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Original Article

Synthesis of phthalimide derivatives and evaluation of their anxiolytic activity

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Abstract

Based on the molecular modeling studies on two major families of anxiolytics and sedative hypnotics (benzodiazepines and barbiturates) a series of phthalimide derivatives were prepared from corresponding di-methyl phthalate derivatives in two steps, namely trans-amidification and ring closer of di-methyl phthalate with urea and base catalysed condensation of 4-bromobenzyl chloride and benzoyl chloride with resulting imide. The designed compounds were synthesized with satisfactory yields and chemical structures were confirmed using IR, NMR and Mass spectrophotometery. Among the compounds that were tested for anxiolytic activity using elevated plus-maze technique, N-benzoyl-phthalimide increased the time spent and the number of entries into the open arms at doses of 0.5 mg/kg (P<0.05).

Keywords: Phthalimide derivatives; Elevated plus-maze; Anxiety

INTRODUCTION

The structure-activity relationship for classical 5-phenyl-1,4-benzodiazepine-2-one anxiolytic agents has been described by Sternbach and other investigators (1). In general, the minimum requirements for binding of 5-phenyl-1,4-benzodiazepine-2-one derivatives to the benzodiazepine receptor includes an aromatic or heteroaromatic ring (Fig. 1, ring A), believed to participate in π/π stacking with aromatic amino acid residues of the receptor. An electronegative group (halogen, nitro) substituted at the 7-position markedly increases functional anxiolytic activity (2).

The barbiturates have a different pharmacologic profile from that of the benzodiazepines.

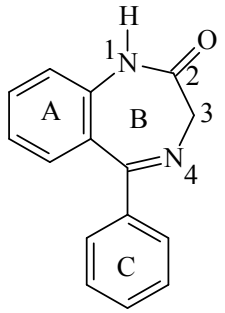

Fig. 1. General structure of 1,4 benzodiazepines.

Depending on the compound, dose, and route of administration, the barbiturates can produce different degree of CNS depression and therefore are used as sedatives, hypnotics, anticonvulsants, or anesthetics. Hundreds of barbiturates have been synthesized on a trial-and-error basis (3). In 1951, Sandberg (4) made his fundamental postulation that, to possess

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good hypnotic activity, a barbituric acid must be a weak acid and must have a lipid/water partition coefficient between certain limits. Therefore, only 5,5-disubstituted barbituric acids, the 5,5-disubstituted thiobarbituric acids, and the 1,5,5-trisubstituted barbituric acids possess acceptable hypnotic, anticonvulsant or anesthetic activity (Fig. 2).

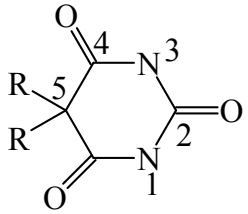
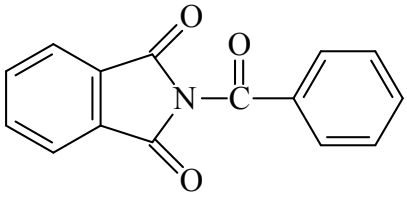


Fig. 2. General structure of barbiturates.

N-Benzoyl-phthalimide (Fig. 3) resemble both classic benzodiazepine and barbituric acid structures. It consists of a tricyclic hydrophobic structure comparable to that of benzodiazepines and possesses a conjugated ureid functional group as can be found in barbiturates. To find out more about conformational similarities, modeling studies were performed. Displaying, mani-pulation, and super-imposition of molecules were done with program Nemesis version 2. Results confirmed that size and tri-dimentional structures of benzodiazepine and phthalimide backbones are highly similar. In the light of these evidences, preparation and evaluation of anxyolitic activity of some phthalimide derivatives were aimed.

Fig. 3. Backboon of phthalimide derivatives.



MATERIALS AND METHODS

Chemistry

All reagents and solvents employed were of general purpose grade. Melting points were determined on an electrothermal 9200 apparatus and are uncorrected. Infra-red spectra were obtained as solid via a diffuse reflectance accessory using KBr matrix, on a Perkin Elmer 1420 series. ^{1}H-NMR spectra were recorded on a Bruker FT-80 spectrometer as dilute solutions in $CDCl_3$ or DMSO-d_6 with tetramethysilane as internal standard. Mass spectra were recorded on a trio 1000 Fisons mass spectrometer.

Initial three dimensional structures were built using Nemesis program, the primary structure of each compound was then subjected to conformational analysis where appropriate freely rotatable bonds, either as a pair or individually were selected and rotated in a stepwise sequence through 360 degrees. At the end of each conformational analysis step, by default, the conformer with the lowest van der waals energy was displayed and minimized. Final conformer of 5-phenyl-1,4-benzodiazepine-2-one was then superimposed onto N-Benzoyl-Phthalimide derivatives. The matched pair was viewed at different positions i.e. top and side elevations, and the geometry of the pair was characterized by measuring the distances between atoms of interest. Due to the QSAR studies cited above, the A/B/C rings of benzodiazepine were used for superimpositions in this work. Superimposition of the benzodiazepine rings on corresponding rings of N-Benzoyl-Phthalimide gave a suitable overlap (Fig. 4).

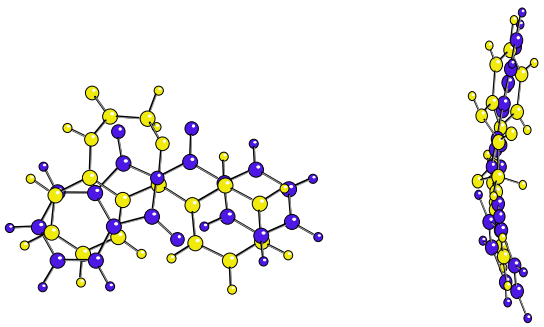
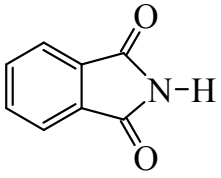


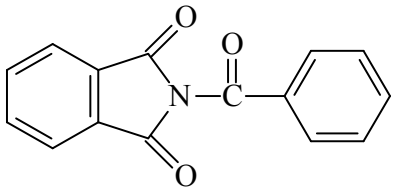
Fig. 4. Superimposition of benzodiazepine backboon (gray) on N-Benzoyl-Phthalimide (black) viewed from different positions.

Phthalimide



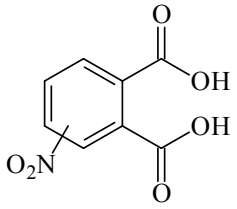
Dry urea (15 g, 0.25 mol) was added stepwise to a mixture of dimethyl phthalate (19.4 g, 0.1 mol) in 50 ml sodium methoxide and was stirred at reflux condition for 6 h. The resulting white suspension was concentrated under reduced pressure at 50 °C. The residue was dissolved in 100 ml of ice/water mixture and neutralized with diluted HCl. Percipitates were recrystalised from ethanol to give the imide, as white crystals (9.19 g, 62.5%), m.p. 228-230 °C (233-234 °C was reported in references 5 and 6); ν_{max} 3200 ν_{max} 1720 (C=O), 1600 (C=C, Ar) cm^{-1}; δ_H (80 MHz; $CDCl_3$), 11.2-10.8 (1H, br s, NH), 7.8 (4H, s, ph-H).

N-Benzoyl-Phthalimide



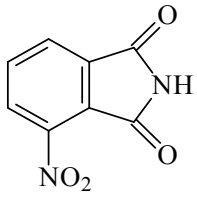
A mixture of phthalimide (1.5 g, 0.01 mol), benzoyl chloride (1.4 g, 0.01 mol) and potassium carbonate (5 g) in dry acetone (50 ml) was stirred at reflux for 6 h. After that, TLC monitoring showed the absence of starting material; the mixture was filtered and the filtrate evaporated at 60 °C under reduced pressure. The residue was dissolved in ethyl acetate, washed with water (2 × 20 ml), dried ($MgSO_4$) and concentrated at 60 °C under reduced pressure to give a white powder (0.5 g, 20%). Crystallization for an analytical sample from ethanol gave the N-Benzoyl-Phthalimide, m.p. 162-164 °C (168 °C was reported in refernce 7; M, 251. $C_{15}H_9NO_3$ requires M, 251]; ν_{max} 3035 (N-H), 1650-1800 (C=O), 1600 (C=C, Ar); δ_H (80 MHz, $CDCl_3$), 8.3-7.4 (9H, m, Ph-H)

3-Nitro Phthalic acid



To a preheated mixture of phthalic anhydride, (100 g, 0.675 mol) and concentrated sulfuric acid (100 ml) was added a mixture of fuming nitric acid (42 ml) and concentrated sulfuric acid (30 ml) dropwise and mixture was stirred at 100–110 °C for 1 h. To the resulting mixture was added 180 ml concentrated nitric acid and stirred for 2 h at 110 °C. Resulting mixture was added to 300 ml of crash ice to furnish the mixture of 3- and 4-nitro compounds as a wet cake. The wet cake was stirred in 40 ml of water to dissolve the unwanted 4-nitrophthalic acid, and filtered. Solid was recrystallised in boiling water to deposit 43 g (30%) of the 3- nitro phthalic acid as yellow solid. m.p. 212 °C (208-210 °C was reported in references 5 and 6); ν_{max} 3500-2500 (OH), 1720-1680 (C=O), 1600 (C=C, Ar), 1550–1350 (NO_2); δ_H (80 MHz, DMSO-d6), 8.4 – 8.3 (1H, d, J=8.9, CNO_2-C<u>H</u>), 8.2–8.1 (1H, d, J=8.9, CO-C-C<u>H</u>-CH), 7.9–7.7 (1H, t, J=8.9, CO-C-CH-C<u>H</u>).

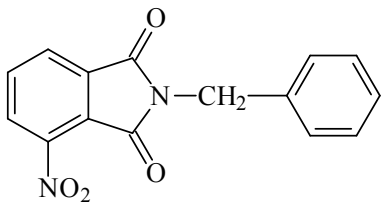
3-Nitro-Phthalimide



A mixture of 3- nitro phthalic acid (21.1 g, 0.01 mol) and urea (6 g, 0.1 mol) was refluxed in ethylene glycol mono methyl ether (40 ml) for 4 h. The resulting mixture

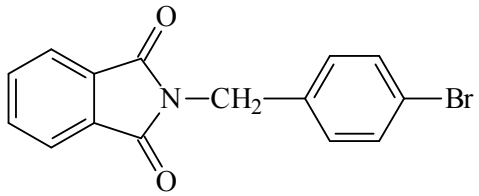
was transferred to a beaker containing crashed ice (150 ml) to deposit 3- nitro phthalimide as a yellow powder which was recrystalized in ethyl acetate. (13 g, 67%). m.p. 223 °C; ν_{max} 3200-3000 (NH), 1780-1680 (C=O), 1600 (C=C, Ar), 1550–1350 (NO_2); δ_H (80 MHz, DMSO-d6), 11.8–11.6(1H, br, NH), 8.2–7.9 (3H, m, Ph –H).

N-Benzyl 3-Nitro-Phthalimide



A mixture of 3- Nitro-phthalimide (5.76 g, 0.03 mol) and benzyl chloride (3.85 g, 0.03 mol) was refluxed in dry acetone (40 ml) containing potassium carbonate (4 g, 0.26 mol) for 3 h. The mixture was filtered and filterate was concentrated at reduced pressure to yeild N-benzyl 3- nitro-phthalimide as yellow crystal (1.13 g 13.3%). m.p. 100 °C; [Found: M, 282. $C_{15}H_{10}N_2O_4$ requires M , 282]; ν_{max} 3020 (CH), 1700 (C=O), 1600 (C=C, Ar), 1530–1370 (NO_2); δ_H (80 MHz, $CDCl_3$), 8–7.6 (3H, m, CNO2-CH-CH-CH), 7.5–7.1 (5H, m, Ph-H), 4.9 (2H, s, CH2).

N-(4-Bromobenzyl)-phthalimide



A mixture of phthalimide (0.44 g, 0.003 mol) and 4-bromobenzyl bromide (0.75 g, 0.003 mol) was refluxed in dry acetone containing potassium carbonate for 1 h. Mixture was filtered and the filtrate was concentrated at reduce pressure to produce N-(4-bromobenzyl) phthalimide as white crystals (0.4 g, 42.19%). m.p. 137-139 °C; [Found: M, 317. $C_{15}H_{10}BrNO_2$ requires M, 317]; ν_{max} 3020 (CH), 1770-1690 (C=O), 1600 (C=C, Ar), 1530–1370 (NO_2); δ_H (80 MHz, DMSO-d6), 8.1–7.6 (4H, m, Ph-H), 7.6–7.3 (2H, d, J=4.5 CH-CBr-CH), 7.4–7.2 (2H, d, J=4.5 CH-CH-CBr-CH-CH).

Pharmacology

Animals

Male NMRI mice (Pasteur, Tehran) weighing 25-30 g were housed in a cage with controlled room temperature at 22-25 ^oC. Food and water were available *ad libitum*. Tests were performed only after the mice had been acclimatized to the above environment for at least 7 days. All experiments were carried out between 09:00 and 13:00 h. Each mouse received a single i.p. injection of drug or vehicle and was tested once in the elevated plus-maze (EPM).

Elevated plus-maze

The EPM test is described in details elsewhere (8-11). Briefly, the apparatuscomprised of two open arms (35 × 5 cm) and two closed arms (30 × 5 × 15 cm) that extended from a common central platform (5 × 5 cm). The floor and the walls of each arm were wooden and painted black. The entire maze was elevated to a height of 50 cm above floor level as validated and described by Lister (10). Testing was conducted in a quiet room that was illuminated only by a dim light. Mice were given a single ip dose of various test compounds or diazepam (Sobhan Pharmaceutical Co. Iran) 30 min before their placement on the EPM. To begin a test session, mice were placed on the open arm facing the center of the maze. An entry into an arm was defined as the animal placing all four paws over the line marking that area. The number of entries and the time spent in the open and closed

arms were recorded during a 5-min test period. The percentage of open arm entries (100 × open/total entries) was calculated for each animal. Between each trial, the maze was wiped clean with a damp sponge and dried with paper towels.

Statistics

Statistical analysis was performed using one-way analysis of variance (ANOVA) with post hoc Tukey test. $P<0.05$ was considered significant. All data are expressed as mean ± standard error of mean (S.E.M.).

RESULTS

Elevated plus-maze results

In order to determine the anxiolytic effects of N-benzoyl-phthalimide on the EPM, various doses (0.5-10 mg/kg) of the compound was used. N-benzoyl-phthalimde at 0.5, 1, 2, 5 and 10 mg/kg significantly increased the number of entries into the open arms (Fig. 5).

There was no significant difference between the anxiolytic effects of 5 doses of the N-benzoyl-phthalimde. N-benzoyl-phthalimde at lower doses than 0.5 mg/kg did not produce significant changes in the number of entries and the time spent in the open arms ($P<0.05$, Fig. 5 and Fig. 6).

The other test compounds (N-benzyl phthalimide and N-(4-bromo) benzyl phthalimide) did not significantly alter any of the measured parameters on the EPM (Fig. 7).

Injected doses of the other compounds did not cause any recognizable changes in the EPM factors in comparison with control group.

DISCUSSION

The aim of the present study was to evaluate the anxiolytic action of somenewly synthetized phthalimide derivatives. In this study diazepam was used as a positive control drug. Diazepam produced significant increases in open arm time and in number of entries into the open arms.

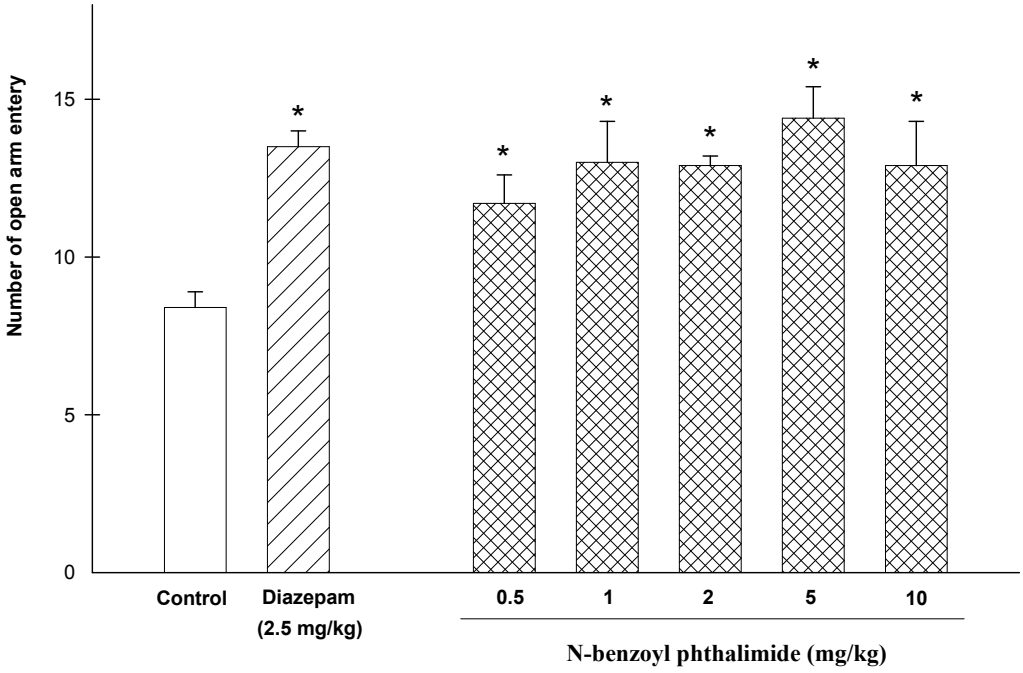


Fig. 5. Effects of diazepam and N-benzoyl phthalimide on the open arm entries of the EPM during a 5 min test in mice. Data are presented as mean values (± S.E.M.) from group of 6 mice. *$P<0.05$ compared with vehicle-treated control.

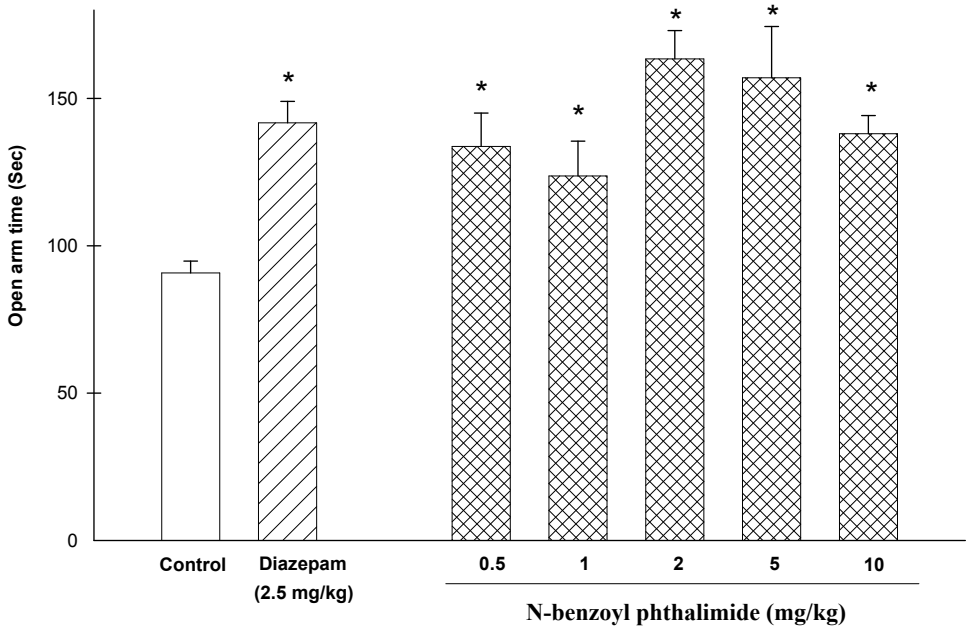


Fig. 6. Effects of diazepam and N-benzoyl phthalimide on the time spent in the open arms of the EPM during a 5 min test in mice. Data are presented as mean values (± S.E.M.) from group of 6 mice. **P*<0.05 compared with vehicle-treated control.

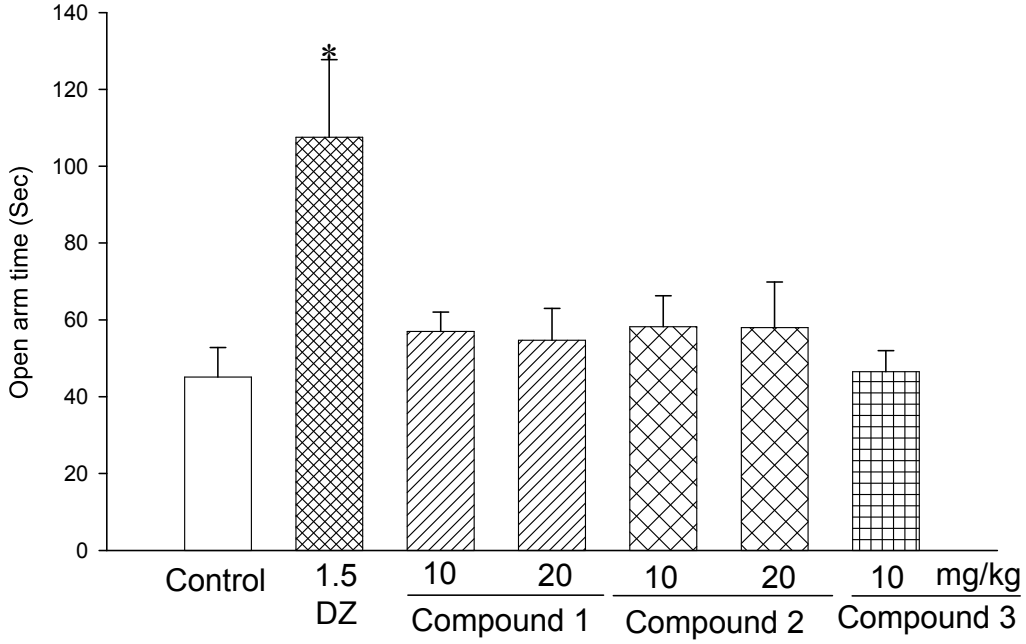


Fig. 7. Effects of diazepam (DZ) and N-benzyl 3-nitro phthalimide (compound 1), N-benzyl phthalimide (compound 2) and N-(4-bromo) benzyl phthalimide (compound 3) on the time spent in the open arms of the EPM during a 5 min test in mice. Data are presented as mean values (± S.E.M.) from group of 6 mice. **P*<0.05 compared with vehicle-treated control.

These data are consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce robust anxiolytic effects in a variety of anxiolytic screening procedures (7-10). Comparing the anxiolytic activities of tested compounds, the highest activity was observed with N-benzoyl phthalimide. This is in favor with the molecular modeling results, which showed a good match between diazepam and N-benzoyl phthalimide. N-benzyl 3-nitro phtalimide, N-benzyl phtalimide and N- (4-bromo benzyl phtalimide) are distorted from planarity due to the change of C=O group of N-benzoyl "which is a favorite anxiolytic agent with a dose of 0.5 mg/kg" to CH_2 group. This distortion probably prevents the accommodation of the compound with its receptor and makes the compound ineffective as an anxiolytic agent.

ACKNOWLEDGEMENT

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