JPMC

Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II, Mayur Vihar, Phase-I
Delhi - 110 091 (India)
Tel: 91-11-22754205, 45796900,
Fax: 91-11-22754205
E-mail: redflowerppl@vsnl.net
Website: www.rfppl.co.in

The Journal of Pharmaceutical and Medicinal Chemistry (JPMC) (ISSN: 2395-6615) is an influential international peer-reviewed semiannual journal that publishes original research and review articles as well as letters in all areas of chemistry (Analytical Chemistry, Inorganic Chemistry, Organic and Bioorganic Chemistry, Biochemistry, Theoretical and Applied Physical Chemistry, Pharmaceutical Chemistry, Applied and Materials Chemistry, Chemical Technology, Agro Chemical Technology, Pharmaceutical Technology, Colloid Chemistry, Interfacial Chemistry, Surface Chemistry, Chemical Engineering, Green Chemistry, etc.). JPMC is the primary vehicle for the publication pertinent to the overlapping areas of behavioral pharmacology, neuro-pharmacology and analgesia, cardiovascular pharmacology, pulmonary, gastrointestinal and urogenital pharmacology, endocrine pharmacology, immune-pharmacology and inflammation and molecular and cellular pharmacology

Subscription Information

India

Individual (1 year): Rs.13500

Life Subscription (Valid for 10 Years): Rs.135000

Institutional (1 year): Rs.15000

Rest of the World

Individual (1 year) USD100

Insitutional (1 year) USD360

Payment methods

Bank draft / cashier & order / check / cheque / demand draft / money order should be in the name of **Red Flower Publication Pvt. Ltd.** payable at **Delhi**.

International Bank transfer / bank wire / electronic funds transfer / money remittance / money wire / telegraphic transfer / telex

1. **Complete Bank Account No.** 604320110000467
2. **Beneficiary Name (As per Bank Pass Book):** Red Flower Publication Pvt. Ltd.
3. **Address:** 41/48, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091(India)
4. **Bank & Branch Name:** Bank of India; Mayur Vihar
5. **Bank Address & Phone Number:** 13/14, Sri Balaji Shop,Pocket II, Mayur Vihar Phase- I, New Delhi - 110091 (India); Tel: 22750372, 22753401. **Email:** mayurvihar.newdelhi@bankofindia.co.in
6. **MICR Code:** 110013045
7. **Branch Code:** 6043
8. **IFSC Code:** BKID0006043 (used for RTGS and NEFT transactions)
9. **Swift Code:** BKIDINBBDOS
10. **Beneficiary Contact No. & E-mail ID:** 91-11-22754205, 45796900, E-mail: redflowerppl@vsnl.net

Online You can now renew online using our RFPPL renewal website. Visit www.rfppl.com and enter the required information and than you will be able to pay online.

Send all Orders to: **Red Flower Publication Pvt. Ltd.,** 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091(India). Phone: 91-11-22754205, 45796900, Fax: 91-11-22754205, E-mail: redflowerppl@vsnl.net, Website: www.rfppl.co.in

Journal of Pharmaceutical and Medicinal Chemistry

JPMC

July - December 2015
Volume 1, Number 2

Contents

Original Articles

Revised Rates for 2015 (Institutional)

Title	Frequency	Rate (Rs): India	Rate ($):ROW
Dermatology International	2	4500	280
Gastroenterology International	2	5000	360
Indian Journal of Agriculture Business	2	4500	300
Indian Journal of Anatomy	2	6000	260
Indian Journal of Ancient Medicine and Yoga	4	7000	330
Indian Journal of Anesthesia and Analgesia	2	5000	600
Indian Journal of Anthropology	2	10500	500
Indian Journal of Applied Physics	2	3500	400
Indian Journal of Biology	2	3000	170
Indian Journal of Cancer Education and Research	2	6500	500
Indian Journal of Communicable Diseases	2	7500	58
Indian Journal of Dental Education	4	4000	288
Indian Journal of Forensic Medicine and Pathology	4	14000	576
Indian Journal of Forensic Odontology	4	4000	288
Indian Journal of Genetics and Molecular Research	2	6000	262
Indian Journal of Law and Human Behavior	2	5000	500
Indian Journal of Library and Information Science	3	8000	600
Indian Journal of Maternal-Fetal & Neonatal Medicine	2	8000	400
Indian Journal of Mathematics and Statistics	2	5000	200
Indian Journal of Medical & Health Sciences	2	6000	120
Indian Journal of Obstetrics and Gynecology	2	5000	200
Indian Journal of Pathology: Research and Practice	2	10000	915
Indian Journal of Plant and Soil	2	5000	1700
Indian Journal of Preventive Medicine	2	6000	250
Indian Journal of Reproductive Science and Medicine	4	3000	180
Indian Journal of Scientific Computing and Engineering	2	4000	280
Indian Journal of Surgical Nursing	3	3000	70
Indian Journal of Trauma & Emergency Pediatrics	4	8500	302
International Journal of Agricultural & Forest Meteorology	2	8000	800
International Journal of Food, Nutrition & Dietetics	2	4000	900
International Journal of History	2	6000	500
International Journal of Neurology and Neurosurgery	2	9000	276
International Journal of Political Science	2	5000	400
International Journal of Practical Nursing	3	3000	70
International Physiology	2	6500	240
Journal of Animal Feed Science and Technology	2	4000	280
Journal of Cardiovascular Medicine and Surgery	2	9000	238
Journal of Orthopaedic Education	2	4500	190
Journal of Pharmaceutical and Medicinal Chemistry	2	15000	350
Journal of Psychiatric Nursing	3	3000	70
Journal of Social Welfare and Management	4	7000	276
Meat Science International	2	5000	500
Microbiology and Related Research	2	6000	150
New Indian Journal of Surgery	4	7000	360
Ophthalmology and Allied Sciences	2	5000	150
Otolaryngology International	2	4500	300
Pediatric Education and Research	4	6500	150
Physiotherapy and Occupational Therapy Journal	4	8000	360
Urology, Nephrology and Andrology International	2	6500	350

Terms of Supply:

1. Advance payment required by Demand Draft payable to Red Flower Publicaion Pvt. Ltd. payable at Delhi.
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 10% discount.
4. Claim must be made within six months from issue date.

Order from

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India), Tel: 91-11-22754205, 45796900, Fax: 91-11-22754205. E-mail: redflowerppl@vsnl.net, redflowerppl@gmail.com, Website: www.rfppl.co.in

Journal of Pharmaceutical and Medicinal Chemistry
Volume 1 Number 2, July - December 2015

Original Article

Studies on 1-(2, 4-Dimethoxy-5-[3-(Substituted-Phenyl)Acryloyl]Phenyl)-4-(Substituted-Phenyl)But-2-En-1-Ones

Asif Husain

Abstract

A series of 1-(2, 4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones (**3a-e**) were synthesized and evaluated for their antimicrobial actions. Resorcinol was used as starting material for the preparation of 1, 1´-(4, 6-dihydroxy-1, 3-phenylene) diethanone (**1**), which then treated with dimethylsulphate to obtain 1-(5-acetyl-2, 4-dimethoxyphenyl)-1-ethanone (**2**). Compound (**2**) was reacted with substituted aromatic aldehydes in ethanol in presence of KOH to furnish the title compounds (**3a-e**). The structures of the synthesized compounds were confirmed on the basis of ^{1}H-NMR, Mass and elemental analysis results. The antimicrobial activity (minimum inhibitory concentration; *MIC*) of the title compounds was determined against some selected bacterial and fungal strains. Two compounds, **3d** and **3e**, showed good antimicrobial activity against *S. aureus* and *E. coli*.

Keywords: Chalcone; MIC; Antibacterial; Antifungal.

Author Affilation: *Sr. Asst. Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard (Hamdard University), New Delhi- 110 062, India.

Reprint Request: Asif Husain, Sr. Asst. Professor, Department of Pharmaceutical Chemistry, Faculty of pharmacy, Jamia Hamdard University, New Delhi-110 062, India.
Email: drasifhusain@yahoo.com
ahusain@jamiahamdard.ac.in

Introduction

The incidences of bacterial and fungal infections are increasing day by day, and the problem is further complicated due to increasing microbial resistance to a number of available antimicrobial drugs [1,3]. Different factors like HIV-infection, cancer, immuno-compromised host, immunosuppressive therapy, age, filthy place, etc. contribute to already existing problem. Therefore, more effective antimicrobial agents with broad spectrum of activity are required to combat the situation.

Among different compounds that have been explored for developing antimicrobial agents, chalcones have played an important role [4]. Chalcones have attracted considerable attention due to their important biological actions including antimicrobial action [5,6]. Further, chalcone derivatives have special place in natural as well as in synthetic chemistry because this system is a frequently encountered structural motif in a number of pharmacologically important compounds [5-8]. Resorcinol has been chemically explored to obtain a variety of compounds of potential pharmaceutical interest [9,10]. In view of these points and in continuation of our work on bischalcones [10-12] it was considered worthwhile to synthesize some new resorcinol derivatives; (1-(2,4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl) but-2-en-1-ones, as antimicrobial agents.

Materials and Methods

Chemistry

Melting points are uncorrected and were recorded in liquid paraffin bath using open end capillaries.

^{1}H-NMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$; chemical shift (δ) values are reported in parts per million (*ppm*). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; m, multiplet. Mass spectroscopic analyses for compounds were performed on a JEOL JMS-D 300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer and were in range of ±0.4% for each element analyzed (C,H,N). Thin-layer chromatography was carried out to monitor the reactions using silica gel G as stationary phase and iodine chamber and UV lamp were used for visualization of TLC spots. The reaction involved in synthesis is given in *scheme 1.*

Synthesis of 1,1´-(4,6-Dihydroxy-1,3-phenylene) diethanone (1)

It was prepared from resorcinol following literature method [10]. It gave a violet colour with ethanolic ferric chloride solution; positive test for phenols. Yield 72%; m.p. 184-186°C. ^{1}H NMR ($CDCl_3$, *ä,* ppm): 2.65 (s, 6H, 2´-$COCH_3$), 6.65 (s, 1H, H-2), 8.15 (s, 1H, H-5).

Synthesis of 1-(5-acetyl-2,4-dimethoxyphenyl)-1-ethanone (2) [11]

A mixture of 1,1´-(4,6-dimethyl-1,3-phenylene)diethanone (**1**) (2.5 mmol), dimethylsulphate (5 mmol) and anhydrous potassium carbonate (11.25 g) in dry acetone (100 mL) was refluxed for 6 h. The contents were then filtered, concentrated to a small volume and poured onto crushed ice. A solid mass separated out which was filtered, washed with water, dried and then crystallized from methanol: dichloromethane mixture to give shiny needles of **3** (it did not give violet colour with ethanolic ferric chloride solution; negative test for phenols). Yield 74%, m.p. 164-166°C. ^{1}H NMR ($CDCl_3$) δ 2.61 (s, 6H, 2´-$COCH_3$), 3.93 (s, 6H, 2´-OCH_3), 6.34 (s, 1H, H-2), 8.37 (s, 1H, H-5). MS: *m/z* 222 (M^+), 207, 177, 175, 149. Anal calcd. for $C_{12}H_{14}O_4$: C, 64.85; H, 6.35. Found: C, 64.71; H, 6.22.

General procedure for synthesis of 1-(2, 4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones (3a-e) [11]

A mixture of compound **2** (5 mmol) in ethanol (20 mL), an aromatic aldehyde (10 mmol) and a solution of potassium hydroxide (3 g) in distilled water (5 mL) was stirred for 2 h at room temperature and then left overnight. It was poured into cold water and acidified with HCl, a solid mass separated out which was filtered, washed with water, sodium bicarbonate solution (2% w/v in water) and again with water. It was crystallized to give **3a-e** (It gave a red colour with conc. sulphuric acid; positive test for chalcones, and no colour with ethanolic ferric chloride solution; negative test for phenols).

1-(2,4-Dimethoxy-5-[3-(3-methylphenyl) acryloyl] phenyl)-4-(3-methylphenyl)but-2-en-1-one (3a). Yield: 51%, m.p. 196-198°C. ^{1}H NMR ($CDCl_3$) *d* 2.41 (s, 6H, 2´-CH_3), 3.93 (s, 6H, 2´-OCH_3), 6.41 (s, 1H, H-3'), 7.35 (d, 2H, 2xH-a), 7.47 (m, 2H, 2xH-5), 7.72 (dd, 2H, 2xH-4), 7.97 (d, 2H, 2xH-b), 8.14 (dd, 2H, 2xH-6), 8.19 (s, 1H, H-6'), 8.32 (s, 2H, 2xH-2). MS: *m/z* 426 (M^+). Anal calcd. for $C_{28}H_{26}O_4$: C, 78.85; H, 6.14. Found: C, 78.74; H, 5.96.

1-(2,4-Dimethoxy-5-[3-(4-hydroxyphenyl) acryloyl]phenyl)-4-(4-hydroxyphenyl)but-2-en-1-one (3b). Yield: 55%, m.p. 232-233°C. ^{1}H NMR ($CDCl_3$) *d* 3.96 (s, 6H, 2´-OCH_3), 6.48 (s, 1H, H-3'), 6.92 (d, 4H, 2´H-3,5), 7.28 (d, 2H, 2xH-a), 7.53 (d, 4H,2´H-2,6), 7.79 (d, 2H, 2xH-b), 8.22 (s, 1H, H-6'). MS: *m/z* 430 (M^+). Anal calcd. for $C_{26}H_{22}O_6$: C, 72.55; H, 5.15. Found: C, 72.42; H, 5.23.

1-(2,4-Dimethoxy-5-[3-(2-nitrophenyl) acryloyl] phenyl) -4-(2-nitrophenyl)but-2-en-1-one (3c). Yield: 60%, m.p. 216-218°C. ^{1}H NMR ($CDCl_3$) *d* 3.97 (s, 6H, 2´-OCH_3), 6.53 (s, 1H, H-3'), 7.08-7.31 (m, 4H, 2´H-3,5), 7.43-7.56 (m, 6H, 2xH-4,6,a), 7.86 (d, 2H, 2xH-b), 8.14 (s, 1H, H-6'). MS: *m/z* 488 (M^+). Anal calcd. for $C_{26}H_{20}N_2O_8$: C, 63.93; H, 4.13; N, 5.74. Found: C, 64.11; H, 3.86; N, 5.62.

1-(2,4-Dimethoxy-5-[3-(3-chlorophenyl) acryloyl] phenyl)-4-(3-chlorophenyl)but-2-en-1-one (3d). Yield: 62%, m.p. 200-202°C. ^{1}H NMR ($CDCl_3$) *d* 3.96 (s, 6H, 2´-OCH_3), 6.51 (s, 1H, H-3'), 7.24 (d, 2H, 2xH-a), 7.55 (m, 2H, 2xH-5), 7.89 (dd, 2H, 2xH-4), 8.01 (d, 2H, 2xH-b), 8.17 (s, 1H, H-6'), 8.26 (dd, 2H, 2xH-6), 8.41 (s, 2H, 2xH-2). MS: *m/z* 466 (M^+), 467 (M^++1). Anal calcd. for $C_{26}H_{20}Cl_2O_4$: C, 66.82; H, 4.31. Found: C, 66.65; H, 4.27.

1-(2,4-Dimethoxy-5-[3-(2,4-dichlorophenyl) acryloyl] phenyl)-4-(2,4-chlorophenyl)but-2-en-1-one (3e). Yield: 56%, m.p. 186-188°C. ^{1}H NMR ($CDCl_3$) *d* 3.93 (s, 6H, 2´-OCH_3), 6.48 (s, 1H, H-3'), 7.21-7.58 (m, 8H, 2xH-3,5,6 + 2xH-), 7.83 (d, 2H, 2xH-b), 8.16 (s, 1H, H-6'). MS: *m/z* 550 (M^+), 551 (M^++1), 552 (M^++2). Anal calcd. for $C_{27}H_{20}Cl_4O_4$: C, 58.93; H, 3.66. Found: C, 58.65 H, 3.52.

Antimicrobial activity

The synthesized compounds were evaluated for their antimicrobial activity [13,14] against three

bacterial strains and two fungal strains at a concentration of 100 µg/mL by cup plate method. Compounds inhibiting growth of one or more of the test microorganisms were further tested for their minimum inhibitory concentration (*MIC*).

Antibacterial activity

The compounds were screened for their in vitro antibacterial activity [13] against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) bacterial strains at a concentration of 100 µg/mL by cup plate method. Ciprofloxacin was used as standard drug for comparison. Freshly prepared liquid agar medium (25 mL/petridish) was poured into each petridishes and the plates were dried by placing in an incubator at 37°C for 1 h. Then standardized culture of microorganism was spread on each petridishes by L-shaped spreader. Wells (6 mm) were made using an agar punch and, each well was labeled accordingly. A control (solvent) was also included in the test. The test compound and standard drug solutions (100 µg/mL) were made in dimethylsulfoxide (DMSO) and added in each well separately and petridishes kept aseptically for 1h for diffusion of the sample. After the completion of diffusion, all the petridishes were kept for incubation at 37°C for 24 h and then diameter of the zone of inhibition was measured in mm (Table 1).

Compounds inhibiting growth of one or more of the test microorganisms were further tested for their minimum inhibitory concentration (*MIC*) by turbidity method. A solution of the compounds (100 µg/mL) was prepared in DMSO and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The inoculum consisting of an overnight broth culture of microorganisms was added to separate tubes. The tubes were incubated at 37° for 24 h and examined for turbidity. The highest dilution (lowest concentration) required to arrest the growth of bacteria was regarded as *MIC*. Results are presented in Table 2.

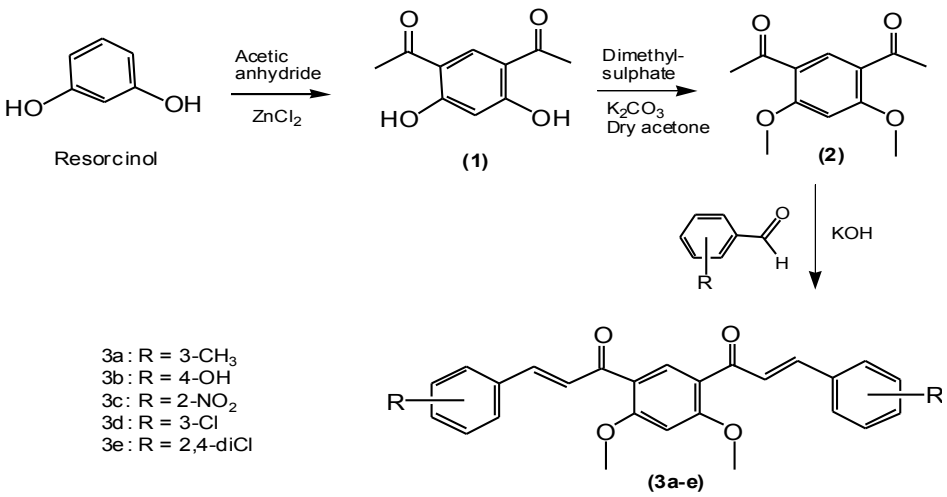
Antifungal activity

In vitro antifungal activity of the synthesized compounds was determined against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) by agar diffusion method [14]. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media (20 mL) was poured into each petridish and the plates were dried by placing in an incubator at 37°C for 1 h. Wells were made using an agar punch and, each well was labeled accordingly. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The test compounds and standard drug (Griseofulvin) solutions (100 µg/mL) were made in dimethylsulfoxide (DMSO) and added in each well separately and petridishes kept aseptically for 1h for diffusion of the sample. After the completion of diffusion, all the petridishes were kept for incubation at 37°C for 3-4 days and then diameter of the zone of inhibition was measured in mm *(Table 1)*. Compounds inhibiting growth of one or more of the fungal strains were further tested for their minimum inhibitory concentration (*MIC*). A solution of the compounds (100 µg/mL) was prepared in DMSO and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The tubes were inoculated with approximately $1.6x10^4$-$6x10^4$ c.f.u. mL^{-1} and incubated for 48 h at 37°C and examined for growth. The lowest concentration (highest dilution) required to arrest the growth of fungus was regarded as *MIC*. Results are presented in *Table 2*.

Results and Discussion

Chemistry

The protocol for synthesis of title compounds is presented in *Scheme-1.* The starting material, 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**), prepared from resorcinol [10], was treated with dimethylsulphate in dry acetone in presence of anhydrous potassium carbonate to get 1,1´-(4,6-dimethyl-1,3-phenylene) diethanone (**2**) [11], which gave negative ferric chloride test showing the absence of phenolic-hydroxyl group. Compound (**2**) was then condensed with different aromatic aldehydes in presence of potassium hydroxide following Claisen-Schmidt reaction conditions to furnish 5 new bischalcones (**3a-e**). These compounds gave a red colour with conc. sulphuric acid; positive test for chalcones, and no colour with ethanolic ferric chloride solution; negative test for phenolic-hydroxyl group. The structures of the synthesized compounds were further supported by ^{1}H NMR, Mass spectral data and elemental analysis results.



Scheme 1: protocol for synthesis of title compounds (3a-e).

The ^{1}H NMR spectrum of 1, 1´-(4,6-dimethyl-1, 3-phenylene)diethanone (**2**) [11] showed a singlet at *ä* 2.61, which could be accounted for six protons of two acetyl groups. The two methoxyl groups appeared as singlet at δ 3.93. The ring protons, H-2 and H-5, gave singlet at δ 6.34 and 8.37, respectively. The mass spectrum of the compound (**2**) showed molecular ion peak at *m/z* 222.

The ^{1}H NMR spectra of the title compounds (3a-e) revealed the presence of two methoxyl groups as singlet at around δ 3.9, and two -CH=CH- groups as two doublets at around δ 7.3 and δ7.9 integrating for two CH-α and two CH-β protons, respectively. Chalcone ring protons H-3' & H-6'appeared as singlet at δ 6.5 and δ 8.1, respectively. Other signals were observed at appropriate δ values integrating for the protons of two substituted phenyl rings. The mass spectra of bischalcones showed the presence of molecular ion peak in reasonable intensities. In case of compounds having phenyl rings with chloro-substituents (**3d** & **3e**), the molecular ion peak or their fragments having chloro-group appeared as cluster of peaks. Elemental analyses values of the synthesized compounds were found within ±0.4% of theoretical values.

Antimicrobial activity

The title compounds (3a-e) were screened for their in vitro antibacterial activity against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) bacterial species, and antifungal activity against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404). The antimicrobial screening data showed that two compounds, 1-(2,4-dimethoxy-5-[3-(3-chloroyphenyl) acryloyl]phenyl)-4-(3-chlorophenyl)but-2-en-1-one 3d and 1-(2,4-dimethoxy-5-[3-(2,4-dichlorophenyl) acryloyl]phenyl) -4-(2,4-chlorophenyl) but-2-en-1-one **3e**, exhibited good activity against *S. aureus* & *E. coli* with *MIC*-12.5 ìg/mL. Rest of the compounds showed moderate to low antimicrobial activities. The standard drugs showed *MIC* values of 6.25 ìg/mL (Table 1 & 2).

An analysis of results indicated that the title compounds 3a-e were appreciable in their antibacterial and antifungal actions. Presence of chloro group(s) found to increase the antimicrobial activity of the bischalcones.

Table 1: Preliminary antibacterial and antifungal activities of the title compounds (3a-e).

Compd.	Substituent (R)	Antibacterial activity#			Antifungal activity#	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. nige*
3a	3-Methyl	+	-	-	+	-
3b	4-Hydroxy	++	+	++	++	-
3c	2-Nitro	+	-	+	+	+
3d	3-Chloro	+++	+++	+	++	-
3e	2,4-Dichloro	+++	+++	++	++	+
	Standard-1†	++++	++++	++++	nt	nt
	Standard-2†	nt	nt	nt	++++	++++

#Zone of inhibition: - = < 5 mm (insignificant or no activity), + = 5-9 mm (weak activity), ++ = 10-14 mm (moderate activity), +++ = 15-20 mm (good activity), ++++ = > 20 mm (excellent activity).

†Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin, nt = not tested.

Table 2: Antibacterial and antifungal activities (*MIC*, mg/mL) of the title compounds (**3a-e**).

Compd.	Substituent (R)	Antibacterial activity			Antifungal activity	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
3a	3-Methyl	50	>100	>100	>100	>100
3b	4-Hydroxy	25	50	25	25	>100
3c	2-Nitro	50	>100	50	50	50
3d	3-Chloro	12.5	12.5	50	25	>100
3e	2,4-Dichloro	12.5	12.5	25	25	50
Standard-1†		6.25	6.25	6.25	nt	nt
Standard-2†		nt	nt	nt	6.25	6.25

nt = not tested; †Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin.

Conclusion

A series of 1-(2,4-dimethoxy-5-[3-(substituted-phenyl)acryloyl]phenyl)-4-(substituted-phenyl)but-2-en-1-ones (**3a-e**) were successfully synthesized starting from resorcinol. The antimicrobial studies showed that the synthesized compounds were having significant antibacterial and antifungal activities. Presence of chloro group(s) increased the antimicrobial activity of the bischalcones. These derivatives may be further explored to develop potential antimicrobial agents.

References

1. Chu DTW, Plattner JJ, Katz L., New directions in antibacterial research. J. Med. Chem. 1996; 39: 3853-3874.
2. Davies J., Bacteria on the rampage. Nature 1996; 383 : 219-220.
3. Baddley JW, Moser SA., Emerging fungal resistance. Clin. Lab. Med. 2004; 24: 721-724.
4. Cushnie TP, Lamb AJ., Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 2005; 26: 343-356.
5. Nowakowska Z., A review of anti-infective and anti-inflammatory chalcones. Eur. J. Med. Chem. 2007; 42: 125-137.
6. Batovska DI, Todorova IT., Trends in utilization of the pharmacological potential of chalcones. Curr. Clin. Pharmacol. 2010; 5: 01-29.
7. Lin YM, Zhou Y, Flavin MT, Zhou LM, Nie W, Chen FC., Chalcones and flavonoids as anti-tuberculosis agents. Bioorg. Med. Chem. 2002; 10: 2795-2802.
8. Sharma M, Chaturvedi V, Manju YK, Bhatnagar S, Srivastava K, Puri SK, Chauhan PM., Substituted quinolinyl chalcones and quinolinyl pyrimidines as a new class of anti-infective agents. Eur. J. Med. Chem. 2009; 44: 2081-2091.
9. Soliman K, Ohad N, Ramadan M, Maayan S, Snait T, Jacob V., Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. Bioorg. Med. Chem. 2005; 13: 433-441.
10. Khan MSY, Sharma S, Husain A., Synthesis and antibacterial evaluation of new flavonoid derivatives from 4,6-diacetyl resorcinol. Scientia Pharmaceutica 2002; 70: 287-294.
11. Husain A, Javed S, Mishra R, Rashid M, Bhutani R., Synthesis and microbiological evaluation of some new Bischalcones. Pharmacophore 2011; 2(6): 316-325.
12. Husain A, Ahmad A, Ibraheem AIM, Mishra R, Rashid M., Synthesis and antimicrobial activity of bischalcones derivatives. Med. Chem. Res. 2013; 22: 1578-1586.
13. Colle JG, Duguid JP, Fraser AG, Marmion BP., Laboratory strategies in diagnosis", In: Mackie TJ, MacCartney JE, eds. Practical Medical Microbiology, 13th ed, London, Churchill-Livingstone, 1989.
14. Varma RS., Antifungal Agents: Past, Present and Future Prospects. National Academy of Chemistry & Biology, Lucknow, India, 1998.

Journal of Pharmaceutical and Medicinal Chemistry

Library Recommendation Form

If you would like to recommend this journal to your library, simply complete the form below and return it to us. Please type or print the information clearly. We will forward a sample copy to your library, along with this recommendation card.

Please send a sample copy to:

Name of Librarian

Library

Address of Library

Recommended by:

Your Name/ Title

Department

Address

Dear Librarian,

I would like to recommend that your library subscribe to the **Journal of Pharmaceutical and Medicinal Chemistry**. I believe the major future uses of the journal for your library would provide:

1. useful information for members of my specialty.
2. an excellent research aid.
3. an invaluable student resource.

I have a personal subscription and understand and appreciate the value an institutional subscription would mean to our staff.

Should the journal you're reading right now be a part of your University or institution's library? To have a free sample sent to your librarian, simply fill out and mail this today!

Stock Manager

Red Flower Publication Pvt. Ltd.

48/41-42, DSIDC, Pocket-II, Mayur Vihar, Phase-I

Delhi - 110 091 (India)

Tel: 91-11-22754205, 45796900, Fax: 91-11-22754205

E-mail: redflowerppl@gmail.com, redflowerppl@vsnl.net

Website: www.rfppl.co.in

Journal of Pharmaceutical and Medicinal Chemistry
Volume 1 Number 2, July - December 2015

Original Article

Assessment of Veterinary Vaccine Safety by Using Spreadsheet Software

Sai Mahesh Reddy Avula*, Venkata Subba Reddy Avula**

Abstract

Assessment of veterinary vaccine safety is often done by veterinarians in animal houses in biological units using conventional methods which is a basically a complex and time consuming. In today's era of Smart phones, tablet PCs and Netbooks where a spreadsheet program in the form of Excel is readily accessible by most veterinarians, it should be possible to adapt the various laborious steps involved in the said method to a spreadsheet program. In this article we have designed a spreadsheet program based on the steps involved in conventional method for assessment of vaccine safety.

Keywords: Smart phones; Netbooks; Excel; Spreadsheet.

Introduction

Assessment of veterinary vaccine safety is often done by veterinarians in animal houses in biological units. The more reliable method we use for the assessment of vaccine safety is conventional method which is described below. The calculations involved in that assessment are complex, confusing, time consuming and less easily reproducible. In today's era of Smart phones, tablet PCs and Notebooks where a spreadsheet program in the form of Excel is readily accessible by most veterinarians, it should be possible to adapt the various laborious steps involved in the said method to a spreadsheet program. This way the laborious steps involved in the method can be easily calculated in a simple, clear, faster, reproducible, accurate and user-friendly manner. The aim of this article is to design a spreadsheet program based on the steps involved in conventional method for assessment of vaccine safety and check the accuracy of such spreadsheet program (Figure 1) for the extremes of values in the assessments.

Author Affilation: *The College of Veterinary Science, Gannavaram-521102, Krishna District, Andhra Pradesh, India.**Andhra Medical College, Maharanipeta, Visakhapatnam-530002, Andhra Pradesh, India.

Reprint Request: Saimahesh Reddy Avula, Teaching Faculty, Dept. of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Gannavaram-521102., Krishna District, Andhra Pradesh, India
E-mail: maheshreddy041@gmail.com

Materials and Methods

The spreadsheet program is based on the various steps for evaluation of vaccine safety by conventional method [2]. According to which 20 animals, those preferably do not have antibodies against rabies virus have to be selected for the safety test. Each animal has to be administered with vaccine by a recommended route a double dose of the vaccine. Observe the animals at least daily for 14 days and clinical signs have to be noted down daily in a prescribed form. The clinical signs to be noted down are:

1. Ruffled fur
2. Hunched back
3. Slow movements
4. Loss of alertness
5. Circular movements

6. Shaky movements
7. Trembling
8. Convulsions
9. Paresis
10. Paralysis
11. Moribund state

The spreadsheet program used was Microsoft Excel version 2013. One representative study is presented to help understand the usefulness of this program. The findings of the experiment along with the calculation are presented in the sheet which was based on the said method.The logical data

Fig. 1: Snapshot taken from the actual spreadsheet of safety assessment(Spreadsheet software)

Vaccine safety calculator		Example of a chart used to record clinical Signs in the rabies vaccine potency test									
		days after challenge of Rabies vaccine									
S.No	Clinical signs	6	7	8	9	10	11	12	13	14	Total no. of mice with the specified sign
1	Ruffled fur	0	1	1	1	1	1	1	1	1	1
2	Hunched back	0	0	0	0	0	0	0	0	0	0
3	Slow movements	0	0	0	0	0	0	0	0	0	0
4	Loss of alertness	0	0	0	0	0	1	1	1	1	1
5	Circular movements	0	0	0	0	0	0	0	0	0	0
6	Shaky movements	0	0	0	0	0	0	0	0	0	0
7	Trembling	0	0	0	0	0	0	0	1	1	1
8	Convulsions	0	0	0	0	0	0	0	0	0	0
9	Paresis	0	0	0	0	0	0	0	0	0	0
10	Paralysis	0	0	0	0	0	0	0	0	0	0
11	Moribund state	0	0	0	0	0	0	0	0	0	0
12	Total no. of mice with atleast one of the sign										3
										percent of animals showing rabies signs	15
										Result of rabies safety test	PAS

Created by Dr. Sai Mahesh Reddy Avula and Venkata Subba Reddy Avula

incorporated in this spreadsheet is (in L17 cell) =IF(L16<=15, "PASS","FAIL").

Results

A spreadsheet program for the vaccine safety assessment is presented. It may be noted that the calculations in the spreadsheet program are accurate from the representative examples provided above. If the percent of the mice showing rabies signs exceeds 15 then the test is said to be failed alternatively the vaccine complies with the test if no or only less than 15 percent animals shows notable signs of disease or dies from causes attributable to the vaccine.

Discussion

A spreadsheet is a computer application that simulates an accounting worksheet. It displays multiple cells which together make up a grid comprising of rows and columns. It can be used to store, process, analyse and graphically represent data. A formula entered in a cell in the spreadsheet defines how the content of that cell is to be calculated from the contents of any other cell(s) each time the content of the other cell(s) is updated. VisiCalc is historically regarded as the first spreadsheet application (year 1979). The spreadsheet application which is most popular currently is familiar to most of us as Microsoft Excel [1]. Although an Excel spreadsheet is ideal for arranging entries in columns and rows, that's not its main purpose. One can do that with a table in a word processing application like Microsoft Word. What makes a spreadsheet so powerful is that it can perform calculations using various values from the spreadsheet. It was hoped that this program which was freely usable was useful to all veterinary doctors who are working at biological units.

Conclusion

The difficulty which usually concerns veterinarians at biological units is the vaccine safety

assessment. We often encountered various problems which were listed below: (a) the calculations involved in the assessment are time consuming. (b) The calculations appear confusing when reviewed at a later date. (c) It is often difficult to explain in the theses or discussions how a final figure was arrived at. With a goal to simplify the calculation, we have explored the possibility of adapting the conventional method to a spreadsheet. With this, the calculations happen automatically and they are reproducible in the form of a printout which can easily explain how the final figure was arrived at in the theses and discussions. The spreadsheet makes the whole process of assessment faster and user friendly. The program was thoroughly tested for the entire range of values which are possible. One representative study was also presented to help understand the applicability of this program.

References

1. http://office.microsoft.com/en.us/excel
2. British Pharmacopoeia Commission, 2015.

Instructions to Authors

Submission to the journal must comply with the Guidelines for Authors.

Non-compliant submission will be returned to the author for correction.

To access the online submission system and for the most up-to-date version of the Guide for Authors please visit:

http://www.rfppl.co.in

Technical problems or general questions on publishing with JPMC are supported by Red Flower Publication Pvt. Ltd's Author Support team (http://www.rfppl.co.in)

Alternatively, please contact the Journal's Editorial Office for further assistance.

Publication-in-Charge
Journal of Pharmaceutical and Medicinal Chemistry
Red Flower Publication Pvt. Ltd.
48/41-42, DSIDC, Pocket-II
Mayur Vihar Phase-I
Delhi – 110 091
India
Phone: 91-11-22754205, 45796900, Fax: 91-11-22754205
E-mail: redflowerppl@gmail.com, redflowerppl@vsnl.net
Website: www.rfppl.co.in

Journal of Pharmaceutical and Medicinal Chemistry
Volume 1 Number 2, July - December 2015

Original Article

Amide Based Mutual Prodrug: Synthesis and Antimicrobial Evaluation

Asif Husain*, Aftab Ahmad, Shah Alam Khan***, Nadir Islam***

Abstract

An amide-based mutual prodrug (NA-D) was synthesized by condensing nalidixic acid with dapsone with an aim of preparing a useful drug, which could show broad spectrum of antimicrobial activity. Its structure was established on the basis of elemental analysis, ^{1}H NMR and Mass spectral data results. The mutual prodrug (NA-D) was also evaluated for in-vitro antibacterial and antifungal activity.

Keywords: Dapsone; Amide; Prodrug; Bacterial; Fungal.

Introduction

Prodrug research is an important and fruitful area of research. A prodrug may be defined as a biologically inactive derivative of a drug that requires a chemical or enzymatic metabolism within the body to release the active drug [1]. Prodrugs may have improved pharmacokinetic, pharmacodynamic, physicochemical, and other properties over the parent molecule [1-3]. In a prodrug, generally the carrier group (promoiety/linker) used is inert or biologically inactive molecule [3]. However, in some cases the prodrug consists of two pharmacologically active agents joined together as a single molecule. In such prodrugs, each acts as promoiety for the other agent [4]. These prodrugs are known as mutual prodrugs [4,5]. Mutual prodrug concept has been successfully applied to a number of NSAIDs to get compounds with improved pharmacological profile including reduced GIT toxicity [1-6].

Microbial resistance to antibiotics has become a huge problem in treating infections in recent times [7]. Tuberculosis (TB) is a global emergency and is amongst the worldwide health threats today [8]. Searching new compounds, which could combine a non specific activity against a broad spectrum of microbes and low toxicity, seems to be a promising way to tackle the problem. Nalidixic acid (1,8-naphthyridine derivative) was the first synthetic quinolone derivative introduced for the treatment of UTI (urinary tract infections) in 1963 [9]. It is particularly effective against gram-negative bacteria particularly *Escherichia coli* and resistant to most of the pseudomonas species [10,11]. The derivatives of nalidixic acid also show significant antimicrobial activities [12].

On the other hand, dapsone is one of the important antimicrobial agents used to kill *M. leprae*. Dapsone derivatives also show potential antimicrobial activities [13,14].

In view of these observations and in continuation of our work on mutual prodrugs [5,15], it was considered worthwhile to synthesize a mutual

Author Affilation: *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard University, New Delhi-110 062, India. **Jeddah Community College, King Abdul Aziz University, Jeddah 21589, Kingdom of Saudi Arabia. ***Department of Pharmacy, Oman Medical College, Muscat, Sultanate of Oman.

Reprint Request: Asif Husain, Sr. Asst. Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi-110 062, India.
Email: drasifhusain@yahoo.com,
ahusain@jamiahamdard.ac.in

prodrug chemically combining nalidixic acid with dapsone in a single structure with an aim of preparing a promising antimicrobial compound which might act with effectiveness on both the gram-positive and gram-negative bacteria, and also have antifungal activity.

Materials and Methods

Melting points were taken in open capillary tubes and are uncorrected. Dry solvents were used throughout the study. Microanalysis of the compounds was done on Perkin-Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. ^{1}H NMR spectrum was recorded on Bruker spectropsin DPX-300MHz with tetramethylsilane as internal standard in solvent $CDCl_3$. Mass spectrum was recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV. Spectral data are consistent with the assigned structure. The progress of the reaction was monitored on TLC, which was performed on silica gel. Iodine chamber and UV-lamp were used for visualization of TLC spots. The reaction involved in synthesis is given in *scheme 1.*

Synthesis

Nalidixic acid (464 mg; 2 mmol) (**1**) was dissolved in dry pyridine (5 mL) and dapsone (249 mg; 1 mmol) (**2**) was also dissolved separately in dry pyridine (4 mL). Both the solutions were mixed together and stirred magnetically. Phosphorous oxychloride (0.9 mL) was added dropwise maintaining the temperature 0-5° C while stirring. The contents were stirred for another half-hour and left overnight. It was poured into ice cold water and a solid mass separated out, which was filtered, washed with plenty of water, dried and crystallized from methanol to give TLC pure reddish-brown crystals of the mutual prodrug (NA-D).

In vitro antibacterial activity

The antibacterial activity of NA-D was evaluated against four bacterial strains, gram positive - *Staphylococcus aureus* (MTCC 96) & *Bacilluus subtilis* (MTCC 121), and gram negative - *Escherichia coli* (MTCC 1652) & *Klebsiella pneumonia* (ATCC 13883). The assay was carried out following the turbidity method [16]. Nalidixic acid was used as a standard drug. A solution of NA-D/standard drug was prepared in dimethylformamide (DMF) and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile stoppered test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The nutrient broth, which contained logarithmic serially two fold diluted amount of test compounds and control were inoculated with approximately $5x10^5$ c.f.u. of actively dividing bacterial cells. The cultures were incubated at 37°C for 24 h and the growth was monitored. The highest dilution (lowest concentration) at which the growth of bacteria arrested was taken as minimum inhibitory concentration (*MIC*).

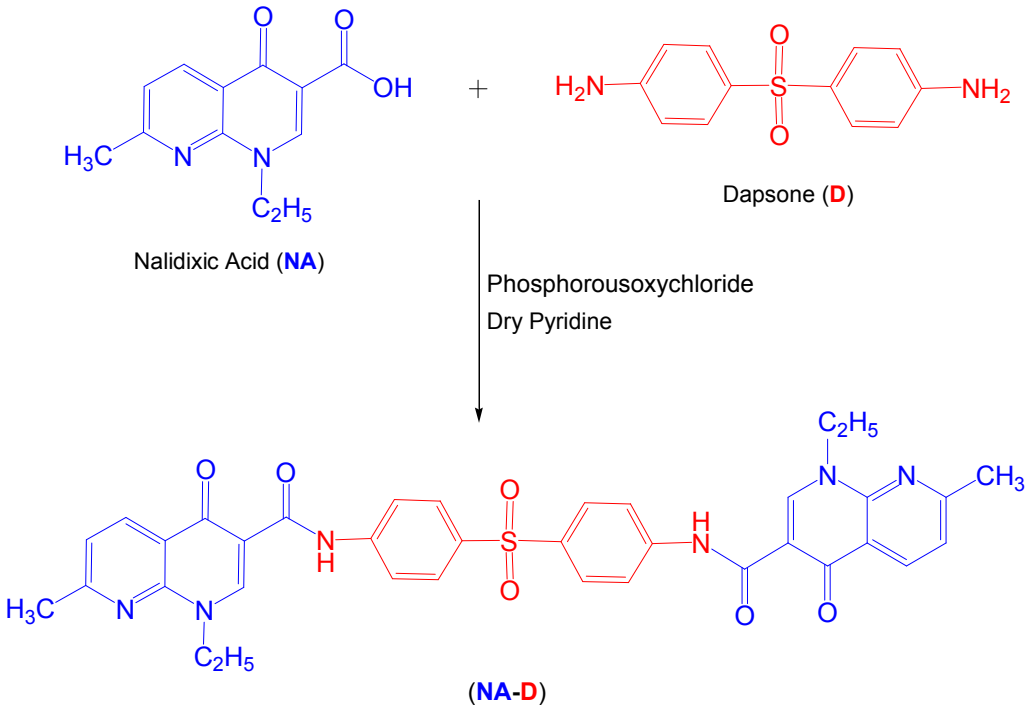
In vitro antifungal activity

Antifungal activity was evaluated against three fungal strains; *Candida albicans*, *Aspergillus niger* and *Rhizopus oryza [17,18]*. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was utilized to prepare a suspension of spores of fungal strain for lawning. A loopful of the fungal strain was transferred to 3 mL saline to obtain a suspension. The nutrient broth, which contained logarithmic serially two fold diluted amount of NA-D/standard drug and control was inoculated with approx. $1.6x10^4$-$6x10^4$ c.f.u./mL. The cultures were incubated at 37°C for 48 h and the growth was monitored. Griseofulvin was used a s standard drug for comparison. The highest dilution (lowest concentration) at which the growth of fungi arrested was regarded as minimum inhibitory concentration (*MIC*).

Results and Discussion

Synthesis

Nalidixic acid was condensed with dapsone in minimum quantity of dry pyridine in presence of phosphorous oxychloride ($POCl_3$) following single step synthesis method (*Scheme 1*). Usual work up of the reaction mixture followed by crystallization from methanol furnished the mutual prodrug (*NA-D*) as reddish-brown crystals, Melting Point: 214-216° C, Rf value: 0.67 (Toluene: Ethyl acetate: Formic acid, 5:4:1), Yield: 54 %.



Scheme 1: Protocol for synthesis of mutual Prodrug (NA-D)

Structure establishment of the mutual prodrug (NA-D)

NMR spectrum: The ^{1}H NMR spectrum of the mutual prodrug (NA-D) showed a triplet and a quartet located at d 1.55 and d 4.81 arising from the methyl and methylene group of ethyl moieties ($2xC_2H_5$) in nalidixic acid. There was a broad singlet located at d 2.75 integrating for the methyl groups ($2xCH_3$) of nalidixic acid skeleton. Four protons of the dapsone ring appeared as doublets ($2xA_2B_2$ pattern) each at δ 7.67 and δ 8.02. There were two *ortho*-coupled doublets each at δ 7.62 and δ 8.63 arising from the two *ortho*-coupled protons of the nalidixic acid system. A singlet located at d 8.95 could be accounted for the lone proton of the nalidixic acid system. Two broad singlets located each at δ 9.81 and 9.98 could be accounted for 2x -NH- protons of the dapsone moiety.

Mass spectrum: The mass spectrum of the mutual prodrug (**NA-D**) showed a molecular ion peak located at m/z 676.

Elemental analysis: The values were found within ±0.4% of the theoretical values, $C_{36}H_{32}N_6O_6S$, Calculated C, 63.89; H, 4.77; N, 12.42, Found C, 63.54; H, 4.62; N, 12.58.

Microbiology

The synthesized mutual prodrug (**NA-D**) was evaluated for its in vitro antibacterial activity against the bacterial strains gram positive (*Staphylococcus aureus* & *Bacilluus subtilis*), gram negative (*Escherichia coli* & *Klebsiella pneumonia*), and in vitro antifungal activity against *Candida albicans*, *Rhizopus oryza*, and *Aspergillus niger*. Minimum inhibitory concentration (*MIC*) was determined and results showed that the mutual prodrug (*NA-D*) was good against *B. subtilis* & *E. coli* with *MIC* 12.5 mg/mL, and significant activity against *S. aureus* with *MIC* 25 µg/mL. Nalidixic showed *MIC* 3.12 mg/mL against *E. coli*, *MIC* 6.25 mg/mL against *S. aureus* & *B. subtilis*, and *MIC* 12.5 µg/mL against *K. pneumonia*.

In antifungal assay, the mutual prodrug (*NA-D*) showed good activity against *C. albicans* with *MIC*12.5 mg/mL, and appreciable activity against *R. oryza* with *MIC* 25 µg/mL. Griseofulvin showed *MIC* 6.25 mg/mL against all the tested three fungal strains.

In vitro and in vivo hydrolysis studies are under progress in our laboratory to assess the fate of the *NA-D* in the system.

Conclusion

Nalidixic acid and dapsone were condensed together through an amide-linkage (-CONH-) to get the mutual prodrug (*NA-D*). Spectral and analytical data were found in agreement with the proposed structure. In-vitro antibacterial activity of NA-D

against some selected bacteria showed good antibacterial and significant antifungal activities with *MIC* ranging from 12.5-25mg/mL. In vitro and in vivo hydrolysis studies are required to assess the fate of the *NA-D* in the system. The present work shows the pharmaceutical potential of mutual prodrugs.

Acknowledgements

One of the authors (AH) is thankful to Hamdard National Foundation (HNF), New Delhi for financial support.

References

1. Jarkko Rautio, Hanna Kumpulainen, Tycho Heimbach, Reza Oliyai, Dooman Oh, Tomi Järvinen & Jouko Savolainen Prodrugs: design and clinical applications. Nature Reviews Drug Discovery 2008, 7: 255-70.
2. Jana S, Mandlekar S, Marathe P. Prodrug design to improve pharmacokinetic and drug delivery properties: challenges to the discovery scientists. Curr Med Chem. 2010; 17(32): 3874-908.
3. Kristiina M. Huttunen, Hannu Raunio and Jarkko Rautio, Prodrugs—from Serendipity to Rational Design, Pharmacological Reviews, 2011, 63(3): 750-71.
4. Bhosle, D.; Bharambe, S.; Gairola, Neha; Dhaneshwar, Suneela S. Mutual Prodrug concept: fundamentals and applications, Ind. J. Pharm. Sci., 2006; 68(3): 286.
5. Asif Husain, Priyanka Ahuja, M. Shaharyar, Aftab Ahmad, Ibraheem Ahmed I. Mkhalid, M.M. Alam, M. Akhter, and M.S. Zaman, Synthesis, biological activities and pharmacokinetics studies of a mutual prodrug of aceclofenac and paracetamol. *Med. Chem. Res.*, 2014; 23(3): 1077-1083.
6. Halen PK, Murumkar PR, Yadav MR, Giridhar R., Prodrug designing of NSAIDs. Mini Rev. Med. Chem, 2009; 9(1): 124-39.
7. Davies J. Nature, 1996; 383: 219-20.
8. Janin YL. Antituberculosis drugs: Ten years of research. Bioorg Med Chem. 2007; 15(7): 2479-513.
9. G.Y. Leisher, E.J. Froelich, M.D. Gruett, J.H. Bailey, P.R. Brundage, J. Med. Pharm. Chem. 5 (1962) 1063e1065.
10. G.C. Crumplin, J.T. Smith, Antimicrob. Agents Chemother. 1975; 8 (3).
11. N. Aggarwal, R. Kumar, C. Srivastava, P. Dureja, J.M. Khurana, J. Agric. Food Chem. 2010, 58.
12. Zhang Y, The magic bullets and tuberculosis drug targets, Annu. Rev. Pharmacol. Toxicol. 2005; 45: 529-64.
13. Asif Husain, Ausaf Ahmad, M. Mujeeb, M. Akhter, New amides of sulphonamides: Synthesis and biological evaluation, J. Chil. Chem. Soc., 2010; 55(1): 74-77.
14. P. D. Mehata, Synthesis and biological activity studies of some thiazolidinones and azetidinone, Indian J. Pharm. Sci., 2006; 68: 101-103.
15. Asif Husain, Aftab Ahmad, Shah Alam Khan, Mohammad Sarafroz. Synthesis and evaluation of a mutual prodrug. *Int. J. Pharmacy*, 2015; 5(2): 389-392.
16. Colle JG, Duguid JP, Fraser AG, Marmion BP. Laboratory strategies in diagnosis. In: Mackie TJ, MacCartney JE, eds. Practical Medical Microbiology, 13th ed. London: Churchill Livingstone, 1989; 601–649.
17. Khan ZK. In vitro and vivo screening techniques for bioactivity screening and evaluation. In: *Proc. Int*. Workshop UNIDO-CDRI, 1997; 210–11.
18. Varma RS, ed. Antifungal Agents: Past, Present and Future Prospects. Lucknow, India: National Academy of Chemistry & Biology, 1998.

Journal of Pharmaceutical and Medicinal Chemistry
Volume 1 Number 2, July - December 2015

Original Article

A Simple and Validated Rp-Hplc Method for the Simultaneous Estimation of Metformin and Dapagliflozin in Bulk and Pharmaceutical Dosage Forms

Syeda Kulsum*, G.Vidyasagar, Tasneem Mohammed*****

Abstract

A simple, rapid, specific, accurate and precise reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Metformin and Dapagliflozin in tablet dosage form. An Inertsil ODS C18 column having 150 x 4.6mm id in Isocratic mode with mobile phase containing Acetonitrile : phosphate buffer (70:30 %v/v pH: 3.0) was used. The flow rate was 1.0ml/min and effluents were monitored at 240nm.) The retention time of Metformin and Dapagliflozin was 2.463min and 3.760min respectively. The concentration curves of Metformin and Dapagliflozin were linear in the concentration range of 50µg/ml-250 µg/ml and 5µg/ml-25µg/ml of Metformin and Dapagliflozin respectively. The developed method was validated for specificity, precision, linearity, accuracy, LOD, LOQ, robustness. Recovery of Metformin and Dapagliflozin in formulations was found to be in the range of 97.0% - 98.0% and 100%-103% respectively confirms the non-interferences of the excipients in the formulation. Due to its simplicity, rapidness and high precision, the proposed HPLC method may be used for the simultaneous determination of these two drugs in pharmaceutical dosage forms.

Keywords: RP-HPLC; Metformin and Dapagliflozin.

Author Affilation: Department of Pharmaceutical Analysis and Quality Assurance, *MRM College of Pharmacy, Chintapallyguda, Ibrahimpatnam Hyderabad. **Veerayathan Institute of Pharmacy, Jakhania Bhuj-Mandvi Road, Kutch Gujarat. ***Ibn Sina National college for medical sciences, Al mahjar, Jeddah, KSA.

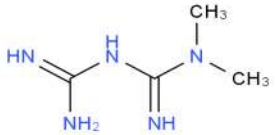
Reprint Request: Syeda kulsum, Department of Pharmaceutical Analysis and Quality Assurance, MRM College of Pharmacy, Chintapallyguda Ibrahimpatnam Hyderabad.

E-mail: syedakulsum@gmail.com

Introduction

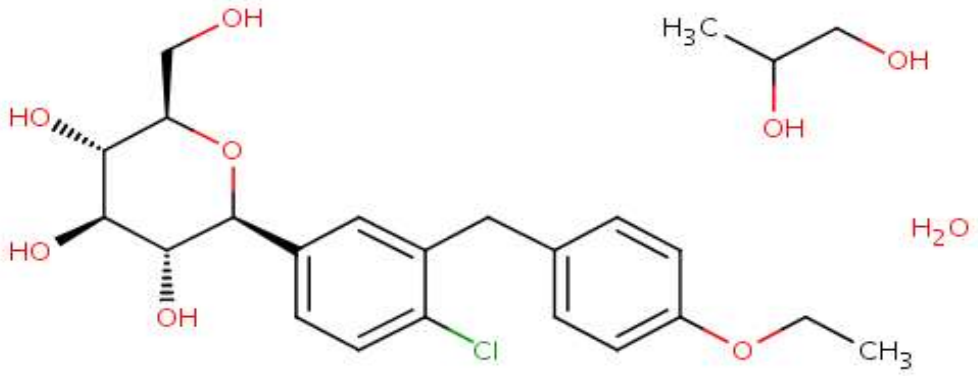
Metformin is chemically 1-carbamimidamido-N, N-dimethylmethanimidamide. It decreases blood glucose levels by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improving insulin sensitivity by increasing peripheral glucose uptake and utilization.

These effects are mediated by the initial activation by metformin of AMP-activated protein kinase (AMPK), a liver enzyme that plays an important role in insulin signaling, whole body energy balance, and the metabolism of glucose and fats. Activation of AMPK is required for metformin's inhibitory effect on the production of glucose by liver cells. Increased peripheral utilization of glucose may be due to improved insulin binding to insulin receptors The rare side effect, lactic acidosis, is thought to be caused by decreased liver uptake of serum lactate, one of the substrates of gluconeogenesis. In those with healthy renal function, the slight excess is simply cleared [1].



Metformin

Dapagliflozin: Dapagliflozin(2S,3R,4R,5S,6R)-2-{4-chloro-3-[(4-ethoxyphenyl)methyl]phenyl}-6-(hydroxymethyl)oxane-3,4,5-triol propane-1,2-diol hydrate. Dapagliflozin is indicated for the management of diabetes mellitus type 2, and functions to improve glycemic control in adults when combined with diet and exercise. Dapagliflozin is a sodium-glucose cotransporter 2 inhibitor, which prevents glucose re-absorption in the kidney. Using dapagliflozin leads to heavy glycosuria (glucose excretion in the urine), which can lead to weight loss and tiredness [2].



Dapagliflozin

Several analytical procedures have been proposed for the quantitative estimation of Metformin and Dapagliflozin separately and in combination with other drugs. High performance liquid chromatography methods for estimation of metformin [3] alone and in combination with glibenclamide and pioglitazone are also available. Dapagliflozin [4] is estimated by UV and HPLC have also been reported.

To our knowledge simple and economical analytical method for simultaneous determination of Metformin and Dapagliflozin has not been reported so far. So attempt was taken to develop and validate an economic, rapid reversed-phase high performance liquid chromatographic method for the quality control of Metformin and Dapagliflozin in pharmaceutical preparations with lower solvent consumption along with the short analytical run time that leads to an environmentally friendly chromatographic procedure and will allow the analysis of a large number of samples in a short period of time. The method was validated and found to be accurate, precise and reproducible.

Materials and Methods

Apparatus

Waters e2695Alliance HPLC system connected with PDA Detector 2998 and Empower2 Software. The drug analysis data were acquired and processed using Empower2 software running under Windows XP on a Pentium PC.

Other Apparatus:: Electronic balance, Sonicator, 0.45µ membrane filter

Reagents and Chemicals

Pharmaceutical grade Metformin and Dapagliflozin were kindly supplied as a gift sample by Astra zenaca Pharma, India. Acetonitrile was of HPLC grade and collected from E. Merck, Darmstadt, Germany. Orthophosphoric acid was of analytical reagent grade supplied by Fischer Scientific Chemicals. Water HPLC grade was obtained from a Milli-QRO water purification system.

Commercial Formulation

Metformin and Dapagliflozin Tablets available in the market as XIGDUO XR in composition of Metformin and Dapagliflozin. The samples were properly checked for their manufacturing license numbers, batch numbers, production, expiry dates and stored properly.

Procedure

Preparation of phosphate buffer

2.95 grams of potassium dihydrogen phosphate and 5.45 grams of potassium hydrogen phosphate was weighed and taken into a 1000ml beaker, dissolved and diluted to 1000ml with HPLC water and pH was adjusted to 3 with ortho phosphoric acid. The resulting solution was sonicated and filtered.

Preparation of mobile phase

Mix a mixture of above buffer 30 ml (30%) and 70 ml of ACN (HPLC grade-70%) and degassed in ultrasonic water bath for 5 minutes. Filter through 0.22 µ filter under vacuum filtration.

Diluents preparation

Mobile phase was used as the diluent.

Preparation of the individual metformin standard preparation

10 mg of metformin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 1 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the individual Dapagliflozin standard preparation

10 mg of Dapagliflozin working standard was accurately weighed and transferred into a 10 ml clean dry volumetric flask and add about 1 ml of diluent and sonicate to Dissolve it completely and make volume up to the mark with the same solvent (Stock solution).Further pipette out 1.0 ml from the above stock solution into a 10 ml volumetric flask and was diluted up to the mark with diluent.

Preparation of the Metformin and Dapagliflozin standard and sample solution

Sample solution preparation

10 mg of metformin and 10 mg Dapagliflozin tablet powder were accurately weighed and transferred into a 10 ml clean dry volumetric flask, add about 1 ml of diluent and sonicate to dissolve it completely and making volume up to the mark with the same solvent(Stock solution). Further pipette 10ml of the above stock solution into a 100ml volumetric flask and was diluted up to the mark with diluent.

Standard solution preparation

10 mg metformin and 10 mg Dapagliflozin working standard was accurately weighed and transferred into a 10ml clean dry volumetric flask and add about 1 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette out 1ml of the above stock solution into a 10ml volumetric flask and was diluted up to the mark with diluent.

Development and Validation of Hplc Method

Present study was conducted to obtain a new, affordable, cost-effective and convenient method for HPLC determination of metformin and Dapagliflozin in tablet dosage form. The experiment was carried out according to the official specifications of USP–30, ICH- 1996 and Global Quality Guidelines-2002. The method was validated for the parameters like system suitability , selectivity, linearity, accuracy, precision, LOD, LOQ, and robustness [5,6,7].

System Suitability

System suitability study of the method was carried out by six replicate analysis of solution containing 100% target concentration of metformin and Dapagliflozin. Various chromatographic parameters such as retention time, peak area tailing factor, theoretical plates (Tangent) of the column and resolution between the peaks were determined and the method was evaluated by analyzing these parameters.

Selectivity

Selectivity test determines the effect of excipients on the assay result. To determine the selectivity of the method, standard sample of metformin and of Dapagliflozin were injected first. Then commercial product, blank and excipients solution were run in the instrument one after another.

Linearity

Linearity of the method was determined by constructing calibration curves. The linearity study was performed for the concentration of 50 ppm to 250 ppm metformin and 5ppm to 25ppm Dapagliflozin. Each measurement was carried out in six replicates and the peak areas of the chromatograms were plotted against the concentrations to obtain the calibration curves and correlation coefficients.

Accuracy (Recovery Studies)

To check the degree of accuracy of the method, recovery studies were performed in triplicate by standard addition method at 50%, 100% and 150%. Known amounts of standard metformin and

Dapagliflozin were added to pre-analyzed samples and were subjected to the proposed HPLC method.

Precision

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The sample solution was prepared in the same manner as described in sample preparation. Percentage relative standard deviation (%RSD) was found to be less than 2% for within a day and day to day variations, which proves that method is precise.

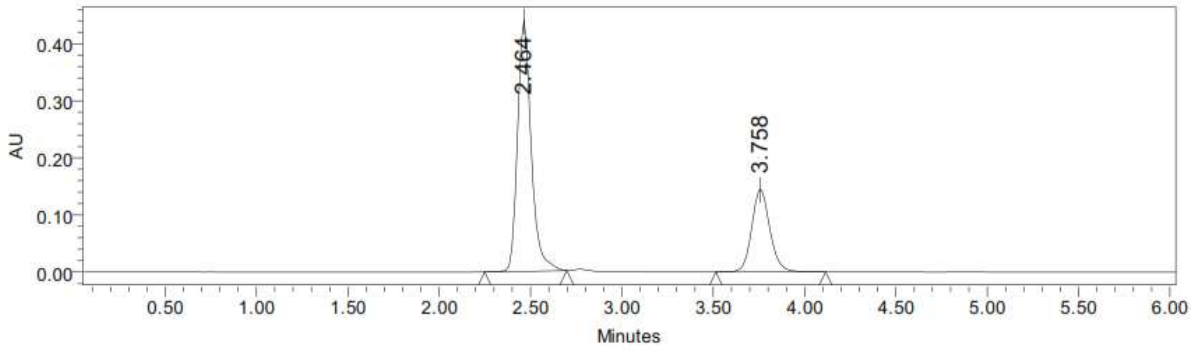
Robustness of Method

To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters were done. The effect of change in flow rate, temperature, on the retention time and tailing factor were studied. The method was found to be unaffected by flow and temperature variation.

Results and Discussion

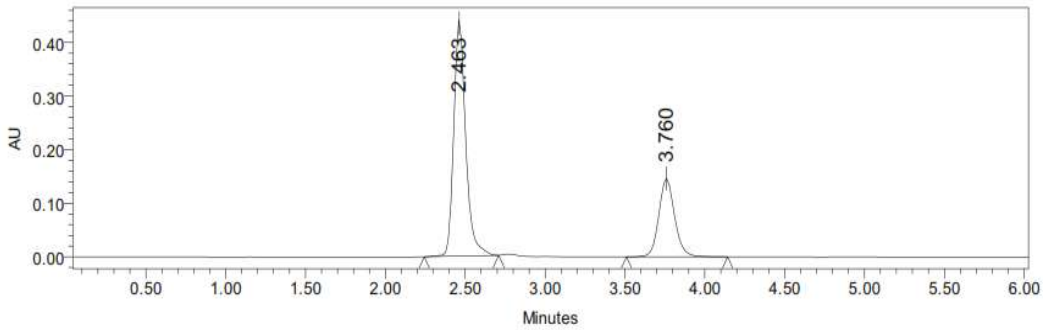
Results of system suitability study are summarized in Table 1. Six consecutive injections of the standard solution showed uniform retention time, theoretical plate count, tailing factor and resolution for both the drugs which indicate a good system for analysis.

Fig. 1: Typical chromatogram of metformin and dapagliflozin in standard preparation.



	Name	Retention Time	Area	Height	USP Tailing	USP Plate Count
1	Metformin	2.464	2288873	435418	1.2	3124.1
2	Dapagliflozin	3.758	1003783	144483	1.1	2366.2

Fig. 2: Typical chromatogram of metformin and dapagliflozin in marketed formulation.



	Name	Retention Time	Area	Height	USP Tailing	USP Plate Count
1	Metformin	2.463	2288873	435418	1.3	3137.6
2	Dapagliflozin	3.760	1006018	145772	1.1	2367.9

Table 1: Result of system suitability tests of Metformin and Dapagliflozin

Column	:	Inertsil RP C18 (4.5×150 mm) 5.0 µm
Column temperature	:	Ambient
Wavelength	:	240 nm
Mobile phase ratio	:	70:30 Acetonitrile : phosphate buffer
Flow rate	:	1 ml/min
Auto sampler temperature	:	Ambient
Injection volume	:	10µl
Run time	:	10.0 minutes

Chromatograms shown in figure 1 and figure 2 explain that retention time for standard sample and commercial product of Metformin and Dapagliflozin are same. This proves that, excipients have no effect on the analytical method. On the other hand, blank peak did not overlap drug peak. So the method is highly selective. A linear relationship between peak areas (average peak areas of six replicates) versus concentrations was observed for Metformin and Dapagliflozin in the range of 50 µg/ml to 250 µg/ml for Metformin and 5 µg/ml to 25 µg/ml for Dapagliflozin concentrations. Correlation coefficient was 0.999 for both the drugs which prove that the method is linear.

Precision was evaluated by carrying out six independent sample preparation of a single lot of formulation. The intermediate precision performed for %RSD of Metformin and Dapagliflozin was found to be 0.63 and 0.92 respectively. The method is highly precise as % RSD of peak area was less than 2% in all tests.

The accuracy study was performed for 50%, 100% and 150 % for Metformin and Dapagliflozin. Each level was injected in triplicate into chromatographic system. The area of each level was used for calculation of % recovery. Results are tabulated in Table 2 and Table 3.

Table 2: Showing accuracy results for Metformin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	3955152	5	4.96	99.91%	99.56%
100%	7893687	10	9.98	99.18%	
150%	12057868	15	15.02	99.60%	

Table 3: Showing accuracy results for Dapagliflozin

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	15532	0.5	0.99	99.53%	
100%	30089	1.0	1.05	99.38%	99.47%
150%	46635	1.5	1.495	99.52%	

Results of accuracy study are presented in table 2 and Table 3. The measured value was obtained by recovery test. Percentage concentration of both the drugs was compared against the recovery amount. % Recovery was 99.56% for Metformin and 99.47% for Dapaglifloxin. All the results indicate that the method is highly accurate.

The results of robustness of the present method showed that small changes were made in the flow rate and temperature did not produce significant changes in analytical results which are presented in Table 4 and Table 5 . As the changes are not significant we can say that the method is robust.

Table 4: Results for robustness test of Metformin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	2590	1.39
2	1	2294	1.27
3	1.2	2146	1.26

Table 5: Results for robustness test of Dapagliflozin

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5435	1.04
2	1	4891	1.03
3	1.2	4781	1.04

Conclusion

The new HPLC method developed and validated for simultaneous determination of Metformin and Dapagliflozin pharmaceutical dosage forms and assured the satisfactory precision and accuracy and also determining lower concentration of each drug in its solid combined dosage form by RP-HPLC method. The method was found to be simple, accurate, economical and rapid and they can be applied for routine analysis in laboratories and is suitable for the quality control of the raw materials, formulations, dissolution studies and can be employed for bioequivalence studies for the same formulation.

References

1. Drug bank : dapaglifloxin (DBO6292)
2. Drug bank : metformin (DB00331)
3. Chhetri H P, Thapa P,Schepdael A V, Simple Method of Extraction and Determination of metformin in Human Plasma by High – Performance Liquid Chromatography using UV detection,2002; 27(5): 841-4.
4. Manasa. Sanagapati *, K. Dhanalakshmi , G. Nagarjunareddy and S. Sreenivasa, Development And Validation Of A Rp-Hplc Method For The Estimation Of Dapagliflozin In Api, International Journal of Pharmaceutical Sciences And Research,27:270-2.
5. Beckett A H and Stanlake J B , practical pharmaceutical chemistry, 4th edition CBS publicaters and distributers, New Delhi, part two 281-307.
6. Nash R A and Wachter A H, Pharmaceutical process validation, An international 3rd edition,Revised and Expanded,129, Marcel Dekker Inc.,New York ,507-522.
7. Green J M, A practical guide to analytical method validation, anal chem. News Feat 305A-309A.

Journal of Pharmaceutical and Medicinal Chemistry
Volume 1 Number 2, July - December 2015

Original Article

Synthesis and Microbiology of Diesters Derived From 1, 1´-(4, 6-Dihydroxy-1, 3-Phenylene) Diethanone

Asif Husain

Abstract

A series of 4, 6-diacetyl-1, 3-di(substituted-phenyl carbonyloxy)benzenes or diesters (**2a-f**) were prepared and screened for in vitro antibacterial and antifungal activities. 1, 1´-(4,6-Dihydroxy-1, 3-phenylene)diethanone (**1**) was treated with different aromatic acids in dry pyridine in presence of phosphorous oxychloride to obtain the diesters (**2a-f**). The structures of the synthesized compounds were established on modern analytical techniques. The antimicrobial activity (minimum inhibitory concentration; *MIC*) of the title compounds was determined against some selected bacterial and fungal strains. The compounds showed appreciable antimicrobial activity against the tested microbes. Presence of halogen group(s) was found to increase the antimicrobial activity of the diesters.

Keywords: Resorcinol; Ester; MIC; Antimicrobial.

Introduction

There is a great interest in the discovery of new antimicrobial compounds in recent times due to the development of new strains of bacteria resistant to a variety of currently available antimicrobial treatments [1,2]. Antimicrobial resistance refers to microbes that have developed the ability to inactivate, bypass or block the inhibition or lethal mechanism of the antimicrobial agents [3]. The resorcinol derivatives are quite important compounds for both synthesis and pharmacological screening [4,5]. A variety of synthesized resorcinol derivatives have been experimentally shown to exert various important biological actions including antimicrobial activities[4-9].

In view of these points and in continuation of our work on resorcinol derivatives [6-9], it was considered worthwhile to synthesize some new diacetyl-resorcinol based diesters and to evaluate their antibacterial and antifungal activities.

Author Affilation: *Sr. Asst. Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi-110 062, India..

Reprint Request: Asif Husain, Sr. Asst. Professor, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi-110 062, India.
Email: drasifhusain@yahoo.com,
ahusain@jamiahamdard.ac.in

Materials and Methods

Synthesis

Melting points were recorded in liquid paraffin bath using open end capillaries and are uncorrected. PMR spectra were recorded on Bruker spectropsin DPX-300 MHz in $CDCl_3$; chemical shift (*ä*) values are reported in parts per million (*ppm*). The splitting pattern abbreviations are as follows: s, singlet; d, doublet; dd, double doublet; m, multiplet. Mass spectra were recorded on a JEOL JMS-D 300 instrument. Elemental analyses were performed on a Perkin-Elmer 240 analyzer. Thin-layer chromatography (TLC) was carried out to observe the progress of synthesis using silica gel G as

stationary phase and iodine chamber and UV chamber were used for locating the spots of compounds. The reaction sequence is presented in *scheme 1*.

Synthesis of 1, 1´-(4,6-Dihydroxy-1, 3-phenylene) diethanone (1)

It was prepared from resorcinol following literature method[8]. It gave a violet colour with ethanolic ferric chloride solution; positive test for phenols. Yield 72%; m.p. 184-186°C. ^{1}H NMR ($CDCl_3$, *ä*, ppm): 2.65 (s, 6H, 2´-$COCH_3$), 6.65 (s, 1H, H-2), 8.15 (s, 1H, H-5).

General procedure for synthesis of diesters (2a-f) [9]

To a solution of **1** (2 mmol; 0.388gm) in dry pyridine (10 mL) was added a solution of aromatic acid (4 mmol) in dry pyridine (5 mL). The contents were stirred for a few minutes and then phosphorous oxychloride (0.5 mL) was added drop-wise into it. Stirring was continued for another 2h and the reaction mixture poured into ice cold water containing a little quantity of HCl. A solid mass separated out which was filtered, washed with water and dried. It was crystallized from methanol: dichloromethane mixture to furnish TLC pure compounds **2a-f**. It did not give colour with ethanolic ferric chloride solution.

4, 6-Diacetyl-1, 3-di(2-hydroxyphenyl carbonyloxy) benzene (2a). Yield 58 %; m.p. 137–139 °C; R*f* 0.71; ^{1}H NMR ($CDCl_3$) δ ppm: 2.61 (s, 6H, 2x –$COCH_3$), 5.86 (s, 2H, 2x –OH), 7.18 (s, 1H, H-2), 7.32–7.81 (m, 8H, 2x H-3‘,4‘,5‘,6‘), 8.32 (s, 1H, H-5); MS: m/z 434 (M^+), 435 (M^++1); Anal calc. for $C_{24}H_{18}O_8$; C 66.36, H 4.18; Found C 66.17, H 4.15.

4,6-Diacetyl-1,3-di(2-acetoxyphenyl carbonyloxy) benzene (2b). Yield 60 %; m.p. 152–154 °C; R*f* 0.74; ^{1}H NMR ($CDCl_3$) δ ppm: 2.35 (s, 6H, 2x –$COCH_3$), 2.63 (s, 6H, 2x –$COCH_3$), 7.22 (s, 1H, H-2), 7.36–7.83 (m, 8H, 2x H-3‘,4‘,5‘,6‘), 8.41 (s, 1H, H-5); MS: *m/z* 518 (M^+), 519 (M^++1); Anal calcd. for $C_{28}H_{22}O_{10}$: C, 64.86; H, 4.28; Found: C, 64.72; H, 3.97.

4,6-Diacetyl-1,3-di(4-fluorophenyl carbonyloxy) benzene (2c). Yield: 63%; m.p. 129-131°C; R*f* 0.76; ^{1}H NMR ($CDCl_3$) δ ppm: 2.59 (s, 6H, 2´-$COCH_3$), 7.16 (s, 2H, H-2), 7.24-7.51 (m, 4H, 2x H-3‘,5‘), 7.78 (d, 4H, 2x H-2‘,6‘), 8.34 (s, 1H, H-5); MS: *m/z* 438 (M^+), 439 (M^++1); Anal calcd. for $C_{24}H_{16}F_2O_6$: C, 65.76; H, 3.68. Found: C, 65.62; H, 3.55.

4 ,6-Diacetyl-1,3-di(2-bromophenyl carbonyloxy) benzene (2d). Yield: 55%; m.p. 118-119°C; R*f* 0.67; ^{1}H NMR ($CDCl_3$) δ ppm: 2.63 (s, 6H, 2x –$COCH_3$), 7.22 (s, 1H, H-2), 7.46-7.68 (m, 6H, 2x H-3‘,4‘,5‘), 7.87-8.16 (m, 2H, 2x H-6‘), 8.41 (s, 1H, H-5); MS: *m/z* 559 (M^+), 560 (M^++1), 561 (M^++2); Anal calcd. for $C_{24}H_{16}Br_2O_6$: C, 51.46; H, 2.88. Found: C, 51.23; H, 2.75.

4, 6-Diacetyl-1,3-di(4-bromophenyl carbonyloxy) benzene (3e). Yield: 58%; m.p. 140-142°C; R*f* 0.70; ^{1}H NMR ($CDCl_3$) δ ppm: 2.61 (s, 6H, 2x –$COCH_3$), 7.19 (s, 1H, H-2), 7.28 (d, 4H, 2x H-3‘,5‘), 7.85 (d, 4H, 2x H-2‘,6‘), 8.28 (s, 1H, H-5); MS: *m/z* 559 (M^+), 560 (M^++1), 561 (M^++2); Anal calcd. for $C_{24}H_{16}Br_2O_6$: C, 51.46; H, 2.88. Found: C, 51.38; H, 2.76.

4, 6-Diacetyl-1,3-di(2,4-dichlorophenyl carbonyloxy) benzene (3f). Yield: 54%; m.p. 134-136°C; R*f* 0.72; ^{1}H NMR ($CDCl_3$) δ ppm: 2.64 (s, 6H, 2x –$COCH_3$), 7.26 (s, 1H, H-2), 7.34-8.08 (m, 6H, 2x H-3‘,5‘,6‘), 8.44 (s, 1H, H-5); MS: *m/z* 539 (M^+), 540 (M^++1), 541 (M^++2); Anal calcd. for $C_{24}H_{14}Cl_4O_6$: C, 53.36; H, 2.61. Found: C, 53.22 H, 2.54.

Microbiology

The synthesized compounds were evaluated for their in vitro antimicrobial activity [10,11] against three bacterial strains and two fungal strains at a concentration of 100 µg/mL by cup plate method. Compounds inhibiting growth of one or more of the test microorganisms were further tested for their minimum inhibitory concentration (*MIC*).

Antibacterial activity [10]

In vitro antibacterial activity of the title compounds was determined against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), and *Pseudomonas aeruginosa* (ATCC-27853) at a concentration of 100 mg/mL by cup plate method. Freshly prepared liquid agar medium (25 mL/ petridish) was poured into each petridishes and the plates were dried by placing in an incubator at 37°C for 1 h. Then standardized culture of microbes was spread on each petridishes by a spreader. Wells (6 mm) were made using an agar punch and each well was labeled accordingly. A control (solvent) was also included in the test. Ciprofloxacin was used as standard drug for comparison. The test compound and standard drug solutions (100 µg/mL) were made in dimethylsulfoxide (DMSO) and added in each well separately and petridishes kept aseptically for 1h for diffusion of the sample. After the completion of diffusion, all the petridishes were kept for incubation at 37°C for 24 h and then diameter of the zone of inhibition was measured in mm (Table 1).

Compounds inhibiting growth of one or more of the test microorganisms were further evaluated for their minimum inhibitory concentration (*MIC*) by turbidity method. A solution of the compounds (100 µg/mL) was prepared in DMSO and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The inoculum consisting of an overnight broth culture of microorganisms was added to separate tubes. The tubes were incubated at 37° for 24 h and examined for turbidity. The highest dilution (lowest concentration) required to arrest the growth of bacteria was regarded as *MIC*. Results are presented in *Table 2*.

Antifungal activity [11]

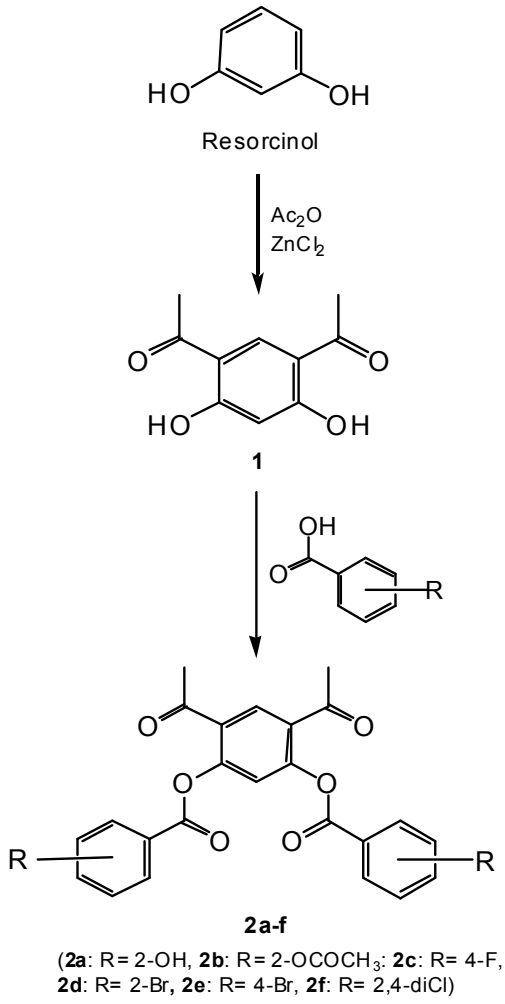
In vitro antifungal activity of the title compounds was determined against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404) by agar diffusion method. Sabourands agar media was prepared by dissolving peptone (1 g), D-glucose (4 g) and agar (2 g) in distilled water (100 mL) and adjusting pH to 5.7. Normal saline was used to make a suspension of spore of fungal strain for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. Agar media (20 mL) was poured into each petridish and the plates were dried by placing in an incubator at 37°C for 1 h. Wells were made using an agar punch and, each well was labeled accordingly. A control was also prepared in triplicate and maintained at 37°C for 3-4 days. The test compounds and standard drug (Griseofulvin) solutions (100 µg/mL) were made in dimethylsulfoxide (DMSO) and added in each well separately and petridishes kept aseptically for 1h for diffusion of the sample. After the completion of diffusion, all the petridishes were kept for incubation at 37°C for 3-4 days and then diameter of the zone of inhibition was measured in mm (Table 1).

Compounds inhibiting growth of one or more of the fungal strains were further tested for their minimum inhibitory concentration (*MIC*). A solution of the compounds (100 µg/mL) was prepared in DMSO and a series of doubling dilutions prepared with sterile pipettes. To each of a series of sterile test tubes a standard volume of nutrient broth medium was added. A control tube containing no antimicrobial agent was included. The tubes were inoculated with approximately $1.6x10^4$-$6x10^4$ c.f.u. mL^{-1} and incubated for 48 h at 37°C and examined for growth. The highest dilution (lowest concentration) required to arrest the growth of fungus was regarded as *MIC* (Table 2).

Results and Discussion

Synthesis

The title compounds were synthesizes through multistep synthesis as given in **Scheme-1**. Resorcinol, the starting material, was reacted with acetic anhydride in presence of anhydrous zinc chloride to obtain 1,1´-(4,6-dihydroxy-1,3-phenylene) diethanone (**1**) [8] Compound **1** was then reacted with 2 moles of different aromatic acids in presence of phosphorous oxychloride to furnish the desired diesters (**2a-f**). The diesters did not give colour with ethanolic ferric chloride solution indicating the absence of free phenolic (-OH) group. The structures of the synthesized compounds were established on the basis of ^{1}H NMR, Mass spectral data and elemental analysis data.



Scheme 1: Protocol for synthesis of title Compounds (2a-f)

In general, the ^{1}H NMR spectra of the title compounds (*2a-f*) revealed the presence of two acetyl groups as singlet at around δ 2.6. Resorcinol ring protons, H-2 and H-5, appeared as two singlet at around δ 7.2 and δ 8.4, respectively. Other signals were observed at appropriate δ values integrating for the protons of two substituted phenyl rings (H-2′/3′/4′/5′/6′). The mass spectra of diesters showed the presence of molecular ion peak in reasonable intensities. Elemental analyses values of the synthesized compounds were found within ±0.4% of theoretical values.

Microbiology

The title compounds (*2a-f*) were screened for their in vitro antibacterial activity against *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) bacterial species, and antifungal activity against *Candida albicans* (ATCC-10231) and *Aspergillus niger* (ATCC-16404). The antimicrobial screening data showed that the compound 2f, 4,6-diacetyl-1,3-di(2,4-dichlorophenyl carbonyloxy)benzene, exhibited good activity against *S. aureus*, *E. coli* and *C. albicans* with *MIC*-12.5 µg/mL. Similar type of activity was also shown by the compound; 4,6-diacetyl-1,3-di(4-fluorophenyl carbonyloxy)benzene (**2c**), against *S. aureus* and *E. coli* with *MIC*-12.5 µg/mL. Another compound, 4,6-diacetyl-1,3-di(4-bromophenyl carbonyloxy)benzene (**2e**), displayed significant activity against *S. aureus* with *MIC*-12.5 µg/mL. The standard drugs showed *MIC* values of 6.25 µg/mL (Table 1 & 2).

An analysis of results indicated that the title compounds 2a-f were significant in their antibacterial and antifungal actions. Presence of halogen group(s) was found to increase the antimicrobial activity of the diesters as evident by compound **2c, 2e** and 2f.

Table 1: Preliminary antibacterial and antifungal activities of the title compounds (2a-f).

Compd.	Substituent (R)	Antibacterial activity[#]			Antifungal activity[#]	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	2-OH	-	-	-	+	-
2b	2-$OCOCH_3$	+	-	+	++	-
2c	4-F	+++	+++	+	++	+
2d	2-Br	++	++	+	+	-
2e	4-Br	+++	++	++	++	+
2f	2,4-diCl	+++	+++	+	+++	++
Standard-1[†]		++++	++++	++++	nt	nt
Standard-2[†]		nt	nt	Nt	++++	++++

[#]Zone of inhibition: - = < 5 mm (insignificant or no activity), + = 5-9 mm (weak activity), ++ = 10-14 mm (moderate activity), +++ = 15-20 mm (good activity), ++++ = > 20 mm (excellent activity).
[†]Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin, nt = not tested.

Table 2: Antibacterial and antifungal activities (*MIC*, µg/mL) of the title compounds (2a-f).

Compd.	Substituent (R)	Antibacterial activity			Antifungal activity	
		S. aureus	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
2a	2-OH	>100	>100	>100	50	>100
2b	2-$OCOCH_3$	50	>100	50	25	>100
2c	4-F	12.5	12.5	50	25	50
2d	2-Br	25	25	50	50	>100
2e	4-Br	12.5	25	25	25	50
2f	2,4-diCl	12.5	12.5	50	12.5	25
Standard-1[†]		6.25	6.25	6.25	Nt	Nt
Standard-2[†]		Nt	nt	nt	6.25	6.25

nt = not tested; [†]Standard-1 = Ciprofloxacin, Standard-2 = Griseofulvin.

Conclusion

Six new diesters (**2a-f**) derived from 1,1´-(4,6-dihydroxy-1,3-phenylene)diethanone (**1**) were successfully synthesized. The antimicrobial studies showed that the synthesized compounds were having appreciable antibacterial and antifungal activities. Presence of halogen group(s) was found to increase the antimicrobial activity of the diesters. It is conceivable that further derivatization of the active compounds may result in potential antimicrobial agents.

Acknowledgement

The author is thankful to the Department of

Biochemistry, Faculty of Science, Jamia Hamdard, for antimicrobial activity determination.

References

1. Davies J., Bacteria on the rampage. Nature 1996; 383: 219-220.
2. Chu DTW, Plattner JJ, Katz L., New directions in antibacterial research. J. Med. Chem. 1996; 39: 3853-3874.
3. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am. J. Med. 2006; 34: 64-73.
4. Soliman K, Ohad N, Ramadan M, Maayan S, Snait T, Jacob V., Chalcones as potent tyrosinase inhibitors: the importance of a 2,4-substituted resorcinol moiety. Bioorg. Med. Chem. 2005; 13: 433-441.
5. Mohammed Sardaryar Khan, Asif Husain and Sanjay Sharma, New 4,6-Diacetyl resorcinol Mannich bases: Synthesis and biological evaluation, *Acta Poloniae Pharmaceutica (Drug Research)*, 2010; 67(3): 261-266.
6. Husain A, Javed S, Mishra R, Rashid M, Bhutani R., Synthesis and microbiological evaluation of some new Bischalcones. Pharmacophore 2011; 2(6): 316-325.
7. Asif Husain, Mohd Rashid, Ravinesh Mishra and Deepak Kumar, Bis-chalcones and Flavones: Synthesis and antimicrobial activity, *Acta Poloniae Pharmaceutica (Drug Research)*, 2013; 70(3) 443-449.
8. Khan MSY, Sharma S, Husain A., Synthesis and antibacterial evaluation of new flavonoid derivatives from 4,6-diacetyl resorcinol. Scientia Pharmaceutica 2002; 70: 287-294.
9. Husain A, Ahmad A, Ibraheem AIM, Mishra R, Rashid M., Synthesis and antimicrobial activity of bischalcones derivatives. Med. Chem. Res. 2013; 22: 1578-1586.
10. Colle JG, Duguid JP, Fraser AG, Marmion BP., Laboratory strategies in diagnosis", In: Mackie TJ, MacCartney JE, eds. Practical Medical Microbiology, 13th ed, London, Churchill-Livingstone, 1989.
11. Varma RS., Antifungal Agents: Past, Present and Future Prospects. National Academy of Chemistry & Biology, Lucknow, India, 1998.

Revised Rates for 2016 (Institutional)

Title	Frequency	Rate (Rs): India	Rate ($):ROW
Dermatology International	2	5000	500
Gastroenterology International	2	5500	550
Indian Journal of Agriculture Business	2	5000	500
Indian Journal of Anatomy	3	8000	800
Indian Journal of Ancient Medicine and Yoga	4	7500	750
Indian Journal of Anesthesia and Analgesia	2	7000	700
Indian Journal of Anthropology	2	12000	1200
Indian Journal of Biology	2	4000	400
Indian Journal of Cancer Education and Research	2	8500	850
Indian Journal of Communicable Diseases	2	8000	800
Indian Journal of Dental Education	4	4500	450
Indian Journal of Forensic Medicine and Pathology	4	15500	1550
Indian Journal of Forensic Odontology	2	4500	450
Indian Journal of Genetics and Molecular Research	2	6500	650
Indian Journal of Law and Human Behavior	2	5500	550
Indian Journal of Library and Information Science	3	9000	900
Indian Journal of Maternal-Fetal & Neonatal Medicine	2	9000	900
Indian Journal of Medical & Health Sciences	2	6500	650
Indian Journal of Obstetrics and Gynecology	2	7000	700
Indian Journal of Pathology: Research and Practice	2	11500	1150
Indian Journal of Plant and Soil	2	5500	550
Indian Journal of Preventive Medicine	2	6500	650
International Journal of Food, Nutrition & Dietetics	2	5000	500
International Journal of History	2	6500	650
International Journal of Neurology and Neurosurgery	2	10000	1000
International Journal of Political Science	2	5500	550
International Journal of Practical Nursing	3	5000	500
International Physiology	2	7000	700
Journal of Animal Feed Science and Technology	2	4100	410
Journal of Cardiovascular Medicine and Surgery	2	9100	910
Journal of Forensic Chemistry and Toxicology	2	9000	900
Journal of Microbiology and Related Research	2	8000	800
Journal of Orthopaedic Education	2	5000	500
Journal of Pharmaceutical and Medicinal Chemistry	2	16000	1600
Journal of Practical Biochemistry and Biophysics	2	5500	550
Journal of Social Welfare and Management	4	7500	750
New Indian Journal of Surgery	2	7100	710
Ophthalmology and Allied Sciences	2	5500	550
Otolaryngology International	2	5000	500
Pediatric Education and Research	4	7000	700
Physiotherapy and Occupational Therapy Journal	4	8500	850
Urology, Nephrology and Andrology International	2	7000	700
SUPER SPECIALITY JOURNALS			
Indian Journal of Emergency Medicine	2	12000	1200
Indian Journal of Surgical Nursing	3	5000	500
Indian Journal of Trauma & Emergency Pediatrics	2	9000	900
International Journal of Pediatric Nursing	2	5000	500
Journal of Community and Public Health Nurisng	2	5000	500
Journal of Geriatric Nursing	2	5000	500
Journal of Medical Images and Case Reports	2	5000	500
Journal of Nurse Midwifery and Maternal Health	2	5000	500
Journal of Organ Transplantation	2	25900	2590
Journal of Psychiatric Nursing	3	5000	500
Psychiatry and Mental Health	2	7500	750
OPEN ACCESS JOURNALS			
Global Research in Engineering		5000	500
Global Research in Food and Nutrition		5000	500
Global Research in Library and Information Science		5000	500
Global Research in Medical Sciences		5000	500
Global Research in Space Science		5000	500

Terms of Supply:

1. Advance payment required by Demand Draft payable to Red Flower Publicaion Pvt. Ltd. payable at Delhi.
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 10% discount.
4. Claim must be made within six months from issue date.

Order from

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India), Tel: 91-11-22754205, 45796900, Fax: 91-11-22754205. E-mail: redflowerppl@vsnl.net, redflowerppl@gmail.com, Website: www.rfppl.co.in

Manuscripts must be prepared in accordance with "Uniform requirements for Manuscripts submitted to Biomedical Journal" developed by international committee of medical Journal Editors.

Types of Manuscripts and Limits

Original articles: Up to 3000 words excluding references and abstract and up to 10 references.

Review articles: Up to 2500 words excluding references and abstract and up to 10 references.

Case reports: Up to 1000 words excluding references and abstract and up to 10 references.

Online Submission of the Manuscripts

Articles can also be submitted online from http://www.rfppl.com (currently send your articles through e-mail attachments).

I) First Page File: Prepare the title page, covering letter, acknowledgement, etc. using a word processor program. All information which can reveal your identity should be here. use text/rtf/doc/PDF files. Do not zip the files.

2) Article file: The main text of the article, beginning from Abstract till References (including tables) should be in this file. Do not include any information (such as acknowledgement, your name in page headers, etc.) in this file. Use text/rtf/doc/PDF files. Do not zip the files. Limit the file size to 400 Kb. Do not incorporate images in the file. If file size is large, graphs can be submitted as images separately without incorporating them in the article file to reduce the size of the file.

3) Images: Submit good quality color images. Each image should be less than 100 Kb in size. Size of the image can be reduced by decreasing the actual height and width of the images (keep up to 400 pixels or 3 inches). All image formats (jpeg, tiff, gif, bmp, png, eps etc.) are acceptable; jpeg is most suitable.

Legends: Legends for the figures/images should be included at the end of the article file.

If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks from submission. Hard copies of the images (3 sets), for articles submitted online, should be sent to the journal office at the time of submission of a revised manuscript. Editorial office: **Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi – 110 091, India, Phone: 91-11-22754205, 45796900, Fax: 91-11-22754205, E-mail: redflowerppl@vsnl.net. Website: www.rfppl.co.in**

Preparation of the Manuscript

The text of observational and experimental articles should be divided into sections with the headings: Introduction, Methods, Results, Discussion, References, Tables, Figures, Figure legends, and Acknowledgment. Do not make subheadings in these sections.

Title Page

The title page should carry

1) Type of manuscript (e.g. Original article, Review article, Case Report)
2) The title of the article, should be concise and informative;
3) Running title or short title not more than 50 characters;
4) The name by which each contributor is known (Last name, First name and initials of middle name), with his or her highest academic degree(s) and institutional affiliation;
5) The name of the department(s) and institution(s) to which the work should be attributed;
6) The name, address, phone numbers, facsimile numbers and e-mail address of the contributor responsible for correspondence about the manuscript; should be mentoined.
7) The total number of pages, total number of photographs and word counts separately for abstract and for the text (excluding the references and abstract);
8) Source(s) of support in the form of grants, equipment, drugs, or all of these;
9) Acknowledgement, if any; and
l0) If the manuscript was presented as part at a meeting, the organization, place, and exact date on which it was read.

Abstract Page

The second page should carry the full title of the manuscript and an abstract (of no more than 150 words for case reports, brief reports and 250 words for original articles). The abstract should be structured and state the Context (Background), Aims, Settings and Design, Methods and Materials, Statistical analysis used, Results and Conclusions. Below the abstract should provide 3 to 10 keywords.

Introduction

State the background of the study and purpose of the study and summarize the rationale for the study or observation.

Methods

The methods section should include only information that was available at the time the plan or protocol for the study was written such as study approach, design, type of sample, sample size, sampling technique, setting of the study, description of data collection tools and methods; all information obtained during the conduct of the study belongs in the Results section.

Reports of randomized clinical trials should be based on the CONSORT Statement (http://www.consort-statement. org). When reporting experiments on human subjects, indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975, as revised in 2000 (available at http://www.wma.net/e/policy/l 7-c_e.html).

Results

Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Do not repeat in the text all the data in the tables or illustrations; emphasize or summarize only important observations. Extra or supplementary materials and technical details can be placed in an appendix where it will be accessible but will not interrupt the flow of the text; alternatively, it can be published only in the electronic version of the journal.

Discussion

Include summary of key findings (primary outcome measures, secondary outcome measures, results as they relate to a prior hypothesis); Strengths and limitations of the study (study question, study design, data collection, analysis and interpretation); Interpretation and implications in the context of the totality of evidence (is there a systematic review to refer to, if not, could one be reasonably done here and now?, What this study adds to the available evidence, effects on patient care and health policy, possible mechanisms)? Controversies raised by this study; and Future research directions (for this particular research collaboration, underlying mechanisms, clinical research). Do not repeat in detail data or other material given in the Introduction or the Results section.

References

List references in alphabetical order. Each listed reference should be cited in text (not in alphabetic order), and each text citation should be listed in the References section. Identify references in text, tables, and legends by Arabic numerals in square bracket (e.g. [10]). Please refer to ICMJE Guidelines (http://www.nlm.nih.gov/bsd/uniform_requirements.html) for more examples.

Standard journal article

[1] Flink H, Tegelberg Å, Thörn M, Lagerlöf F. Effect of oral iron supplementation on unstimulated salivary flow rate: A randomized, double-blind, placebo-controlled trial. J Oral Pathol Med 2006; 35: 540-7.

[2] Twetman S, Axelsson S, Dahlgren H, Holm AK, Källestål C, Lagerlöf F, et al. Caries-preventive effect of fluoride toothpaste: A systematic review. Acta Odontol Scand 2003; 61: 347-55.

Article in supplement or special issue

[3] Fleischer W, Reimer K. Povidone iodine antisepsis. State of the art. Dermatology 1997; 195 Suppl 2: 3-9.

Corporate (collective) author

[4] American Academy of Periodontology. Sonic and ultrasonic scalers in periodontics. J Periodontol 2000; 71: 1792-801.

Unpublished article

[5] Garoushi S, Lassila LV, Tezvergil A, Vallittu PK. Static and fatigue compression test for particulate filler composite resin with fiber-reinforced composite substructure. Dent Mater 2006.

Personal author(s)

[6] Hosmer D, Lemeshow S. Applied logistic regression, 2nd edn. New York: Wiley-Interscience; 2000.

Chapter in book

[7] Nauntofte B, Tenovuo J, Lagerlöf F. Secretion and composition of saliva. In: Fejerskov O, Kidd EAM,

editors. Dental caries: The disease and its clinical management. Oxford: Blackwell Munksgaard; 2003. p. 7-27.

No author given

[8] World Health Organization. Oral health surveys - basic methods, 4th edn. Geneva: World Health Organization; 1997.

Reference from electronic media

[9] National Statistics Online—Trends in suicide by method in England and Wales, 1979-2001. www.statistics.gov.uk/downloads/theme_health/HSQ 20.pdf (accessed Jan 24, 2005): 7-18. Only verified references against the original documents should be cited. Authors are responsible for the accuracy and completeness of their references and for correct text citation. The number of reference should be kept limited to 20 in case of major communications and 10 for short communications.

More information about other reference types is available at www.nlm.nih.gov/bsd/uniform_requirements.html, but observes some minor deviations (no full stop after journal title, no issue or date after volume, etc).

Tables

Tables should be self-explanatory and should not duplicate textual material.

Tables with more than 10 columns and 25 rows are not acceptable.

Table numbers should be in Arabic numerals, consecutively in the order of their first citation in the text and supply a brief title for each.

Explain in footnotes all non-standard abbreviations that are used in each table.

For footnotes use the following symbols, in this sequence: *, ¶, †, ‡‡,

Illustrations (Figures)

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files of minimum 1200x1600 pixel size. The minimum line weight for line art is 0.5 point for optimal printing.

When possible, please place symbol legends below the figure instead of to the side.

Original color figures can be printed in color at the editor's and publisher's discretion provided the author agrees to pay.

Type or print out legends (maximum 40 words, excluding the credit line) for illustrations using double spacing, with Arabic numerals corresponding to the illustrations.

Sending a revised manuscript

While submitting a revised manuscript, contributors are requested to include, along with single copy of the final revised manuscript, a photocopy of the revised manuscript with the changes underlined in red and copy of the comments with the point to point clarification to each comment. The manuscript number should be written on each of these documents. If the manuscript is submitted online, the contributors' form and copyright transfer form has to be submitted in original with the signatures of all the contributors within two weeks of submission. Hard copies of images should be sent to the office of the journal. There is no need to send printed manuscript for articles submitted online.

Reprints

Journal provides no free printed reprints, however a author copy is sent to the main author and additional copies are available on payment (ask to the journal office).

Copyrights

The whole of the literary matter in the journal is copyright and cannot be reproduced without the written permission.

Declaration

A declaration should be submitted stating that the manuscript represents valid work and that neither this manuscript nor one with substantially similar content under the present authorship has been published or is being considered for publication elsewhere and the authorship of this article will not be contested by any one whose name (s) is/are not listed here, and that the order of authorship as placed in the manuscript is final and accepted by the co-authors. Declarations should be signed by all the authors in the order in which they are mentioned in the original manuscript. Matters appearing in the Journal are covered by copyright but no objection will be made to their reproduction provided permission is obtained from the Editor prior to publication and due acknowledgment of the source is made.

Abbreviations

Standard abbreviations should be used and be spelt out when first used in the text. Abbreviations should not be used in the title or abstract.

Checklist

- Manuscript Title
- Covering letter: Signed by all contributors
- Previous publication/ presentations mentioned, Source of funding mentioned
- Conflicts of interest disclosed

Authors

- Middle name initials provided.
- Author for correspondence, with e-mail address provided.
- Number of contributors restricted as per the instructions.
- Identity not revealed in paper except title page (e.g.name of the institute in Methods, citing previous study as 'our study')

Presentation and Format

- Double spacing
- Margins 2.5 cm from all four sides
- Title page contains all the desired information. Running title provided (not more than 50 characters)
- Abstract page contains the full title of the manuscript
- Abstract provided: Structured abstract provided for an original article.
- Key words provided (three or more)
- Introduction of 75-100 words
- Headings in title case (not ALL CAPITALS). References cited in square brackets
- References according to the journal's instructions

Language and grammar

- Uniformly American English
- Abbreviations spelt out in full for the first time. Numerals from 1 to l0 spelt out
- Numerals at the beginning of the sentence spelt out

Tables and figures

- No repetition of data in tables and graphs and in text.
- Actual numbers from which graphs drawn, provided.
- Figures necessary and of good quality (color)
- Table and figure numbers in Arabic letters (not Roman).
- Labels pasted on back of the photographs (no names written)
- Figure legends provided (not more than 40 words)
- Patients' privacy maintained, (if not permission taken)
- Credit note for borrowed figures/tables provided
- Manuscript provided on a CDROM (with double spacing)

Submitting the Manuscript

- Is the journal editor's contact information current?
- Is the cover letter included with the manuscript? Does the letter:

1. Include the author's postal address, e-mail address, telephone number, and fax number for future correspondence?
2. State that the manuscript is original, not previously published, and not under concurrent consideration elsewhere?
3. Inform the journal editor of the existence of any similar published manuscripts written by the author?
4. Mention any supplemental material you are submitting for the online version of your article.

Contributors' Form (to be modified as applicable and one signed copy attached with the manuscript)

Revised Rates for 2015 (Institutional)

Title	Frequency	Rate (Rs): India	Rate ($):ROW
Dermatology International	2	4500	280
Gastroenterology International	2	5000	360
Indian Journal of Agriculture Business	2	4500	300
Indian Journal of Anatomy	2	6000	260
Indian Journal of Ancient Medicine and Yoga	4	7000	330
Indian Journal of Anesthesia and Analgesia	2	5000	600
Indian Journal of Anthropology	2	10500	500
Indian Journal of Applied Physics	2	3500	400
Indian Journal of Biology	2	3000	170
Indian Journal of Cancer Education and Research	2	6500	500
Indian Journal of Communicable Diseases	2	7500	58
Indian Journal of Dental Education	4	4000	288
Indian Journal of Forensic Medicine and Pathology	4	14000	576
Indian Journal of Forensic Odontology	4	4000	288
Indian Journal of Genetics and Molecular Research	2	6000	262
Indian Journal of Law and Human Behavior	2	5000	500
Indian Journal of Library and Information Science	3	8000	600
Indian Journal of Maternal-Fetal & Neonatal Medicine	2	8000	400
Indian Journal of Mathematics and Statistics	2	5000	200
Indian Journal of Medical & Health Sciences	2	6000	120
Indian Journal of Obstetrics and Gynecology	2	5000	200
Indian Journal of Pathology: Research and Practice	2	10000	915
Indian Journal of Plant and Soil	2	5000	1700
Indian Journal of Preventive Medicine	2	6000	250
Indian Journal of Reproductive Science and Medicine	4	3000	180
Indian Journal of Scientific Computing and Engineering	2	4000	280
Indian Journal of Surgical Nursing	3	3000	70
Indian Journal of Trauma & Emergency Pediatrics	4	8500	302
International Journal of Agricultural & Forest Meteorology	2	8000	800
International Journal of Food, Nutrition & Dietetics	2	4000	900
International Journal of History	2	6000	500
International Journal of Neurology and Neurosurgery	2	9000	276
International Journal of Political Science	2	5000	400
International Journal of Practical Nursing	3	3000	70
International Physiology	2	6500	240
Journal of Animal Feed Science and Technology	2	4000	280
Journal of Cardiovascular Medicine and Surgery	2	9000	238
Journal of Orthopaedic Education	2	4500	190
Journal of Pharmaceutical and Medicinal Chemistry	2	15000	350
Journal of Psychiatric Nursing	3	3000	70
Journal of Social Welfare and Management	4	7000	276
Meat Science International	2	5000	500
Microbiology and Related Research	2	6000	150
New Indian Journal of Surgery	4	7000	360
Ophthalmology and Allied Sciences	2	5000	150
Otolaryngology International	2	4500	300
Pediatric Education and Research	4	6500	150
Physiotherapy and Occupational Therapy Journal	4	8000	360
Urology, Nephrology and Andrology International	2	6500	350

Terms of Supply:

1. Advance payment required by Demand Draft payable to Red Flower Publicaion Pvt. Ltd. payable at Delhi.
2. Cancellation not allowed except for duplicate payment.
3. Agents allowed 10% discount.
4. Claim must be made within six months from issue date.

Order from

Red Flower Publication Pvt. Ltd., 48/41-42, DSIDC, Pocket-II, Mayur Vihar Phase-I, Delhi - 110 091 (India), Tel: 91-11-22754205, 45796900, Fax: 91-11-22754205. E-mail: redflowerppl@vsnl.net, redflowerppl@gmail.com, Website: www.rfppl.co.in

Subject Index

Author Index

Name	Page No
A. Lakshmana Rao	53
Aftab Ahmad	95
Amol Chandekar	29
Asif Husain	95
Asif Husain	105
Asif Husain	85
Atul tripathi	29
Bhaskar Banerjee	29
Chetan H. Borkhataria	47
D. N. Prasad	13
Dhaval V. Patel	47
Dhruv Dev	13
Farah Iram	105
Farah Iram	85
G.Vidyasagar	99
J.Padmapreetha	35
Jyoti Parkash	13
K. Kamalanathan	35
K. Selvaraju	35
K. Srikanth Kumar	53
K.S.G. Arulkumaran	35
K.V. Sowmya	71
Kalariya Manisha V.	5
Kalpesh A. Patel	47
Karun Arora	105
Karun Arora	85
Kiran Shukla	29
M. Ambiga	35
M.V. Basaveswara Rao	53
Md Azhar Iqbal	105
Md Azhar Iqbal	85
Mohammad Shahnaz	13
Nadir Islam	95
Neeraj Upmanyu	29
Nilesh K. Patel	47
Pooja Joshi	13
Prajapati Rakesh P.	5
Rahul Raj S	71
Ravi A. Manek	47
Sadaf Ali	105
Sai Mahesh Reddy Avula	91
Sajid Ali	85
Sandeep V. Nathwani	47
Shah Alam Khan	95
Shanmuganathan S	71
Sheth Navin R.	5
Surya Prakash Gupta	29
Syeda kulsum	99
Tasneem Mohammed	99
Venkata Subba Reddy Avula	91
Virendra Patel	29
X Fatima Grace	71

Introducing a new sister concerned company of ***Red Flower Publication Pvt. Ltd.***

RF Library Services Pvt. Ltd.

RF Library Services Pvt. Ltd. is a global market leader in managing professional information. We develop and deliver innovative services that enable the use of knowledge to its full extent. As the only information Service Company globally we play a key role in today's complex information marketplace. Founded in 1985 as a registered company under sub-section (2) of section 7 of the Companies Act, 2013 and rule 8 of the Companies (Incorporation) Rules, 2014, the business draws on more than a decade of experience within the information industry. With this knowledge, we satisfy the needs of thousands of customers from over 30 countries. We are a division of Red Flower Publication Pvt. Ltd.

Where we are based?

RF Library Services Pvt. Ltd is located in Delhi-91 in India.

RF Library Services Pvt. Ltd.
D-223/216, Laxmi Chambers, Laxmi Nagar,
Near Laxmi Nagar Metro Station,
Delhi-110092(India)
Tel: 011-22756995, Fax: 011-22756995
E-mail: rflibraryservices@vsnl.net, rflibraryservices@gmail.com
Wesite: www.rf-libraryservices.com