SYNTHESIS AND ANTIOXIDANT ACTIVITY OF SOME NEW HYDROXY-(SUBSTITUTED4-HYDROXY-PHENYL)OR(SUBSTITUTED-NAPHTHALEN-1-YL)-ACETICACID2-(1,3-DIOXO-1,3-DIHYDRO-ISOINDOL-2-YL)-ETHYLESTERS DERIVATIVES

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Summary

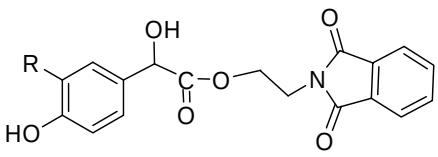
Seven new Hydroxy-(substituted 4-hydroxy-phenyl) or (substituted-naphthalen-1-yl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl esters(6_{a-g}) were synthesized by reacting with substituted Mandelic acids(2a-2e) with N-(2-hydroxy ethyl)Phthalamide. All these compounds were characterized by 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 methyl,methoxy and electron attracting groups such as chloro substituent, at position 3, 6 and 7of mandelic acid derived phthalamides,Considerably enhanced the activities when compared to the mandelic acid derived phthalamides having no substituents on the rings.

Key words: Mandelic acid derived phthalamides, Antioxidant activity, Lipid peroxidation

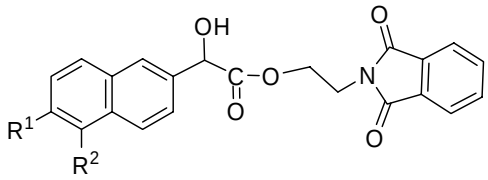
Introduction

Compounds bearing mandelic acid moiety are reported to possess a number of interesting biological ativities such as antibiotic(1),antichlonergic(2), antimicrobial(3-7), antithrombic(8), anticancer(9-11), anti-HIV(12-13), antihypertensive(14), anticonvulsant(15), antipsychotic(16), and immunomodulatories(17). In previous studies we reported the synthesis and antioxidant(18) activities of large series of mandelic acid derivatives. On the basis of these reports and as a continuation of our research program on mandelic acid derivatives,we report here the synthesis of novel mandelic acid derivativies to evaluate their antioxidant properties.

In connection with these studies, a series of new mandelic acid derived phthalamides was prepared by using DCC (N,N-Dicyclo Hexyl Carbidamide),1-4dioxane as catalyst and appropriate substituted mandelicacids with the corresponding N-(2-hydroxy ethyl)Phthalamide for evaluation of their biological activities.



(6a-6e)



(6f-6g)

Fig 1. Structures of Hydroxy-(substituted 4-hydroxy-phenyl) or (substituted-naphthalen-1-yl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl esters(6_{a-g})

Earlier studies on some synthesized 3,4-dihydroxy mandelic acid dopamide and 4-hydroxy mandelic acid dopamide which shows antioxidant property & their in vitro effects on lipid peroxidation in the rat liver(18).Hence, some new Hydroxy-(substituted 4-hydroxy-phenyl) or (substituted-naphthalen-1-yl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl esters 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 with out further purification. Purity of compounds was checked by TLC using precoated Aluminium plates with Silica gel-G and the spots were detected by iodine vapour. 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 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 Hydroxy-(substituted 4-hydroxy-phen(substituted-naphthalen-1-yl acetic acid2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl esters(6_{a-g})

STEP -1:

General Method for the synthesis of 2-(3-substituted-4-hydroxyphenyl), 2-(substituted-naphthalen-1-yl)-2-hydroxyethanoic acids (2a-2g)

To a mixture of substituted phenol, α-napthol or β-napthol (5,8 grams, 40 m.moles) & glyoxylic acid monohydrate (5.8 gms, 60 mmole) in water (20 ml) was added 2M KOH (50 ml, 0.10 mol) under cooling. The mixture was stirred at room temperature for 4 hrs, then neutralized with 2M HCl (50 ml, 0.10 mol) extracted with Et_2O (3 x 40 ml). The combined extracts were washed with H_2O & dried over $MgSO_4$. Evaporation of the solvent gave the crude product in good yield. Recrystallised from Et_2O- toluene(20:80)

STEP-2:

General Method for the synthesis of N-(2-Hydroxyethyl)phthalimide (5)

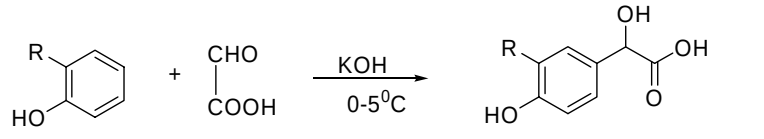
A mixture of phthalic anhydride (6.94g, 42.29 mmol) and ethanolamine (3.75g, 42.42 mmol) and triethyl amine (0.7 mmol) in toluene (500 ml) was heated under reflux for 4 hrs under azeotropic removal of water using Dean-stark apparatus. The reaction mixture was concentrated at reduced pressure, added ethylacetate to the residue and the organic phase was washed with 1N HCl solution (20 ml) to eliminate the unreacted triethylamine& dried over $MgSO_4$, concentrated to yield the N-(2-hydroxyethyl)phthalimide as a white crystalline solid. TLC :{n-hexane : ethylacetate 7 : 3} R_f :0.46,Melting Point-140^{0}C,Yield-90%

STEP-3:

General Method for the synthesis of Hydro-(substituted 4-hydroxy-phenyl) or (substituted-naphthalen-1-yl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl esters (6a-6g).

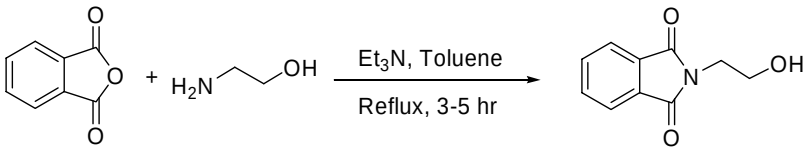
A mixture of substituted mandelic acid (3.5g, 20mmol) and N-(2-hydroxy ethyl)phthalamide (4g, 20 mmol) in 1,4-Dioxan (20ml) were taken in a round bottomed flask under nitrogen. Added N,N-dicyclohexyl carbodimide (4.5g, 20 mmol) to the mixture at room temperature and stirred for 48 hrs. The byproduct was precipitated out and then filtered. The filtrate thus obtained was extracted with chloroform. The combined layer was rotavapoured under reduced pressure. The product obtained was recrystallized from ethyl acetate and confirmed by TLC (30%). and purified by column chromatography on silica gel using hexone / ethylacetate (7:3) as eluent to yield the products.

STEP - 1



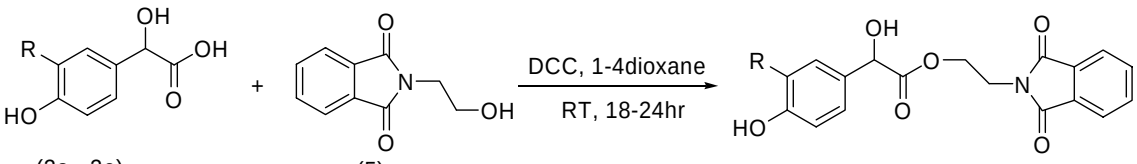
Substituted Phenols (1a-1e) | Glyoxylic acid | Substituted Mandelic acids (2a-2e)

STEP - 2



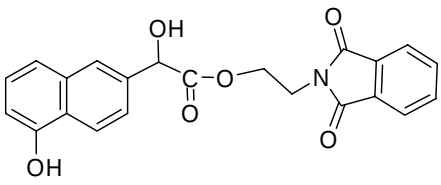
Phathalic anhydride **(3)** | Ethanolamine **(4)** | N-(2-hydroxyethyl)phthalamide **(5)**

STEP - 3

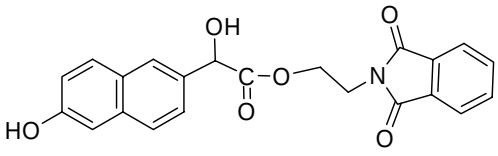


(2a - 2e) | (5) | (6a-6e)

Following the same procedure, other derivatives also synthesized



(6f)



(6g)

Anti-oxidant activity

Preparation of Rat Brain Homogenate

Wistar rats (180-200gm) 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(19),Tissue 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

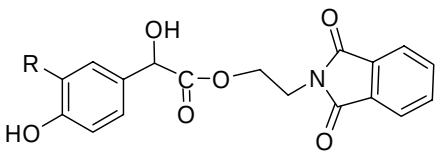
The incubation mixture contained in a final volume of 1ml, brain homogenate (500µl), KCl(150 mM) and ethanol (10 ml) or test compound dissolved in ethanol. Peroxidation was initiated by adding to give the final concentration stated, Fe^{2+}(200 µM)(20-21). After incubating for 20 minutes at $37^{O}C$, 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 $80^{O}C$ for 15 minutes.

Samples were cooled and centrifuged at 1000 rpm for 10 minutes. The absorbance of the supernatant was measured at 532 nm(22-23). The amount of lipid peroxidation was determined using the molar extinction coefficient of $1.56X10^{5}M^{-1}cm^{-1}$ 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 (24).

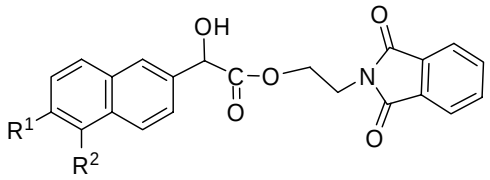
Results and Discussion

Chemical data of the compounds (6_{a-g})

Table 1. Chemical data of the compounds (6_{a-g})



(6a-6e)



(6f-6g)

Compound Number	R	M.P ^{0}C	Yield %	Formula
6a	H	108	62	$C_{18}H_{15}O_6N$
6b	Cl	104	60	$C_{18}H_{14}O_6NCl$
6c	OCH_3	116	75	$C_{19}H_{17}O_6N$
6d	CH_3	118	68	$C_{19}H_{17}O_5N$
6e	NO_2	123	55	$C_{18}H_{14}O_7N_2$

Compound	R^1	R^2	M.P ^{0}C	Yield %	Formula
6f	H	OH	118	70	$C_{22}H_{17}O_6N$
6g	OH	H	106	67	$C_{22}H_{17}O_6N$

Spectral data of the compounds(6_{a-g})

Compd 6a : Hydroxy-(4-hydroxy-phenyl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr) υ 3340(OH), 2925(C-H, Ar) 2854 (CH_2), 1719(C=O), 1294 (C-N), 1459(C-C) Cm^{-1} $^{.1}$HNMR(300MHz, d_6-DMSO):δ 3.92 (s, 4H, C_2H_4), 4.91 (s,1H, CH-OH), 6.78 (d, J = 8.4 Hz, 2H, Ar-H), 7.21(d, J = 8.9 Hz, 2H, Ar-H) 7.69 – 7.78 (dd, J = 8, 2Hz, 4H, Ar-H), 8.97 (br, 2H, OH). MS (ESI):341 (M^+)

Compound 6b : Hydroxy-(3-chloro-4-hydroxy-phenyl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr):υ 3327(OH), 2927(C-H, Ar) 2850 (CH_2), 1626(C=O), 1438 (C-N), 793(C-Cl) $Cm^{1.1}$HNMR (300MHz, d_6-DMSO):δ 4.18 (s, 4H, C_2H_4), 4.97 (s,1H, CH-OH), 6.92 (d, J = 7.5 Hz, 1H, Ar-H), 7.17(d, J = 7.6 Hz, 1H, Ar-H) 7.38 (s, 1H, Ar-H). MS (ESI):375 (M^+)

Compound 6c : Hydroxy-(3-methoxy-4-hydroxy-phenyl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr):υ 3470(OH), 2928(C-H, Ar) 2854 (CH_2), 1681(C=O), 1315(C-N),1448(C-C) $Cm^{1.1}$HNMR(300MHz, d_6-DMSO):δ 3.98(s, 3H, OCH_3),4.22 (s, 4H, C_2H_4), 5.98 (s,1H, CH-OH), 7.13 (s, 1H, Ar-H),7.21(d, J = 7.6 Hz, 1H, Ar-H) 7.71 (m, 1H, Ar-H),8.02-8.12(d, J = 7.8 Hz, 4H, Ar-H), 5.5 (br, 1H, OH), 9.42(br, 1H, OH).MS (ESI):341 (M^+)

Compound 6d : Hydroxy-(3-methyl-4-hydroxy-phenyl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr):υ 3270(OH), 2928(C-H, Ar) 2850 (CH_2), 1626(C=O), 1311 (C-N),1439(C-C) Cm^{-1}.HNMR (300MHz, d_6-DMSO):δ 2.12 (s, 3H, CH_3), 3.91 (s, 4H, C_2H_4), 4.82 (s,1H, CH-OH), 6.69 (d, J=7.8 Hz, 1H, Ar-H), 6.94-7.08 (m, 3H, Ar-H), 7.82-7.94 (dd, J=3,8Hz, 4H, Ar-H), 8.82 (br, 2H, OH).MS (ESI):354 (M^+)

Compound 6e : Hydroxy-(3-nitro-4-hydroxy-phenyl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr):υ 3472 (OH), 2948(CH_2), 2888 (C-H, Ar), 1767 (C=O), 1318 (C-N), 1429(C-C) Cm^{-1}.^{1}HNMR (300MHz, d_6-DMSO):δ 3.85 (s, 4H, C_2H_4),4.25 – 4.80 (br, 1H, CHOH), 7.08 (d, J = 7.9 Hz, 1H, Ar-H), 7.49 – 7.52 (m, 2H, Ar-H), 7.79 (s, 2H, OH), 7.98 – 8.02 (dd, J = 3, 7.9 Hz, 4H, Ar-H).MS (ESI):379 (M^++3)

Compound 6f : Hydroxy-(5-hydroxy-naphthalen-1-yl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr):υ 3383(OH), 2962(CH_2), 2921 (C-H, Ar), 1656 (C=O),1598(C-C) Cm^{-1}.^{1}HNMR (300MHz, d_6-DMSO):δ 4.01 (s, 4H, C_2H_4), 5.40 (s, 1H, CHOH), 6.92 (m, 2H, Ar-H), 7.42 (d, J = 8Hz, 2H, Ar-H),7.21 – 7.45 (m, 2H, Ar-H), 7.78 (dd, J = 3,7.8 Hz, 4H, Ar-H). MS (ESI):393 (M^++2)

Compound 6g : Hydroxy-(6-hydroxy-naphthalen-1-yl)-acetic acid 2-(1,3-dioxo-1,3-dihydro-isoindol-2-yl)-ethyl ester

IR (KBr):υ 3326(OH), 2927(C-H, Ar), 2850 (CH_2), 1626 (C=O), 1311 (C-N),1439(C-C) Cm^{-1}.^{1}HNMR (300MHz, d_6-DMSO):δ 3.76 (s, 4H, C_2H_4), 4.9 (s, 1H, CHOH), 6.78 (d, J=7.9Hz, 2H, Ar-H), 6.82 (d, J = 8.1Hz, 2H, Ar-H), 6.99 (m, 2H, Ar-H), 8.08 (br, 2H, OH). MS (ESI):391 (M^+)

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

Table 2. Anti- oxidant activity of the compounds (6_{a-g})

Compound number	% inhibition at 100 µm
6_a	76.6
6_b	77.4
6_c	85.9
6_d	73.5
6_e	71.2
6_f	84.4
6_g	76.8
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 (6_a) exhibited 76.6% activity. The Methoxy substituted derivative (6_c) showed highest activity (85.9%) and is comparable to α-tocopherol.Influence of substitution by nitro groups showed less activity (71.2%) compared to unsubstituted derivative (76.6%).Substitution with electron donating groups such as 5-NO_2 and 5-CH_3 showed decreased activity compared to unsubstituted compound..Perusual 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 5th position on the phenyl ring. It was also observed that with the increse in the size of the aryl(napthyl) group, the activity has also increased.

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