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Evaluation of Antioxidant, Analgesic and Anti-inflammatory Activity of 2-(4-Aminophenyl)Benzimidazole-based Schiff Bases

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Abstract:
Background: Benzimidazoles are a group of hetrocyclic compounds containing fused benzene ring with an imidazole ring. It has been shown benzimidazolesretain important biological activity including antioxidant, analgesic and anti-inflammatory activity. In order to find analogues with improved activity, 13 benzimidazole derivatives which previously synthesized by our group were biologically investigated.
Materials and Methods:This study is a pilot attempt to explore the antioxidant, analgesic and anti-inflammatory activity of the 13 compounds. The in vitroantioxidant potential was investigated using DPPH free radical scavenging assay. Two models of pain assessment i.e. thermally induced pain by hot plate and chemically induced pain by acetic acid were used to evaluate the analgesic activity of the compounds under test. However, formalin induced pain and inflammation in mouse paw was employed as a model to assess the anti-inflammatory activity.
Results:Mice treated at dose of 50mg/kg of compounds 6, 10 and 1a produced a significant reduction in the percentage of writhings response to acetic acid injection to 45%, 18.7% and 48% of control respectively. Comparatively the same dose of Aspirin (50mg/kg) reduced the responses to 8% from the control. Benzimidazole derivatives 1, 3, 6, 7, 9, 10, 11, 12 and 1a produced a significant increase in the response time of animals to heat upon putting on hot plate surface. Compounds 1, 6, 12 and the main compound (1a) in a dose of 50 mg/kg produced similar effect or higher than that produced by morphine at a dose of 25kg/mg, i.e. almost half potency of morphine. Compounds 1a and 6 were selected to test further for anti-inflammatory activity by formalin test. Both compounds caused great significant reduction in phase I and II responses. Most of the tested compounds showed highly significant scavenging activity compared to the standard ascorbic acid. Furthermore, compounds 4, 6, 9 and 11 were reported to have scavenging activity above 75%.
Key Word: Benzimidazole, Schiff base, Antioxidant, Analgesic, Anti-inflammatory.

I. INTRODUCTION

Benzimidazole refers to a fusion product between benzene and an imidazole ring [1]. It was found compounds containing benzene fused heterocyclic nucleus, i.e. benzimidazole have many biological activities including anti-cancer[2], anti-viral[3], anti-bacterial[4-6], antitubercular[7], anti-fungal[8], anti-helmintic[9], anti-inflammatory[10], anti-histaminic[11], proton pump inhibitor[12], anti-oxidant[13], anti-hypertensive[14], anti-coagulant[15] and immunomodulatory[16]properties.Schiff bases on the other hand have a broad spectrum of medical activity as antimicrobial[17], anticancer[17,18], antitubercular, anti-inflammatory, analgesic, anticonvulsant, antioxidant, and anthelmintic[19]. They are generally synthesized by reacting carbonyl compounds with primary or secondary amines[20,21].

This work presents an attempt to screen the anti-oxidant, analgesic and anti-inflammatory activity of benzimidazole-based Schiff bases. Their chemical synthesis was reported in a previous work[22]. Therefore, the synthesized benzimidazole derivatives were tested on lab mice to state whether they can be promising therapeutic agents. Different pharmacological models were used to study their possible antioxidant, analgesic and anti-inflammatory activity.

II. MATERIAL AND METHODS

Chemicals and Drugs:

o-Phenylenediamine, *p*-aminobenzoic, aromatic aldehydes, DPPH, acetic acid, carboxymethylcellulose and formalin were all commercially available chemical grade. The solvents were used without further purification. Aspirin and Morphine sulphate were provided from Sigma-Aldrich.

Animals:

Male and female Albino mice of weight (25-35 g) were used for different experiments. Mice were bred in the animal house of the University of Tripoli, where each group was housed separately in a cage. Standard food pallet diet and water were available *ad lib*. The animals were kept at constant room temperature (20-25^0 C), with 12 hours dark/light cycle. The institutional animal ethical committee (IAEC) has approved the protocol to conduct experiments on these animals.

Synthesis of 2-(4-aminophenyl)benzimidazole-based Schiff bases:

Synthesis and characterization of the titled compounds were previously reported by our group[22]. As shown in scheme 1, the 2-(4-aminophenyl)benzimidazole (1a) was obtained in 60% yield by refluxing of *o*-phenylenediamine and *p*-aminobenzoic acid in acidic medium (4N HCl). Reaction of 2-(4-aminophenyl)benzimidazole (1a) with different aromatic aldehydes in methanol under refluxing conditions gave the corresponding 2-substituted benzimidazole-based Schiff bases (1-12).

Scheme 1: Synthetic scheme for the title compounds.

Antioxidant Activity Evaluation:

The antioxidant activity of the compounds was evaluated by DPPH method as this method is widely used to test the free radical scavenger ability of compounds and also to evaluate their antioxidant activity[23].Solutions from synthesized benzamidazoles were prepared in a concentration of 1mg/1ml. Aliquot of 90 µl from each solution was added into a 1 ml of freshly prepared 0.2 µM DPPH solution in methanol. The solutions were incubated at 37 ^{0} C for 20 min. The absorbance was read at 517 nm using double beam spectrophotometer. Ascorbic acid (1mM) was used as positive control. Percentage scavenging activity of the synthesized compounds to ascorbic acid was calculated from the following formula:

SA (DPPH) (%) = A_c-A_t \A_c * 100%

Where; A_c = absorbance of control (ascorbic acid) solution, A_t= absorbance of test solution.

Drug administration:

Test compounds or drugs were suspended in 1% w/v carboxymethylcellulose. Control and test animals (n≥4) were injected intraperitoneally either by test compound or vehicle control in dose 50mg/kg or various concentration of Aspirin or Morphine. Volume of injection was fixed at 1ml/100g. Tests for analgesic and anti-inflammatory activity were performed 30 minutes after test or drug administration[23].

Analgesic and anti-inflammatory activity Evaluation:

a. Chemically induced pain by Acetic acid

Albino mice (treated and control groups) were injected intraperitoneally with 1ml/100g of 3% acetic acid, the number of writhings was recorded for a period of 10 minutes ignoring the period of first 10 minutes after injection of acetic acid[24].

b. Formalin induced pain and inflammation test

Formalin 5% in saline was injected into the dorsal surface of mouse hind paw. The time that mouse spent licking the paw was recorded. The response pattern was two distinct periods of intensive licking activity; an early (0-5 min after injection) and a late response (20-30 min after injection)[25]. The early phase is a sign for analgesic activity while the late phase is a parameter points toward the anti-inflammatory activity[26].

c. Thermally induced pain by hot plate

The mice were placed individually on the hot plate at 55-56°C. End point was recorded when the animal licked its fore-limbs or jumped out of the plate. The cut-off time of the experiment was 30 seconds, this test is a useful approach to assess the centrally analgesic activity[27].

Statistical analysis:

Descriptive statistical analysis was applied on the parameters of different samples using SPSS (software package, version 10) to find out whether the observed samples normally distributed using Kolmogorov-Smirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, treatments were compared by applying One-way ANOVA (one dependent variable) or Two-way ANOVA (two dependent variables) Post-Hoc tests (LSD and Duncan tests) was applied. If the parameters are not normally distributed, treatments were compared by applying the Mann-Whitny two samples (non-matched) test. The differences were considered to be significant at $p<0.05$.

III. RESULT AND DISCUSSION

Antioxidant Activity:

It has been recognized that many diseases such as cancer, cardiovascular disease and diabetes are contributed to chronic inflammation. It is also widely thought reactive oxygen species are produced during inflammation[28]. Antioxidants are agents that conquer the free radicals through the intervention at one of the three major steps of free radical mediated oxidative process[29]. Therefore agents with antioxidant properties could help in reducing the burden and the destructive effects of inflammation and accompanied pain. Benzimidazoles were reported to have antioxidant activity[30], thus we promoted to explore the free radical scavenging activity of our previously synthesized benzimidazoles. DPPH radical scavenging assay was used to evaluate the antioxidant activity.

Most of the tested compounds showed higher than 50% scavenging activity compared to the standard ascorbic acid (table1). Furthermore compounds 4, 6, 9 and 11 scored activity above 75%. These results indicate the ability of the compounds to reverse free radical scavenging activity. Therefore they might protect cells and molecular components from the destructive action of oxidation processes and lipid peroxidation[31].

Table 1:DPPH scavenging capacity of the tested compounds as a percentage to ascorbic acid activity

Test compound	Molecular weight	Percentage of antioxidant scavenging activity
Main compound (1a)	342.35	21.9± 2.1
1	297.35	62.5 ± 3.3
2	313.35	21.9 ± 3.0
3	340.42	21.0 ± 1.1
4	331.8	78.1 ± 4.2
5	327.38	65.6 ± 3.2
6	311.38	81.3 ± 4.4
7	343.88	59.37 ± 3.6
8	323.39	59.87 ± 6.1
9	287.32	81.3 ± 2.9
10	303.30	21.87 ± 1.4
11	313.35	84.3 ± 2.6

12	313.35	1± 1.1

Values are the means of triplicate experiments ± standard deviation.

Analgesic and anti-inflammatory activity

Benzimidazoles were previously reported to have various biological activities including the analgesic effects[32]. Analgesic activity testing models are broadly fall into two categories; for centrally acting drugs (morphine like action) and the other one is for peripherally acting analgesics (aspirin like action). The peripheral analgesic and anti-inflammatory activity was tested using chemically induced pain and inflammation by acetic acid. On the other hand, the central analgesic activity of the compounds was tested using hot plate model[33].

a. Effect on acetic acid induced pain and inflammation

It has been found that some benzimidazoles retain significant activity against acetic acid induced pain[34].

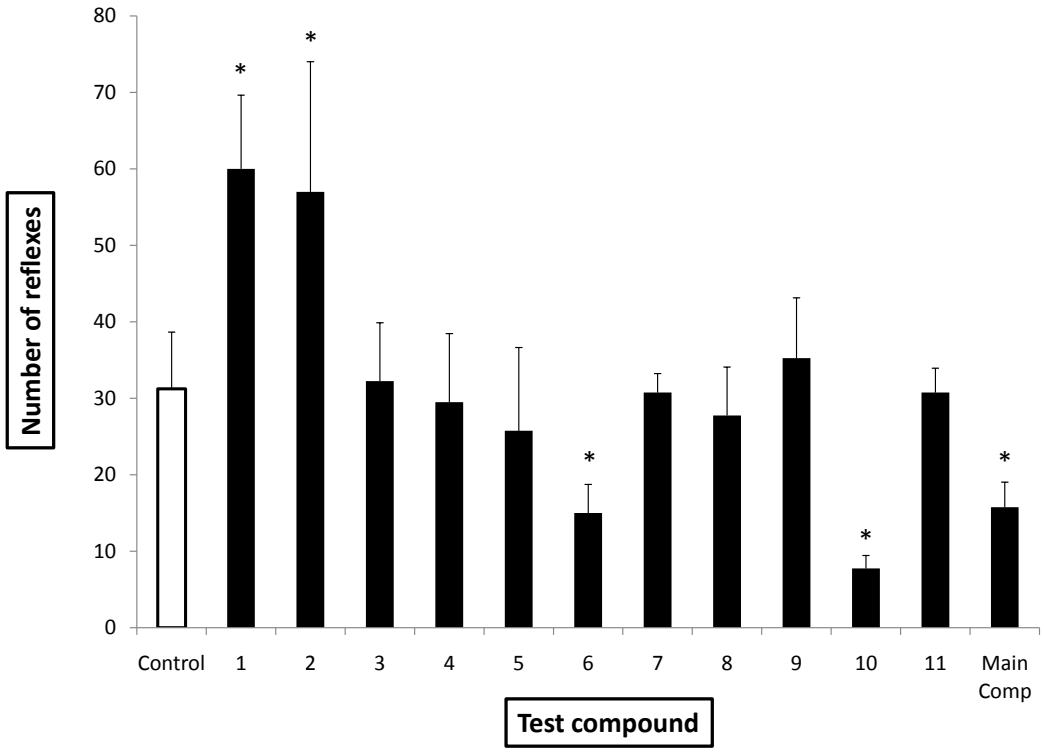

Figure 1: Effect of the tested benzimidazoles on chemically induced pain and inflammation by acetic acid.

Some of tested benzimidazole derivatives showed significant reduction in the number of writhing reflex upon acetic acid injection compared to the vehicle control (figure 1). Compounds 6, 10 and the main compound (1a) produced significant decrease in writhings. This result goes hand in hand with what was previously reported by our group[35]. The significant inhibition produced by some of the tested benzimidazoles led us to compare it to Aspirin, the classical non-steroidal anti-inflammatory drug (NSAID) (figure 2). Animals treated at dose of 50mg/kg of compounds 6, 10 and the main compound produced reduction in percentage of writhings to 45%, 18.7% and 48%respectively of control. Comparatively the same dose of Aspirin reduced the reflexes to 8%. This indicates a considerable analgesic effect to these compounds and could be an incurring evidence to carry out more testes to elucidate their other pharmacological aspects. On the contrary, compounds 1 and 2 resulted in an unexpected significant increase in the number of writhings which may be due to increasing the sensitivity of animals to pain and inflammation.

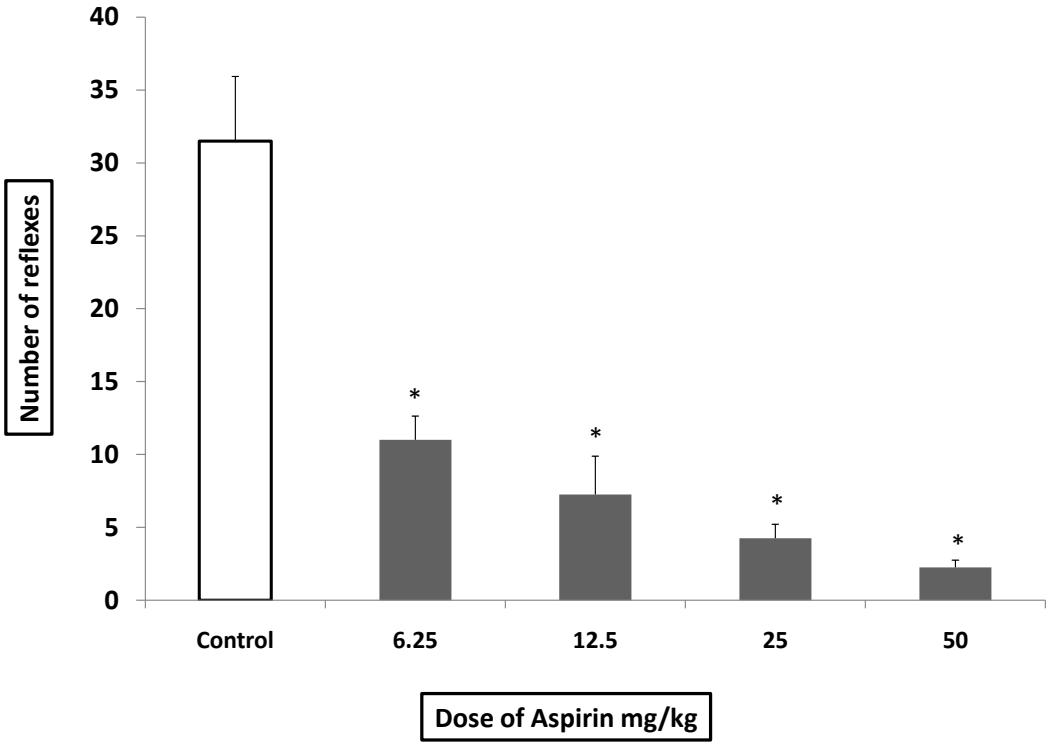

Figure 2: Effect of Aspirin on chemically induced pain and inflammation by acetic acid.

The promising results of acetic acid test encouraged us to further investigate the centrally acting analgesic activity of the under test benzimidazoles. Hot plate is used as a model of thermally induced pain. As can be seen in figure 3, benzimidazole derivatives 1, 3, 6, 7, 9, 10, 11, 12 and the main compound produced a significant increase in the response time of animals to heat upon putting on hot plate surface. So these encouraging results promote us to compare them with the standard morphine (figure 4).

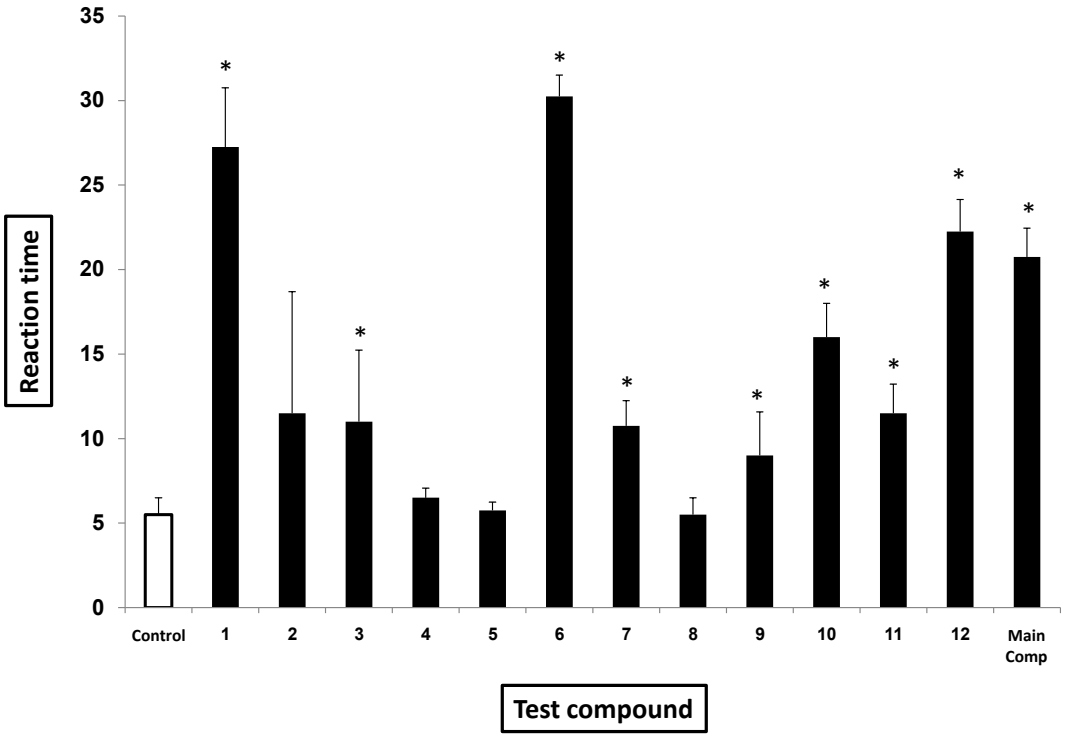

Figure 3: Effect of the tested bezamidizole derivatives on thermally induced pain by hot plate.

Compounds 1, 6, 12 and the main compound (1a) in a dose of 50 mg/kg produced similar effect or higher than that produced by morphine at a dose of 25kg/mg, i.e. almost half potency of morphine.

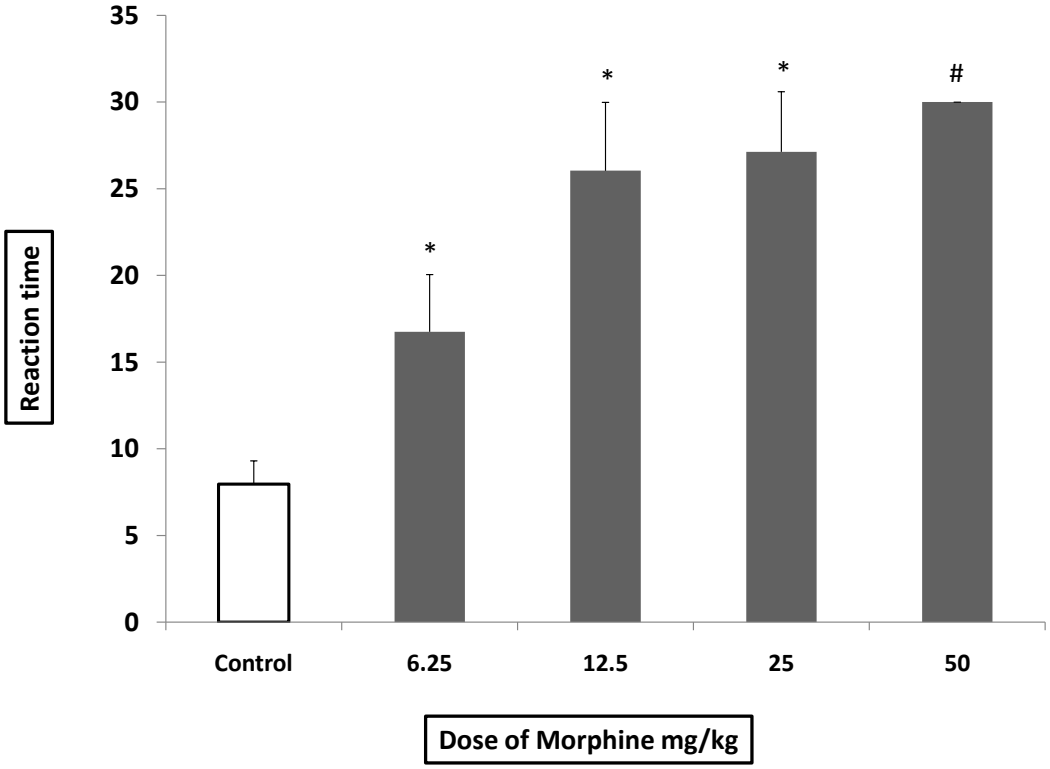

Figure 4: Effect of morphine on thermally induced pain.
# indicates the reaction time was longer than the cut off time (30 seconds).

As the main compound (1a) and compounds 6 showed both significant effects on both models of pain i.e. chemically and thermally induced pain, therefore they were selected for carrying out formalin test to assess their potential anti-inflammatory activity. The two distinct periods of licking as a response to formalin injection exerted by animal indicates the analgesic activity at phase I (after 5 minutes) and anti-inflammatory activity during phase II (after 20 minutes)[25,26]. Both compounds caused great reduction in phase I and II responses (figures 5 and 6). However the effect of main compound (1a) was greater than compound 6 in reducing the number of mouse licking its paw, this outcome strengthening the results generated from the acetic acid test that pointed to analgesic activity retained by these compounds. In addition the significant decrease of number of lickings during phase II leads to expect a possible good anti-inflammatory activity of these two benzimidazole derivatives.

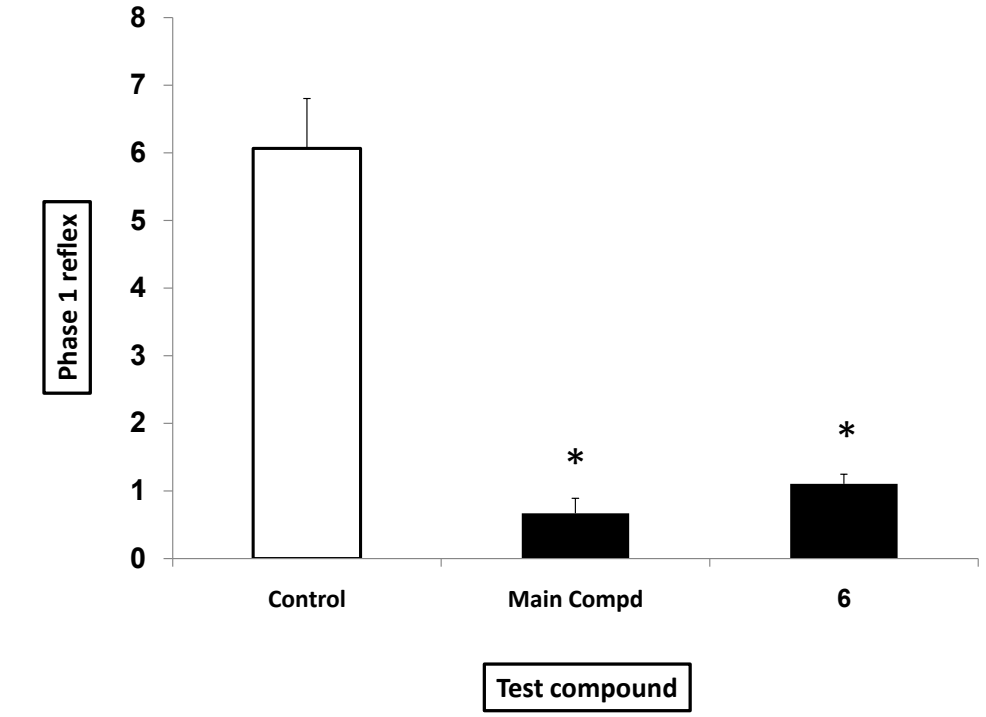

Figure 5: Formalin test phase I indicating the effect of main compound (1a)and compound 6 on paw lickings by animal in the period (0-5 minutes) after formalin injection.

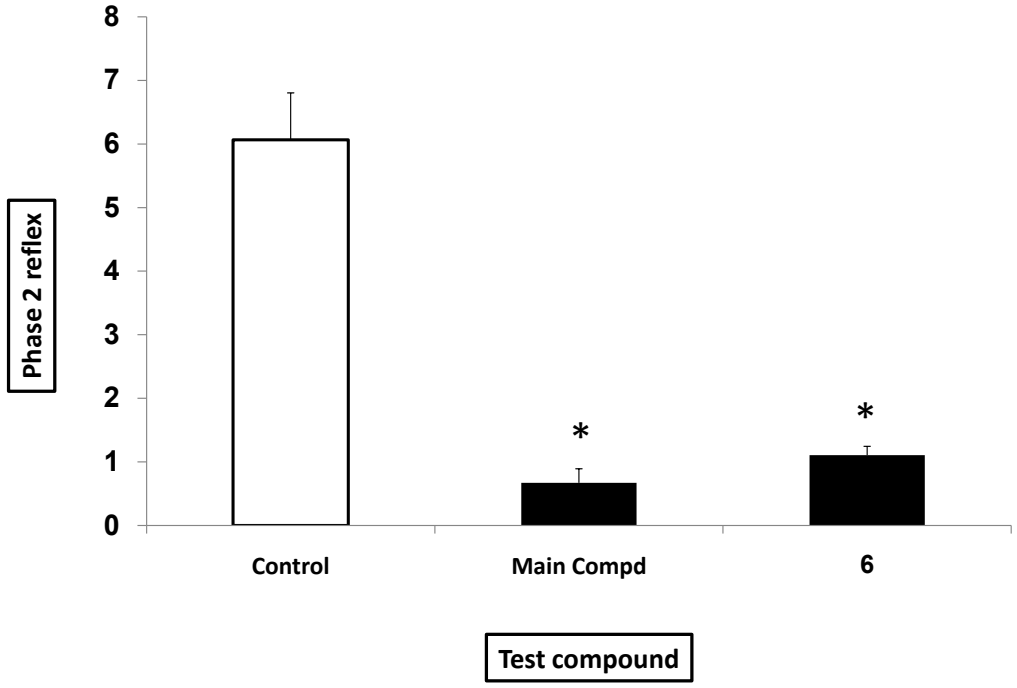

Figure 6: Formalin test phase II indicating the effect of main compound (1a) and compound 6 on paw lickings by animal in the period (0-5 minutes) after formalin injection.

IV. CONCLUSION

To conclude, some of the tested benzimidazole Schiff bases showed noticeable effects in different analgesic and anti-inflammatory models. So these compounds may worth further pharmacological screening to elucidate the mechanisms of action and to establish toxicity data. Likewise, compounds showed considerable anti-radical potentials and thus warranted further studies.

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