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SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW CHEMICAL ENTITIES BASED ON PARACETAMOL AND THEIR IBUPROFEN CONJUGATES AS NOVEL AND SUPERIOR ANALGESIC AND ANTI-INFLAMMATORY CANDIDATES

Abbas Ahmadi,[1,*] Mohsen Khalili,[2] Sepideh Ahmadian,[1] Nadia Shahghobadi,[1] and Babak Nahri-Niknafs[1]

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p-Aminophenol had been evaluated in the past as an analgesic–antipyretic drug, but its N-acetylated derivative acetaminophen (paracetamol) was found to be the most suitable therapeutically. Acetaminophen is still one of the most popular drugs, which is frequently used for the relief of acute and chronic pain. In this study, new analogs of paracetamol with dimethyl and ethyl substitutions in the phenyl moiety and sulfonamide, which was modified by inserting morpholine, were synthesized (compounds **4** and **5**). Then, their conjugated compounds with ibuprofen (**6**) were synthesized (**8** and **9**) and the analgesic and anti-inflammatory activities of these new drugs (**4, 5, 8, 9**) were evaluated in formalin-induced edema test (as a model of acute and chronic chemical pain and also paw edema inflammation) on rats. The results were compared to paracetamol, ibuprofen (as standards), and control (saline) groups. Results showed that the new synthesized derivatives exhibit higher analgesic and anti-inflammatory effects compared to acetaminophen alone for compounds **4, 5, 8** and **9** or ibuprofen for compounds **4, 8** and **9.** Moreover, it was concluded that chemical-structural binding of the potent synthesized drugs (**4** and **5**) with ibuprofen (**6**) produced new superior anti-nociceptive and anti-inflammatory drugs (**8** and **9**).

Keywords: paracetamol, sulfonamide-morpholine analogs, ibuprofen, non-steroidal anti-inflammatory agents, acute and chronic pain

INTRODUCTION

Acetaminophen (4-hydroxyphenyl acetamide, paracetamol) is a derivative of 4-aminophenol which shows analgesic and antipyretic properties. It is useful in the treatment of pain such as headache, toothache, rheumatism and neuralgia [1]. In addition, it has some weak anti-inflammatory effect, which can be attributed to the fact that it is only a weak inhibitor of COX in the presence of high concentration of peroxides that are found in inflammatory lesions [2].

Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, are the most commonly prescribed drugs in the world with anti-inflammatory, antipyretic, antithrombotic and analgesic properties, but their use continues to be limited due to their undesirable side effects mainly on the gastrointestinal tract. NSAIDs are known to have inhibitory activity with respect to both isoforms of the cyclooxygenase (COX) enzymes [3]. They vary considerably in their tendency to cause gastric erosions and ulcers. Gastric damage by these agents is usually caused by at least two distinct mechanisms. First, by inhibiting the cytoprotective COX-1 in the stomach, and second, by physical contact and ion-trapping mechanism. The use of pro-drugs to temporarily mask acidic group of NSAIDs has been suggested as an approach to decrease the gastrointestinal toxicity due to direct contact effects [4, 5].

It has also been reported that conversion of the carboxylic group containing NSAIDs to ester and amide functions makes them more selective toward COX-2 enzyme. Taking into account the aforementioned report and the potential cardiovascular dangers posed by COXIBs, as well as the cost and time involved in the discovery of new drugs, we decided to convert some common NSAIDs into *p*-amidophenol derivatives [6].

More analgesic effects by N-phenyl-acetamide sulfonamide derivatives with morpholine group were reported as a result of applying non-classical bioisosterism and replacing the phenolic hydroxyl group of acetaminophen with a modi-

[1] Department of Chemistry, Faculty of Science, Islamic Azad University, Karaj Branch, P. O. Box: 31485 – 313, Karaj, Iran.
[2] Neurophysiology Research Center, Shahed University, Tehran, Iran.
[*] e-mail: a-ahmadi@kiau.ac.ir; ahmadikiau@yahoo.com.

fied non-oxidizable sulfonamide. By inserting morpholine group [7, 8], new morpholine-sulfonamide derivatives with dimethyl (2,6-dimethyl aniline, 2,6-xylidine, **1′**) and ethyl (2-ethyl aniline, **1″**) substitutions in phenyl moiety of acetamide with many pharmacological properties [9, 10] were synthesized in this study. Then, for improving the pharmacokinetic profiles and obtaining non-hepatotoxic mutual pro-drug analogs [11], these drugs (**4** and **5**) were coupled with commonly used carboxylic group containing NSAIDs like ibuprofen (**6**) for producing new analogues (**8** and **9**). The analgesic and anti-inflammatory effects of compounds **4, 5, 8,** and **9** were evaluated in formalin edema test (as a model of acute and chronic chemical pain and also paw edema inflammation) [12, 13] on rats and the results were compared to paracetamol**,** ibuprofen (as standards), and control (saline) groups.

EXPERIMENTAL PART

Chemistry

Materials and methods. All chemicals, including chlorosulfonic acid, aniline, 2,6-dimethyl aniline, 2-ethyl aniline, thionyl chloride, morpholine, acetaminophen, and ibuprofen were purchased from Merck Chemical Co. (Darmstadt, Germany). Melting points (uncorrected) were determined with a digital ElectroThermal Melting Point apparatus (Model 9100, Electrothermal Engineering Ltd., Essex, UK). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 300 MHz (AMX model, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded with a Thermo Nicolet FT-IR (Nexus-870 model**,** Nicolet Instrument Corp, Madison, Wisconsin, USA.) spectrophotometer. Mass spectra (MS) were recorded using an Agilent Technologies 5973 (Wilmington, USA) spectrometer with mass selective detector (MSD). Elemental analyses were carried out using a Perkin-Elmer Model 2400 CHN elemental analyzer.

Synthesis (Scheme 1)

N-(2,6-Dimethylphenyl)-acetamide (2) and N-(2-ethylphenyl)-acetamide (2′). These compounds were prepared from 2,6-dimethyl aniline (**1**) or 2-ethyl aniline (**1′**) and acetic anhydride in glacial acetic acid as solid compounds (compound **2**: m.p., 178 – 180°C; compound **2′**: m.p., 110 – 112°C) following a published method [14, 15].

2,6-Dimethylphenyl-1-acetylamine-4-sulfonyl chloride (3) and 2-ethylphenyl-1-acetylamine-4-sulfonyl chloride (3′). Chlorosulfonic acid (9.12 g, 5.2 ml, 28.27 mmol) was slowly added to 14.80 mmol of N-(2,6-dimethylphenyl)-acetamide (**2**) or N-(2-ethylphenyl)-acetamide (**2′**). The mixtures were stirred and heated at 80°C for 2 h. After completion of the reaction (monitored by TLC), the desired compounds were obtained by adding water to the reaction mixtures. Then, solid products were filtered through a Buckner funnel and doubly washed with water.

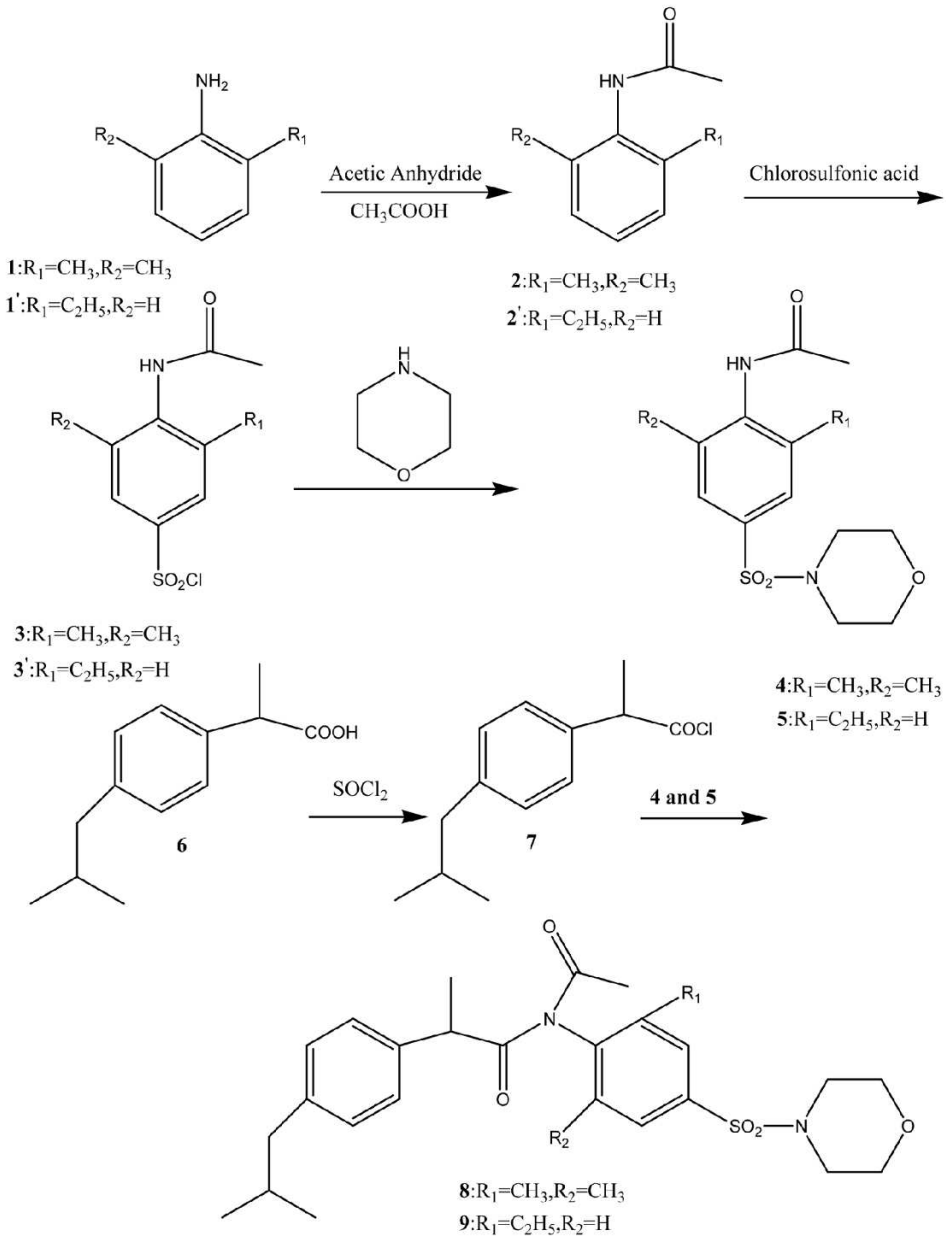
Compound 3: white-yellowish solid; yield, 70%; m.p., 138 – 140°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 3276, 2987, 1664, 1517, 1370, 1170, 618; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 2.49 (3H, s), 2.31 (3H, s), 2.63 (3H, s), 7.13 (1H, s), 7.26 (3H, s), 7.91 – 7.94 (1H, m); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 15.1, 23, 127.8, 136.1, 141.5, 144.9, 169.8; MS, *m/z* (I_{rel}, %): 261 (62), 219 (100), 246 (19), 203 (28), 162 (33); Found (%): C, 45.97; H, 4.68; N, 5.29; $C_{10}H_{12}ClNO_3S$; Anal. calcd. (%): C, 45.89; H, 4.62; N, 5.35.

Compound 3′: violet solid; yield 74%; m.p., 90 – 92°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 3304, 2972, 1665, 1518, 1375, 1189, 622; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 1.27 – 1.32 (3H, m), 2.25 (3H, s), 2.67-2.74 (2H, m), 7.27 – 7.77 (3H, m), 8.57 (1H, s); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 13.2, 24.3, 121.6, 123.3, 129.5, 136.2, 142.3, 142.6, 168.7; MS, *m/z* (I_{rel}, %): 261 (56), 219 (100), 246 (19), 204 (29), 162 (35); Found (%): C, 45.79; H, 4.58; N, 5.41; $C_{10}H_{12}ClNO_3S$; Anal. calcd. (%): C, 45.89; H, 4.62; N, 5.35.

N-[4-(Morpholin-4-ylsulfonyl)-2,6-dimethylphenyl]acetamide (4) and N-[4-(Morpholin-4-ylsulfonyl)-2-ethylphenyl]acetamide (5). Morpholine (5.0 mmol) in 5ml of methylene chloride was added to a solution of sulfonyl chloride derivatives **3** and **3′** (2.14 mmol) in 50 ml methylene chloride. The reaction mixtures were stirred for about 1 h at room temperature, until termination of the reactions as observed by TLC. The sulfonamide derivatives (**4** and **5**) were isolated by addition of 50 ml methylene chloride and extraction with 10% aq. HCl and brine. The organic layers were dried over anhydrous Na_2SO_4 and evaporated at a reduced pressure to give the corresponding sulfonamide derivatives in good yields.

Compound 4: white powder, m.p., 150 – 152°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 3247, 2867, 1647, 1526, 1342, 1145, 726; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 2.23 (3H, s), 2.27 (3H, s), 2.43 (3H, s), 3.13-3.19 (4H, m), 3.69 – 3.74 (4H, m), 7.16 (1H, s), 7.86 (1H, s); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 15.1, 23, 45.1, 66.1, 127.7, 134.4, 136.1, 142.3, 168.9; MS, *m/z* (I_{rel}, %): 312 (17), 269 (8), 226 (14), 279 (14), 167 (34), 149 (100); Found (%): C, 53.91; H, 6.50; N, 8.89; $C_{14}H_{20}N_2O_4S$; Anal. calcd. (%): C, 53.83; H, 6.45; N, 8.97.

Compound 5: white powder; m.p., 140 – 144°C; IR spectrum in KBr (ν_{max}, cm^{-1}): 3338, 2968, 1668, 1520, 1346, 1160, 731; ^{1}H NMR spectrum in $CDCl_3$ (δ, ppm): 1.25 – 1.3 (3H, m), 2.22 (3H, s), 2.61 – 2.7 (2H, m), 2.96 – 3.03 (4H, m), 3.72 – 3.75 (4H, m), 7.27 – 7.54 (3H, m), 8.17 – 8.19 (1H, m); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 13.4, 24.3, 45.9, 66.1, 122.4, 126.5, 127.7, 132.8, 133.9, 139.7, 168.6; MS, *m/z* (I_{rel}, %): 312 (17), 269 (8), 226 (14), 279 (14), 167



Scheme 1. Synthesis of title compounds and their intermediates.

(34), 149 (100); Found (%): C, 53.74; H, 6.39; N, 8.90; $C_{14}H_{20}N_2O_4S$; Anal. calcd. (%): C, 53.83; H, 6.45; N, 8.97.

4-[2-(4-isobutylphenyl) propanoyl chloride (7). This compound was prepared from ibuprofen (**6**) and thionyl chloride in dry toluene at 80°C as light yellow liquid following a published method [16].

N-acetyl-N-(2,6-dimethyl-4-(morpholinosulfonyl)phenyl)-2-(4-isobutylphenyl)-propanamide (8) and N-acetyl-N-(2-ethylphenyl-4-(morpholinosulfonyl)phenyl)-2-(4-isobutylphenyl)-propanamide (9). A stirred mixture of sodium hydride (60% dispersion, 2 g, 0.05 mol) in 80 ml DMF was heated and sulfonamide-morpholine-acetamide derivatives (**4** and **5**, 0.05 mol) in 30 ml DMF were added dropwise. When the hydrogen evolution ceased, the reaction mixtures were cooled and ibuprofen acid chloride (**7**, 0.06 mol) in 20 ml DMF was added dropwise. The mixture was heated at 110°C with stirring for one week. After completion of the reaction as monitored by TLC, the solvent was evaporated and the mixture was poured into crushed ice and water, and extracted with 5% NaOH and ether. Then the organic layer was neutralized with 5% HCl and dried over Na_2SO_4. Finally, the solvent was evaporated for obtaining the desired compounds.

Compound 8: white powder; yield, 45%; m.p., 190 – 194°C, IR spectrum in KBr (ν_{max}, cm^{-1}): 3367, 2951, 1640, 1599, 1550, 1412, 1334, 1052, 787 cm^{-1}; ^1H NMR

spectrum in $CDCl_3$ (δ, ppm): 0.84 – 0.85 (6H, m), 1.25 (3H, s), 1.75 – 1.80 (1H, m), 2.35 – 2.37 (2H, m), 2.55 (9H, s), 2.96 – 3.03 (4H, m), 3.24 – 3.34 (1H, m), 3.72 – 3.75 (4H, m), 6.94 – 7.19 (4H, m), 8.27 (1H, s), 8.54 (1H, s); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 13.6, 15.7, 20.2, 22.2, 29.7, 39.4, 44.3, 66.3, 127.2, 128, 132.3, 137.5, 143, 148.9, 169.7, 177.7; MS, *m/z* (I_{rel}, %): 501 (7), 467 (26), 368 (74), 339 (26), 313 (57), 299 (36), 269 (100), 257 (79); Found (%): C, 64.85; H, 7.29; N, 5.55; $C_{27}H_{36}N_2O_5S$; Anal. calcd. (%): C, 64.77; H, 7.25; N, 5.60.

Compound 9: white powder; yield, 43%; m.p., 200 – 204°C, IR spectrum in KBr (ν_{max}, cm^{-1}): 3367, 2951, 1640, 1599, 1550, 1412, 1364, 1058, 787; ^1H NMR spectrum in $CDCl_3$ (δ, ppm): 0.85 – 0.86 (6H, m), 1.21 (3H, s), 1.43 – 1.44 (3H, m), 2.26 (1H, m), 2.48 (3H, s), 2.96 – 3.03 (4H, m), 3.11 – 3.36 (4H, m), 3.72 – 3.75 (4H, m), 3.24-3.34 (1H, m), 7.06 – 7.52 (4H, m), 7.9 – 8.15 (3H, m); ^{13}C NMR spectrum in $CDCl_3$ (δ, ppm): 13.6, 15.6, 20.5, 22.4, 29.7, 39.2, 40.1, 44.3, 66.5, 122.2, 123.4, 127.7, 128.3, 132.3, 134.4, 137.7, 143.1, 148.6, 169.7, 177.5; MS: *m/z* (I_{rel}, %): 501 (8), 467 (22), 452 (37), 423 (48), 395 (60), 382 (55), 368 (97), 354 (60), 339 (26), 313 (57), 299 (36), 269 (100), 257 (79); Found (%): C, 64.70; H, 7.22; N, 5.64; $C_{27}H_{36}N_2O_5S$; Anal. calcd. (%): C, 64.77; H, 7.25; N, 5.60.

Pharmacology

Experimental animals. Eighty-four adult male Wistar rats (Pasteur's Institute, Tehran), weighing 230 ± 15 g at the beginning of the experiment, were 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 at 25°C. 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 in the Research Council of Shahed University of Medical Sciences (Tehran, Iran).

Formalin induced pain test. In the test control, formaldehyde solution (50 µl, 2.5%) was subcutaneously injected into the plantar surface of rat hind paw. Then the animal was placed in a Plexiglas chamber (30 × 30 × 30 cm^3), with a mirror at 45°C angle underneath for accurate observation. In treatment groups, the drugs acetaminophen, ibuprofen and their synthesized derivatives (100 mg/kg) were administered intraperitoneally 30 min prior to the formaldehyde injection [12].

Prior to the experiments, all animals were brought to the test chamber 5 times at 5 min intervals to adapt them with the environment. The behavioral pain reactions due to formalin injection were detected and recorded for 1 h. The scores for pain reaction were as follows: 0, normal weight bearing on the injected paw; 1, limping during locomotion or resting the paw lightly on the floor; 2, elevation of the injected paw; 3, licking or biting of the injected paw, or grooming. The first 15 min after formalin injection is known as the early (I) or acute chemical phase, and the period of 15 – 60 min is known as the second (II) or chronic phase. The chronic phase can further be divided into initial (15 – 40 min) and late (40 – 60 min) periods.

Formalin induced inflammation in paw edema test. Formaldehyde solution (50 µl, 3 %) as a phlogistic agent was applied to sub plantar surface of rat right hind paw. Then, the superior-inferior paw diameter was measured using a caliper before (zero time) and at 1, 2 and 3 h after the formalin injection. Percentage growth of paw edema in each test group was calculated by the following formula:

[(*paw diameter in zero time*)–(*paw diameter at each other test time*)]/(*paw diameter at zero time*) × 100%.

Finally, the difference of paw diameter percentage between control and treatment groups was determined and subjected to statistical analysis [13].

Statistical Analysis

Sigma stat 3.5 software was used as the method of statistical analysis. The measured data were presented as means ± SEM. Comparisons were carried out with one way analysis of variances (ANOVA) followed by post-hoc Tukey test with $p < 0.05$ as the level of significance.

RESULTS AND DISCUSSION

Chemistry

New sulfonamide-morpholine derivatives of acetaminophen (**4** and **5**) were synthesized by reaction of N-phenyl acetamide analogs with chlorosulfonic acid and morpholine. For increasing potency and reducing side effects, these compounds were coupled with commonly used carboxylic group containing NSAIDs like ibuprofen. This compound was converted into the acid chloride form through the treatment with thionyl chloride under anhydrous conditions and reacted with *p*-acetylamine-benzenesulfonyl morpholine compounds (**4** and **5**) to obtain new conjugated drugs (**8** and **9**). The synthesized compounds conformed to the assigned structures, as deduced based on their spectral data. Compounds **2, 2′** and **4** were synthesized by known procedures [14 – 16]. Spectroscopic (IR, ^1H and ^{13}C NMR, MS) and elemental (CHN) data confirmed the structure of the newly synthesized compounds (**4, 5, 8, 9**). The purity of each compound was checked by TLC with ethyl acetate – hexane as the eluent.

Pharmacology

Analgesic activity of paracetamol, ibuprofen and their newly synthesized derivatives in formalin induced

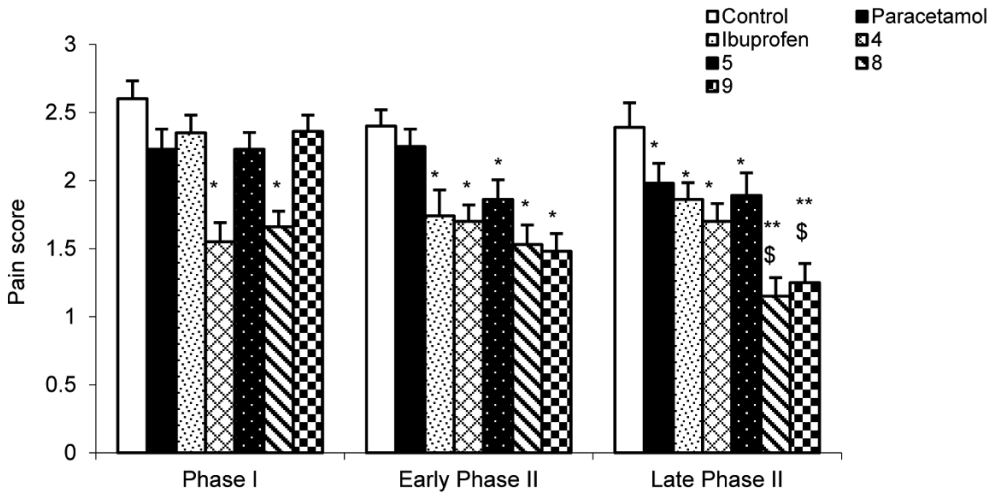


Fig. 1. Comparative effects of all drugs in acute chemical and chronic formalin induced pain test. Bars show mean ± SEM pain score; asterisks *, ** ($p < 0.05$ and 0.01, respectively) indicate the difference from control group; and $ ($p < 0.05$) indicates the difference from other treatment

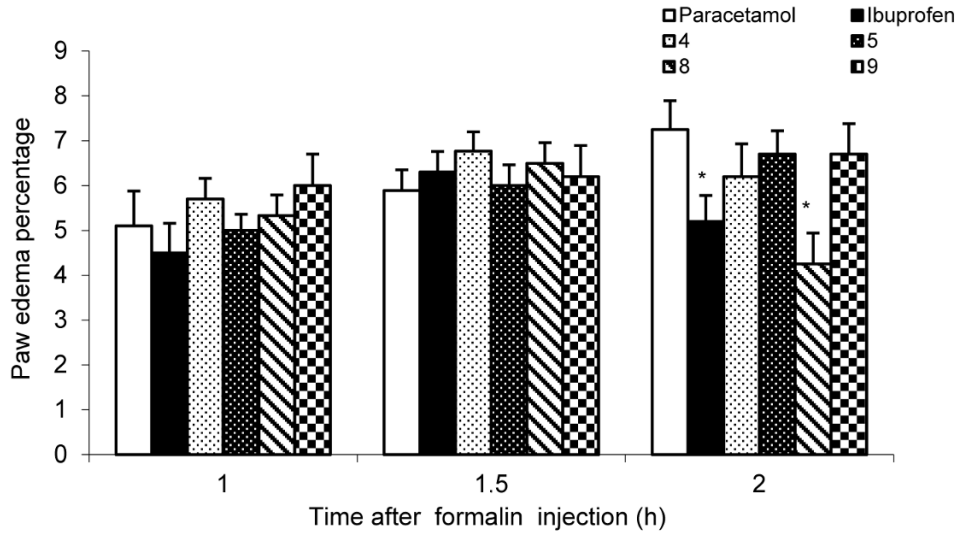


Fig. 2. Anti-inflammatory effects of all drugs in formalin-induced rat paw edema test. Bars show the mean ± SEM of paw edema percentage in animal groups; asterisks * ($p < 0.05$) indicate the difference from the acetaminophen treated group ($n = 12$ in each group test).

edema test. The results showed that new drugs (**4** and **8**) could diminish the acute formalin induced pain (phase I) by 40.38 and 36.15%, respectively. These compounds gave more significant results than those in other treatment animal groups (Figure 1). It should be noted that drugs **4** and **8** produced better and more analgesic effects than other analogs in this phase (acute chemical pain). New drugs (**4, 5, 8, 9)** also better alleviated early chronic pain in phase II as compared to acetaminophen. No significant differences, however, were found between their analgesic activities. In late chronic pain phase, the new drugs (**4, 5, 8, 9)** better reduced the formalin induced pain as compared to other groups, while superior analgesic effects were observed due to administration of **8** and **9** (51.88 and 47.48%, respectively).

Anti-inflammatory effect of paracetamol, ibuprofen and their newly synthesized derivatives in paw edema model. The anti-inflammatory effects of all drugs against acute paw edema induced by phlogistic agent (formalin) are shown in Figure 2. As it was indicated, all drugs exhibited the same anti-phlogistic effects at 1 and 1.5 h after formalin injection. However, compounds **6** and **8** markedly reduced the inflammation (28.28 and 41.38 %, respectively) 2 hours after formalin injection.

DISCUSSION

As is known, 4-hydroxyacetanilide was originally synthesized in 1878. Its first clinical use was reported in 1893. It

was introduced worldwide in the 1950s as an antipyretic and analgesic drug and is still one of the most popular drugs for relief of acute and chronic pain in recent years with few side effects [17, 18]. In a continuing effort to develop new analgesic drug candidates with allowance for the beneficial reported pharmacological properties of N-phenyl-acetamide sulfonamide derivatives with morpholine group [7], the design, synthesis and pharmacological evaluation of new analogs (**4, 5, 8, 9**) were planned by structural modification on the prototype paracetamol as reported in this paper.

In this study, the nature of N-phenyl acetamide moiety was modified by introducing 2,6-xylidine and ethyl aniline with various pharmacological properties [9, 10], instead of aniline, because of electron donating properties and dipole moments of alkyl groups [19]. It could be anticipated that this modification would play effective role in decreasing pain and inflammation as compared to the case of aniline (without any substitutions). Also for improving the pharmacokinetic profiles and obtaining non-hepatotoxic mutual pro-drug analogs [11], the newly synthesized drugs (**4** and **5**) were coupled with commonly used NSAID (ibuprofen, **6**) for obtaining new more analgesic and anti-inflammatory derivatives (**8** and **9**).

Results showed that the newly synthesized derivatives (**4, 5, 8, 9**) could exert higher analgesic (especially in chronic formalin induced pain test) and anti-inflammatory effects (especially pronounced 2 h after formalin injection) compared to acetaminophen alone. If the central and peripheral origins of acute and chronic formalin pain and inflammation are considered [20], it seems that the main effective mechanisms in this in this case are as follows: reduction of central prostaglandins due to COX3 inhibition [21], suppression of pain signal transduction in spinal cord level via endogenous opioid system activity [22], and hyperactivity of cannabinoid system [23].

On the other hand, the results of the formalin induced pain and inflammation tests indicated that compound **4** and **5** were more effective compared to acetaminophen, which is related to higher electron donating properties and dipole moments of alkyl groups at the phenyl ring. Undoubtedly, this effect was more pronounced for compound **4** than for compound **5.** This is due to two methyl groups rather than one ethyl group substituted at the phenyl ring, which leads to more pronounced analgesic effects as compared to that of ibuprofen.

However, the best results in this test were obtained for compounds **8** and **9** formed by structural binding of compounds **4** and **5** with ibuprofen. The effect and anti-inflammatory activity of **8** were more pronounced than for **9,** which is due to the aforementioned substitution of methyl groups at phenyl ring in the drug molecule.

Since the potent COX2 inhibition or blockage of pain signal transduction in spinal cord level is the chief mechanism involved in analgesic and anti-inflammatory action of NSAIDs [24], it could be anticipated that an additional mechanism was responsible for superior activity of these double binding drugs as compared to other experimental compounds.

CONCLUSION

It can be concluded that joining alkyl groups (especially, two methyl groups) and sulfonamide-morpholine groups to the phenyl moiety of acetaminophen (**4** and **5**, especially **4**) and the chemical-structural binding of these potent drugs with ibuprofen (**6**) leads to new supra anti-nociceptive and anti-inflammatory drugs (**8** and **9**).

The best results were observed when two methyl groups were added to the phenyl ring of paracetamol. Conjugation of this new drug with ibuprofen showed the better activity than that of all other experimental drugs. Therefore, it can be concluded that this chemical binding of compounds can produce the third generation of drugs with higher therapeutic potential in alleviating the chronic pain and inflammation.

Conflict of Interest

This research is not a part of our normal lecturing, employment, consultation and involvement. No institution will require any rights from this work too. In addition, no patent has been applied. No commercial right has been given to any company and/or institution, or it will not be done later either.

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