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## Thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole derivatives of isonicotinic acid hydrazide: synthesis and their biological evaluation

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**Abstract:** A series of thiazolidin-4-one (**2a–h**; **3a–h**), azetidin-2-one (**4a–h**) and 1,3,4-oxadiazole (**5a–h**) derivatives of isonicotinic acid hydrazide (INH) were synthesized in order to obtain new compounds with potential anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation activities. The structures of the new compounds were supported by their IR, <sup>1</sup>H-NMR and mass spectral data. All compounds were evaluated for their anti-inflammatory activity by the carrageenan-induced rat paw edema test method. Eleven of the new compounds, out of 32, showed very good anti-inflammatory activity in the carrageenan-induced rat paw edema test, with significant analgesic activity in the tail immersion method together with negligible ulcerogenic action. The compounds, which showed less ulcerogenic action, also showed reduced malondialdehyde content (MDA), which is one of the by-products of lipid peroxidation. The study showed that the compounds inhibited the induction of gastric mucosal lesions and it can be suggested from the results that their protective effects may be related to inhibition of lipid peroxidation in the gastric mucosa.

**Keywords:** anti-inflammatory activity; analgesic activity; ulcerogenic activity; lipid peroxidation.

### INTRODUCTION

The currently available non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, flurbiprofen, fenbufen and naproxen, exhibit gastric toxicity. Long-term use of these drugs has been associated with gastro-intestinal (GI) ulceration, bleeding and nephrotoxicity.<sup>1</sup> The pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis from arachidonic acid

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by inhibiting the enzyme cyclooxygenases (COXs).<sup>2,3</sup> Recently, it was discovered that COX exists in two isoforms, COX-1 and COX-2, which are regulated differently.<sup>4-6</sup> COX-1 provides cytoprotection in the gastrointestinal (GI) tract, whereas inducible COX-2 mediates inflammation.<sup>7-9</sup> Since most of the NSAIDs on the market show greater selectivity for COX-1 than COX-2,<sup>10</sup> chronic use of NSAIDs may elicit appreciable GI irritation, bleeding and ulceration.

The GI damage from NSAIDs is generally attributed to two factors, *i.e.*, local irritation by the carboxylic acid moiety common to most NSAIDs (topical effect); and decreased tissue prostaglandin production, which undermines the physiological role of the cytoprotective prostaglandins in maintaining GI health and homeostasis.<sup>11</sup> The incidence of clinically significant GI side effects due to NSAIDs is high (30 %) and causes some patients to abandon NSAID therapy.<sup>12</sup> Thus, the discovery of COX-2 provided the rationale for the development of drugs devoid of GI disorders while retaining clinical efficacy as anti-inflammatory agents. However, recent reports showed that selective COX-2 inhibitors (coxibs) could lead to adverse cardiovascular effects.<sup>13</sup> Therefore, the development of novel compounds having anti-inflammatory and analgesic activity with improved safety profiles is still a necessity.

Synthetic approaches based on chemical modification of NSAIDs have been undertaken with the aim of improving their safety profiles. A literature survey revealed that derivatization of the carboxylate function of NSAIDs resulted in retained anti-inflammatory activity with reduced ulcerogenic potential.<sup>14-17</sup> It has also been reported in the literature that certain compounds bearing a thiazolidin-4-one, azetidin-2-one or 1,3,4-oxadiazole nucleus possess significant anti-inflammatory activity.<sup>18-20</sup>

In view of the important biological properties of the thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole nuclei, it was planned to suitably incorporate the above-mentioned ring systems into the isoniazide moiety to explore the possibilities of some altered biological actions. Hence, the thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole derivatives reported herein were designed and synthesized. They were found to possess an interesting profile of anti-inflammatory and analgesic activity with significant reduction in their ulcerogenic risks to the stomach.

## EXPERIMENTAL

### *Materials, methods and instrumentation*

All the solvents were of AR grade and were obtained from Merck, CDH and S.D. Fine chemicals. Melting points were determined in open capillary tubes and are uncorrected. All the compounds were subjected to elemental analysis (CHN) and the measured values agreed within  $\pm 0.4$  % with the calculated ones. Thin layer chromatography was performed on silica gel G (Merck). The spots were developed in an iodine chamber and visualized with an ultra-violet lamp. The solvent systems used were benzene:acetone (8:2, v/v) and toluene:ethyl ace-

tate:formic acid (5:4:1, v/v). Ashless Whatman No. 1 filter paper was used for vacuum filtration. The IR spectra were recorded in KBr pellets on a (BIO-RAD FTS 135) WIN-IR spectrophotometer. The FAB mass spectra of all the compounds were recorded on a JEOL SX102/DA-600 mass spectrometer using argon/xenon (6 kV, 10 mA) as the FAB gas. The  $^1\text{H}$ -NMR spectra were recorded on a Bruker model DPX 300 FT-NMR spectrometer in  $\text{CDCl}_3$  using tetramethylsilane ( $\text{Me}_4\text{Si}$ , TMS) as an internal standard. The chemical shifts are reported in the  $\delta$  ppm scale.

*General procedure for the preparation of the new (E)-N'-(substituted benzylidene)-isonicotinohydrazides (1a–h)*

To an equimolar methanolic solution of isonicotinic acid hydrazide (0.1 mol) and substituted benzaldehyde (0.1 mol), a few drops of glacial acetic acid were added. The mixture was then refluxed on water bath for 5–6 h. It was then allowed to cool, poured onto crushed ice and recrystallised from methanol.

*General procedure for the preparation of the new N-(2-(substituted phenyl)-4-oxothiazolidin-3-yl)isonicotinamides (2a–h)*

A mixture of **1** (0.01 mol) and thioglycolic acid (0.01 mol) was heated on an oil-bath at 120–125 °C for 12 h. The reaction mixture was cooled and treated with 10 % sodium bicarbonate solution. The product was isolated and recrystallised from methanol–dioxane (4:1).

*General procedure for the preparation of new 2-(2-(2-substituted phenyl)-3-(isonicotinamido)-4-oxothiazolidin-5-yl)acetic acids (3a–h)*

A mixture of **1** (0.01 mol) and thiomalic acid (0.01 mol) was heated on an oil-bath at 120–125 °C for 12 h. The reaction mixture was cooled and treated with 10 % sodium bicarbonate solution. The product was isolated and recrystallised from methanol–dioxane (4:1).

*General procedure for the synthesis of N-(3-chloro-2-(2-substituted phenyl)-4-oxoazetidin-1-yl)isonicotinamide (4a–h)*

A solution of **1** (0.01 mol) in dioxane (20 mL) was added to a well-stirred mixture of chloroacetyl chloride (0.012 mol) and triethylamine ( $\text{Et}_3\text{N}$ ) (0.012 mol) in dioxane (10 mL) at 0–5 °C. The reaction mixture was then stirred for 8 h, kept for 2 days at room temperature and then treated with cold water. The solid thus obtained was filtered, washed with water and recrystallised from methanol.

*General procedure for the synthesis of 1-(2-(2-substituted phenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl)ethanone (5a–h)*

A mixture of **1** (0.003 mol) and acetic anhydride (10 mL) was heated under reflux for 4 h. After the reaction mixture attained room temperature, the excess acetic anhydride was decomposed by water and the mixture was stirred for further 30 min. The separated product was filtered, washed with water, dried and recrystallised from appropriate solvent systems.

*Biological evaluation*

*Animals.* Adult Wistar strain rats of either sex weighing 150–200 g were used for the evaluation of the anti-inflammatory, ulcerogenic and lipid peroxidation activities, whereas Swiss albino mice weighing 25–30 g were used for assessing the analgesic activity. The animals were allowed food and water *ad libitum* except during the experiments. They were housed in a room at  $25 \pm 2$  °C, and  $50 \pm 5$  % relative humidity with a 12 h light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiments. All the experimental protocols were performed with permission from the Institutional Animal Ethics Committee (IAEC), form No. 520. The animals were obtained from the Central Animal House

Facility, Hamdard University, New Delhi-110062, India. Registration number and date of registration of the Animal House Facility (173/CPCSEA, 28, JAN-2000). All the test compounds and the reference drug were administered orally suspended in 0.5 % carboxymethyl cellulose (CMC) solutions.

#### *Anti-inflammatory activity*

The synthesized compounds were evaluated for their anti-inflammatory activity using the carrageenan-induced hind paw edema method.<sup>21</sup> The animals were randomly allocated into groups of six animals each and were fasted for 24 h before the experiment with free access to water. The control group received only 0.5 % carboxymethyl cellulose solution. The standard drug naproxen was administered orally at a dose of 10 mg kg<sup>-1</sup>. The test compounds were administered orally at an equimolar oral dose relative to 10 mg kg<sup>-1</sup> naproxen. Into the sub-plantar region of the right hind paw of each rat, 0.1 ml of 1 % carrageenan solution in saline was injected subcutaneously, 1 h after the administration of the test compounds or the standard drug. The right hind paw volume was measured before and after 3 and 4 h of carrageenan treatment by means of a plethysmometer. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\text{Percent edema inhibition} = (V_c - V_t / V_c) \times 100$$

where  $V_t$  represents the mean increase in paw volume in rats treated with test compounds and  $V_c$  represents the mean increase in paw volume in the control group of rats.

#### *Analgesic activity*

The analgesic activity was evaluated by the tail immersion method.<sup>22</sup> Swiss albino mice allocated into different groups consisting of six animals of either sex in each, weighing 25–30 g, were used for the experiment. The analgesic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 10 mg kg<sup>-1</sup> naproxen. The test compounds and the standard drug were administered orally as a suspension in carboxymethyl cellulose solution in water (0.5 %, w/v). The analgesic activity was assessed before and 4 h after the administration of test compounds and standard drug. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55±0.5 °C. The time in seconds for tail withdrawal from the water was taken as the reaction time with a cut-off time of immersion set at 10 s for both the control as well as the treated group of animals.

#### *Acute ulcerogenicity*

Acute ulcerogenicity was determined according to the method of Cioli *et al.*<sup>23</sup> The animals were allocated into different groups consisting of six animals in each group. The ulcerogenic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 30 mg kg<sup>-1</sup> naproxen. The control group received only 0.5 % carboxymethyl cellulose solution. Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with a normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5, redness, 1.0, spot ulcers, 1.5, hemorrhagic streaks, 2.0, ulcers < 3, 3.0, ulcers > 3 but ≤ 5. The mean score of each treated group minus the mean score of control group was regarded as the severity index of gastric mucosal damage.

### Lipid peroxidation

Lipid peroxidation in the gastric mucosa was determined according to the method of Ohkawa *et al.*<sup>24</sup> After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 ml of 1.15 % ice-cold KCl solution. The homogenate was supplemented with 0.2 ml of 8.1 % sodium dodecyl sulphate (SDS), 1.5 ml of acetate buffer (pH 3.5) and 1.5 ml of 0.8 % thiobarbituric acid (TBA). The mixture was heated at 95 °C for 60 min. After cooling, the reactants were supplemented with 5 ml of the mixture of *n*-butanol and pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was removed and the absorbance measured at 532 nm using a UV/Vis spectrophotometer. The results were expressed as nmol MDA 100 mg<sup>-1</sup> tissue, using an extinction coefficient  $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$ .

### Statistical analysis of data

The data are expressed as mean  $\pm$  standard error of mean (SEM). In the anti-inflammatory, ulcerogenic and lipid peroxidation studies, statistical differences between the treatments and the standard were tested by one-way ANOVA followed by the Dunnett multiple comparison test. A value of  $p < 0.01$  was considered significant. In analgesic activity study, the statistical differences in treatments and standard were tested by the paired Student's *t*-test.

## RESULTS AND DISCUSSION

### Chemistry

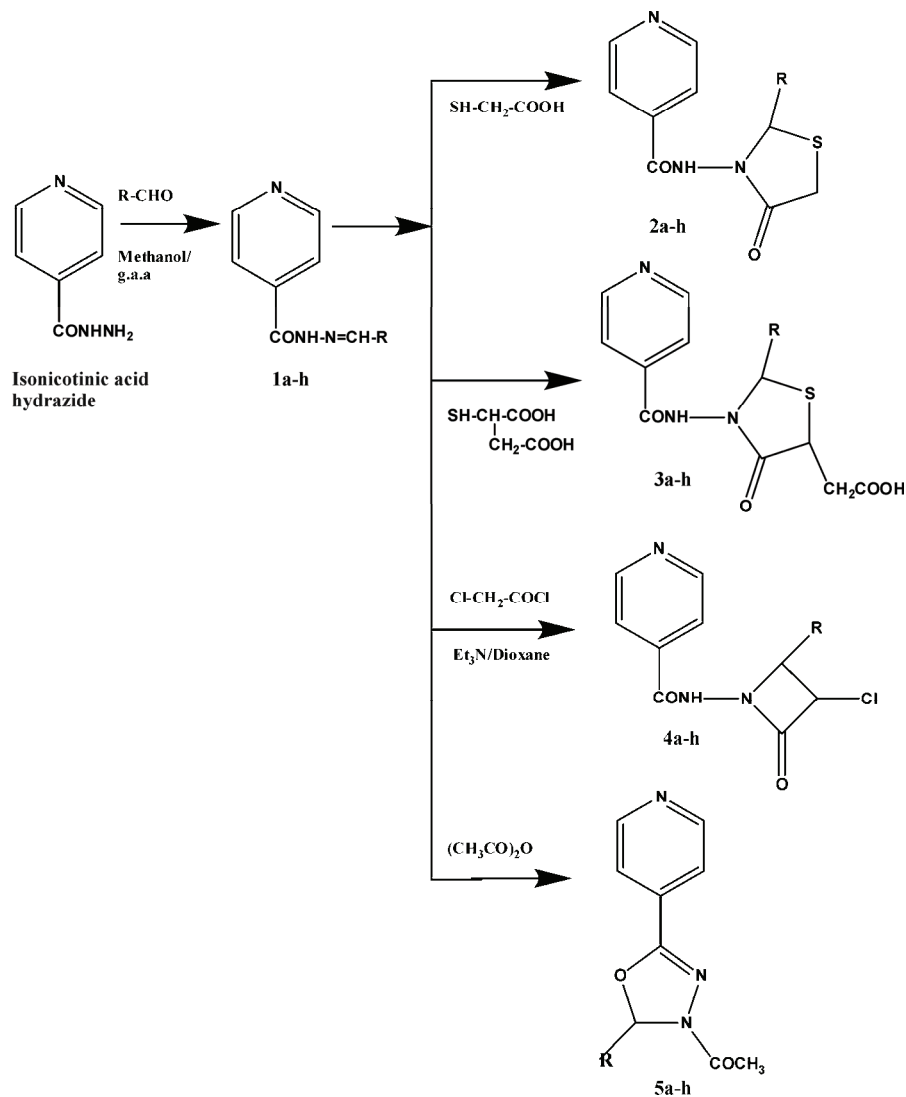
The key intermediates used in the synthesis of thiazolidin-4-ones **2a–h** and **3a–h**, azetidin-2-one **4a–h** and 1,3,4-oxadiazole derivatives **5a–h**, (*E*)-*N'*-(2-substituted benzylidene)isonicotinohydrazides **1a–h** were prepared starting from isonicotinic acid hydrazide. The reaction of isonicotinic acid hydrazide with substituted benzaldehyde in refluxing methanol with few drops of glacial acetic acid gave the (*E*)-*N'*-(2-substituted benzylidene)isonicotinohydrazides **1a–h**. In the present study, the reaction of the substituted benzylidene isonicotinohydrazides **1a–h** with thioglycolic acid, thiomalic acid, chloroacetyl chloride and acetic anhydride in presence of various reagents gave the new thiazolidin-4-ones **2a–h** and **3a–h**, azetidin-2-one **4a–h** and 1,3,4-oxadiazole derivatives **5a–h**, respectively.

The synthesis routes to the compounds are outlined in Scheme 1. The analytical and spectral data of the newly prepared compounds are given in the supplementary material to this paper.

The IR spectra of compounds **1a–h** showed absorption peaks at 3300, 1680 and 1600 cm<sup>-1</sup> due to N–H, C=O and –N=CH stretching vibrations. The appearance of the stretching of the C=O of thiazolidinone,  $\beta$ -lactam ring and the acetyl of the oxadiazole ring at 1700, 1745 and 1660 cm<sup>-1</sup>, respectively, in the spectra of the derivatives, together with the C=O stretching at 1680 cm<sup>-1</sup> confirmed the formation of the compounds **2a–h**, **3a–h**, **4a–h** and **5a–h**.

The <sup>1</sup>H-NMR spectra of compounds **1a–h** revealed a multiplet at  $\delta$  7.72 and 8.63 ppm for pyridine and  $\delta$  7.12–7.15 ppm for the aromatic ring and singlets at  $\delta$  6.1 and 7.9 ppm for –NH and –N=CH, respectively. The disappearance of the singlet peak of –N=CH and presence of a singlet peak at  $\delta$  3.5, 10.0, 7.7 and 7.19

ppm of  $-\text{CH}_2$  of thiazolidinone,  $\text{COOH}$ ,  $-\text{N}-\text{CH}$  and  $-\text{CH}$  oxadiazole, respectively, proved that these compounds participated in the cyclisation reaction and formed the desired compounds.



Scheme 1. The synthesis routes to the title compounds.

#### Anti-inflammatory activity

The anti-inflammatory activities of the synthesized compounds **2a-h**, **3a-h**, **4a-h** and **5a-h** were evaluated by the carrageenan-induced paw edema method of Winter *et al.*<sup>15</sup> The compounds were tested at a  $10 \text{ mg kg}^{-1}$  oral dose and were

compared with the standard drug naproxen at the same oral dose. The tested compounds showed anti-inflammatory activity ranging from 31.81–84.54 % (Table I), whereas the standard drug naproxen showed 81.86 % inhibition after 4 h.

TABLE I. Biological data of isonicotinic acid hydrazone derivatives (anti-inflammatory and analgesic activities of the test compounds with reference to the control. Ulcerogenic and lipid peroxidation were compared with reference to the standard drug, *i.e.*, naproxen. Data were only analyzed by the Student's *t*-test for *n* = 6)

Compd.	R	Anti-inflammatory activity (% inhibition $\pm$ SEM)	Analgesic activity (% inhibition $\pm$ SEM)	Ulcerogenic activity (severity index $\pm$ SEM)	(nmol MDA content $\pm$ SEM)/100 mg tissue
Control	—	—	—	0.000 $\pm$ 0.00	3.25 $\pm$ 0.005
Naproxen	—	81.86 $\pm$ 3.71 <sup>c</sup>	71.42 $\pm$ 1.31 <sup>a</sup>	2.250 $\pm$ 0.11	9.04 $\pm$ 0.24
2a	<i>o</i> -C <sub>6</sub> H <sub>4</sub> Cl	78.00 $\pm$ 2.53 <sup>c</sup>	70.32 $\pm$ 1.31 <sup>a</sup>	0.583 $\pm$ 0.18 <sup>a</sup>	5.715 $\pm$ 0.14 <sup>a</sup>
2b	<i>p</i> -C <sub>6</sub> H <sub>4</sub> Cl	64.64 $\pm$ 3.79 <sup>c</sup>	59.09 $\pm$ 3.60 <sup>c</sup>	2.750 $\pm$ 0.19 <sup>c</sup>	8.808 $\pm$ 0.06 <sup>c</sup>
2c	<i>o</i> -C <sub>6</sub> H <sub>4</sub> OH	57.57 $\pm$ 3.45 <sup>c</sup>	65.15 $\pm$ 1.51 <sup>c</sup>	1.000 $\pm$ 0.31 <sup>c</sup>	7.904 $\pm$ 0.19 <sup>c</sup>
2d	<i>m</i> -C <sub>6</sub> H <sub>4</sub> OH	34.08 $\pm$ 3.47 <sup>c</sup>	44.05 $\pm$ 3.76 <sup>c</sup>	1.667 $\pm$ 0.24 <sup>c</sup>	6.715 $\pm$ 0.16 <sup>a</sup>
2e	<i>p</i> -C <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub>	39.54 $\pm$ 2.27 <sup>c</sup>	31.81 $\pm$ 3.71 <sup>c</sup>	2.140 $\pm$ 0.11 <sup>c</sup>	8.155 $\pm$ 0.14 <sup>c</sup>
2f	<i>p</i> -C <sub>6</sub> H <sub>4</sub> F	82.81 $\pm$ 2.17 <sup>a</sup>	71.57 $\pm$ 1.85 <sup>a</sup>	0.666 $\pm$ 0.16 <sup>a</sup>	5.608 $\pm$ 0.02 <sup>a</sup>
2g	<i>o</i> -C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	84.24 $\pm$ 0.95 <sup>a</sup>	72.42 $\pm$ 1.31 <sup>a</sup>	0.510 $\pm$ 0.08 <sup>a</sup>	5.876 $\pm$ 0.13 <sup>a</sup>
2h	<i>p</i> -C <sub>6</sub> H <sub>4</sub> N(CH <sub>3</sub> ) <sub>2</sub>	65.15 $\pm$ 1.51 <sup>c</sup>	59.23 $\pm$ 3.71 <sup>c</sup>	2.427 $\pm$ 0.14 <sup>c</sup>	8.128 $\pm$ 0.06 <sup>c</sup>
3a	<i>o</i> -C <sub>6</sub> H <sub>4</sub> Cl	77.27 $\pm$ 1.92 <sup>c</sup>	70.45 $\pm$ 3.26 <sup>a</sup>	0.833 $\pm$ 0.24 <sup>c</sup>	6.110 $\pm$ 0.04 <sup>a</sup>
3b	<i>p</i> -C <sub>6</sub> H <sub>4</sub> Cl	63.63 $\pm$ 1.96 <sup>c</sup>	54.28 $\pm$ 3.76 <sup>c</sup>	2.466 $\pm$ 0.20 <sup>c</sup>	8.742 $\pm$ 0.17 <sup>c</sup>
3c	<i>o</i> -C <sub>6</sub> H <sub>4</sub> OH	68.93 $\pm$ 3.60 <sup>c</sup>	62.85 $\pm$ 1.65 <sup>c</sup>	0.917 $\pm$ 0.12 <sup>c</sup>	7.788 $\pm$ 0.10 <sup>c</sup>
3d	<i>m</i> -C <sub>6</sub> H <sub>4</sub> OH	31.81 $\pm$ 3.45 <sup>c</sup>	47.27 $\pm$ 2.73 <sup>c</sup>	2.835 $\pm$ 0.18 <sup>c</sup>	8.110 $\pm$ 0.13 <sup>c</sup>
3e	<i>p</i> -C <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub>	56.81 $\pm$ 1.94 <sup>c</sup>	66.46 $\pm$ 2.38 <sup>c</sup>	0.814 $\pm$ 0.24 <sup>c</sup>	7.337 $\pm$ 0.16 <sup>c</sup>
3f	<i>p</i> -C <sub>6</sub> H <sub>4</sub> F	75.00 $\pm$ 2.53 <sup>c</sup>	70.04 $\pm$ 1.64 <sup>a</sup>	0.541 $\pm$ 0.10 <sup>a</sup>	6.854 $\pm$ 0.14 <sup>a</sup>
3g	<i>o</i> -C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	82.27 $\pm$ 1.84 <sup>a</sup>	72.72 $\pm$ 1.66 <sup>a</sup>	0.516 $\pm$ 0.11 <sup>a</sup>	5.788 $\pm$ 0.10 <sup>a</sup>
3h	<i>p</i> -C <sub>6</sub> H <sub>4</sub> N(CH <sub>3</sub> ) <sub>2</sub>	59.09 $\pm$ 3.60 <sup>c</sup>	49.54 $\pm$ 2.27 <sup>c</sup>	2.413 $\pm$ 0.10 <sup>c</sup>	8.371 $\pm$ 0.12 <sup>c</sup>
4a	<i>o</i> -C <sub>6</sub> H <sub>4</sub> Cl	70.45 $\pm$ 3.26 <sup>c</sup>	64.80 $\pm$ 1.28 <sup>c</sup>	2.683 $\pm$ 0.10 <sup>c</sup>	7.874 $\pm$ 0.15 <sup>c</sup>
4b	<i>p</i> -C <sub>6</sub> H <sub>4</sub> Cl	68.93 $\pm$ 3.60 <sup>c</sup>	59.61 $\pm$ 1.00 <sup>c</sup>	1.751 $\pm$ 0.16 <sup>c</sup>	5.192 $\pm$ 0.22 <sup>a</sup>
4c	<i>o</i> -C <sub>6</sub> H <sub>4</sub> OH	84.54 $\pm$ 1.94 <sup>a</sup>	73.06 $\pm$ 2.61 <sup>a</sup>	0.517 $\pm$ 0.25 <sup>a</sup>	6.651 $\pm$ 0.17 <sup>a</sup>
4d	<i>m</i> -C <sub>6</sub> H <sub>4</sub> OH	44.05 $\pm$ 3.76 <sup>c</sup>	57.14 $\pm$ 2.20 <sup>c</sup>	1.836 $\pm$ 0.11 <sup>c</sup>	7.327 $\pm$ 0.19 <sup>c</sup>
4e	<i>p</i> -C <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub>	54.28 $\pm$ 3.45 <sup>c</sup>	44.19 $\pm$ 2.76 <sup>c</sup>	2.432 $\pm$ 0.24 <sup>c</sup>	8.418 $\pm$ 0.11 <sup>c</sup>
4f	<i>p</i> -C <sub>6</sub> H <sub>4</sub> F	77.14 $\pm$ 2.20 <sup>c</sup>	71.12 $\pm$ 1.24 <sup>a</sup>	0.750 $\pm$ 0.18 <sup>c</sup>	5.62 $\pm$ 0.29 <sup>a</sup>
4g	<i>o</i> -C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	76.50 $\pm$ 1.82 <sup>c</sup>	69.54 $\pm$ 3.19 <sup>c</sup>	0.817 $\pm$ 0.12 <sup>c</sup>	8.364 $\pm$ 0.24 <sup>c</sup>
4h	<i>p</i> -C <sub>6</sub> H <sub>4</sub> N(CH <sub>3</sub> ) <sub>2</sub>	72.72 $\pm$ 1.66 <sup>c</sup>	52.48 $\pm$ 3.76 <sup>c</sup>	1.342 $\pm$ 0.23 <sup>c</sup>	7.821 $\pm$ 0.14 <sup>c</sup>
5a	<i>o</i> -C <sub>6</sub> H <sub>4</sub> Cl	82.86 $\pm$ 1.84 <sup>a</sup>	70.45 $\pm$ 3.26 <sup>a</sup>	0.750 $\pm$ 0.14 <sup>c</sup>	7.51 $\pm$ 0.68 <sup>c</sup>
5b	<i>p</i> -C <sub>6</sub> H <sub>4</sub> Cl	69.23 $\pm$ 3.71 <sup>c</sup>	62.69 $\pm$ 1.65 <sup>c</sup>	1.967 $\pm$ 0.17 <sup>c</sup>	7.441 $\pm$ 0.12 <sup>c</sup>
5c	<i>o</i> -C <sub>6</sub> H <sub>4</sub> OH	63.46 $\pm$ 2.38 <sup>c</sup>	55.32 $\pm$ 1.96 <sup>c</sup>	1.834 $\pm$ 0.22 <sup>c</sup>	7.652 $\pm$ 0.17 <sup>c</sup>
5d	<i>m</i> -C <sub>6</sub> H <sub>4</sub> OH	51.28 $\pm$ 3.61 <sup>c</sup>	42.45 $\pm$ 3.16 <sup>c</sup>	2.667 $\pm$ 0.14 <sup>c</sup>	8.345 $\pm$ 0.19 <sup>c</sup>
5e	<i>p</i> -C <sub>6</sub> H <sub>4</sub> OCH <sub>3</sub>	31.81 $\pm$ 3.41 <sup>c</sup>	57.80 $\pm$ 2.84 <sup>b</sup>	1.684 $\pm$ 0.26 <sup>b</sup>	6.791 $\pm$ 0.13 <sup>a</sup>
5f	<i>p</i> -C <sub>6</sub> H <sub>4</sub> F	76.92 $\pm$ 1.58 <sup>b</sup>	71.72 $\pm$ 1.66 <sup>a</sup>	0.667 $\pm$ 0.18 <sup>a</sup>	6.338 $\pm$ 0.20 <sup>a</sup>
5g	<i>o</i> -C <sub>6</sub> H <sub>4</sub> NO <sub>2</sub>	83.80 $\pm$ 1.85 <sup>a</sup>	72.52 $\pm$ 1.00 <sup>a</sup>	0.412 $\pm$ 0.11 <sup>a</sup>	5.742 $\pm$ 0.17 <sup>a</sup>
5h	<i>p</i> -C <sub>6</sub> H <sub>4</sub> N(CH <sub>3</sub> ) <sub>2</sub>	68.26 $\pm$ 2.31 <sup>b</sup>	61.82 $\pm$ 1.65 <sup>b</sup>	1.327 $\pm$ 0.20 <sup>b</sup>	7.155 $\pm$ 0.22 <sup>b</sup>

<sup>a</sup> *p* < 0.0001; <sup>b</sup> *p* < 0.001; <sup>c</sup> *p* < 0.01



The anti-inflammatory activities of the thiazolidin-4-one derivatives (**2a–h** and **3a–h**) were in the range 34.08–84.24 %. When the CH<sub>2</sub> of thiazolidin-4-one moiety (**2a–h**) at the position 5 was substituted by CH<sub>2</sub>COOH (**3a–h**), no substantial change was observed in the activity. It was observed that thiazolidin-4-one derivatives having *o*-Cl, *p*-F and *o*-NO<sub>2</sub> phenyl group showed better or equivalent activity (78.00, 82.81, 84.24 (in series **2**), 77.27, 75.00 and 82.27 % (in series **3**), respectively) to that of the standard drug. The other thiazolidin-4-one derivatives showed moderate activity.

The anti-inflammatory activities of the azetidin-2-one derivatives were between 44.05 and 84.54 %. The highest activity (84.54 %) was found with the azetidin-2-one derivative **4c** having an *o*-hydroxyphenyl group at the position 4. It was observed that the azetidin-2-one derivative having a *p*-dimethylaminophenyl group (**4h**) also showed good activity, *viz.* 72.72 % as did those having an *o*-chloro, *p*-fluoro and *o*-nitrophenyl group (70.45, 77.14 and 76.50 %, respectively). The other derivatives showed moderate activity.

The 1,3,4-oxadiazole derivatives of isonicotinic acid hydrazide showed anti-inflammatory activities ranging from 31.81 to 83.80 %. The highest activity (83.80 %) of the oxadiazole derivative was found for compound **5g** having a *o*-nitrophenyl group at the position 2. When this group was replaced by the *o*-chloro phenyl group, the activity was found to be decreased but equivalent to that of the standard drug (82.86 %). Furthermore, the oxadiazole derivative having a *p*-fluorophenyl group (**5f**) at the position 2 also showed good activity (76.92 %). The remaining oxadiazole derivatives showed moderate activity.

#### *Analgesic activity*

The thiazolidin-4-one derivatives **2f**, **2g** and **3g** showed analgesic activities ranging from 71.57 to 72.72 %, *i.e.*, better than that of the standard drug naproxen (71.42 %). The presence of a *o*-nitrophenyl group, at the position 2 of the thiazolidinone ring (**2g** and **3g**) gave the maximum activity (72.42 and 72.72 %, respectively). When this group was replaced by *o*-chlorophenyl (**2a** and **3a**) and *p*-fluorophenyl (**2f** and **3f**), the activity was found to be slightly decreased (70.32–71.57 %). When these groups were replaced by *p*-OCH<sub>3</sub> and *m*-OH phenyl group (**2e** and **3d**), the activity decreased drastically (31.81 and 47.27 %, respectively). The results show that an electron-withdrawing group increased the analgesic activity of the compounds.

When the thiazolidin-4-one nucleus was replaced by the azetidin-2-one nucleus, the analgesic activity of most of the compounds decreased, except for compound **4c** having an *o*-hydroxylphenyl group at the position 4, which exhibited the highest activity (73.06 %). 1,3,4-Oxadiazole derivatives (**5a–h**) were also screened and showed 42.45–72.52 % analgesic activity. The compounds having a *p*-fluorophenyl (**5f**) and a *o*-nitrophenyl group (**5g**) at the position 2 of the oxa-



diazole nucleus showed a slight difference in their activity (71.72 and 72.52 %). There was a decrease in the activity when these groups were replaced by an *m*-hydroxyphenyl group (**5d**).

#### *Acute ulcerogenesis*

The tested compounds showed significant reductions in ulcerogenic activity, ranging from  $0.417 \pm 0.08$  to  $2.835 \pm 0.18$ , whereas the standard drug naproxen exhibited a high severity index ( $2.250 \pm 0.11$ ). The ulcerogenic activity of the thiazolidin-4-one derivatives **2a–h** and **3a–h** ranged from  $0.510 \pm 0.08$  to  $2.835 \pm 0.18$ . The compounds with a *o*-nitrophenyl group (**2g** and **3g**) showed minimum ulcerogenic activity ( $0.510 \pm 0.08$  and  $0.516 \pm 0.11$ , respectively). Moreover, their anti-inflammatory activity was found to be high *viz.* 84.24 and 82.27 %, respectively. The other three thiazolidinone derivatives **2a**, **2f** and **3f** also showed reductions in ulcerogenic activity ( $0.583 \pm 0.08$ ,  $0.666 \pm 0.16$  and  $0.541 \pm 0.10$ , respectively) in comparison to the standard drug.

The azetidin-2-one derivatives **4c**, **4f** and **4g** also showed reductions in ulcerogenic activity ( $0.517 \pm 0.11$ ,  $0.750 \pm 0.14$  and  $0.817 \pm 0.13$ ). The 1,3,4-oxadiazole derivatives showed minimal ulcerogenic activity, when compared to the thiazolidin-4-one and azetidin-2-one derivatives. Compound **5g** having a *o*-nitrophenyl group showed the minimum severity index ( $0.412 \pm 0.11$ ).

#### *Lipid peroxidation*

It was reported in the literature that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a by-product of lipid peroxidation.<sup>25</sup> Therefore, an attempt was made to correlate the decrease in ulcerogenic activity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation.

The lipid peroxidation was measured as nmol of MDA per 100 mg of tissue. The naproxen (standard drug) showed the maximum lipid peroxidation ( $9.04 \pm 0.24$ ), whereas the control group showed  $3.25 \pm 0.05$ . It was found that all the cyclised derivatives showing lower ulcerogenic activity also exhibited reduced lipid peroxidation (Table I). Thus, these studies showed that the synthesized compounds inhibited the induction of gastric mucosal lesions and the results further suggested that their protective effect might be related to the inhibition of lipid peroxidation in the gastric mucosa.

#### CONCLUSIONS

Various thiazolidin-4-one, azetidin-2-one and 1,3,4-oxadiazole derivatives of isonicotinic acid hydrazide were prepared with the objective of developing better anti-inflammatory molecules with minimum ulcerogenic activity. It was interesting to note that six of the cyclised compounds, **2f**, **2g**, **3g**, **4c**, **5a** and **5g**, were

found to have anti-inflammatory activity greater than that of the standard drug (naproxen, 81.86 %) at 10 mg kg<sup>-1</sup> *p.o.* Furthermore, five compounds, **2a**, **3a**, **4f**, **4g** and **5f**, exhibited anti-inflammatory activity equivalent to the standard drug in the carrageenan-induced paw edema test in rats. When these compounds were subjected to the analgesic activity test, they showed increased activity over the reference drug.

The presence of *p*-fluorophenyl and *o*-nitrophenyl groups at the 2<sup>nd</sup> position of the thiazolidinone nucleus increased the anti-inflammatory activity whereas the presence of *m*-hydroxyphenyl and *p*-methoxyphenyl groups decreased the anti-inflammatory activity. It was further noted that the presence of *o*-hydroxy, *o*-chloro and *o*-nitrophenyl groups at the and positions 4 and 2 of azetidinone and oxadiazole nucleus, respectively, increased the anti-inflammatory activity compared to the standard drug, whereas presence of the *p*-fluorophenyl group showed anti-inflammatory activity slightly less than that of the standard drug.

These compounds tested for ulcerogenic activity showed a significant decrease in activity compared to that of the standard drug. It was noted that the oxadiazole derivatives showed maximum reduction in ulcerogenic activity followed by the thiazolidinone and azetidinone derivatives. It was further concluded that the presence of *p*-fluorophenyl and *o*-nitrophenyl groups at the positions 2, 4 and 2 of thiazolidinone, azetidinone and oxadiazole nucleus, respectively, showed maximum anti-inflammatory and analgesic activity, minimum ulcerogenic activity together with minimum lipid peroxidation.

#### SUPPLEMENTARY MATERIAL

Analytical and spectral data of the synthesized compounds are available electronically at <http://www.shd.org.rs/JSCS/>, or from the corresponding author on request.

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#### ИЗВОД

ТИАЗОЛИДИН-4-ОНСКИ, АЗЕТИДИН-2-ОНСКИ И 1,3,4-ОКСАДИАЗОЛСКИ ДЕРИВАТИ ХИДРАЗИДА ИЗОНИКОТИНСКЕ КИСЕЛИНЕ: СИНТЕЗА И ЊИХОВА БИОЛОШКА АКТИВНОСТ

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Синтетисана је серија нових тиазолидин-4-онских (**2a-h** и **3a-h**), азетидин-2-онских (**4a-h**) и 1,3,4-оксадиазолских (**5a-h**) деривата хидразида изоникотинске киселине (INH) са циљем да се испита њихова анти-инфламаторна, аналгетичка и улцерогена активност и активност према пероксидацији липида. Структура синтетисаних једињења одређена је на основу IC, NMR и масених спектра. Анти-инфламаторна активност једињења одређена је

тестом карагеном идукованог отока на шапи пацова. Од 32 испитана једињења, 11 показује врло добру анти-инфламаторну активност и изражену аналгетичку активност, уз незнатну улцерогену активност. Једињења која показују најмању улцерогену активност доводе до снижавања количине малондиалдехида (MDA), који је споредни производ липидне пероксидације. Резултати показују да једињења инхибирају индукцију оштећења слузокоже желуца и може се претпоставити да је њихова заштитна улога повезана са инхибицијом липидне пероксидације у слузокожи желуца.

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