ANTIOXIDANT AND ANTIDEPRESSANT EFFECTS OF FOUR NOVEL BUPROPION ANALOGUES

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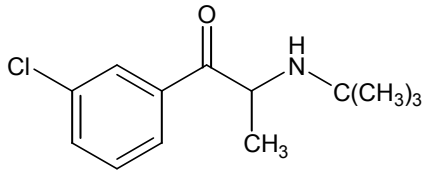
Summary

In order to introduce new antioxidant and antidepressant compounds and to study the relation between the structure and the effect of bupropion, we investigated the antioxidant and antidepressant effects of its new analogues (A-D). The antidepressant effects were investigated using forced swimming test in mice. Taking the previous studies into consideration, doses of 2.5, 5, 10, 15, and 20 mg/kg of bupropion and its analogues were selected and injected intraperitoneally into male BALB/c mice at three different times of 23.5, 5, and 1 hours before the main swimming test. Only in the dose of 15 mg/kg, bupropion reduced significantly the duration of immobility ($p< 0.05$). Analogue A in all doses other than 2.5 mg/kg, analogue B in the doses of 15 and 20 mg/ kg, analogue C in all doses (it had the most effect in the dose of 15 mg/kg) and analogue D only in the dose of 20 mg/kg showed significant effects on the duration of immobility. To differentiate between the antidepressant effects and locomotor activity effects, the open field test was used. In this test, only analogue C in the dose of 20 mg/kg significantly increased total locomotion ($p< 0.05$). Finally, the results showed that compound C in the dose of 15 mg/kg had the most effect in comparison with the other compounds and bupropion. Regarding the structure of the analogues, compound C contains the cyclic structure of piperidine which has probably produced a better effect. The tested compounds also showed good antioxidant activity in DPPH Radical-Scavenging Activity. Compound C showed the most antioxidant activity. Its IC_{50} was 63 ± 2.1 mg ml^{-1}.

Key words: Bupropion, Antidepressant, Forced Swimming Test, Open field test, Antioxidant activity, DPPH.

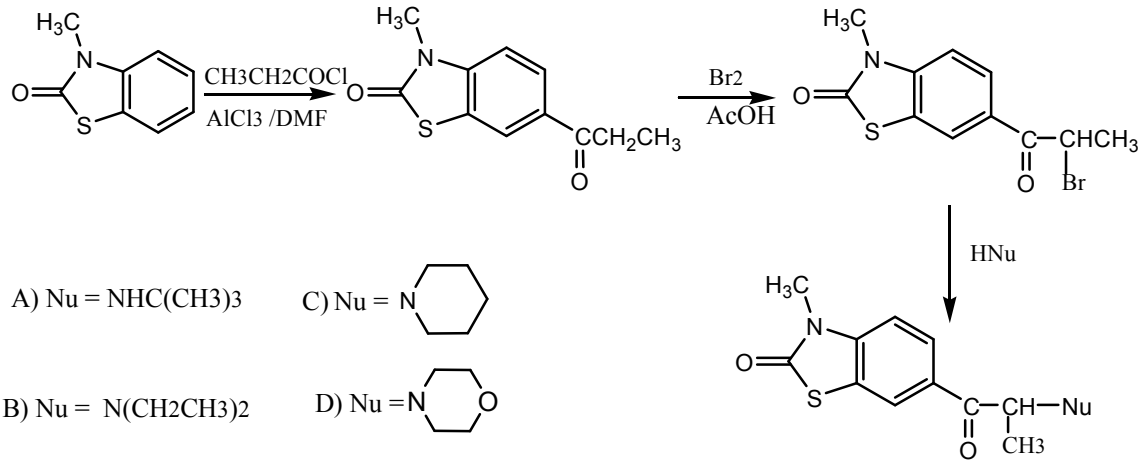
Introduction

Depression is a common, chronic and potentially debilitating illness that has tempered the human condition since the beginning of recorded history (1). It is a potentially life threatening disorder that affects hundreds of millions of people all over the world. It can occur at any age from childhood to late life and is a tremendous cost to society as this disorder causes severe distress and disruption of life and, if left untreated, can be fatal (2).The area of pharmacotherapy of depression started in the 1950s, with landmark publications and discoveries that still govern the manner in which we treat depression. There are currently 22 medications have received FDA approval in USA for the treatment of antidepressants (1). They can be grouped into four categories: Tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs) and miscellaneous. Bupropion is an antidepressant with a unique mechanism of action essentially lacking cardiovascular, anticholinergic and sexual dysfunction effects but incidence of seizures limited the use of bupropion as a first line agent (3). Bupropion is a trimethylated monocyclic phenylaminoketone that is an effective antidepressant in humans (4).



Bupropion

Its mechanism of action may be related to inhibition of norepinephrine and dopamine reuptake. Its usual dosage is 100 mg three times daily (1). Clinical trials comparing bupropion 300-750 mg/d with placebo show it to be superior to placebo inefficacy and as well tolerated. Bupropion, in controlled clinical trials, is as effective as amitriptyline or imipramine, with fewer side effects. Bupropion appears to be safe and effective in both adult and geriatric depressed patients. Although it appears to be safer and equally efficacious when compared with currently used antidepressants, it has not been tested by routine clinical use (5). In our previous work we synthesized 2-Benzothiazolinone analogues A-D of bupropion (6,7) (scheme 1) and in this paper we report antidepressant effects of these analogues. 2-Benzothiazolinone could be prepared from 2-aminothiophenol and urea in fusion condition. Reaction with dimethyl sulfate in NaOH solution afforded our starting methylated materials. 3-methyl-6-propionyl-2-benzothiazolinone could be prepared either by reaction of propionic acid in PPA (in higher yield) or by Friedel Crafts reaction of propionyl chloride in DMF/AlCl3 (in lower one). Bromination of this compound in glacial acetic acid afforded 2-bromopropionyl analog in good yield. Reaction of appropriate amine with the latter gave desired compounds A-D (6,7).



Scheme 1. Synthesis of 2-benzothiazolinone derivatives.

The antioxidant and antidepressant activities of compounds (A-D) were performed in order to understand some structure activity relationships in 2-benzothiazolinone series.

Materials and methods

Animals: Male BALB/c mice, weighing 20-30 g, were kept in the animal house of Mashhad University of Medical Sciences, in colony rooms with 12/12 h light/dark cycle at 22 ± 2°C. The animals had free access to food and water.

Preparation of samples: Test agents were dissolved in DMSO. The solution was injected intraperitoneally with a constant volume of 10 ml/kg. Doses used are shown in figure 1. Control animals were given DMSO of the same volume as the test agent. Six mice were used for each dose.

Forced Swimming Test: This test was performed essentially as previously described 26 groups of mice (n = 6/ dose) were individually introduced into a cylinder (13 cm in diameter) containing water (13 cm deep, 25°C) and left there for 15 min (habituation). Mice were then dried and returned to their home cage. Twenty four hours later, mice were replaced in the cylinder containing water (13 cm deep, 25°C) and left there for 6 min; the total duration of immobility in each mouse was measured during the last 4 min (test). Mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads above water. Test compounds were administered i.p. 23.5, 5 and 1 h before the test session. The effect of drug was expressed as the doses that produced a statistically significant reduction in the duration of immobility as compared with control (8-10).

Open-Field test: The test room was illuminated at the same intensity at the colony room. Each mouse was placed in the center of the open field, made of white wood had a floor of 100× 100 cm divided by red lines into 25 squares of 20× 20 cm and walls (50 cm high) were also painted in white, and mice behaviors were observed for 5 min. The parameters evaluated were the total number of squares crossed, the number of outer squares (those adjacent to the walls) crossed, and the number of inner squares crossed; the three measures were referred to as total, peripheral, and central locomotion, respectively (11).

DPPH radical-scavenging activity: The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of samples (12-15). Different concentrations of samples were added, at an equal volume, to methanolic solution of DPPH (100 μM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. Vitamin C, BHA and quercetin were used as standard controls. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Statistical Analysis: The data were expressed as mean ± SEM and tested with analysis of variance followed by the multiple comparison test of Tukey-Kramer.

Results

Forced Swimming Test: The results are shown in Figure 1. Bupropion was used as a standard antidepressant. Bupropion reduced the duration of immobility only at 15 mg/kg ($p<0.05$). Analogue A in all doses other than 2.5 mg/kg, analogue B in the doses of 15 and 20 mg/ kg, analogue C in all doses (it had the most effect in the dose of 15 mg/kg), and analogue D only in the dose of 20 mg/kg showed significant effects on the duration of immobility.

Open-Field test: To differentiate between the antidepressant effects and locomotor activity effects, the open field test was used. In this test, only analogue C in the dose of 20 mg/kg significantly increased total locomotion ($p < 0.05$).

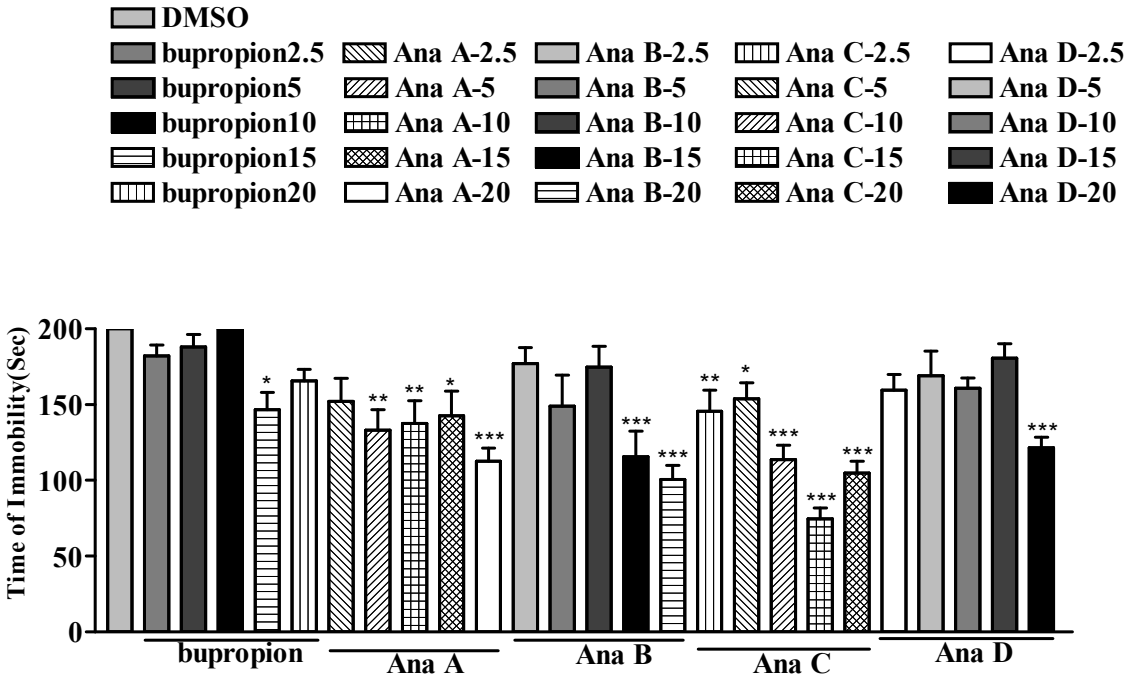


Figure 1: The effect of bupropion and its analogues (**A-D**) on immobility time in the forced swimming test. Agents were administered to mice intraperitoneally 23.5, 5 and 1h prior to test. Values are the mean ± S.E.M. for 6 mice, *P<0.05 , **P< 0.01 and ***P<0.001, as compared to control, Tukey-Kramer.

DPPH radical-scavenging activity: It was found that the radical- scavenging activities of all the compounds increased with increasing concentration. IC_{50} for DPPH radical scavenging activity was in the order: piperidino (63 ± 2.1) > tert-butylamino (181 ± 11) > diethylamino

(247 ± 14.7) > morpholino (450 ± 19) mg ml^{-1}, respectively. The IC_{50} values for ascorbic acid, quercetin and BHA were 5.05 ± 0.12, 5.28 ± 0.43 and 53.96 ± 2.13 μg ml^{-1}, respectively.

Discussion

The forced swim test (FST) was developed by Porsolt and colleagues (16) in the rat and subsequently, in the mouse. This test is the most widely used tool for assessing antidepressant activity preclinically (17). The compounds were tested by the porsolt swimming test method in order to identify potential antidepressant activity. Bupropion was used as a standard antidepressant. Analogue C in the dose of 15 mg/kg had the most effect in comparison with the other compounds and bupropion. Regarding the structure of the analogues, compound C contains the cyclic structure of piperidine which has probably produced a better effect. In 2-benzothiazolinone series the activity was in the order of piperidino > diethylamino > tert-butylamino and > morpholino analog. In 2-benzoxazolinone series the following order were observed: diethylamino > piperidino > tert-butylamino and > morpholino analog (10). It improves in both series, diethylamino and piperidino derivatives were the most active compounds and were more potent than Bupropion at 15 mg/kg. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (18). DPPH is a stable nitrogen-centered free radical the color of which changes from violet to yellow upon reduction by either the process of hydrogen- or electron-donation. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (19, 20). It was found that the radical-scavenging activity of extract increased with increasing concentration. IC_{50} for DPPH radical-scavenging activity was 380 ± 12 μg ml^{-1}. In series, 2-benzothiazolinone and 2-benzoxazolinone analogs, the piperidino derivatives were the most active and morpholino derivatives had the least activity. Totally, 2-benzothiazolinone derivatives had more antioxidant activity than 2-benzoxazolinone analogs (10).

Conclusion

In both series of 2- benzothiazolinone and 2-benzoxazolinone analogs of Bupropion, piperidino derivatives had the best antioxidant and antidepressant activities.

Acknowledgements

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References

1. Koda-Kimble MA, Young LY. In: Applied therapeutics: the clinical use of drugs, 7th ed., Philadelphia, Lippincott Williams & Wilkines. 2005: 78.9.
2. Bondy B. Pathophysiology of depression and mechanisms of treatment. Dialogues. Clinical Neuroscience. 2002; 4: 7-20.
3. Helms RA, Quan DJ. In: Text book of therapeutics, drug and disease management, 8th ed, Philadelphia, Lippincott Williams & Wilkines, 2006: 1432.
4. Bourin M, Colombel MC, Redrobe JP, Nizard J, Hascoet M, Baker GB. Evaluation of efficacies of different classes of antidepressants in the forced swimming test in mice at different ages. Progress in Neuro Psychopharmacology & Biological Psychiatry. 1998; 22: 343-351.

5. Dufresne RL, Weber SS, Becker RE. Bupropion hydrochloride. Drug Intell Clin Pharm 1984; 18(12): 957-964.
6. Ebrahimzadeh MA, Moussavi Z, Haji-aghaee R. Synthesis of 6-carbamido and 6-acylo-2-benzoxazolinone and 2-benzothiazolinone derivatives. Chemistry: an Indian journal 2004; 1(5): 334-337.
7. Moussavi Z, Ebrahimzadeh MA, Javadian M, Saeedi A Synthesis of 3-methyl-6-[2-(substitutedamino) propionyl]-2-benzoxazolinone and 2-benzothiazolinone derivatives. Chemistry: an Indian journal. 2003; 1(4): 258-261.
8. Sakakibara H, Ishida K, Grundmann O, Nakajima J, Seo S, Butterweck V, Minami Y, et al. Antidepressant effect of extracts from Ginkgo biloba leaves in behavioral models. Biol Pharm Bull. 2006; 29: 1767-1770.
9. Yamada J, Sugimoto Y, Yamada S. Involvement of dopamine receptors in the anti-immobility effects of dopamine re-uptake inhibitors in the forced swimming test. Eur J Pharmacol. 2004; 504: 207-211.
10. Hadizadeh F, Ebrahimzadeh MA, Hosseinzadeh H, Motamed-Shariaty V, Salami S, Bekhradnia AR. Antidepressant and antioxidant activities of some 2-benzoxazolinone derivatives as Bupropion analogues. Pharmacologyonline 2009; 1: 331-335.
11. Pardon M, Perez-Diaz F, Joubert C. Cohen-Salmon C. Age-dependent effects of a chronic ultramild stress procedure on open-field behaviour in B6D2F1 female mice. Physiol Behav. 2000; 70: 7-13.
12. Ebrahimzadeh MA, Pourmorad F, Hafezi S. Antioxidant Activities of Iranian Corn Silk. Turkish Journal of Biology 2008; 32: 43-49.
13. Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activities of methanol extract of *Sambucus ebulus* L. Flower. Pakistan Journal of Biological Sciences 2009;12 (5): 447-450.
14. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Jafari M. Free radical scavenging activity and antioxidant capacity of *eryngium caucasicum trautv.* and *froriepia subpinnata,* Pharmacologyonline 2008; 3: 19-25.
15. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Fazelian M, Eslami B. In vitro Antioxidant and Free Radical Scavenging Activity of *Diospyros lotus* and *Pyrus boissieriana* growing in Iran. Pharmacognosy Magazine 2009; 4(18): 122-126.
16. Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Ther. 1977; 229: 327-336.
17. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends in Pharmacological Science 2002; 23: 238-245.
18. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM. Antioxidant activity of methanol extract of *Ferula assafoetida* and its Essential oil composition, Grasas y Aceites, 2009; 60(4): 405-412.
19. Ebrahimzadeh MA, Bahramian F. Antioxidant activity of *Crataegus pentaegyna subsp. elburensis* fruits extracts used in traditional medicine in Iran. Pakistan Journal of Biological Sciences 2009; 12(5): 413-419.
20. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Bahramian F. In vitro antioxidant activity of *Phytolacca americana* berries. Pharmacologyonline 2009; 1: 81-88.