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SYNTHESIS, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF SOME NOVEL MANNICH DERIVATIVES OF 3-(BENZOFURAN-2-YL)-1-(4-HYDROXY-3-METHOXYPHENYL)PROP-2-EN-1-ONE

Kantlam Ch[1], Srinivasa Murthy M[2*] and Narsimha Reddy Y[3,]

[1]Research Scholar, School of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kakinada - 533 003 Andhra Pradesh, India.

[2]Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Near Ramoji Film City, Deshmukhi, Nalgonda - 508 284, Telangana State, India. Ph: +91- 9866399799, +91-8790568321

[3]Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal - 506 009, Telangana State, India.

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ABSTRACT

Purpose: The present research work is aspired to describe the synthesis, characterization and antibacterial activity of novel Mannich derivatives (4a-4f) derived from apocynin.

Methodology/ Approach: In the present work, a novel chalcone was prepared by Claisen-Schmidt condensation of apocynin with benzo[b]furan aldehyde. Mannich bases were prepared by reaction of 3-(benzofuran-2-yl)-1-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one with formaldehyde and 2° amines.

Findings: All the newly synthesized Mannich base derivatives were characterized by their IR, NMR and Mass spectral data. Further all these final compounds were evaluated for their antibacterial activity using norfloxacin as standard reference. It was observed that among the final derivatives (4a-4f), compounds incorporated with morpholino, piperazino and piperidino moieties (4c-4e) exhibited excellent antibacterial activity, while the compound (4f) with pyyrolidino substituent showed similar promising activity and other compounds (4a-4b) possessed moderate activity.

Original Value : The review of literature revealed that conversion of various acyclic conjugated α,β-unsaturated ketones e.g. chalcones, into the corresponding Mannich bases was often accompanied by increased bioactivity both *in vitro* and *in vivo*. These facts prompted us to synthesize novel Mannich base derivatives having better biological activity. All the synthesized compounds were screened for their *in vitro* antibacterial activity.

Conclusion: The study revealed that compounds (**4c), (4d)** and **(4e)** derivatives exhibited highest activity against the tested bacteria.

Key words: *Apocynin; Chalcone; Mannich derivative; Antibacterial activity.*

INTRODUCTION

Nature has been a resource of medicinal agents for thousands of years and a remarkable number of recent drugs were isolated from natural sources. Plants used as traditional medicine contain a wide range of active chemical substances such as apocynin used to treat various diseases. Clinical microbiologists have an immense interest in screening of medicinal plants for antimicrobial activities and phytochemical as potential new therapeutics. Among them, apocynin is a promising, plant derived, nonsteroidal anti-inflammatory compound that has been studied in different *in vitro* systems as well as *in vivo* models for chronic inflammatory diseases. The potential value of apocynin has been proved in the treatment of several experimental inflammatory diseases such as rheumatoid arthritis[1]. It is a potent inhibitor of NADPH oxidase-dependent reactive oxygen species (ROS) production in activated human PMNs. Apocynin was also isolated from *Picrorhiza kurroa*, a small plant that grows at high altitudes in the western Himalayas. *P. kurroa* was used for ages as a treatment for liver and heart problems, jaundice and asthma.

α,β-Unsaturated ketones are biogenetic precursors of flavonoids in higher plants and also known chemically as chalcones, in which the two aromatic rings are joined by a three carbon chain[2]. They display a wide range of pharmacological properties, including cytotoxicity towards cancer cell lines[3,4], antimitotic[5], antimutagenic[6], antibacterial[7], antiviral[8], anti-inflammatory[9], antiulcerative[10] and hepatoprotective activities[11].

The review of literature revealed that conversion of various chalcones, into the corresponding Mannich bases, was often accompanied by increased bioactivity both *in vitro* and *in vivo*[12-14]. Also, Mannich bases, which have been recently synthesized from heterocyclic chalcones, exhibited very potent activity against some tumor cell lines[15-18]. The broad range of biological applications of chalcones and Mannich bases encouraged us to focus on the present research work,

*Correspondence : email : msmurthy1093@gmail.com

which is aspired to do the synthesis, characterization and antibacterial activity of novel Mannich derivatives (**4a-4f)** derived from apocynin.

MATERIALS AND METHODS

All chemicals used for the synthesis of Mannich base derivatives were available commercially and used as received. According to standard procedures, the solvents were purified prior to use. Melting points were found on open capillary melting point apparatus and are uncorrected. Thin-layer chromatography (TLC) analysis was done by using Merck pre-coated Plates and the spots were identified by UV light. The Infrared (IR) spectra were recorded by using Perkin Elmer FT-IR spectrophotometer. NMR spectra were recorded at 400 MHz by Varian MR-400 MHz instrument using tetramethylsilane (TMS) as an internal standard and DMSO-d_6 as solvent. The Mass spectra were obtained using Agilent ion trap Mass Spectrophotometer.

***Preparation of (E)-3-(Benzofuran-2-yl)-1-(4-hydroxy-3-methoxyphenyl)prop-2-en-1-one (3)**:*

To methanol solution containing apocynin **1** (1.80 mmol), sodium hydroxide (7.20 mmol) was added and followed by Benzo[b]furan aldehyde **2** (1.90 mmol) and the contents were stirred at room temperatures for 24 h. The reaction mixture was diluted with water and acidified to pH 3 using 1N HCl and extracted with ethyl acetate. The organic layer was washed with water followed by brine solution, dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain the pure yellow color compound **3**. Yield: 98%; M.p: 168-170 °C; IR (KBr): υ_{max} 3390 (Ar-OH), 2965(SP^3-CH), 1647(ketone C=O), 1607, 1574, 1519(C=C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.78 (m, 3H, Ar-H), 7.70 (s, 1H, Ar-H), 7.58 (d, 1 H, *J* = 16.0 Hz,=C-H), 7.50 (d, 1 H, *J* = 16.0 Hz,=C-H), 7.38 (t, 1 H, *J* = 8.2 Hz, Ar-H), 7.02 (d, 1 H, *J* = 8.4 Hz, Ar-H), 6.10 (br.s, 1 H, Ar-OH), 4.00 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ 56.2, 106.9, 111.6, 115.5, 117.4, 121.0, 123.3, 123.6, 124.7, 127.4, 131.5 (3C), 151.5, 151.9, 157.3, 158.3, 189.7; EI-MS: m/z (rel.abund.%) 343.18 (M+,100); EI-MS: m/z (rel.abund.%) 296.13 (M+,100).

General experimental procedure for the preparation of novel Mannich derivatives (4a – 4f):

To a stirred solution of compound **3** (0.68m mol) in ethanol (1 mL), paraformaldehyde (1.0 mmol) and corresponding amines a-f (0.67 mmol) were added at reflux temperature and stirred for 12 h. The reaction medium was poured into water and extracted with ethyl acetate. The organic layer was washed with 6N HCl (10 mL), water and followed by brine solution, dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain the pure compounds. Yields of the products varied between 84 to 95%.

(E)-3-(Benzofuran-2-yl)-1-(3-((dimethylamino) methyl)-4-hydroxy-5-methoxyphenyl) prop-2-en-1-one (4a):

Yield: 84%; M.p: 185-190 °C; IR (KBr): υ_{max} 3426(Ar-OH), 3064, 2928(SP^3-CH), 2847(OCH_3), 1650(C=O), 1592(C=C), 1457(aliphatic-C-H), 1279, 1159(C-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.80 (d, *J* = 1.2 Hz, 1H, Ar-H), 7.72 (d, *J* = 6.0 Hz, 1H, Ar-H), 7 .70 (s,1H, Ar-H), 7.66 (d, *J* = 6.6 Hz, 2H, Ar-H), 7.54 (s,1H, Ar-H), 7.48 (s, 1H, Ar-H), 7.44 (d, *J* = 6.0 Hz, 1H,=C-H), 7.32 (t, *J* = 6.0Hz, 1H,=C-H), 3.87 (s, 3 H, OCH_3), 3,82 (s, 2 H, Ar-CH_2), 2.34 (s, 6 H, *N,N*-dimethyl); ESI-MS: m/z, 352.15 (M+1).

(E)-3-(Benzofuran-2-yl)-1-(3-((diethylamino)methyl)-4-hydroxy-5-methoxyphenyl)prop-2-en-1-one (4b):

Yield: 87%; M.p: 190-195°C; IR (KBr): υ_{max} 3435(Ar-OH), 3312, 2986, 2952(SP^3-CH), 2842(OCH_3), 1670(C=O), 1621, 1605, 1558, 1520, 1504(C=C), 1481, 1425, 1395(aliphatic-C-H),1285, 1256, 1168(C-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): 7.80 (d, *J* = 16.6 Hz, 1H, Ar-H), 7.72 (d , *J* = 18.2 Hz, 1H, Ar-H), 7.68-7.60 (m, 3H, Ar-H), 7.50-7.38 (m, 3H, Ar-H), 7.30 (d, *J* = 10.2Hz, 1H, =C-H), 3.90 (s, 2H, Ar-CH_2), 3.80 (s, 3H, OCH_3), 2.60 (q, *J* = 6.8Hz, 4H, N-CH_2), 1.0 (t, *J* = 6.6 Hz, 6H, aliphatic-CH_3); ESI-MS: m/z, 380.2 (M+1).

(E)-3-(Benzofuran-2-yl)-1-(4-hydroxy-3-methoxy-5-(morpholinomethyl)phenyl)prop-2-en-1-one (4c):

Yield: 95%; M.p: 198-200 °C IR (KBr): υ_{max} 3425(Ar-OH), 3069, 2937(SP^3-CH), 2845(-OCH_3), 1731-1654(C=O), 1593(C=C), 1454(aliphatic-C-H), 1286, 1160, 1116(C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 7.80 (d, *J* =12.4 Hz, 1H, Ar-H), 7.73 (d, *J* = 6.4 Hz, 1H, Ar-H), 7.70 (s,1H, Ar-H), 7.68 (d, *J* = 3.2 Hz, 1H, Ar-H), 7.65 (s, 1H, Ar-H), 7.56 (d, *J* =1.6 Hz, 1H, Ar-H), 7.50 (s,1H, Ar-H), 7.45 (d, *J* = 6.2 Hz, 1H, =C-H), 7.30 (d, *J* = 6.0 Hz, 1H, =C-H), 3.90 (s, 3H, OCH_3), 3.75 (s, 2H, Ar-CH_2), 3.68-3.64 (m, 4H, cyclic O(CH_2)$_2$), 2,56-2,54 (m, 4H, cyclic N-(CH_2)$_2$); ESI-MS: m/z, 394.2 (M+1).

(E)-3-(Benzofuran-2-yl)-1-(4-hydroxy-3-methoxy-5-(piperazin-1-ylmethyl)phenyl)prop-2-en-1-one (4d):

Yield: 92%; M.p: 200-205 °C IR (KBr): υ_{max}3441(Ar-OH), 3063, 2935(SP^3-CH), 2820(OCH_3), 1653(C=O), 1592(C=C-), 1456, 1415(aliphatic-C-H), 1337, 1283, 1159, 1092(C-N) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d6): δ 7.78 (d, *J* =12.4 Hz, 1H,Ar-H), 7.72 (d, *J* = 6.4 Hz, 1H,Ar-H), 7.70 (s, 1H,Ar-H), 7.64-7.63 (m, 2H,Ar-H), 7.54 (s,1H,Ar-H), 7.48 (s,1H,Ar-H), 7.45 (s,1H,Ar-H), 7.42 (d, *J* = 6.2 Hz,1H,=C-H), 7.30 (d, *J* = 6.2 Hz, 1H,=C-H), 4.06-3.79 (m, 13H, OCH_3+ benzylic Ar-C**H_2**, cyclic N-(CH_2)$_2$-N-(CH_2)$_2$); ESI-MS: m/z, 393.2 (M+1).

(E)-3-(Benzofuran-2-yl)-1-(4-hydroxy-3-methoxy-5-(piperidin-1-ylmethyl)phenyl)prop-2-en-1-one (4e):

Yield: 90%; M.p: 225-230 °C IR (KBr): υ_{max} 3424(Ar-OH), 3098, 2960(SP^3-CH), 2830(OCH_3), 1653(C=O), 1590(C=C), 1454(aliphatic -C-H), 1323, 1287, 1160(C-N) cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.80 (s,1H,Ar-H), 7.72 (d, *J* = 6.0 Hz, 1H,Ar-H), 7.69 (s, 1H,Ar-H), 7.64 (d, *J* = 6.8 Hz, 2H,Ar-H), 7.52 (d, *J* =1.6 Hz, 1H,Ar-H), 7.48 (s,1H,Ar-H), 7.42 (d, *J* = 6.0 Hz, 1H,=C-H), 7.32 (d, *J* = 6.0 Hz, 1H,=C-H), 3.86 (s, 3H, OCH_3), 3.82 (s, 2H, Ar-CH_2), 2.78-2.62 (m, 4H, cyclic N-(CH_2)$_2$), 1.72-1.68 (m, 4H, cyclic N-C-(CH_2)$_2$), 1.44-1.40 (m, 2H, cyclic N-C-C-CH_2); ESI-MS: m/z, 392.2 (M+1).

(E)-3-(Benzofuran-2-yl)-1-(4-hydroxy-3-methoxy-5-(pyrrolidin-1-ylmethyl)phenyl)prop-2-en-1-one (4f):

Yield: 85%; M.p: 205-210 °C; IR (KBr): u_{max} 3426(Ar-OH), 3098, 3028, 2937(SP^3-CH), 2855, 2805(OCH_3), 1655(C=O), 1592(C=C), 1458, 1429(aliphatic-C-H), 1288, 1161, 1067(C-N), cm^{-1}; ^{1}H NMR (400MHz, DMSO-d_6): δ 7.78 (d, *J* = 1Hz,1H, Ar-H), 7.73 (d, *J* = 6.0 Hz,1H, Ar-H), 7.71-7.64 (m, 3H, Ar-H), 7.52 (s,1H, Ar-H), 7.46 (s,1H, Ar-H), 7.45 (d, *J* = 6.0Hz, 1H,=C-H), 7.29 (d, *J* = 6.0 Hz, 1H,=C-H), 3.98 (s, 2H, Ar-CH_2), 3.85 (s, 3H, OCH_3), 2.70-2.62 (m, 4H, cyclic N-$(CH_2)_2$,), 1.80 (m, 4 H, cyclic N-C-**$(CH_2)_2$**); ESI-MS: m/z, 378.15 (M+1).

Biological Assay

The antibacterial activity of the synthesized Mannich base derivatives (4a-4f) was determined by disc diffusion method[19-20] using Norfloxacin as standard reference against two Gram negative bacterial strains viz., *Escherichi coli and Pseudomonas aeruginosa* and two Gram positive bacterial strains viz., *Staphylococcus aureus* and *Staphylococcus pyogenes*. The newly synthesized Mannich base derivatives (4a-4f) were dissolved in dimethylsulphoxide to get 25 µg/mL concentrated solution. The nutrient agar media was prepared by using the yeast extract (5g), bactotryptone (10g), sodium chloride (10g), agar (20g) and pH was adjusted to 7.4. The petri plates were filled with nutrient agar at room temperature and dried at 37°C for 18 h. The growing culture of the four bacterial strains at 37°C was diluted in sterile broth. 1 mL of culture medium was added to nutrient agar media for giving a final bacterial count of 1×10^6 cell/ mL. The paper discs used for the bacterial assay were prepared by using the Whatman no.41 filter paper and sterilized by ultraviolet light. Paper discs were dipped in test solution and standard solution and placed on to the agar surface developed with microorganism growth at equal distance (6-7 mm). The plates were incubated at 37°C for 24 h in an inverted position. The activity was observed by measuring zone of inhibition. The growth of inhibition was estimated with reference to control.

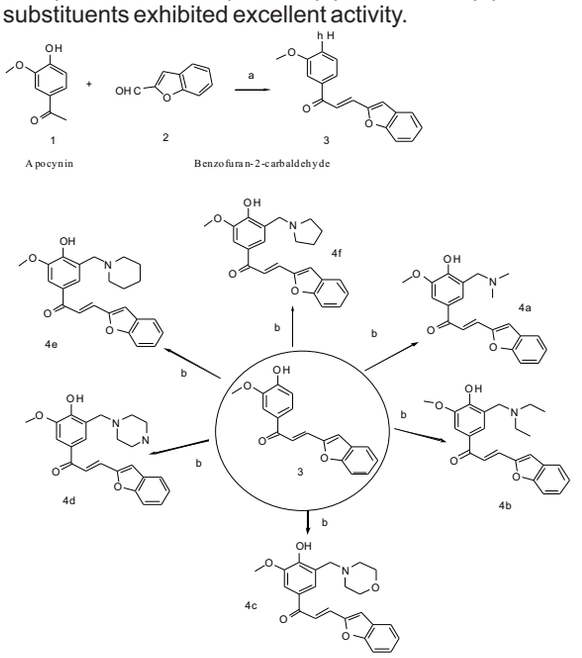
RESULTS AND DISCUSSIONS

Chemistry

The newly synthesized Mannich derivatives (4a-4f) described in this paper were prepared according to the synthetic Scheme 1. The Claisen-Schmidt condensation of apocynin (1) with benzofuran-2-carboxaldehyde (2) was carried out in presence of sodium hydroxide in methanol at room temperature for 24 h to obtain chalcone derivative (3) in 98% yield. The condensation of 3-(benzofuran-2-yl)-1-(4-hydroxy-3-methoxy-phenyl)prop-2-en-1-one (3) using para-formaldehyde and corresponding 2° amines in ethanol at reflux temperature resulted in the formation of Mannich derivatives (4a-4f). The structures of the synthesized compounds were confirmed by IR, NMR and Mass spectral data. All the aliphatic and aromatic protons were observed at expected regions. The IR and NMR data for the derivatives (4a-4f) are in agreement with the assigned structures. The Mass spectra of compounds showed (M+1) peaks, which are in agreement with their respective molecular formula.

Antibacterial Activity

The antibacterial activity of (4a-4f) was determined by the disc diffusion method using norfloxacin (25 µg/ml) as the reference antibiotic[20]. The synthesized compounds were screened against two Gram negative bacterial strains viz., *Escherichia coli and Pseudomonas aeruginosa* and two Gram positive bacterial strains viz., *Staphylococcu. aureus* and *Staphylococcus pyogenes.* The outcome of the results are presented in the Table-1, it is evident from the results that compounds (4c), (4d) and (4e) derivatives exhibited highest activity against the tested bacteria. The compound (4f) was found to be equipotent active against the tested bacteria. The rest of the compounds (4a) and (4b) were found to be moderately active against the tested microorganisms. It is observed from the above antibacterial data that within the Mannich derivatives (4a-4f), compounds incorporated with morpholino, piperazino and piperidino substituents exhibited excellent activity.



Scheme 1. *Synthesis of novel Mannich derivatives* ***(4a-4f)***

Experimental Conditions: *a) Apocynin,Benzofuranaldehyde, NaOH, MeOH, rt, 24 h; b) Para-formaldehyde,2°Amines, Ethanol, Reflux temperature,12 h.*

Table-1: *Results of Antibacterial Bioassay of Compounds* ***(4a-4f)*** *(Concentration Used 25 µg/mL of DMSO)*

Compd No.	R	Gram negative		Gram positive	
		E.coli MTCC 443	*P.aeruginosa MTCC 424*	*S.aureus MTCC 96*	*S.pyogenes MTCC 442*
		Zones of Inhibition of compounds **(4a –4f)** in mm			
4a	*N,N*-Dimethylamino	16	14	15	14
4b	*N,N*-Diethylamino	17	13	16	13
4c	Morpholino	28	22	27	22
4d	Piperazino	27	23	26	23
4e	Piperidino	28	23	27	23
4f	Pyrrolidino	25	19	25	19
Standard Drug	**Norfloxacin** *(25 µg/mL of DMSO)*	25	19	25	19

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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