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Synthesis and Biological Evaluation of N-Cinnamoyl and Mandelate Metformin Analogues

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A series of N,N-dimethyl-N1-[3-(substituted phenyl)-1-oxo-2-propenyl]biguanides were synthesized by coupling a solution of metformin in pyridine with different cinnamoyl chloride derivatives in ether for 3 h in addition to synthesis of some five molecules of metformin-mandelates. All the synthesized cinnamoyl metformins and a few metformin-mandelates were characterized by IR, NMR and Mass spectroscopic techniques. All the synthesized compounds were also evaluated for their antioxidant activity by DPPH scavenging method and nitric oxide scavenging method. All the compounds exhibited good antioxidant activity.

Keywords: Antioxidants; Metformin-mandelates, Cinnamoyl metformins, Nitric oxide, DPPH.

INTRODUCTION

A continuous demand for a search for antioxidant molecules either by semi-synthetic or by synthetic means is inevitable. Double bond of cinnamic acid derivatives probably participates in stabilizing the radical by resonance. Increasing the number of hydroxy and methoxy substituents increases the potency of antioxidant side-chain and conjugated double bond in the side chain could have a stabilizing effect by resonance on the phenoxy radical. Hydroxy cinnamic acids are the most widely represented phenolic acids in food vegetables, strengthening their potential role as nutritional antioxidants [1]. It was discovered that cinnamic acid, a naturally occurring aromatic fatty acid of low toxicity, has a long history of human exposure [2]. Cinnamic acid exhibited several pharmacological activities, including antimicrobial and anti-inflammatory activity as well as platelet aggregation and its quartenary ammonium salts were reported to exhibit plant growth retardant activity [3]. Several hydroxylated cinnamic acid derivatives were found to have inhibitory effect on mutagenesis, in a dose dependent manner [2,4].

In present study, certain cinnamic acid and mandelic acids were prepared and coupled with metformin, to synthesize novel cinnamic acid and mandelic acid derivatives, could result in different pharmacological effects like anti-inflammatory, cardioprotective, neuroprotective, anticonvulsant and anti-hypertensive properties which are having oxidation properties as the causative factor.

EXPERIMENTAL

Melting points were determined in open capillaries on a melting point apparatus (Tempo) & were uncorrected. FTIR spectra were recorded using Thermo Nicolet 670 spectrometer. ^{1}H NMR spectra of the compounds in deuteriated dimethyl sulphoxide were recorded on GEMINI-300 MHz & AVANCE-200 MHz. All chemicals used are of AR grade. Purity of the compounds was checked by thin layer chromatography, using aluminium plates, precoated with silicagel GF 254. Ethanol was used as the eluent. The spots were visualized in the ultraviolet light chamber.

General method for synthesis of cinnamic acids: A mixture of substituted aldehydes (5 g, 0.33 mol), malonic acid (7.5 g, 0.72 mol) dissolved in pyridine (15 mL) and piperidine (0.25 mL) was heated under reflux for 2 h on a water bath. The reaction mixture was allowed to cool and poured into the excess of water, containing enough HCl. The solid mass, which separated out was filtered washed with little water, dried and recrystallized from glacial acetic acid and alcohol. By following the same procedure, different analogues of cinnamic acid were prepared. In present work, we have synthesized 12 substituted cinnamic acids (Fig. 1).

General method for synthesis of N-[3-(substituted phenyl)-2-propenyl-1-amido]metformin: A mixture of substituted cinnamic acid (0.137 g, 1.0 mmol) and ether (10 mL) and thionyl chloride (10 mL) were taken in an iodine flask

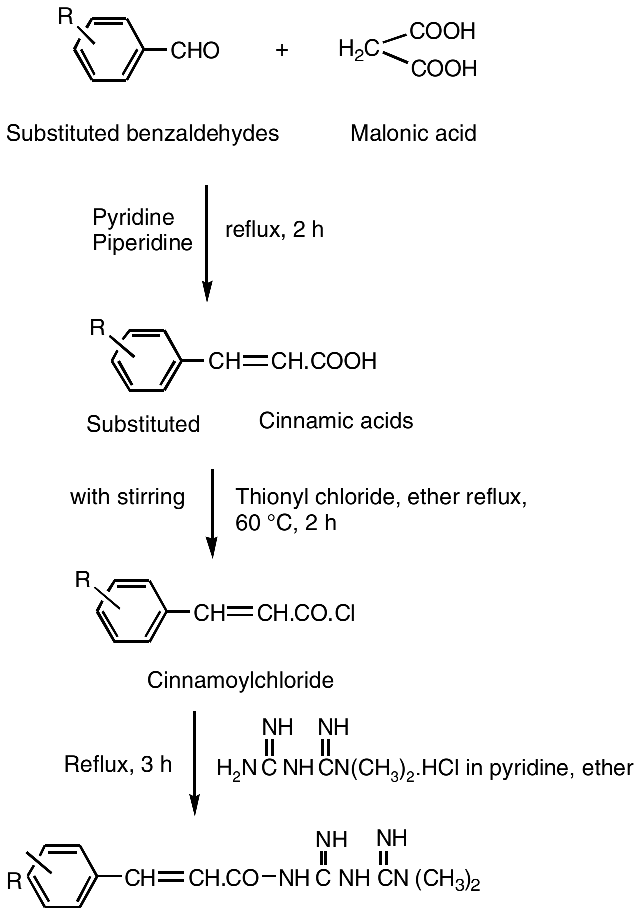

Fig. 1. Synthesis of substituted cinnamic acids

and heated under reflux at 60 °C for 2 h, with stirring. Then the reaction mixture was allowed to be concentrated under reduced pressure. To the distillate, add ether (20 mL) and a solution of metformin (330 mg, 2 mmol) in pyridine (5 mL). The reaction mixture in the flask was heated under reflux for 3 h. Again the reaction mixture was concentrated under reduced pressure. To the distillate, add 20 mL of ethanol and 100 mL distilled water. The byproduct was precipitated out and filtered. The product obtained was recrystallized by a mixture of ethanol:water (1:5) and later was confirmed by TLC. Better yields were obtained by using thionyl chloride. In present work, about 12 cinnamic acids were condensed analogues with metformin synthesized by following the above procedure.

General method for the synthesis of mandelic acid derivatives: A mixture of substituted mandelic acid (0.175 g, 1 mmol) and ether (10 mL) and thionyl chloride (10 mL) were taken in an iodine flask and heated under reflux at 60 °C for 2 h, with constant stirring. Then the reaction mixture was allowed to be concentrated under reduced pressure. To the distillate, add ether (20 mL) and a solution of metformin (330 mg, 2 mmol) in pyridine (5 mL). The reaction mixture in the flask was heated under reflux for 3 h. Again the reaction mixture was concentrated under reduced pressure. To the distillate, add 20 mL of ethanol and 100 mL distilled water. The byproduct was precipitated out and filtered. The product obtained was recrystallized from a mixture of ethanol:water (1:5), later was confirmed by TLC. In present work, about 5 mandelic acid condensed derivatives were synthesized with metformin, by following the above procedure better yields were obtained by using thionyl chloride (Fig. 2).

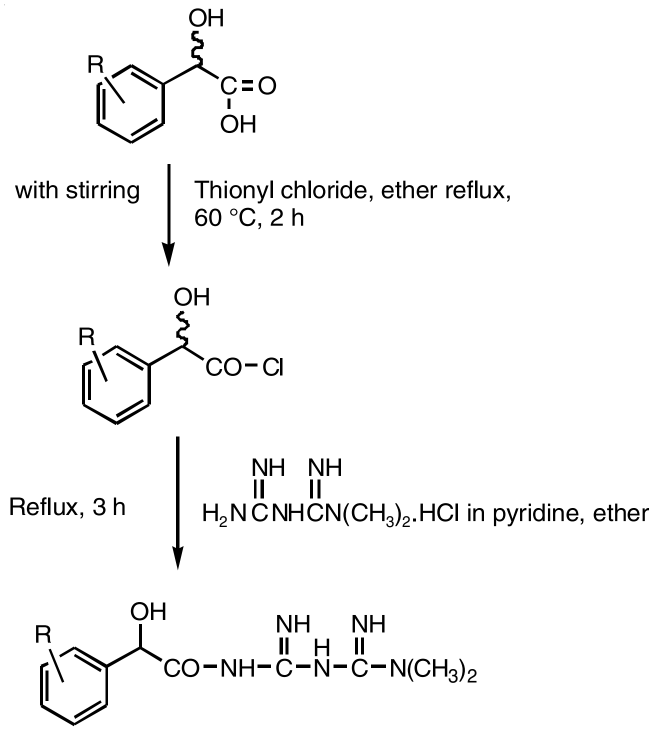

Fig. 2. Scheme of synthesis

Antioxidant screening of cinnamoyl and mandelate-metformins

Diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging activity: 0.1 mM solution of DPPH was prepared in ethanol. To this solution, 3 mL of the test solution was added at different concentration (25-800 μg/mL). Equal amount of distilled water was added to the control. The mixture was shaken well and incubated at room temperature (23 ± 2 °C) for 0.5 h. The absorbance was read at 517 nm using a spectrophotometer.

Nitric oxide (NO) scavenging: Nitric oxide scavenging activity was measured by using a spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffered saline (0.25 g of sodium dihydrogen phosphate, 0.253 g of disodium hydrogen phosphate and 0.82 g of sodium chloride were dissolved in sufficient quantity of water to produce 100 mL) was mixed with different concentrations of the ethanolic extracts (25-800 μg/mL) dissolved in distilled water and incubated at 25 °C for 0.5 h. A control without test compound but with equivalent amount of distilled water was taken. After 0.5 h, 1.5 mL of the incubation solution was removed and diluted with 1.5 mL of Griess reagent (1 % w/v sulphanilamide, 2 % v/v phosphoric acid and 0.1 % w/v naphthyl ethylene diamine dihydrochloride).

The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylene diamine was measured at 546 nm.

Determination of lipid peroxidation: Malondialdehyde were measured as an index of lipid peroxidation. Peroxidation was induced with Fe^{2+} (0.02 mM), ascorbate (1 mM) and H_2O_2 (0.5 mM) in a final volume of 0.5 mL. Test compounds were added into the reaction mixtures at indicated concentrations just before the addition of lipid substrates. Malondialdehyde was measured by its reaction with thiobarbituric acid at 532 nm [5].

RESULTS AND DISCUSSION

An attempt was made to synthesize certain novel cinnamic acid and mandelic acid derivatives. Substituted cinnamic acids were prepared by reacting a mixture of malonic acid, pyridine and piperidine with the corresponding substituted aldehydes, heated under reflux for 2 h. Metformin hydrochloride was allowed to condense with the above prepared substituted cinnamic acids (**1-12**), in the presence of thionyl chloride, ether and pyridine to obtain the desired new series of compounds **1a-12a**.

Twelve compounds were synthesized, with the yields generally 50-90 %. *p*-Methyl, *p*-methoxy, vanillinyl and *p*-isopropyl derivatives were obtained at highest yields (80-90 %) and remained analogs were produced at lowest yields (50-70 %).

Substituted mandelic acids **13-17** (Table-1) were prepared by reacting a mixture of KOH solution with the corresponding substituted benzene and glyoxalic acid monohydrate under cooling (0-5 °C). Metformin hydrochloride was allowed to condense with the above prepared substituted mandelic acids, in the presence of thionyl chloride, ether and pyridine to obtain the desired new series of compounds **13a-17a**. Five compounds were prepared with the yields generally 60-90 %. α-Nitro, α-phenol and 2-chloro derivatives were obtained at high yields (80-90 %) and remained analogs were produced at 60-70 %.

Antioxidant studies

Effect of compounds 1a-17a on ferrous induced lipid peroxidation in rat brain homogenate: Out of seventeen compounds, all the compounds were tested for the inhibitory effect on iron induced lipid peroxidation. Among these derivatives, the unsubstituted (**1a**) showed 41.0 % activity. Highest activity, showed with *p*-fluoro derivative (**9a**) (55.10 %). Substitution with *p*-chloro (**7a**) 54.0 %, *p*-hydroxy (**11a**) 53.08 %, 3,4,5-trimethoxy (**8a**) 50.49 %, *p*-methoxy (**3a**) 49.02 % and PDMAB (**10a**) 45.68 % derivatives showed significant activity.

Substituted with α-naphthol (**17a**) 43.99 %, 3,4-di(OCH_3) (**6a**) 41.0 %, unsubstituted 41.0 %, 2-chloro (**13a**) 39.44 %, *o*-cresol (**14a**) 39.82 %, *p*-CH_3 38.14 %, *p*-isopropyl (**5a**) 35.28 %, showed moderate activity and substitution with 2-nitro (**16**), *p*-diethylamino- (**12a**), α-phenol (**15a**) and vanillinyl (**4a**) derivatives showed less activity (Table-1).

Reduction of DPPH by test compounds: Among the seventeen compounds all the compounds were tested for the reduction of DPPH. Among the 17 compounds, the unsubstituted derivative (**1a**) showed 38.1 % activity. 2-Chloro derivative showed highest activity 54.02 %.

Substitution with *p*-chloro (**7a**) 46.33 %, α-naphthol (**17a**) 45.19 %, *p*-fluoro (**9a**) 45.0 %, 3,4,5-tri(OCH_3) (**8a**) 43.81 % and *para*-isopropyl (**5a**) 42.73 % showed significant activity. Substitution with *o*-cresol (**14a**) 40.0 %, *p*-dimethylamino- (**10a**) 38.6 %, unsubstituted (**1a**) 38.1 %, vanillinyl (**4a**) 35.06 % and 2-nitro (**16a**) 33.25 % derivative showed moderate activity. Substitution with *p*-CH_3 (**2a**), *p*-OCH_3 (**3a**), 3,4-di(OCH_3) (**6a**), α-phenol (**15a**) derivatives showed less activity (Table-1).

Scavenging of NO free radical: Out of 17 compounds, all compounds were tested for their ability to scavenge NO at 100 μm concentrations. Unsubstituted derivative (**1a**) showed an activity of 64.33 %. Test compounds containing different substituents on the phenyl ring were also evaluated for NO scavenging activity.

Substitution with Cl^- derivative at *para* position (**7a**) resulted in increased activity (76.12 %) which is the highest activity. Substitution with *p*-OCH_3 (**3a**) 71.52 %, 3,4-di(OCH_3) (**6a**) 72.06 %, *p*-F (**9a**) 66.46 %, *p*-dimethylamino- (**10a**) 66.01 %, 2-chloro (**13a**) 65.90 %, *o*-cresol (**14a**) 68.48 % and α-naphthol (**17a**) 65.70 % derivatives showed significant activity. Substitution with *p*-CH_3 62.30 %, vanillinyl (**4a**) 56.12 %, *p*-isopropyl (**5a**) 58.43 %, α-phenol (**15a**) 59.27 %, 2-nitro (**16a**) 63.26 %, *p*-OH (**11a**) 56.52 %, showed moderate activity and substitution with *p*-diethylamino- (**12a**) showed less activity (Table-1).

TABLE-1
ANTIOXIDANT ACTIVITY DATA OF N-CINNAMOYL AND MANDELATE METFORMIN ANALOGUES

Compd.	R	m.f.	Reaction time (h)	Yield (%)	Inhibition (%) at 100 μm		
					Lipid peroxidation	DPPH radical	NO scavenging
1	H	$C_{13}H_{17}N_5O$	5	72.00	41.00	38.1	64.33
2	4-CH_3	$C_{14}H_{19}N_5O$	5	80.00	38.14	32.0	62.3
3	4-OCH_3	$C_{14}H_{19}N_5O_2$	5	81.90	49.02	32.7	71.52
4	Vanillinyl	$C_{14}H_{19}N_5O_3$	5	92.70	33.72	35.06	56.12
5	Isopropyl	$C_{16}H_{21}N_5O$	5	82.80	35.28	42.73	58.43
6	3,4-di-(OCH_3)	$C_{15}H_{21}N_5O_3$	6	63.90	41.00	30.03	72.06
7	4-Cl	$C_{13}H_{19}N_5OCl$	5	58.60	54.00	46.33	76.12
8	3,4,5-tri(OCH_3)	$C_{16}H_{23}N_5O_4$	5	55.80	50.49	43.81	64.90
9	4-F	$C_{13}H_{16}N_5OF$	5	66.60	55.10	45.0	66.46
10	4-$N(CH_3)_2$	$C_{15}H_{22}N_6O$	5	60.30	45.68	38.6	66.01
11	4-OH	$C_{13}H_{17}N_5O_2$	6	56.70	53.08	56.52	56.52
12	4-$N(C_2H_5)_2$	$C_{17}H_{26}N_6O$	6	63.09	31.06	37.92	37.92
13	2-Cl	$C_{12}H_{16}N_5O_2$	5	87.50	39.44	54.02	65.90
14	*o*-Cresol	$C_{17}H_{21}N_5O_3$	6	62.50	39.82	40.0	68.48
15	α-Phenol	$C_{16}H_{19}N_5O_3$	5	76.33	32.49	30.63	59.27
16	2-Nitro	$C_{12}H_{15}N_6O_4$	5	89.20	31.78	33.25	63.26
17	α-Naphthol	$C_{16}H_{17}N_5O_3$	6	92.40	43.99	45.19	65.70

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