Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(24):15-20
(http://www.derpharmachemica.com/archive.html)

Comparative Studies of Anti-inflammatory Activity *In vivo and In silico* Studies of 4-methoxy Benzoin and Benzoin

Thanuja B[1*], Ayyaddurai GK[1], kanagam CC[2]

[1]*Department of Chemistry, Sri Sairam Engineering College, Chennai, Tamil Nadu, India*
[2]*Department of Chemistry, Presidency College, Chennai, Tamil Nadu, India*

ABSTRACT

The Pharmacological activity of the synthesized benzoin (BEN) and 4-methoxy benzoin (4-MB) was studied and characterized by Mass, NMR, IR and single XRD analysis. Parent benzoin showed anti-inflammatory activity was evaluated by formalin induced paw oedema method. Introduction of additional methoxy group in the para position is found to more potent to have anti-inflammatory activity than parent benzoin. The results of the Pharmacological activity were comparatively studied using 1 EQG protein and that their activities were compared.

Keywords: Docking studies, Formalin induced paw oedema, Benzoins, Single crystal XRD

INTRODUCTION

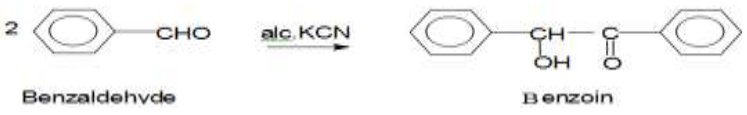
Inflammatory diseases are still one of the main health problems of the world′s population. Several modern drugs are used to treat these disorders but, their prolonged use may cause severe adverse side effects. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. Nowadays synthetic drugs play an important role for the inflammatory diseases [1]. The high cost of acquiring synthetic drugs, their inadequate supplies, the side effects associated with their uses, and the belief that traditional system of medicines hold cure to many disease condition like painful inflammatory conditions have led to a reawakening of interest in the utilization of new products in recent years. There is a need to intensify research into new medicine especially those claimed to have beneficial effects in serious inflammatory disorders [2]. Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases.

However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use. However, no work has been reported on the adequate characterization and anti-inflammatory effects on acute phase of inflammatory activity of 4-Methoxy Benzoin (4-MB) and Benzoin (BEN). The work has done for scientific evaluation to find out the anti-inflammatory effect of 4-MB and BEN in experimental animal model. Benzoin, compound having α-hydroxy ketone as a functional group is expected to have pronounced inflammatory activities [3,4]. We here in report the synthesize of BEN and 4-MB [5,6] having electron donor substituent which may improve the efficiency of anti-inflammatory activities [7]. In the present investigation undergone the docking studies of 4-MB, BEN synthesized compounds with 1EQG protein.

MATERIALS AND METHODS

Synthesis of benzoin

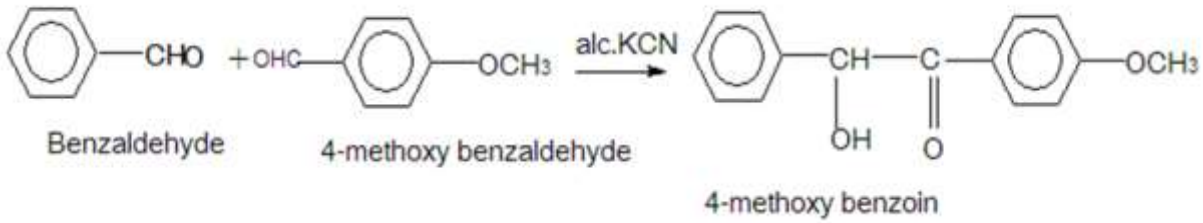
Pure benzaldehyde (47.6 cc., 0.47 mol) 50 g and 5 g of potassium cyanide (96-98%) are taken in a 3 L round-bottomed flask. To this 60 cc. of 95% alcohol are added along with 50 cc. of water. The mixture was subjected to reflux for half an hour. Finally the solution was cooled, filtered and washed with little water. 45-46 g (90-92% of theoretical amount) yield of a dry crude benzoin, which is light yellow, was obtained and its melting point found to be 135°C (Scheme 1).



Scheme 1: Schematic representation of benzoin

Synthesis of 4 -methoxy benzoin (4 MB)

The 4-MB was synthesized by benzoin condensation using 4 g of KCN dissolved in 75 cc of water in a one litre flask. 6.8 g (0.05 mol) of p-anisaldehyde (Scheme 2).



Scheme 2: Schematic representation of 4-methoxy benzoin

RESULTS AND DISCUSSION

Spectral studies

Single crystal X-ray diffraction data was recorded using a Bruker AXS Kappa Apex (II) CCD X-ray diffractometer with MoKα radiation (λ=0.71073 Å) to identify the structure of the grown crystal (Figure 1a and 1b). FTIR spectrum was recorded on a Bruker 66 V Fourier Transform Infra-Red (FTIR) spectrometer by the KBr pellet technique in the range 400-4000 cm^{-1} for the identification of the functional groups. Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectrum was recorded in a Brucker spectrometer of frequency 300 MHz using MeOD as a solvent (Aldrich) and Tetramethylsilane (TMS) (0.03%) as an internal standard.

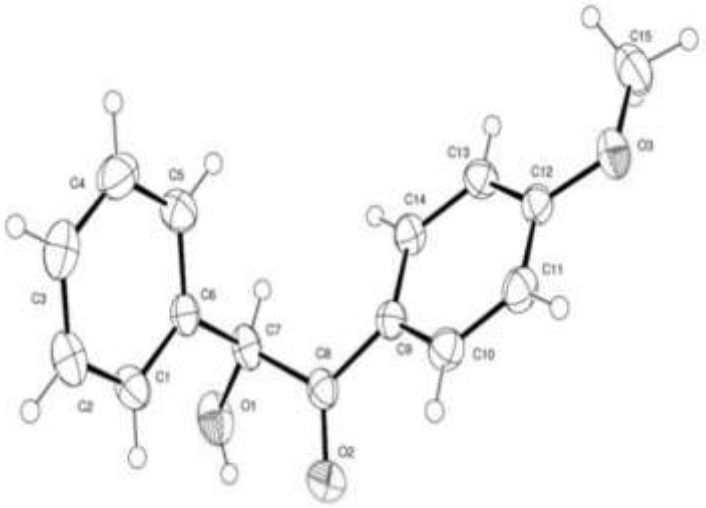


Figure 1a: ORTEP diagram of 4-MB

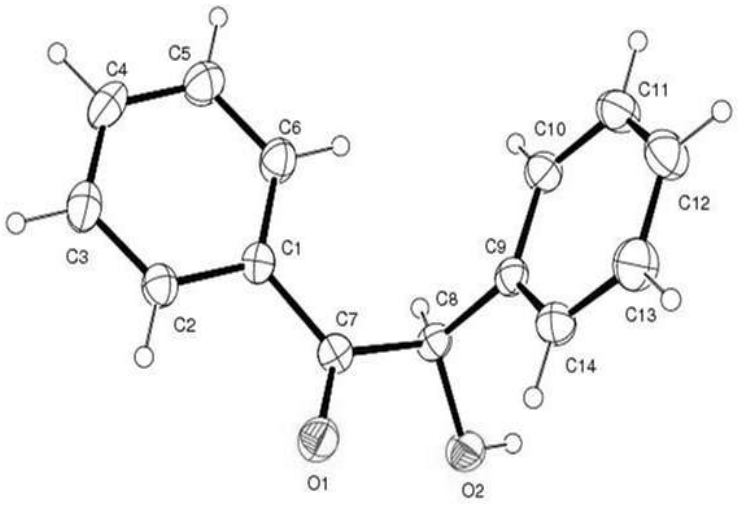


Figure 1b: ORTEP diagram of BEN

Molecular docking studies

Molecular docking study is a method to predict the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Computers and programs (software) are used to predict or simulate the possible reaction (interactions) between two molecules based on the three dimensional structures. This method can therefore be used not only to predict possible binders or inhibitors, but also to predict how strong association existing between the molecules [8]. It is useful to compare the binding strength between groups of compounds or derivatives. Prediction of the binding affinity will be useful to synthesis desired compounds. Because of its ability of predicting binding interactions and orientation, it is being widely used in rational drug design and structure based drug design processes. Molecular docking was performed for synthezised compounds using 1 EQG protein was taken from the protein bank with GLIDE 4.0 and IFD script from Schrödinger, LLC (New York) as a docking engine [9-14].

The anti-inflammatory activities were comparatively studied using different proteins and that their activities were compared. The anti-inflammatory activities of BEN and 4-MB were studied using two proteins 1 EGQ and 1 KPM. The binding interactions of 4-MB with 1 EGQ protein had at least two hydrogen bonding interaction and glide energy is found to be minimum when compared to BEN compounds. The docking diagram (Figure 2) and top score poses were shown in Table 1.

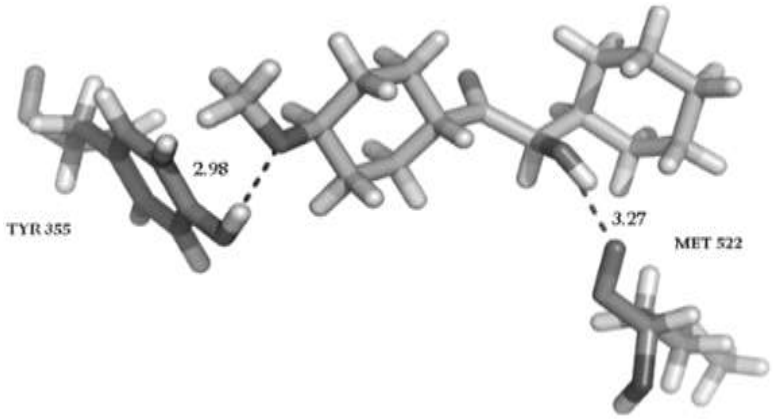


Figure 2: Hydrogen bond interactions of compound 4MB

Table 1: Energy and hydrogen-bond distance parameter for the compounds 4-MB and parent benzoin on binding with 1 EQG, 1 KPM proteins

	Compounds	Docking score	Glide energy (Kcal/Mol)	Hydrogen bond	
				Bond type	Distance (Å)
1 EQG	4-MB	-8.56	-36.78	MET 522 (O-H…O)	3.27
				TYR 355 (O-H…O)	2.98
	BEN	-7.51	-33.19		
1 KPM	4-MB	-4.91	-34.18	GLY 30 (N-H…O)	2.79
	BEN	-6.45	-37.44	GLY 30 (N-H…O)	3.23
				ASP 49 (O-H…O)	2.66

Materials and methods for acute toxicity studies of BEN and 4-MB

Swiss albino male mice, weighing between 25-30 g, 2 mon old obtained from animal house of Pharmacology department, Vels University was used for the experimental study. Six animals were housed, in each cage made up of polypropylene with stainless steel top grill and standard laboratory conditions of temperature 24-28°C, RH, 60-70% and 12 h light dark cycles were maintained. The animals were acclimatized for 7 days before commencement of the experiment in standard laboratory conditions 12 ± 01 h day and night rhythm, maintained at 25 ± 3°C and 50%-70% humidity as per Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) guidelines. Animals were provided with balanced food (Sai meera feeds, Bangalore) and water *ad libitum*. Protocol used in this study for using animals was granted by the ethical committee. (Approval number: XIII/VELS/PCOL/54/2000/CPCSEA/IAEC/08.08.2012).

Anti-inflammatory study of 4 MB and BEN by formalin induced oedema method

Formalin induced paw oedema method was adopted for evaluating anti-inflammatory activity [15-17]. Animals of all the groups were injected with 0.1 ml of 1% formalin in 0.9% normal saline, in the right hind foot under the plantar region. Group I animals (formalin control) administered a suspension of 2% of Carboxy Methyl Cellulose (CMC) p.o., 30 min before to formalin injection. Group II and III administered p.o., 5 and 10 mg/kg of 4-MB respectively, 30 min before to formalin injection. The standard reference group was given to group IV, p.o., an aqueous solution of aspirin (100 mg/kg), 30 min before to formalin injection. The paw volume or the inflammation was quantified in terms of ml i.e., replacement of mercury by oedema using a plethysmometer just before and 30, 60, 120 min after formalin injection. The inhibition percentage of the oedema was compared with the control groups. 4-MB at 5 and 10 mg/kg doses significantly inhibited the oedema in rats by oral administration. The inhibition found to be 50% at 5 mg/kg and 63.15% at 10 mg/kg (Figure 3). The group treated with aspirin showed maximum inhibition of oedema, which was 69.23%. Similarly the compound BEN on oral administration showed 30% inhibition at 25 mg/kg and 50% inhibition at 50 mg/kg was observed (Figure 4). Aspirin treated group showed maximum inhibition of oedema, which was 65.73%. This result confirms that the inhibitory effect of 4-MB and BEN (Tables 2 and 3). The 4-MB exhibited significant dose dependent anti-

inflammatory activity in the acute model of inflammation involving the induction of oedema in rat hind paw, comparable to the reference drug aspirin [18,19]. The reason for this significant effect may be due to the inhibition of cyclooxygenase enzyme which restricts the synthesis of prostaglandin.

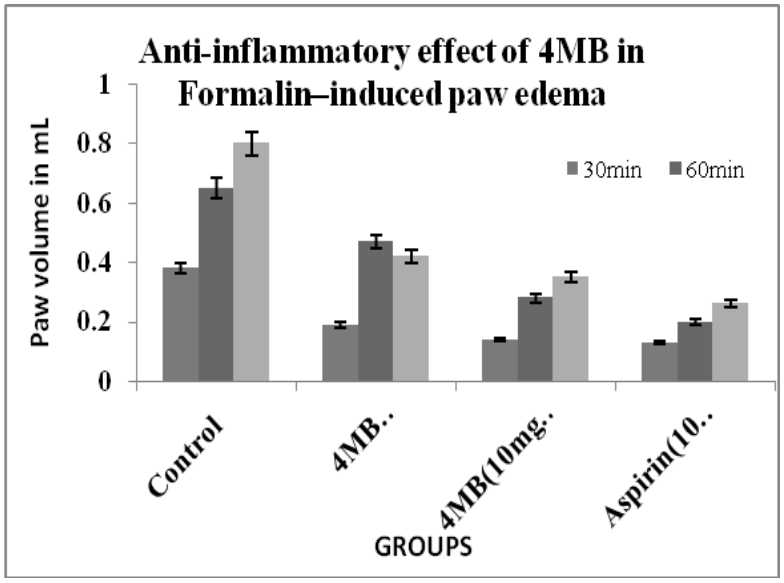


Figure 3: Anti-inflammatory effect of 4MB in formalin–induced paws edema

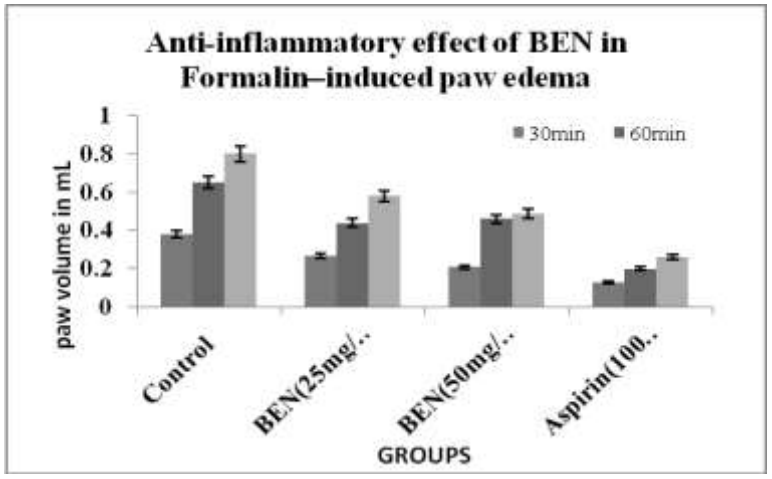


Figure 4: Anti-inflammatory effects of BEN in formalin-induced paw edema

Table 2: Anti-inflammatory effects of 4MB in formalin-induced paw edema

Group	Dose (mg/kg)	Increase of Paw volume in ml and % inhibition		
		30 min	60 min	120 min
Control	----	0.38 ± 0.08	0.65 ± 0.07	0.80 ± 0.09
4MB	5	0.19 ± 0.04** (50%)	0.47 ± 0.06* (27.69%)	0.42 ± 0.04** (47.50%)
4MB	10	0.14 ± 0.03** (63.15%)	0.28 ± 0.04** (56.92%)	0.35 ± 0.04** (56.25%)
Aspirin	100	0.13 ± 0.04** (65.78%)	0.20 ± 0.04** (69.23%)	0.26 ± 0.05** (67.50%)

n=6 animals in each group $^{*}p < 0.05$, $^{**}p < 0.01$

Table 3: Anti-inflammatory effects of BEN in formalin-induced paw edema

Group	Dose (mg/kg)	Increase of Paw volume in ml and % inhibition		
		30 min	60 min	120 min
Control	----	0.38 ± 0.08	0.65 ± 0.07	0.80 ± 0.09
BEN	25	0.27 ± 0.06** (28.94%)	0.44 ± 0.09* (32.30%)	0.58 ± 0.07** (27.50%)
BEN	50	0.21 ± 0.05** (44.73%)	0.46 ± 0.06** (29.23%)	0.49 ± 0.08** (38.75%)
Aspirin	100	0.13 ± 0.04** (65.78%)	0.20 ± 0.04** (69.23%)	0.26 ± 0.05** (67.50%)

n=6 animals in each group $^{*}p < 0.05$ $^{**}p < 0.01$

All the rats were sacrificed after blood collection. The positions, shapes, sizes and colors of internal organs were evaluated. The lung, liver were excised from all rats to visually detect gross lesions, and weighed to determine relative organs' weights and preserved in 10% neutral formalin for histopathological assessment. The tissues were embedded in paraffin, and then sectioned, stained with hematoxylin and eosin and were examined microscopically (Figure 5a and 5b).

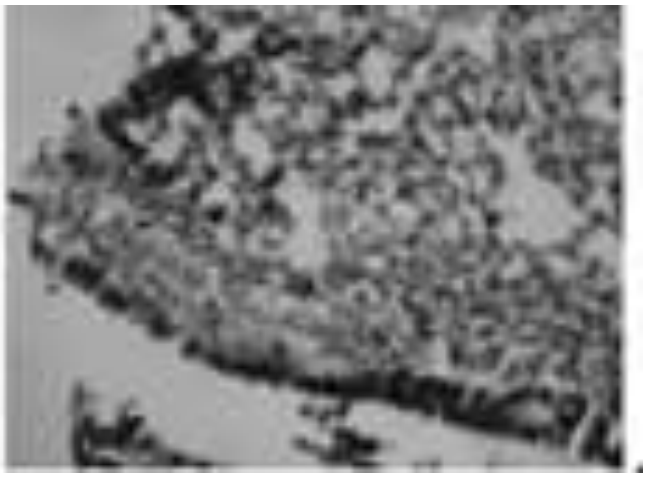


Figure 5a: Photomicrograph lung of rat: 4-MB 5 mg/kg

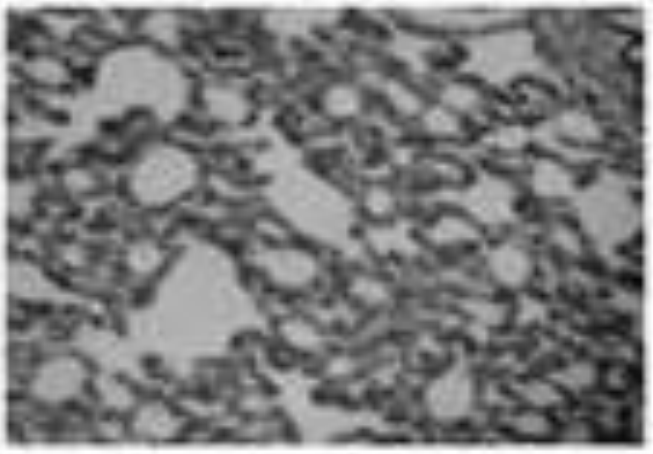


Figure 5b: Photomicrograph lung of rat: BEN 50 mg/kg

CONCLUSION

Core structure of the compounds 4-MB and BEN was found to be same but the degree of interaction and binding site were found to be different. The changes in the activities are due to the presence of electron donor-acceptor groups.

ACKNOWLEDGEMENT

The authors acknowledge the Dr. Anbu, Vels University for animal studies. Dr. Velmurugan, University of Madras for Docking studies and IIT, Chennai for spectral and single crystal XRD studies.

REFERENCES

[1] L. Ferrero-Miliani, O.H. Nielsen, P.S. Andersen, S.E. Girardin, *Clin. ExpImmunol*., **2007**, 147, 227.
[2] P.L. Hordijk, *FEBS. J*., **2006**, 273, 4408.
[3] J. Koprowski, E. Luczak, Krawczyk, *Tetrahedron.*, **2006**, 62, 12363.
[4] M. Bierenstiel, P.J.D. Hondt, M. Schlaf, *Tetrahedron.*, **2005**, 61, 4911.
[5] B. Thanuja, G. Nithya, C. Kanagam, *Photonics Lett. Poland*., **2012**, 4, 41.
[6] B. Thanuja, C. Kanagam, *J. Energy Chem. Engin*., **2014**, 2, 51.
[7] L. Ferrero-Miliani, O.H. Nielsen, P.S. Andersen, S.E. Girardin, *Clin. Exp. Immunol*., **2007**, 147, 227.
[8] I.D. Kuntz, J.M. Blaney, J.M. Oatley, S.J. Langridge, T.E.A. Ferrin, *J. Mol. Biol*., **1982**, 161, 269.
[9] Schrodinger, Glide version 4.0 LLC New York, **2005**.
[10] Schrodinger, Suite 2006, Induced Fit Docking protocol; Prime version 1.5, LLC New York, **2005**.
[11] M.D. Eldridge, C.W. Murray, T.R. Auton, G.V. Paolini, R.P. Mee, *J. Comput. Aided Mol. Des.*, **1997**, 11, 425.
[12] Pymol, DeLano Scientific LLC, San Carlos, California, USA, **1998**-**2004**.

[13] H.M. Berman, *Nature Struct. Biol*., **2000**, 7, 957.
[14] J. Westbrook, Z. Feng, L. Chen, H. Yang, H.M. Berman, *Research*., **2003**, 31, 489.
[15] M.B. Gupta, T.N. Bhalla, G.P. Gupta, C.R. Mitra, K.P. Bhargava, *Eur. J. Pharmacol*., **1969**, 6, 67.
[16] N.M. Gomes, C.M. Rezende, S.P. Fontes, M.E. Matheus, A.C. Pinto, P.D. Fernandes, *J. Ethnopharmacol.*, **2010**, 128, 177.
[17] D. Dhingra, A. Sharma, *Neuro. Psychopharmacol. Biol. Psychiatr*., **2006**, 30, 449.
[18] R.D. Porsolt, A. Bertin, M. Jalfre, *Arch Int. Pharmacodyn. Ther*., **1977**, 229, 327.
[19] L. Englert, D. Taylor, *J. Pharmacol*., **1973**, 49, 243.