Research Article

ORGANOCATALYZED SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF LAPACHOL ANALOGUES

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

Research Complex, Allama Iqbal Open University, Department of Chemistry, Islamabad, Pakistan. Email: shaitasardar123@yahoo.co.in

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ABSTRACT

Organocatalyzed stereoselective synthesis of 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) organocatalyst proved to be an excellent catalyst for asymmetric synthesis of lapachol analogues. Good to high yields and enantioselectivities 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 Pseudomonas aeruginosa and minimum activity against Escherichia coli. The rest of the compounds showed moderate antibacterial activities. The same compound also showed maximum antifungal activity against Candida albicans. Compound 3f (2-hydroxy-3-[4-oxopentan-2-yl]naphthalene-1,4-dione dihydrate) has minimum antifungal activity against Aspergillusflavus. 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 system 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, antitrypanosomal, 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 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 naphthaquinone 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 naphthaquinone.

EXPERIMENTAL

The 1H-NMR and 13C-NMR spectra were recorded using CDCl3 on a Bruker (300 MHz) and (Avance 300 MHz) and their chemical shifts are recorded in parts per million units with respect to tetramethylsilane (TMS) as internal standards. Progress of the reaction was monitored by using pre-coated TLC plates (aluminum sheets, layer thickness 0.2 mm, HF254, Riedel-de-Haen) using n-hexane: ethylacetate (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 Phenomix Lux cellulose-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 1 mmol (0.145 g) of benzalacetone (1) and reacted it with 1 mmol (0.174 g) of naphthaquinone 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.

![Fig. 1: Structures of lapacoles and lapacones](image1)

![Fig. 2: Model reaction of benzalacetone and naphthaquinone](image2)
When there was not further significant increase in the disk diffusion method was used for 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 naphthaquinone (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 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 mixture. The enantiomeric excess of these compounds was also calculated by using chiral Phenomix Lux cellulose-1 column.

**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 naphthaquinone (1mmol) and different α,β unsaturated ketones (1mmol) were reacted at room temperature in dry THF. The progress of the reaction was monitored by TLC and the chromatograms were developed in vanillin spray. The product developed light pink spot in reaction mixture. The reactants were allowed to react on stirring at room temperature with these different combinations. 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 mixture. The enantiomeric excess of these compounds was also calculated by using chiral Phenomix Lux cellulose-1 column.

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. 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 mixture. The enantiomeric excess of these compounds was also calculated by using chiral Phenomix Lux cellulose-1 column.

**Fig. 4: Synthesis of lapacoval analogues**

### Table 1: Optimization of Reaction Conditions

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Cocatalyst</th>
<th>Solvent</th>
<th>Time [h]</th>
<th>Yield [%]</th>
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<tbody>
<tr>
<td>1</td>
<td>I</td>
<td>PhCOOH</td>
<td>DCM</td>
<td>72</td>
<td>N.R</td>
</tr>
<tr>
<td>2</td>
<td>II</td>
<td>PhCOOH</td>
<td>DCM</td>
<td>72</td>
<td>N.R</td>
</tr>
<tr>
<td>3</td>
<td>III</td>
<td>PhCOOH</td>
<td>DCM</td>
<td>72</td>
<td>N.R</td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>PhCOOH</td>
<td>DCM</td>
<td>72</td>
<td>N.R</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>PhCOOH</td>
<td>DCM</td>
<td>72</td>
<td>62</td>
</tr>
<tr>
<td>6</td>
<td>V</td>
<td>PhCOOH</td>
<td>i-PrOH</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>7</td>
<td>V</td>
<td>PhCOOH</td>
<td>MeOH</td>
<td>48</td>
<td>43</td>
</tr>
<tr>
<td>8</td>
<td>V</td>
<td>PhCOOH</td>
<td>THF</td>
<td>48</td>
<td>62</td>
</tr>
<tr>
<td>9</td>
<td>V</td>
<td>TFA</td>
<td>THF</td>
<td>48</td>
<td>72</td>
</tr>
</tbody>
</table>

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. 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 mixture. The enantiomeric excess of these compounds was also calculated by using chiral Phenomix Lux cellulose-1 column.

**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, *Aspergillus flavus* and *Candida albicans*. Sabouraud dextrose agar (SDA) was used to grow fungus for inoculums preparations. Fluconazole 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 was 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 testing 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 levofloxicin was used as a standard reference. The concentration of the drugs and the standards was maintained at 1mg/ml. 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, Entries 1-5). The attempts to react α,β unsaturated ketones with naphthaquinone catalysed by different L-proline amides (I-HV) provided disappointing results (Table, Entries 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 enantioselectivities 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, Entries7,8). Variation of cocatalysts was then investigated and for this purpose different acid i.e. 4-NO₂C₆H₄COOH, PhCOOH, salicylic acid and TFA were tried. Results shows that with 4-NO₂C₆H₄COOH, PhCOOH, salicylic acid provides low yield. Finally TFA was selected as co-catalyst because it effectively catalyses the reaction with good yield (Table 1, Entry 9). Furthermore mol% of the catalyst were also 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 naphthaquinones in good to excellent yields and enantioselectivities (Scheme 2).

Table 2: Physical data of the synthesized compounds

<table>
<thead>
<tr>
<th>Entry</th>
<th>Code</th>
<th>R₁</th>
<th>R₂</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Time [h]</th>
<th>Yield [%]</th>
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<tbody>
<tr>
<td>1</td>
<td>3a</td>
<td>CH₃</td>
<td>Ph</td>
<td>C₂₀H₁₈O₄</td>
<td>320</td>
<td>72</td>
<td>72</td>
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<tr>
<td>2</td>
<td>3b</td>
<td>CH₃</td>
<td>4-NO₂Ph</td>
<td>C₂₁H₁₆O₅</td>
<td>350</td>
<td>72</td>
<td>61</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>Ph</td>
<td>4-MeO Ph</td>
<td>C₂₁H₁₆ClO₄</td>
<td>412</td>
<td>72</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>Ph</td>
<td>4-FPh</td>
<td>C₂₁H₁₆FO₄</td>
<td>400</td>
<td>72</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>3e</td>
<td>CH₃</td>
<td>(CH₂)₂</td>
<td>C₂₁H₁₆BrO₄</td>
<td>287</td>
<td>72</td>
<td>71</td>
</tr>
<tr>
<td>6</td>
<td>3f</td>
<td>CH₃</td>
<td>CH₃</td>
<td>C₂₀H₁₈NO₄</td>
<td>258</td>
<td>48</td>
<td>70</td>
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</tbody>
</table>

The results summarized 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 fluoro-benzaldehydeketone 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 ([2S]-2-amino-3-phenylpropanoic acid) is active in bringing about good enantioselectivities with excellent yield.

Table 3: Enantiomeric excess of the synthesized compounds

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Mobile Phase hexane : i-PrOH</th>
<th>Flow Rate ml/min</th>
<th>Retention time Major [Min]</th>
<th>Retention time Minor [Min]</th>
<th>ee %</th>
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<tbody>
<tr>
<td>3a</td>
<td>85:15</td>
<td>1</td>
<td>5.2</td>
<td>15.7</td>
<td>75</td>
</tr>
<tr>
<td>3b</td>
<td>97:3</td>
<td>1</td>
<td>4.8</td>
<td>10.3</td>
<td>75</td>
</tr>
<tr>
<td>3c</td>
<td>90:10</td>
<td>1</td>
<td>7.4</td>
<td>15.2</td>
<td>71</td>
</tr>
<tr>
<td>3d</td>
<td>85:15</td>
<td>1</td>
<td>4.9</td>
<td>13.4</td>
<td>75</td>
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<tr>
<td>3e</td>
<td>90:10</td>
<td>1</td>
<td>5.2</td>
<td>19.7</td>
<td>63</td>
</tr>
<tr>
<td>3f</td>
<td>90:10</td>
<td>1</td>
<td>5.2</td>
<td>18.6</td>
<td>69</td>
</tr>
</tbody>
</table>

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 initiated by nucleophilic attack of the primary amine to the carbonyl group of substrate 1αβ-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 αβ-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

¹HNMR(300 MHz, CDCl₃, δ ppm): 2.13 (s, 3H), 3.72 (dd, J= 17.7 Hz J= 9.6 Hz 2H), 4.20 (t, J = 6 Hz, 1H), 7.23-7.49 (m, 2H), 7.51-7.63 (m, 2H), 7.65-7.73 (m, 3H), 8.05 (dd, J= 18 Hz J= 6 Hz J = Hz, 2H)

¹CNR: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 dihydride)

\[ \text{HNMR}(300 \text{ MHz, CDCl}_3, \delta \text{ ppm}): 2.72 (s, 3H), 3.4 (m, 2H), 4.92 (dd, \ J = 11.7, 7.4), 7.30-7.39 (m, 2H), 7.42-7.52 (m, 2H), 7.53-7.74 (m, 2H), 7.76-8.32 (m, 2H).
\]

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

\[ \text{HNMR}(300 \text{ MHz, CDCl}_3, \delta \text{ ppm}): 2.76 (s, 3H), 4.3-4.96 (m, 2H), 5.34 (dd, \ J = 8.4, 5.3), 7.33-7.40 (m, 5H), 7.53-7.58 (m, 2H), 7.74 (d, \ J = 8.7 Hz, 2H), 8.11-8.16 (m, 2H), 8.23 (d, \ J = 5.4 Hz, 2H).
\]

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

\[ \text{HNMR}(300 \text{ MHz, CDCl}_3, \delta \text{ ppm}): 2.48 (s, 2H), 5.13 (dd, \ J = 9.6 Hz, 7.4), 6.92-7.14 (m, 3H), 7.40-7.48 (m, 2H), 7.49-7.73 (m, 4H), 7.90-7.96 (m, 2H), 8.07 (dd, \ J = 7.5, 3.9 Hz).
\]

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

\[ \text{HNMR}(300 \text{ MHz, CDCl}_3, \delta \text{ ppm}): 0.85 (s, 3H), 1.90 (s, 6H), 2.38 (m, 2H), 6.66 (d, \ J = 14.1 Hz, 1H), 7.42-7.81 (m, 4H).
\]

3f: (2-hydroxy-3-[1-(4-oxopentan-2-yl)]naphthalene-1,4-dione dihydride)

\[ \text{HNMR}(300 \text{ MHz, CDCl}_3, \delta \text{ ppm}): 1.23 (s, 3H), 1.53 (s, 3H), 2.10 (m, 2H), 4.20 (dd, \ J = 6 Hz, 3.9 Hz, 1H), 7.52-8.0 (m, 4H).
\]

MS: Molecular ion peaks at 375.0.

MS: Molecular ion peaks at 356.0.

MS: Molecular ion peaks at 362.0.

Table 4: Antibacterial activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Sample description</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>Levofloxacin</th>
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<tr>
<td>Escherichia</td>
<td></td>
<td>9.1</td>
<td>6.9</td>
<td>11.4</td>
<td>12.3</td>
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<td>9.3</td>
<td>14.5</td>
</tr>
<tr>
<td>Coli</td>
<td></td>
<td>14.4</td>
<td>17.4</td>
<td>14.5</td>
<td>13.4</td>
<td>11.8</td>
<td>13.1</td>
<td>18.4</td>
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<tr>
<td>Acetobacter</td>
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<td>13.9</td>
<td>11.9</td>
<td>11.8</td>
<td>15.7</td>
<td>12.4</td>
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<td>17.4</td>
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<td>Accett</td>
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<td>9.4</td>
<td>9.1</td>
<td>9.8</td>
<td>7.2</td>
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<td>Staphylococcus aureus</td>
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<td>6.7</td>
<td>11.4</td>
<td>10.8</td>
<td>11.1</td>
<td>15.7</td>
</tr>
<tr>
<td>Klebsiella pneumonia</td>
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<td>7.8</td>
<td>10</td>
<td>8.2</td>
<td>9.1</td>
<td>7.4</td>
<td>6.9</td>
<td>18.3</td>
</tr>
</tbody>
</table>

Table 5: Antifungal activity of the synthesized compounds

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Sample description</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
<th>3e</th>
<th>3f</th>
<th>Fluconazole</th>
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<td>10.5</td>
</tr>
<tr>
<td>Candida albicans</td>
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<td>10</td>
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<td>9.1</td>
<td>7.4</td>
<td>6.9</td>
<td>18.3</td>
</tr>
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