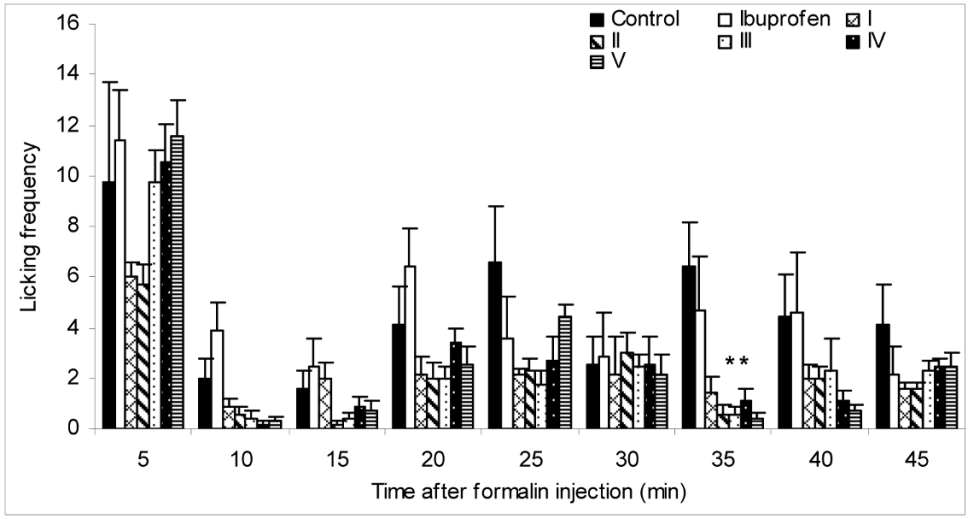

Fig. 3. Licking frequency for control, ibuprofen, and new drugs (**I – V**) in various groups upon formalin injection. Symbol * shows the difference ($p < 0.05$) from control group. Bars show the licking time mean ± SEM for $n = 12$ in each animal group.

sons were carried out as one way analysis of variance (ANOVA) followed by post-hoc Tukey test with *p <D<|0.05 as the level of significance. For nonparametric data, we used Kruskal – Wallis one way ANOVA and post related tests.*

RESULTS

Chemistry

The synthetic strategy of the title compounds is outlined in the scheme 1. 2-(4-isobutyl-phenyl)propionic acid (ibuprofen)reacted with thionyl chloride to yield 2-(4-isobutylphenyl)-propionylchloride (**6**). Acid chloride was further reacted with substituted amino benzotiazoles (**1 – 5**) in dry toluene to get new substituted ibuprofenamides (**I – V**).

Spectroscopic (IR, ^{1}H and ^{13}C NMR, mass spectroscopy) and elemental (CHN) data confirmed the structure of the newly synthesized compounds. The purity of every compound was checked by TLC with ethyl acetate – hexane as the eluent.

Pharmacology

Analgesic activities of ibuprofen and its newly synthesized derivatives in tail immersion test. If the application of newly synthesized drugs **I – V** (100 mg/kg, i.p) could lift up tail immersion pain threshold with respect to control animals, but only derivative **II** produced a significant ($p < 0.05$) analgesic effect (17.61 ± 0.51), exceeding that in control (5.47 ± 0.27) and ibuprofen (9.14 ± 0.25) animal groups (Fig. 1).

Analgesic activities of ibuprofen and its newly synthesized derivatives in formalin test. As shown (Fig.2) the licking time was prominently reduced 30 min after formalin injection for drug **II** and **IV** to 10.98 ± 3.44 and 7.57 ± 3.22 respectively, in comparison with control (37.28 ± 25.31) animals ($p < 0.05$). The analgesic effect of mentioned drugs (i.e., **II** and **IV**) was lasting for 35 min after formalin injection in licking frequency analysis (Fig. 3). Both compounds **II** and **IV** showed a prominent reduction in licking frequency to 0.57 ± 0.29 and 1.14 ± 0.4, respectively, in comparison with control animals (6.42 ± 1.77).

Anti-inflammatory effects of ibuprofen and its newly synthesized derivatives in paw edema model. A marked anti-inflammatory effect of drugs **II** and **III** against acute paw edema is shown in Fig. 4. As can be seen 90 min after formalin injection, derivative **II** could produce more pronounced anti-inflammatory effect (0.193 ± 0.04) than that in control (0.53 ± 0.06) and ibuprofen (0.516 ± 0.07) group ($p < 0.05$). Moreover, compound **III** showed a significant reduction in paw edema at 90 (204 ± 0.01), 150 (0.18 ± 0.02) and 180 (0.16 ± 0.01) min after formalin application, which was more significant than in control and ibuprofen groups respectively: (0.53 ± 0.06 and 0.516 ± 0.07, 0.62 ± 0.06 and 0.44 ± 0.09, and 0.68 ± 0.07 and 0.40 ± 0.01).

DISCUSSION

NSAIDs such as ibuprofen are widely used in the treatment of pain and inflammation, including osteoarthritis and rheumatoid arthritis [5]. However, these drugs exhibit GI toxicity related to free –COOH groups. Therefore, synthetic approaches based upon chemical modification of NSAIDs as

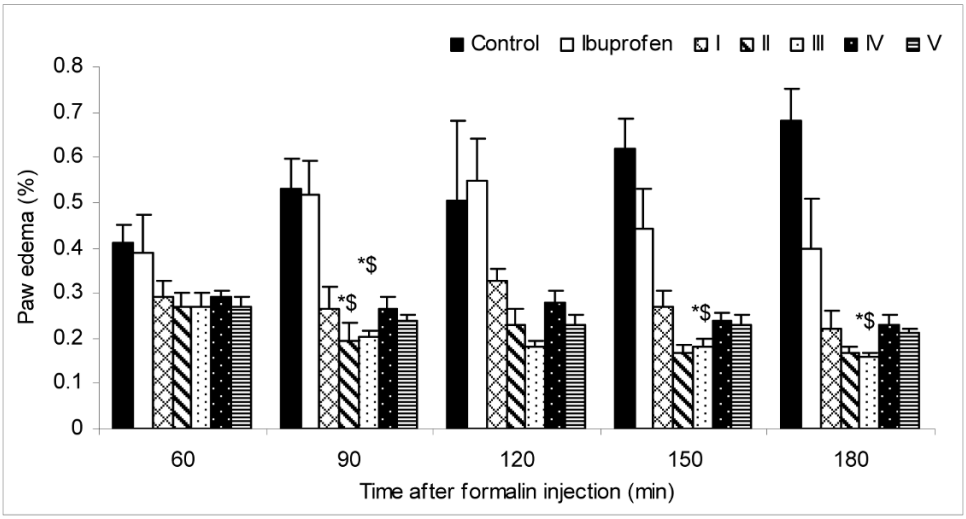

Fig. 4. Comparison of paw edema (%) after formalin injection in groups treated with saline, ibuprofen and new drugs (**I – V**). Symbols * and $ indicate the difference ($p < 0.05$) with control and ibuprofen groups, respectively. Bars show paw edema percentage mean±SEM for $n = 12$ in each group.

potential anti-inflammatory and analgesic agents with less GI toxicity have been undertaken in recent years [17, 18].

These drugs reduce pain and edema by suppressing the formation of prostaglandins, by inhibiting the activity of COX-1 and COX-2enzymes. Selective COX-2 inhibitors elicit less or no GI damage and bleeding compared to conventional NSAIDs. Producing effective NSAIDs with improved safety profile that eliminate the disadvantages of selective COX-2 inhibitors and spare the GI mucosa is a compelling need. Most studies reported that the derivatization of carboxylic acid to amide groups increased anti-inflammatory activity with reduced ulcerogenic potential [19], which was usually related to their potent inhibitory activity against COX-2 but not COX-1 [5].

In this work, in view of various pharmacological (in particular, anti-inflammatory and analgesic) effects of heterocyclic compounds containing substituted 2-amino benzothiazole moiety [8 – 11], the synthesis of a series of hybrid molecules containing important pharmacophore of ibuprofen and substituted 2-aminobenzothiazoles **1 – 5** was undertaken.

Results indicated that all of the new analogs (**I – V**) exhibited more pronounced analgesic and anti-inflammatory activities compared to the control and ibuprofen test groups, especially for diminishing thermal acute pain (for drug **II**) and chronic formalin pain and inflammation (for drugs **II, III** and **IV**).

It seems that, because of the synthesis of hybrid molecules with nitric oxide-releasing group, which favors increased anti-inflammatory activity with reduced GI ulcerogenicity [20, 21] and supports several endogenous GI defense mechanisms (including increase in mucus, bicarbonate secretions, mucosal blood flow, inhibition of the activation of pro inflammatory cells [22 – 24], and beneficial cardiovascular effects such as vasodilation), nitro amino benzothiazole ring in drug **IV** leads to increased anti-inflammatory effects as compared to those of other substituent's.

In addition, the superior analgesic (acute thermal and chemical chronic pains) and anti-inflammatory effects of compound **II** as compared to saline control and other drugs, may be related to higher electron donating and dipole moment activity of this group [25 – 27].

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