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Research Article

ORGANOCATALZED SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF LAPACHOL ANALOGUES

SHAISTA SABIR*, SIDRA NAZ, NAGHMANA RASHID, BILAL MASOOD

Research Complex, AllamaIqbal Open University, Department of Chemistry, Islamabad, Pakistan. Email: shaistasardar123@yahoo.co.in

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ABSTRACT

Organocatalyzedstereoselectivesynthesisof lapachol analogues from the Michael addition of naphthaquinone to different α,β-unsaturated ketones is presented. Different secondary and primary amines were tried to synthesise these analogues. A primary amine**(**(2R)-2-amino-3-phenylpropanoic acid)organocatlyst proved to be an excellent catalyst for asymmetric synthesis of lapacol analogues.Good to high yields and enantioselectivitie were obtained. The synthesized compounds were further screened for antimicrobial activities.The antimicrobial activities were evaluated by Filter paper Disc diffusion Method. The synthesized compounds were screened against different bacteria and fungi. The compound 3b **(**2-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]naphthalene-1,4-dione dihydrate**)** showed maximum activity against *PseudomonasAeruginosa*and minimum activity against*Eschirichia coli.* The rest of the compounds showed moderate antibacterial activities. The same compound also showed maximum antifungal activity against *Candidiaalbicans*. Compound 3f **(**2-hydroxy-3-(4-oxopentan-2-yl)naphthalene-1,4-dione dihydrate**)**has minimum antifungal activity against Aspergilusflavus. The rest of the compounds were moderately active against the two fungal strains.

Keywords: Michael addition, Asymmetric synthesis, Organocatalysis, Antimicrobial agents.

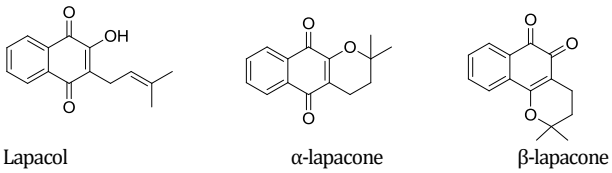
INTRODUCTION

Organocatalysis has been the main focus of chemical research in asymmetric synthesis[1-2]. In the past decade, significant progress has been achieved in asymmetric reactions catalyzed by chiral organic molecules[3]. A large number of organocatalysts have been developed so far, among these chiral bifunctional catalysts combining hydrogen-bond donors and amines are extremely efficient for many asymmetric transformations [4-5]. Chiral primary and secondary amines are extremely powerful reagents and dominated the field of aminocatalysis [6-7].

In organic synthesis the Michael addition of an α,β unsaturated systems is an important carbon- carbon bond forming reaction and the development of enantioselective pathway for this reaction could be an efficient route for the synthesis of biologically active drugs [8-10].

Among the quinone class there are two important isomeric natural products, lapachol and β-lapachone which have attracted substantial interest from scientific community. β-Lapachone is a natural ortho-pyran-naphthaquinone obtained as a minor component of heartwood from the Lapachol trees and is readily obtained in high yield from lapachones by cyclization in concentrated sulphuric acid [11]. Lapachones and its derivatives are of tremendous importance and they often possess biological activities.Lapachones have antibacterial, antifungal, antitrypansomal, antimalarial and antitumor properties and are used in traditional medicines for the treatment of pyrexia, jaundice, and edema[12]. They also have potential clinical utility in the treatment of human leukemia and prostate cancer [13-14].Lapachol and and β-Lapachone derivatives are very active against epimastigote and trypomastigote forms [15]. Consequently, the development of an efficient synthesis to obtain such valuable compounds has attracted great interest, and recently enantioselective reactions of naphthoquinone to electron withdrawing olefins have been reported [16-17].

In this article we will introduce a new asymmetric procedure via a convenient and economical catalyst for the synthesis of lapachol analogues starting from α,β-unsaturated ketones and naphthoquinone.



Lapacol α-lapacone β-lapacone

Fig.1: Structures of lapacoles and lapacones

EXPERIMENTAL

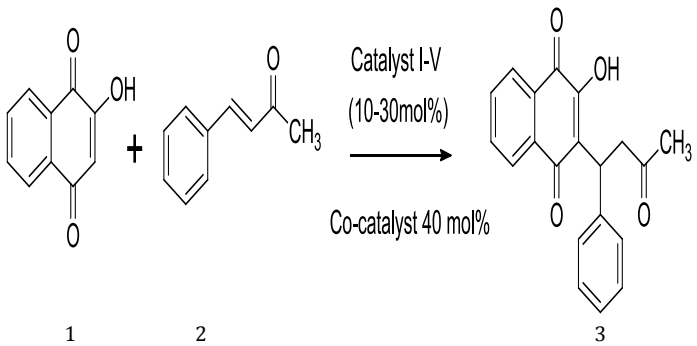
The ^{1}H-NMR and ^{13}C-NMR spectra were recorded using $CDCl_3$on a Bruker(300 MHz)and (Avance 300 MHz) and their chemical shifts are recorded inδ(parts per million) units with respect to tetramethylsilane (TMS) as internal standard.Progress of the reaction was monitored by using pre coated TLC plates(aluminum sheets, layer thickness 0.2mm, HF-254, Riedel-de-Haen) using n-hexane: ethylactate(7:3) as the solvent systems. Chromatograms were detected by UV light (254 and 360 nm) and by the development in the vanillin spray.

Melting points were determined in Gallenkamp (UK) electrothermal melting point apparatus. The HPLC experiments were performed on Perkin Elmer series 200 using a chiral Phenonmix Lux cellolose-1 column. Different combinations of i-propanol and n-hexane were used as eluents.

General procedure

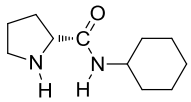
Synthesis of compound 3 and optimization of reaction conditions

The compound **3** was synthesized at different reaction conditions. To start, we took 1mmol (0.145g) of benzalacetone (**1**) and reacted it with 1mmol (0.174g) of naphthoquinone**2** as model reaction in the presence of amine catalysts (I-V) as described in Scheme 1, and their results are summarized in table 1.

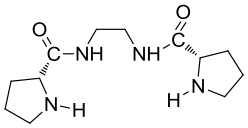


1 2 3

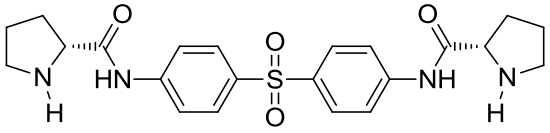
Fig. 2: Model reaction of benzalacetone and naphthaquinone



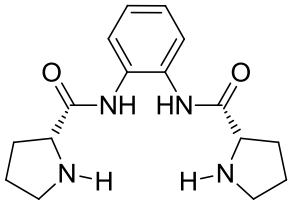
I((2R)-N-{2-[(cyclopentylcarbonyl) [(cyclopentylcarbonyl)amino]ethyl}



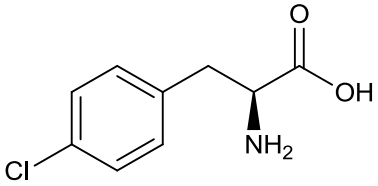
II ((2R)-N-{2 -pyrrolidine-2-carboxamide)- amino]ethyl)



III((2R)-N-{2-[(cyclopentylcarbonyl)amino]sulphonylbiphenyl}pyrrolidine-2-carboxamide)



IV(2*R*)-*N*-{2-[(cyclopentylcarbonyl) amino]phenyl pyrrolidine-2-carboxamide)



V ((2*S*)-2-amino-3-phenylpropanoic acid)

Fig. 3: Organocatalysts screened for lapacol analogues

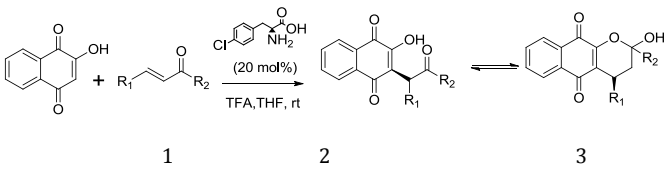
Table 1: Optimization of Reaction Conditions

Entry	Catalyst	Cocatalyst	Solvent	Time [h]	Yield[%]
1	**I**	PhCOOH	DCM	72	N.R
2	**II**	PhCOOH	DCM	72	N.R
3	**III**	PhCOOH	DCM	72	N.R
4	**IV**	PhCOOH	DCM	72	N.R
5	**V**	PhCOOH	DCM	72	62
6	**V**	PhCOOH	*i*-PrOH	48	46
7	**V**	PhCOOH	MeOH	60	43
8	**V**	PhCOOH	THF	48	62
9	**V**	TFA	THF	48	72
10	**V**	4-$NO_2C_6H_4COOH$	THF	48	60
11	**V**	salicylic acid	THF	72	64
12	**V**	TFA	$CHCl_3$	72	58
13	**V**	TFA	MeOH	72	51
14	**V**	TFA	DMF	72	37
15	**V** (10 mol %)	TFA	THF	80	68
16	**V** (20 mol %)	TFA	THF	48	72
17	**V** (30 mol %)	TFA	THF	59	71

A number of different solvents including polar and non-polar were also screened for this reaction. Different combination of solvents, co catalysts and catalysts were also used to enhance the yield of the product. The reactants were allowed to react on stirring at room temperature with these different combinations. The progress of the reaction was monitored by TLC and the chromatograms were developed in vanillin spray. The product developed light pink spot in vanillin spray.When there was not further significant increase in concentration of product, the reaction was stopped. The final product was purified by column chromatography. The columns were packed in silica gel in n-hexane or pet ether. Elution was made with increasing concentration of n-hexane: ethyle acetate.

Synthesis of compounds 3a-3f

After the optimization of reaction conditions the variety of α,β unsaturated ketones were reacted with naphthaquinone to form various lapacole analogues. By using 20 mol% catalyst V, 40 mol% TFA naphtahquinone (1mmol) and different α,β unsaturated ketones (1mmol) were reacted at room temperature in dry THF for the corresponding time. After the formation of the products, the purification was done by column chromatography. Column was packed in n-hexane and eluted with increasing polarities of n-hexane/ethyl acetate mixture. The enantiomeric excess of these compounds was also calculated by using chiral Phenonmix Lux cellolose-1 column.



1 2 3

Fig. 4: Synthesis of lapachol analogues

Antimicrobial Activities

All the synthesised products (3a-3f) were screened for their antifungal and antibacterial activities by disc diffusion method [18]. All the human pathogens including fungi and bacteria were procured from Pakistan Institute of Medical Sciences. The media used for fungal and bacterial growth were purchased from Sigma Aldrich suppliers.

The antifungal assay was done against two fungal strains, *Aspergillusflavus*and*Candida albicans.*Sabouraud dextrose agar (SDA) was used to grow fungus for inoculums preparations.Flucanazole was used as a standard for reference.

Media was prepared by dissolving Sabouraud dextrose agar 6.5gm /100ml in distilled water. Contents were dissolved and were autoclaved at 121°C for 20 minutes. After sterilization media is poured on sterile plates under LFC and allowed to solidify. After solidification the plates were pre-incubated at 37°C for 24 hours to confirm sterility .The plates showing no growth were then used for antifungal activity studies. The disk diffusion method was used for testifying the antibacterial activity as well. The antibacterial assay

was done against *Escherichia coli, Acetobacteraceti, Staphylococcus aureus, Klebsilla pneumonia and Pseudomonas aeruginosa.* These bacteria were maintained on nutrient agar medium at 4°C.. For antibacterial activity the levofloxacine was used as a standard reference. The concentration of the drugs and the standards was maintained at 1mg/g. The zone of inhibitions was measured in mm (mille meters).

RESULTS AND DISCUSSIONS

Chemical Part

All the catalysts tested performed well in the model reaction (Table 1, Enteries 1-5). The attempts to react α,β unsaturated ketones with naphthoquinone catalysed by different L-proline amides (I-IV) provided disappointing results (Table, Enteries 1-4). However primary chiral amine catalyst (V) (20 mol%) in combination with TFA (40 mol%) exhibited good catalytic activity (72% yield, Entry 9). In order to get good yield and enantioselectivity,varieties of parameters are studied. As is known that solvents and acid additives have a notable effect on organocatalytic reactions; therefore, we examined the reaction media and cocatalysts. Reactions in polar solvents, such as MeOH and *i*-PrOH provided low yield and low ee values (Table 1, Enteries7,8). Variation of cocatalysts was then investigated and for this purpose different acid i.e. 4-$NO_2C_6H_4COOH$,PhCOOH, salicylic acid and TFAwere tried. Results shows that with 4-$NO_2C_6H_4COOH$, PhCOOH, salicylic acid provides low yield. Finally TFA was selected as co-catalyst because it effectively catalyses the reaction with good yield (Table 1, Entery 9). Furthermore mol% of the catalyst werealso screened to increase the yield. From 10 mol% to 30 mol% of the catalyst were tried for the synthesis. It was noted that the yield was decreased when 10 mol% of the catalyst was used, also it took longer period of time for completion. With the increase of mol% to 20% the yield was increased in a shorter period of time. When the mol% were further increased to 30 mol%, there was not a significant increase in the yield.

On the basis of above results we further synthesized six derivatives of naphtaquinones in good to excellent yields and enantioselectivities (Scheme 2).

Table 2: Physical data of the synthesized compounds

Entry	Code	R_1	R_2	Molecular formula	Molecular weight	Time [h]	Yield [%]
1	3a	CH_3	Ph	$C_{20}H_{16}O_4$	320	72	72
2	3b	CH_3	4-NO_2Ph	$C_{21}H_{18}O_5$	350	72	61
3	3c	Ph	4-MeO Ph	$C_{20}H_{15}ClO_4$	412	72	63
4	3d	Ph	4-FPh	$C_{20}H_{15}FO_4$	400	72	58
5	3e	CH_3	$N(CH_3)_2$	$C_{20}H_{15}BrO_4$	287	72	71
6	3f	CH_3	CH_3	$C_{20}H_{15}NO_6$	258	48	70

The results summerized in table 2 and table 3 showed that all the reactants provide good to excellent enantiomeric excess and yields. The maximum enantiomeric excess was obtained when 4 fluro-benzaledenacetone was used as a reactant with naphthaquinone. A minimum enantiomeric excess was obtained when nitro substituent was used on phenyl moiety. All the above results proved that the catalyst V **(**(2*S*)-2-amino-3-phenylpropanoic acid)is active in bringing about good enantioselectivities with excellent yield.

Table 3:Enantiomeric excess of the synthesized compounds

Sample Code	MobilePhase hexane : *i*-PrOH	Flow Rate ml/min	Retention time		e.e %
			Major	Minor	
3a	85:15	1	5.2	15.7	75
3b	97:3	1	4.8	10.3	
3c	90:10	1	7.4	15.2	71
3d	85:15	1	4.9	13.4	75
3e	90:10	1	5.2	19.7	63
3f	90:10	1	3.2	18.6	69

Based on the previous reports of primary amine catalysis [19], a catalytic mechanism for the reaction is proposed. Firstly, under the catalysis of protonic acid, the catalytic cycle is initited by nucleophlic attack of the primary amine to the carbonyl group of substrate **1**(α-β-unsaturated ketone). The resultant intermediate A then undergoes dehydration to form iminiumcation B. Reactant **2** (2-hydroxy 1,4Naphthaquinone) attacks from the Re face of the α-β-unsaturated ketone that allows the Michael addition of **1** and **2** to take place. Intermediate **D** provides product through hydrolysis and regenerates catalyst.

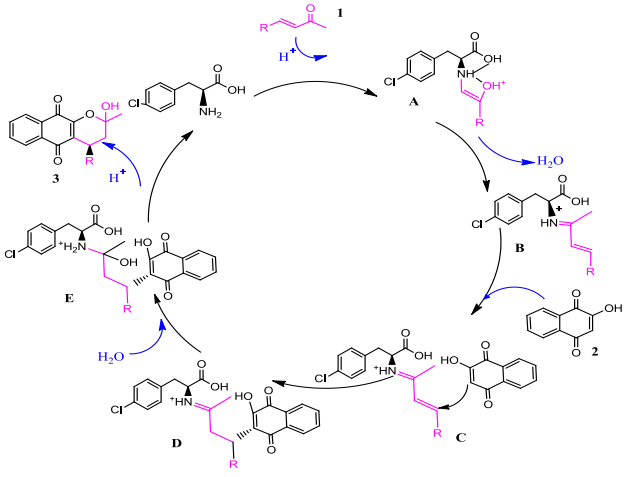


Fig. 5: Proposed catalytic mechanism for primary amine catalysis

Spectral Data 3a.2-hydroxy-3-(3-oxo-1-phenylbutyl)naphthalene-1,4-dione dehydrate

^{1}HNMR(300 MHz, CDCl3, δ ppm)**:** 2.13 (s, 3H), 3.72 (dd, J_1= 17.7 Hz,J_2= 9.6 Hz2H), 4.20 (t, J = 6Hz, 1H), 7.23-7.49 (m, 2H), 7.51-7.63 (m, 2H), 7.65-7.73 (m, 3H), 8.05 (dd, J_1= 18 Hz,J_2= 6 Hz J = Hz, 2H)
^{13}CNMR**:**199.1, 196,141.1**,**135.2**,** 133.0, 128.1, 126, 125.8, 79.7, 53, 47.8, 38.8, 30.1.
EI-MS:Molecular ion peaks at 330.

3b: (2-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]naphthalene-1,4-dione dihydrate)

^{1}HNMR(300 MHz, CDCl3, δ ppm):2.72 (s, 3H), 3.4 (m, 2H), 4.92 (dd, J_1= 11.7 Hz,J_2= 9.3 Hz,2H), 7.32-7.39 (m, 2H), 7.42-7.52 (m, 2H), 7.53-7.74 (m, 2H), 7.76-8.32 (m, 2H)

^{13}CNMR: 206.5, 199, 196, 148.0, 145.2, 135.0, 133.2, 129.0, 124.0, 79.7, 53.1, 47.8, 38.8, 30.0

EI-MS:Molecular ion peaks at 346.1.

3c:(2-hydroxy-3-[1-(4-methoxyphenyl)-3-oxobutyl]naphthalene-1,4-dione dihydrate)

^{1}HNMR(300 MHz, CDCl3, δ ppm)**:** 2.76 (s, 3H), 4.3-4.96 (m, 2H), 5.34 (dd, J_1= 8.4 Hz,J_2= 3.3 Hz,1H), 7.33-7.40 (m, 5H), 7.53-7.58 (m, 2H), 7.74 (d, J= 8.7 Hz, 2H), 8.11-8.16 (m, 2H), 8.23 (d, J= 5.4 Hz, 2H)

^{13}CNMR: 206.5, 199, 196, 157.9, 135.2, 133.0, 129.1, 125.8, 114.4, 79.7, 55.8, 53.1, 47.8, 38.8, 30.1

EI-MS:Molecular ion peaks at 360.

3d:(2-hydroxy-3-[1-(4-flurophenyl)-3-oxobutyl]naphthalene-1,4-dione dihydrate)

^{1}HNMR(300 MHz, CDCl3, δ ppm):4.28 (m, 2H), 5.13 (dd, J_1= 9.6 Hz,J_2= 3.9 Hz,1H), 692-7.14 (m, 3H), 7.40-7.48 (m, 2H), 7.49-7.73 (m, 4H), 7.90-7.96 (m, 2H), 8.07 (dd, J_1= 7.5 Hz,J_2= 3.9 Hz,2H)

^{13}CNMR: 206.5, 199, 196, 160.2, 137.5, 135.2, 133.0, 131.3, 125.8, 115.6, 79.7, 55.8, 53.1, 47.8, 38.8, 30.1

EI-MS:Molecular ion peaks at 348.0.

3e:(2-hydroxy-3-[1-(4-N,Ndimethylphenyl)-3-oxobutyl]naphthalene-1,4-dione dihydrate)

^{1}HNMR(300 MHz, CDCl3, δ ppm)**:** 0.85 (s, 3H), 1.90 (s, 6H), 2.38 (m, 2H), 6.66 (d,J = 14.1 Hz, 1H), 7.42-7.81 (m, 4H).

^{13}CNMR: 206.5, 199, 196, 148.4, 135.2, 133.0, 131.4, 125.8, 113.0, 79.7, 55.8, 53.1, 47.8, 41.3, 38.8, 30.1

EI-MS:Molecular ion peaks at 375.0.

3f(2-hydroxy-3-(4-oxopentan-2-yl)naphthalene-1,4-dione dihydrate)

^{1}HNMR(300 MHz, CDCl3, δ ppm):1.23 (s, 3H), 1.53 (s, 3H), 2.10 (m, 2H), 4.20 (dd, J_1= 6 Hz,J_2= 3.9 Hz,1H), 7.52-8.0 (m, 4H).

^{13}CNMR: 206.5, 199, 196, 125.8, 113.0, 79.8, 55.8, 53.1, 47.6, 30.1, 23.0, 19.2

EI-MS:Molecular ion peaks at 258.0.

Antimicrobial Activities

All the synthesized compounds of the series (**3a-3f**) show excellent antibacterial activity against almost all the test microbes.The compound **3a**,**3b**and **3c**were extremely active against *Acetobacteracceti,* even the antibacterial activity of **3b**is comparable with standard drug.While compounds **3d**, **3e** and **3f**showed activity against*Staphylococcsaureus.*

Table 4:Antibacterial activity of the synthesized compounds

Micro organisms	Sample description						
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**Levofloxacin**
Escherichia Coli	9.1	6.9	11.4	12.3	9.7	9.3	14.5
Acetobecor Acceti	14.4	17. 4	14.5	13. 4	11.8	13.1	18.4
Staphylococcsaureus	13.9	11.9	11.8	15.7	12.4	13.2	17.4
Klebsiella pneumonia	8.7	9.4	9.1	9.8	7.2	8.1	14.4
Pseudomonas Aeruginosa	10.2	9.1	6.7	11.4	10.8	11.1	15.7

Conc: 1mg/ml, Zone of inhibition (mm)

The synthesized compounds (**3a-3f**) were also tested for antifungal activity. *Aspergillusflavus* and *Candidiaalbucans* were the test organism and antifungal fluconazole was used as standard. It is observed that the synthesized compounds also showed good antifungal activity against the test organisms.

Table 5: Antifungal activity of the synthesized compounds

Micro organisms	Sample description						
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**Fluconazole**
Aspergillusflavus	6.4	7.3	7.7	6.8	7.6	6.2	10.5
CandidiaAlbucans	7.8	10	8.2	9.1	7.4	6.9	18.3

CONCLUSION

In summary we have developed a simple, inexpensive, efficient and friendly benign organocatalysed synthesis of lapacol analogues in excellent yield.The catalyst V is introduced for the first time for enantioselective synthesis. The synthesised compounds were evaluated for their antimicrobial activities.Some of the compounds showed very good antibacterial activities.

ACKNOWDEGMENT

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