SYNTHESIS AND ANTIOXIDANT ACTIVITY OF SOME NEW [1-BENZYL-2-PHENYL-SUBSTITUTED]-1H-5, 6-SUBSTITUTED-BENZO (d) IMIDAZOLES DERIVATIVES

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Summary

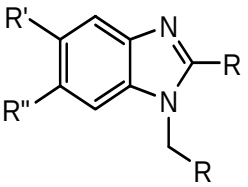
Nine new [1-benzyl-2-phenyl-Substituted]-1H-5, 6-substituted-benzo (d) imidazoles (2_{a-i}) were synthesized by reacting with substituted *o*-phenylene diamine with substituted benzaldehydes. All these compounds were characterized by means of their IR, ^{1}H NMR and Mass Spectroscopic data. Antioxidant activity of these compounds was evaluated by ferrous induced lipid peroxidation in rat brain homogenate. It was found that the compounds possessing electron releasing groups such as dimethyl amino, methoxy and hydroxyl substituent, at position 1 and 2 of benzimidazoles, considerably enhanced the activities when compared to the benzimidazoles having no substituents on the rings.

Key words: 1, 2- diaryl Benzimidazoles, Antioxidant activity, Lipid peroxidation

Introduction

Compounds bearing benzimidazole moiety are reported to possess a number of interesting biological activities such as antitubercular (1), anticancer (2, 3), antihelmintic (4), antiallergic (5, 6), antimicrobial (7-13), antihistaminic (14), anti-HIV (15), antiulcer (16, 17), cardiotonic (18), antihypertensive (19, 20) and neuroleptic (21). In previous studies several authors reported the synthesis and antioxidant (22-24) activities of a large series of benzimidazole derivatives. On the basis of these reports and as a continuation of our research program on benzimidazole derivatives, we report here the synthesis of novel benzimidazole derivatives to evaluate their antioxidant properties.

In connection with these studies, a series of new 1, 2-aryl substituted benzimidazoles was prepared by using citric acid (2(OH) propane-1, 2, 3tricarboxylic acid) as catalyst and appropriate aldehydes with the corresponding *o*-phenylenediamines for evaluation of their biological activities.



(2_{a-i})

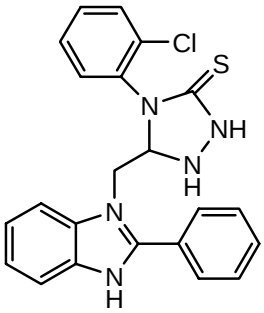


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Fig.1 Structures of [1-benzyl-2-phenyl-Substituted]-1H-5,6-substituted-benzo(d) imidazoles (2_{a-i}) and 5-(2-phenylbenzimidazol-1-yl-methyl)-4-aryl-4H-1,2,4-triazole-3-thione (3)

Earlier studies on some 1-[(thio carbamoyl hydrazine carbonyl)methyl]-2-phenyl-1H-benzimidazole,N-[(2-phenylbenzimidazol-1-yl-methyl)-[1,3,4]-thiadiazole-2-yl arylamines & their in vitro effects on lipid peroxidation in the rat liver (25). Hence, some new [1-benzyl-2-phenyl-Substituted]-1H-5,6-substituted benzo(d)imidazoles have been synthesized and evaluated for their antioxidant activities using ferrous induced lipid peroxidation in rat brain homogenate.

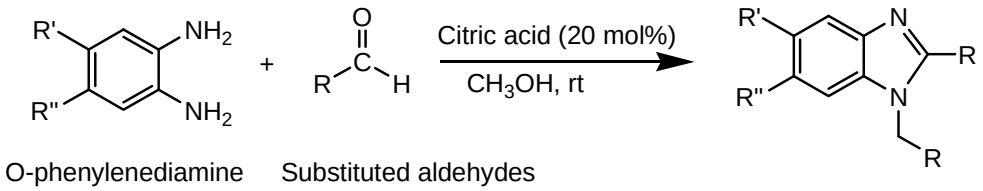
Material and Methods

Melting points were determined in open capillaries on a melting point apparatus (Tempo) and are uncorrected. UV spectra were recorded on UV visible spectrometer (Systronics Ltd., Ahmedabad). FTIR spectra were required using Thermo Nicolet Nexus 670 spectrometer. PMR is obtained using GEMINI-300 MHz and AVANCE-200 MHz Instrument using TMS as internal standard. All chemical shifts were reported as δ (ppm) values. The chemical reagents used in the synthesis were purchased from E. Merck (Darmstadt, Germany) and Aldrich (Milwaukee, MI, USA). All chemicals and solvents were reagent grade and used without further purification. Purity of compounds was checked by TLC using precoated Aluminium plates with Silica gel-G and the spots were detected by iodine vapor. Column chromatography was performed on silica gel (Merck, 60-120 mesh).

Animals

Albino rats (175-200 g) procured from Mahaveer Enterprises, Hyderabad, India were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25±2^{o}C and 50±15% relative humidity with a 12-h light/12-h dark cycle. Rats were fed with a commercial pellet diet (Rayans Biotechnologies Pvt Ltd., Hyderabad) and water *ad libitum*. The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of our institute. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

General procedure for the synthesis of 1-benzyl-2-phenyl-1H-5,6-substituted-benzo(d) imidazoles (2_{a-i})



O-phenylenediamine Substituted aldehydes

Synthesis of 1-benzyl-2-phenyl-1H-5,6-substituted-benzo(d) imidazoles (2_{a-i})

Citric acid (0.02 mmol) was dissolved in 4 mL CH_3OH. To this solution added 1,2-phenylenediamine and 5,6-substituted 1,2-phenylene diamine (1.0 mmol) and substituted aldehydes (2 mmol). The resulting mixture was stirred at room temperature for 3-4 hrs (monitored by TLC).diluted with water, extracted into EtOAc and purified by column chromatography on silica gel using hexone/ethylacetate (7:3) as eluent to yield the products. The chemical data of the compounds (2_{a-i}) are given in table 1.

Antioxidant Activity

Preparation of Rat Brain Homogenate

Wistar rats of either sex were used for the study. Prior to decapitation and removal of the brain, the animals were anesthetized with ether and perfused transcardially with ice-cold normal saline to prevent contamination of brain tissue with blood (26).Tissue was weighed and homogenates (10%, w/v) were prepared in 150 mM KCl and centrifuged at 800 RPM for 10 minutes. The supernatant was used immediately for the study. α-tocopherol was supplied by Sigma Chemical Co. was used as standard.

Iron Induced Lipid Peroxidation

The incubation mixture contained in a final volume of 1mL, brain homogenate (500µl), KCl (150 mM) and ethanol (10 mL) or test compound dissolved in ethanol.

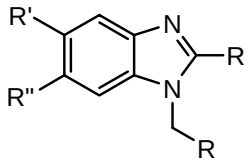
Peroxidation was initiated by adding to give the final concentration stated, Fe^{2+}(200 μM) (27-28). After incubating for 20 minutes at 37^0C, reactions were stopped by adding 2 mL ice-cold 0.25 N HCl containing 15% TCA, 0.38% thiobarbituric acid, and 0.05% BHT, following heating at 80^0C for 15 minutes.

Samples were cooled and centrifuged at 1000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm (29, 30). The amount of lipid peroxidation was determined using the molar extinction coefficient of $1.56X10^5M^{-1}cm^{-1}$ and expressed as thiobarbituric acid reactive substances (TBARS) as described by Braughler *et. al*. Percent inhibition of TBARS formed by test compounds was calculated by comparing with vehicle only control experiments. Iron solutions were prepared fresh in distilled water and used immediately. Since most buffers trap hydroxyl radical or interfere with iron conversion, the reactions were carried out in unbuffered 150 mM KCl. Results are expressed as the means of triplicate experiments (31).

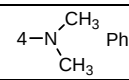
Results and Discussion

Chemical Data of the Compounds (2_{a-i})

Table 1. Chemical data of the compounds (2_{a-i})



Ph=phenyl

Compound Number	R′	R″	R	Reaction time (hr)	M.P (°C)	Yield (%)	Formula
2_a	H	H	Ph	3	135	80	$C_{20}H_{16}N_2$
2_b	H	H	2-OH Ph	5	198	67	$C_{20}H_{16}O_2N_2$
2_c	H	H	4-OH Ph	6	165	63	$C_{20}H_{16}O_2N_2$
2_d	H	H		2	160	80	$C_{24}H_{26}N_4$
2_e	H	H	3-OCH_3, 4-OH Ph	4	188	60	$C_{22}H_{20}O_4N_2$
2_f	H	H	4-OCH_3 Ph	4	130	78.4	$C_{22}H_{20}O_2N_2$
2_g	H	H		4	170	80	$C_{16}H_{12}N_2S_2$
2_h	CH_3	CH_3	Ph	3	165	70	$C_{22}H_{20}N_2$
2_i	Cl	Cl	Ph	2	181	70	$C_{20}H_{14}N_2Cl_2$

Spectral Data of the Compounds (2_{a-i})

Compd 2_a. 1-benzyl-2-phenyl-1H-benzo[d] imidazole
IR (KBr) υ 3030, 2926 (Ar, C-H), 1468 (C=N), 1328 (C-N), 1444 (C-C) cm^{-1} ^1H NMR (300 MHz, $CDCl_3$). δ 5.45 (s,2H,CH_2), 7.12 (dd, J = 8, 2 Hz, 2H,Ar-H) 7.15-7.38 (m, 6H,Ar-H), 7.4-7.48 (m, 3H,Ar-H), 7.70 (dd, J = 8, 2 Hz, 2H,Ar-H), 7.85 (d, J = 8 Hz, 1H,Ar-H). MS (EI) : 284 (M)

Compd 2_b. 1-(2-hydroxybenzyl)-2-(2-hydroxyphenyl)-1H-benzo[d] imidazole
IR (KBr) υ 3228(OH),3048,2926 (Ar, C-H), 1394 (C-O), 1240 (C-N), 1454 (C=N), 1592(C-C) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$+DMSO) δ 5.57 (s, 2H,CH_2), 6.85-7.01 (m, 4H, Ar-H), 7.19-7.36(m, 5H, Ar-H), 7.70-7.80 (m, 2H, Ar-H), 7.92-7.98 (d, J = 7.6 Hz, 1H, Ar- H), 2.52 (br s, 2H,OH MS (ESI) : 317 (M)

Compd2_c. 1-(4-hydroxybenzyl)-2-(4-hydroxy phenyl)-1H-benzo [d] imidazole
IR (KBr) υ 3246(OH), 2923 (Ar, C-H), 1443 (C=N), 1246 (C-N), 1347 (C-O), 1515 (C-C) cm^{-1}.^1H NMR (200 MHz, DMSO) δ 5.35 (s, 2H,CH_2), 6.80-6.91 (m, 4H, Ar-H), 7.11-7.46 (m, 5H, Ar-H) 7.49-7.56 (m, 2H, Ar-H), 7.91-8.01 (d, J = 7.6 Hz, 1H, Ar-H), 10.86 (br, s, 2H)MS (ESI) : 317 (M)

Compd2_d. N_1,N_1-dimethyl-4-1-[4-(dimethylamino)benzyl]-1H-benzo[d]imidazol-2-yl Aniline
IR (KBr)υ 2880, 2800 (Ar, C-H), 1441 (C=N), 1250 (C-N), 1526 (C-C) Cm^{-1}. ^1H NMR(300 MHz, $CDCl_3$) δ 2.93 (s, 6H,OCH_3), 3.01 (s, 6H, OCH_3), 5.37 (s, 2H,CH_2) 6.66-6.75 (m, 4H, Ar-H), 6.95-7.02 (m, 2H, Ar-H), 7.14-7.29 (m, 2H, Ar-H), 7.63 (d, J = 8.8 Hz, 2H, Ar-H), 7.79 (m, Hz, 2H, Ar-H) MS (ESI): 371 (M^+)

Compd2_e. 1-(3-methoxy, 4-hydroxy benzyl)-2-(3-methoxy,4-hydroxyphenyl)-1H-Benzo[d] imidazole.
IR (KBr) υ3399(OH), 2998, 2935,2832 (Ar, C-H),1458 (C=N), 1274 (C-N), 1389 (C-O),1458 (C-C)cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 3.70-3.81 (d, J = 6.2 Hz, 6H,2OCH_3), 5.39-5.42(s,2H,CH_2), 6.49-6.52 (d, J = 7.9 Hz, 1H, Ar-H), 6.60-6.68 (d, J = 8.1 Hz, 1H, Ar-H), 6.90-6.95 (d, J = 8.4 Hz 2H, Ar-H), 7.10-7.30 (m, 5H, Ar-H), 7.69-7.79 (d, J = 8.6 Hz, 1H, Ar-H), 8.40 (s, 1H,OH) 8.95 (s,1H,OH). MS(ESI): 377 (M^+)

Compd 2_f. 1-[4-methoxy benzyl]-2-[4-methoxyphenyl]-1H-benzo[d] imidazole
IR (KBr) υ 3053,2963, 2935 (Ar, C-H), 1459 (C=N), 1294 (C-N), 1382 (C-O), 1459 (C-C) cm^{-1}.^1H NMR (300 MHz, $CDCl_3$) δ 3.77 (s, 3H,OCH_3), 3.83 (s, 3H, OCH_3), 5.45 (s, 2H,CH_2), 6.81 (d, J = 8 Hz, 2H, Ar-H), 6.99 (m, 4H, Ar-H), 7.22 (m, 3H, Ar-H), 7.64 (d, J = 9 Hz, 2H, Ar-H), 7.78 (d, J = 8Hz, 1H, Ar-H)MS (ESI): 345 (M^++3)

Compd 2_g. 2-(2-thionyl)-1-(2-thionylmethyl)-1H-benzo [d] Imidazole
IR (KBr) υ 3058,2926, 2851 (Ar, C-H), 1568 (C=N), 1271 (C-N), 1091 (C-S),1448(C-C)cm^{-1}.^{1}H NMR (300 MHz, DMSO) δ 5.78 (s, 2H,CH_2), 6.87-6.97 (m, 1H, Ar-H),7.10-7.18 (d, J=7.3 Hz, 3H, Ar-H), 7.21-7.29 (m, 1H, Ar-H), 7.39-7.59 (m, 2H, Ar-H), 7.78-7.82 (d, J = 7.9Hz, 3H, Ar-H) MS (ESI) : 297 (M^+)

Compd 2_h. 1-Benzyl-2-phenyl-5,6-dimethyl-1H-benzo[d] Imidazole
IR (KBr) υ 3060 ,2925 ,2853 (Ar, C-H), 1969 (C=C), 1450 (C=N), 1289 (C-N), 1450 (C-C) cm^{-1}.^{1}H NMR (CDCl3, 300 MHz) δ 2.34 (s, 3H,CH_3), 2.41 (s, 3H,CH_3), 5.41 (s 2H,CH_2), 6.91 (s, 1H, Ar-H), 7.09-7.11 (d, J = 8 Hz, 1H, Ar-H), 7.25-7.47 (m, 6H Ar-H), 7.19 (s, 1H, Ar-H), 7.62-7.69 (d, J = 8 Hz, 1H, Ar-H), 7.97 (d, J = 8Hz, 1H, Ar-H), 8.15 (d, J = 8.1 Hz, 1H, Ar-H) MS (ESI) :313 (M^+)

Compd 2_i. 1-Benzyl-2-phenyl-5,6-dichloro-1H-benzo[d] imidazole
IR (KBr) υ 3060, 2925 (Ar, C-H), 1958 (C=C, Ar), 1448 (C=N), 1273 (C-N), 696(C-C) cm^{-1}.^{1}H NMR (300 MHz, $CDCl_3$) δ5.42 (s, 2H,CH_2), 7.09 (d, J =8 Hz, 1H, Ar-H), 7.25-7.29 (d, J= 7.7 Hz, 2H, Ar-H), 7.32-7.64 (m, 6H, Ar-H), 7.9 (s,2H, Ar-H), 8.1 (d, J = 8 Hz, 1H, Ar-H). MS (ESI):354 (M^+)

S=Singlet; dd=Doublet of doublets; m=Multiplet

Table 2. Antioxidant activity of the compounds (2_{a-i})

Compound number	% inhibition at 100 µm
2_a	62.9
2_b	70.7
2_c	72.4
2_d	80.7
2_e	75.0
2_f	76.3
2_g	59.4
2_h	60.9
2_i	71.5
Standard (α-tocopherol)	51.6
Control (vehicle)	-

In this study most of the compounds showed significant antioxidant activity on ferrous induced lipid peroxidation in rat brain homogenate. Compounds unsubstituted derivative (2_a) exhibited 62.9% activity. The 4-N,N-dimethylamino substituted derivative (2_d) showed highest activity (80.7%) and is comparable to α-tocopherol. Influence of substitution by non-phenyl groups like thiophenyl derivative showed less activity (59.4%) compared to unsubstituted derivative (62.9%). Substitution with electron donating groups such as 4-hydroxy, 4-OCH_3 and 4-CH_3 showed increased activity compared to

unsubstituted compound. 4-methoxy derivative (2_f) 76.3% showed increased activity. When this methoxy group is placed ortho to hydroxyl group, vanillinyl derivative (2_e) exhibited slight decrease in activity (75%) compared to methoxy derivative (2_f). Perusal of the data indicates that the ability of compounds to inhibit Fe^{+3} induced lipid peroxidation in rat brain homogenate is influenced mainly by the substitution at 4^{th} position on the phenyl ring. It was also been observed that with the increase in the size of the alkyl group, the activity has also increased.

References

1. V.L. Khairnar. S.R. Lockhande, M.R. Patel and B.G.Khadse, Chemical Abstract., 95, 203833h (1981).
2. L. L .Kruse, D. L. Ladd, P.B. Harrsch, F.L. McCabe, S.M. Mong, L. Faucette and R. Johnson, J. Med. Chem., 32, 409-417 (1989).
3. I. Islam, E.B. Skibo, R.T. Dorr and D.S. Alberts, J.Med.Chem., 34, 2954-2961 (1991).
4. V.J. Habernickel, Drugs made in Germany, 35, 97 (1992).
5. T. Fukuda, T. Saito, S. Tajima, K. Shimohara and K. Ito, Arzneim.-Forsch./Drug Res., 34, 805-810 (1984).
6. H. Nakano. T. Inoue, N. Kawasaki, H. Miyataka, H. Matsumoto. T. Taguchi, N. Inagaki, H. Naagi and T. Satoh, Chem. Pharm. Bull. 47, 1573-1578 (1999).
7. B. Can-Eke, M.O. Puskullu, E. Buyukbingol and M. Iscan, Chemico-Biological Interactions, 113, 65-77 (1998).
8. C. Kus, G. Ayhan-Kilcigil, B. Can-Eke and M. Iscan, Arch. Pharm. Res. 27, 156-163 (2004).
9. G. Ayhan-Kilcigil, C. Kus, T. Coban, B. Can-Eke and M. Iscan, Journal of Enzyme Inhibition and Medicinal Chemistry, 19, 129-135 (2004).
10. H. Goker, G. Ayhan-Kilcigil, M. Tuncbilek, C. Kus, R, Ertan, E. Kendi, S. Ozbey, M. Fort, C. Garcia and A., J. Farre, Heterocycles, 51, 2561-2573 (1999).
11. A. E. Abdel-Rahman, A.M. Mahmoud, G.M. EI-Naggar and H.A. EI-Sherief, Pharmazie 38, S89-S90 (1983).
12. F.S.G. Soliman. S.M. Rida, E.A.M. Badawey and T. Kappe, Arch. Pharm.317, 951-958 (1984).
13. R.A. Coburn, M. T. Clark, R. T. Evans and R. J. Genco, J. Med. Chem. 30, 205-208 (1987).
14. R. Iemura, M. Hori, H. Ohtaka, Chem. Pharm. Bull., 37, 962-966 (1989).
15. A. Chimirri, S. Grasso, A. M. Monforte, P Monforte, M. Zappala, I Farmaco, 46,925-933 (1991).
16. K. Ishihara, T. Ichikawa, Y. Komuro, S. Ohara, K. Hotta, Arzneim.-Forsch.,/Drug Res., 44, 827-830 (1994).
17. D. Y. Graham, A. Mccullough, M. Sklar, J.S. Sontag, W.M. Roufail, R. Stone, R. H. Bishop, N. Gitlin, A.J. Cagliola, R. S. Berman, T. Humphries, Digestive diseases and Sciences, 35, 66-72 (1990).
18. G. Piazzesi, L. Morano, J. C. Ruegg, Arzneim.-Forsch.,/Drug Res., 37, 1141-1143 (1987).

19. I. Wiedemann, H. Peli, H. Justus, S. Adamus, V. Brantl, H. Lohamann, Arzneim.-Forsch.,/Drug Res.**,** 35, 964-969 (1985).
20. K. Kubo, Y. Kohara, E. Imamiya, Y. Sugiura, Y. Inada, Y. Furukawa, K. Nishikawa, T. Naka, J. Med. Chem. 36, 2182-2195 (1993).
21. P.A.J. Janssen, F.T.N. Allewijn, Arzneim.-Forsch.,/Drug Res., 18, 279-282 (1968).
22. B. Can-Eke, M.O. Puskullu, E. Buyukbingol and M. Iscan, Chemico-Biological Interactions, 113, 65-77 (1998).
23. C. Kus, G. Ayhan-Kilcigil, B. Can-Eke and M. Iscan, Arch. Pharm. Res. 27, 156-163 (2004).
24. G. Ayhan-Kilcigil, C. Kus, T. Coban, B. Can-Eke and M. Iscan, Journal of Enzyme Inhibition and Medicinal Chemistry, 19, 129-135 (2004).
25. Guelguen, Archive Pharmacol. Res. 27, 156-163 (2004).
26. B.R. Shiva Kumar, SVR. Koullri, V. Ravindranath, Mol. Cell Biochem., 111, 125-129 (1992).
27. J.M. Braughler, L. A. Duncan, Int. J. Pharm. 100, 93-97 (1993).
28. M. Ciuffi, G. Gentilini, S. Franchi-Micheli, L. Zilletti, Neurochem Res. 16, 43-49 (1991).
29. H. Wiseman and B. Halliwell. FEBS Letters, 332, 159-163 (1993).
30. B. Halliwell and. J.M.C. Gutteridge, Methods Enzymol. Edited by Packerz **3**,186 (1990).
31. M.N.A. Sreejayan Rao, Int. J. Pharm. 100, 93-97 (1999).