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ORIGINAL ARTICLE

Biocatalysts for cascade reaction: porcine pancreas lipase (PPL)-catalyzed synthesis of bis(indolyl)alkanes

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Abstract A cascade reaction between aldehydes and indole catalyzed by lipase from *porcine pancreas* Type II (PPL) in solvent mixture at 50 °C was reported for the first time. Some control experiments had been designed to demonstrate that the PPL was responsible for the cascade reaction. After the optimization of the stepwise process, a series of bis(indolyl)alkanes were prepared in moderate to excellent yields under the catalysis of PPL.

Keywords PPL · Promiscuity · Bis(indolyl)alkanes · Cascade reaction

Introduction

Biocatalysis has received great attention as an efficient and green tool for the production of pharmaceutical, industrial and agricultural chemicals, and intermediates due to mild reaction conditions, wide sources, broad range of substrates and potential use of inexpensive regenerable resources (Feng et al. 2009; Pollard and Woodley 2007; Aleu et al. 2006; Kazlauskas 2005; Schmid et al. 2001). In the recent years, many researchers had successfully utilized these natural catalysts for the construction of C–C (Svedendahl et al. 2005; Xu et al. 2007; Cai et al. 2011; Wu et al. 2010), C–N (Torre et al. 2004; Wu et al. 2005, 2006), C–O (Kitazume et al. 1986), and C–S (Carlqvist et al. 2005; Lou et al. 2008) bonds and insertion of chirality for producing higher value molecules under mild reaction conditions. Indoles and their derivatives possess various biological properties like antibacterial, cytotoxic, antioxidative, and insecticidal activities (Lounasmaa and Tolvanen 2000; Hibino and Choshi 2001; Fukuyama and Chen 1994; Shiri et al. 2009). Especially, bis(indolyl)alkanes and their derivatives have attracted much interest because they can act as highly selective fluorescent molecular sensors for Cu^{2+} cations and also as colon tumor and cancer cell growth inhibitors (Martínez et al. 2008; Guinchard et al. 2007; Safe et al. 2008; Lei et al. 2008).

In the past years, more and more researchers were attracted to study the synthesis of bis(indolyl)methanes and they found that these compounds could be generally synthesized via the cascade reaction between an aldehyde (or acetone) with two equivalents of indole in the presence of a Lewis or protic acid catalyst, such as trichloro-1,3,5-triazine (Sharma et al. 2004), bentonite (Guillermo et al. 2003), $ZrCl_4$ (Zhang et al. 2005), Montmorillonite clay K-10 (Chakrabarty et al. 2004), I_2 (Bandgar and Shaikh 2003), $CeCl_3·7H_2O$ (Silveira et al. 2009), $AlPW_{12}O_{40}$ (Firouzabadi et al. 2006), zeolites (Karthik et al. 2004), $Dy(OTf)_3$/ionic liquid (XI et al. 2004), $FeCl_3$/ionic liquid (Ji et al. 2004), $Sc(OTf)_3$ (Ma et al. 2005), $HClO_4$-SiO_2 (Kamble et al. 2007a), $InCl_3$-NMTA (Pradhan et al. 2005), MW/Lewis acids ($BiCl_3$, $FeCl_3$, $InCl_3$,$CoCl_2$, $ZnCl_2$) (Xia et al. 2004), silica sulfuric acid (SSA) (Zolfigol et al. 2008), NBS (Koshima and Matsusaka 2002), TiO_2 (Mona 2007), $LiClO_4$ (Mehrazma et al. 2006), $NaBF_4$ (Kamble et al. 2007b), Ph_3CCl (Khalafi-Nezhad et al. 2008), metal hydrogen sulfates (Niknam et al. 2006), $H_3PW_{12}O_{40}$ (Azizi et al. 2007), ceric ammonium nitrate (CAN) (Mohit and Pulak 2006), $La(NO_3)_3·6H_2O$ (Jon-Paul-Selvam et al. 2008), and $Fe(DS)_3$ (Wang and Ji 2008). Most of the above attempts were efficient for the synthesis of bis(indolyl)methanes with

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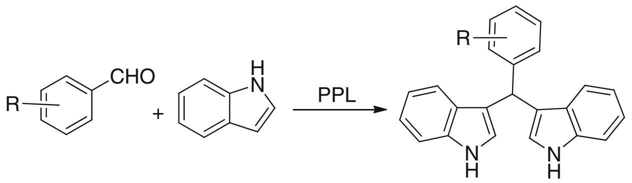


Scheme 1 Lipase catalyzed the reaction between indole and aldehydes

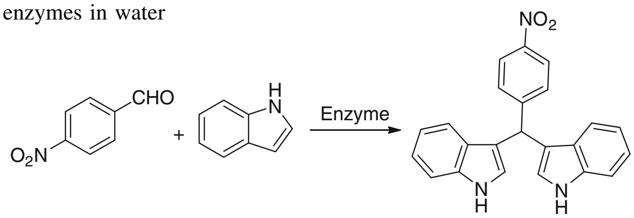
high yields. However, there were still some drawbacks in these catalytic systems including the requirement of heavy metal, drastic conditions for catalyst preparation and also require tedious work-up leading to the generation of large amount of toxic waste.

Biocatalytic promiscuity is ability of the same or different, or induced active site to catalyze more than one chemical transformation which usually differ in the type of bond formation or cleavage and in the catalytic mechanism of bond making or breaking and could provide useful methods in organic synthesis (Humble and Berglund 2011; Hult and Berglund 2007; Gatti-Lafranconi and Hollfelder 2013; Wu et al. 2010; Babtie et al. 2010; Taglieber et al. 2007; Kazlauskas 2005). In recent decades years, many enzymes have been found to be able to catalyze non-natural reactions, such as Aldol (Branneby et al. 2004; Li et al. 2008; Serafimov et al. 2008), Michael (Xu et al. 2007; Lou et al. 2007; Torre et al. 2004; Svedendahl et al. 2005; Carlqvist et al. 2005), Henry (Wang et al. 2010; Purkarthofer et al. 2006; Gruber-Khadjawi et al. 2007), Knoevenagel reaction (Chen et al. 2011; Hu et al. 2012) and Markovnikov additions (Lou et al. 2009; Wu et al. 2005). Exploiting the promiscuous activity of biocatalyst to achieve cascade reactions has become particularly fascinating and remains a great challenge (Klossowski et al. 2013; Wang et al. 2011; Liu et al. 2011; Xue et al. 2012; He et al. 2012). As a part of our research, aimed at developing green chemistry by using enzyme as the reaction catalyst, we focused on the waste-free, general, and safe protocol for the synthesis of bis(indolyl)alkanes by the cascade nucleophilic addition–elimination-Michael addition between aldehydes and indole (Scheme 1).

Results and discussion

Based on our previous research, initial efforts were performed in pure water using *p*-nitrobenzaldehyde and indole as the model reaction and some hydrolases as catalysts at 50 °C for 50 h. Results indicated that porcine pancreas lipase (PPL) displayed the best activity and the yield of bis(indolyl)alkanes was 41 % (Table 1, entry 1). The other six hydrolases, such as lipase from Candida *cylindracea* (CCL), Amano lipase M from M. *javanicus* (MJL), Protease-S“amano”G, D-aminoacylase from *Escherichia coli* (DA), Lipase-PS“amano”SD, and Acylase “Amano” from *Aspergillus oryzae* (AA), showed no or low activity toward this reaction.When the reactants were incubated with denatured PPL or bovine serum albumin (BSA), the yields were practically equal to the background reaction (Table 1, entries 2–4), which could rule out the possibility that the amine residues on the protein surface promoted the process. The control experiments suggested that the tertiary structure and the specific catalytic site of PPL were responsible for the cascade between aldehydes and indole.

Table 1 Reaction of 4-nitrobenzaldehyde and indole under different enzymes in water



Entry	Enzyme	Yield (%)[a]
1	PPL	41
2	Blank	<1
3	BSA	<1
4	PPL[b]	2
5	CCL	6
6	MMJL	4
7	Protease-S“amano”G	<1
8	DA	5
9	Lipase-PS“amano”SD	<1
10	AA	6

Experimental conditions: enzyme 10 mg, *p*-nitrobenzaldehyde 10 mg, indole 15 mg, water 1 ml, 50 °C, 50 h

[a] Determined by HPLC

[b] Pretreated with urea at 100 °C for 8 h

The reaction medium played an important role in enzyme-catalyzed reactions, owing to its effects on enzyme stability and substrate solubility, we investigated the effect of conventional organic solvents on the model reaction (Lou et al. 2009; Xue et al. 2012). The results indicated that the different solvents had significant effects on activity of PPL for the cascade reaction. The reaction afforded a product yield of 41 % in water after 50 h (Table 2, entry 1). The other tested solvents including DMSO, 1,4-dioxane, chloroform, toluene, THF, hexane, and octane gave the low yields (Table 2, entries 2–8).

Considering that the mixed solvent could improve the substrate solubility and enhance the coexistence of substrate, some mixture of water and organic solvents were studied under the same condition. As shown in Fig. 1, better result with a high yield of 80 % was obtained from this cascade reaction in the mixture solvent of water and

Table 2 Effect of solvents on the reaction of 4-nitrobenzaldehyde and indole

Entry	Solvent	Yield (%)[a]
1	Water	41
2	DMSO	<1
3	1,4-Dioxane	<1
4	Chloroform	<1
5	Toluene	3
6	THF	<1
7	Octane	6
8	Hexane	3

Experimental conditions: PPL 10 mg, *p*-nitrobenzaldehyde 10 mg, indole 15 mg, solvent 1 ml, 50 °C, 50 h

[a] Determined by HPLC

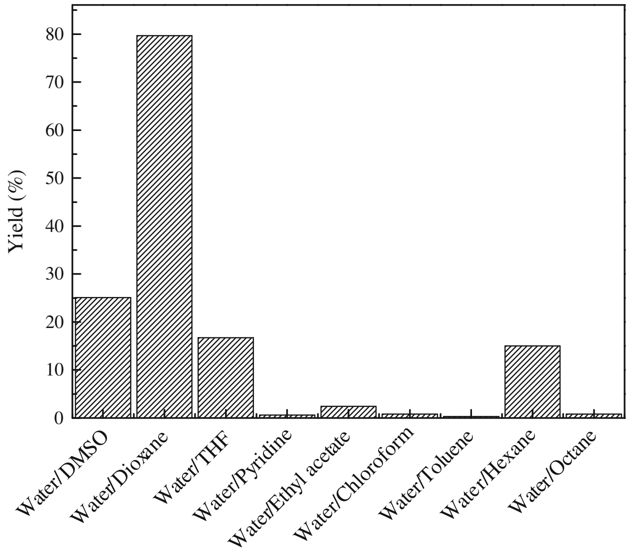


Fig. 1 Effect of solvents mixture on the cascade reaction. The reactions between *p*-nitrobenzaldehyde (10 mg) and indole (15 mg) were carried out in the presence of PPL (10 mg) in 1.0 mL solvent ($V_{\text{water}}/V_{\text{organic solvent}} = 4{:}1$) at 50 °C for 50 h

1,4-dioxane. In other mixture solvents such as pyridine, ethyl acetate, chloroform, toluene, DMSO, THF, hexane, and octane mixed with water, respectively, reactions proceeded very slowly and yields were also far from satisfactory.

Obviously, it was important to optimize the 1,4-dioxane content for the model reaction system. We screened the range of 1,4-dioxane content from 5 to 80 % ($V_{\text{dioxane}}/V_{\text{water+dioxane}}$), and the results were showed in Fig. 2. The rate of the enzymatic reaction can be accelerated by increasing the concentration of 1,4-dioxane and reached the top when using 20–25 % 1,4-dioxane. However, when the 1,4-dioxane content was increased further, the yield remarkably decreased to 1 %. Results indicated that the optimal 1,4-dioxane concentration for the PPL-catalyzed cascade reaction in water was about 20 %.

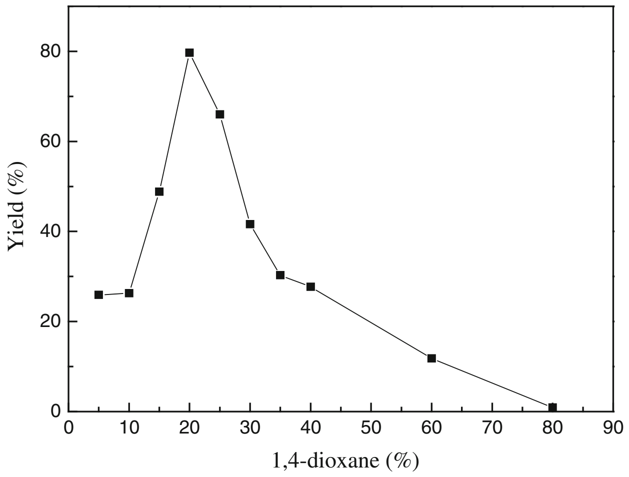


Fig. 2 Influence of 1,4-dioxane concentration on the PPL-catalyzed cascade reaction. The reactions were carried out with *p*-nitrobenzaldehyde (10 mg) and indole (15 mg) in the presence of PPL (10 mg) in 1.0 mL solvent ($V_{\text{water}} + V_{\text{1,4-dioxane}}$) at 50 °C for 50 h

Table 3 Effect of temperature, catalyst amount, and time on the reaction of 4-nitrobenzaldehyde and indole

Entry	T/°C	Catalyst/mg	Time (h)	Yield (%)[a]
1	50	15	50	80
2	30	15	50	21
3	60	15	50	70
4	50	5	50	23
5	50	10	50	71
6	50	15	50	80
7	50	25	50	84
8	50	15	12	36
9	50	15	24	55
10	50	15	72	98

Experimental conditions: PPL 10 mg, *p*-nitrobenzaldehyde 10 mg, indole 15 mg, water 0.8 mL, 1,4-dioxane 0.2 mL

[a] Determined by HPLC

Temperature has certain influence on enzyme catalyzed reactions due to its effects on the rate of the reaction and also on the stability of the enzyme and it was found that the enzyme showed the best yields of 80 % at 50 °C (Table 3, entries 1–3). Then, the effect of the enzyme concentration was also optimized. As the data shown in Table 3, the yield of the product was improved greatly by increasing the enzyme concentration from 5 to 15 mg/mL and reached a plateau after 15 mg/ml. Finally, we investigated the time of the cascade reaction between 4-nitrobenzaldehyde and indole catalyzed by PPL under the optimal conditions and it could be seen that the yield of the product the reaction was high up to 98 % after 72 h.

Having the optimal conditions in hand, we further studied the generality of the PPL catalyzed cascade reaction between indole and other aldehydes, and all results were summarized in Table 4. Most bis(indolyl)alkanes were formed in excellent yields when using benzaldehyde or aromatic aldehydes with an electron-withdrawing substituent such as a nitro group (Table 4, entries 1–3) and chloro-groups (Table 4, entries 5–7) in the presence of PPL. However, the reaction of electron-rich benzaldehydes such as *p*-methylbenzaldehyde, *p*-methoxylbenzaldehyde and hydroxylbenzaldehyde with indole were comparatively inefficient, giving the corresponding bis(indolyl)alkanes in low to moderate yields (Table 4, entries 8–12). Interestingly, aromatic aldehydes with two substitutions also gave moderate to excellent yields with indole (Table 3, entries 13–14). However, alicyclic aldehydes only gave the corresponding bis(indolyl)alkanes in poor yields (Table 4, entry 15). Thus, it can be inferred that the presence of electron withdrawing group on the benzene ring can improve the enzymatic cascade reaction, comparing with the electron donating group or alicyclic aldehydes.

At last, we have selected the reaction of benzaldehyde with indole for the synthesis of bis(3-indolyl)methane as a model reaction and compared the catalytic activity of PPL with other catalysts in terms of reaction time and percentage yields (Table 5). Although the reaction catalyzed by PPL required longer reaction time, the satisfied results were also obtained in terms of yields. More importantly, the present methodology have provided a biocatalytic route for the synthesis of bis(indolyl)alkanes and expanded the application's scope of enzyme.

In conclusion, we successfully developed a facile and inexpensive methodology with low pollution for the synthesis of bioactive bis(indolyl)alkanes using PPL as catalyst while exploring the promiscuous lipase catalyzed system for the cascade reaction. After the optimization of the stepwise process, a series of bis(indolyl)alkanes was prepared in moderate to excellent yields. The hydrolase catalyze this cascade reaction was of practical essential to develop the application of enzymes and in the evolution of new biocatalysts.

Experimental section

Materials and general methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DMX-400 spectrometer at 400 and 125 MHz in $CDCl_3$ and DMSO-d_6, respectively. High resolution mass spectra (HRMS) were run on waters GCT premier mass spectrometer. Chemical shifts are reported in ppm (δ), relative to the internal standard of tetramethylsilane (TMS). IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Analytical HPLC was performed using an Agilent 1,100 series with a reversed-phase Shim-Pack VP-ODS column and a UV detector (250 nm). Eluents were methanol and water. Lipase from *porcine pancreas*, Type II (PPL) was purchased from Sigma-Aldrich, lipase from *candida cylindracea* (CCL) (E.C. 3.1.1.3, 2.08 U/mg) was purchased from Fluka, lipase from *mucor javanicus* (MJL) (E.C. 3.1.1.3 9.9 U/mg) was obtained from Fluka, D-aminoacylase from *Escherichia coli* (DA) (E.C. 3.5.1.81, lyophilized powder, acylase activity 5 MU g-1) was purchased from Amano Enzyme Inc. (Japan), acylase "Amano" from *Aspergillus oryzae* (E.C. 3.5.1.14, lyophilized powder) was purchased from Amano Enzyme Inc. (Japan), Alkaline protease from *Bacillus subtilis* (BSA) (E.C. 3.4.21.14, 10 U/g) was obtained from Wuxi Enzyme Co. Ltd, Wuxi, P. R. China, Protease-S"amano"G, Lipase-PS"amano"SD were purchased from Amano Enzyme Inc. (Japan) and All chemicals were obtained from commercial suppliers. Solvents for column chromatography were not distilled before use.

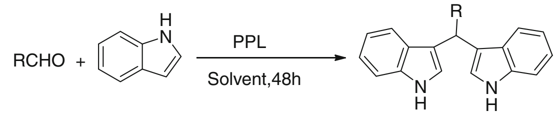
General procedure for the reaction of 4-nitrobenzaldehyde and indole

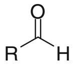
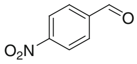
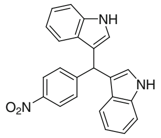
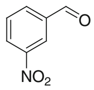
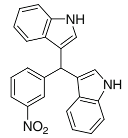
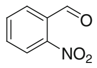
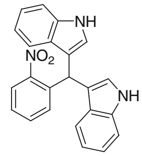
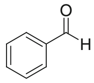
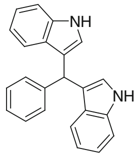
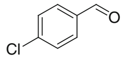
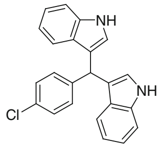
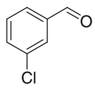
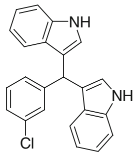
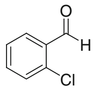
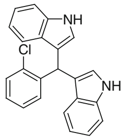
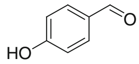
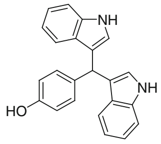
A suspension of 100 mg 4-nitrobenzaldehyde (0.67 mmol), 150 mg indole (1.28 mmol), and 100 mg PPL in 10 ml mixture solvent (8 ml water and 2 ml 1,4-dioxane) was incubated at 50 °C and 200 r.p.m. (orbitally shaken) for 48 h. The products were extraction with 3 × 15 ml ethylacetate. Then the ethylacetate was evaporated under vacuum to dryness. The crude residue was purified by flash column chromatography on silica gel using petroleum/ethyl acetate mixtures. All the known products were characterized by comparing the ^{1}H NMR data with those reported in the literature (Silveira et al. 2009; Chakrabarty et al. 2002; Nadkarni et al. 2008). The structures of new compounds were confirmed by IR, ^{1}H NMR, ^{13}C NMR, and HR-MS.

3,3′-((4-Nitrophenyl)methylene)bis(1H-indole) (Ji et al. 2003) CAS NO. 7501-91-9; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (s, 2H), 8.15 (d, $J = 8.8$ Hz, 2H), 7.61 (d, $J = 8.4$ Hz, 2H), 7.36 (d, $J = 8.4$ Hz, 2H), 7.28 (d, $J = 7.6$ Hz, 2H), 7.05 (t, $J = 7.2$, 15.6, 8.4 Hz, 2H), 6.88 (m, 4H), 6.02 (s, 1H); IR (neat): 3,423, 1,615, 1,592, 1,507, 1,487, 1,456, 1,339, 1,094, 746.

3,3′-((3-Nitrophenyl)methylene)bis(1H-indole) (Das and Das 2012; Yadav et al. 2001) CAS NO. 33948-93-5; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.91 (s, 2H), 8.13 (s, 1H), 8.05 (d, $J = 8.0$ Hz, 1H), 7.83 (d, $J = 8.0$ Hz, 1H), 7.56 (t, $J = 8.4$, 16.0, 7.6 Hz, 1H), 7.37 (d, $J = 8.0$ Hz, 2H), 7.30 (d, $J = 7.6$ Hz, 2H), 7.05 (t, $J = 7.2$, 14.8, 7.6 Hz, 2H), 6.87 (m, 4H), 6.06 (s, 1H); IR (neat): 3,413, 1,612, 1,525, 1,456, 1,348, 1,095, 744.

Table 4 Direct cascade reaction of aldehydes and indoles[a]



Entry		Products	Yield (%)[a]
1			98
2			93
3			89
4			87
5			91
6			89
7			83
8			67

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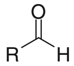
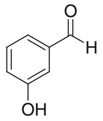
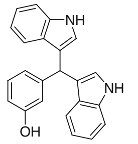
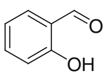
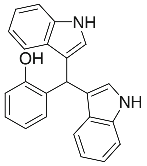
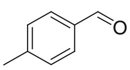
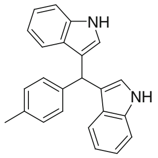
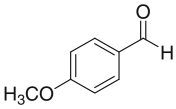
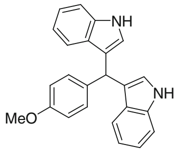
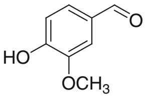
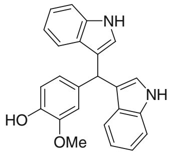
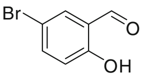
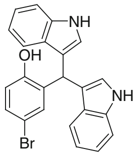
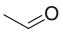
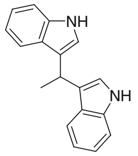
Entry		Products	Yield (%)^{a}
9			63
10			72
11			52
12			50
13			76
14			99
15			38

Reaction conditions: aldehydes 0.1 mmol, indole 0.2 mmol, 1,4-dioxane 0.3 ml, water 1.2 ml, and PPL 15 mg was shaken at 50 °C for 72 h

[a] Determined by HPLC

3,3′-((2-Nitrophenyl)methylene)bis(1H-indole) (Tayebee et al. 2013) CAS NO. 119091-74-6; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.90 (s, 2H), 7.87 (d, $J = 8.0$ Hz, 1H), 7.55 (t, $J = 7.2$, 15.2, 8.0 Hz, 1H), 7.35–7.46 (m, 4H), 7.22 (d, $J = 7.6$ Hz, 2H), 7.05 (t, $J = 7.2$, 14.8, 7.6 Hz, 2H), 6.88 (t, $J = 14.4$, 7.2 Hz, 2H), 6.77 (s, 2H), 6.41 (s, 1H); IR (neat): 3,412, 1,615, 1,600, 1,522, 1,456, 1,353, 1,346, 1,095, 743.

3,3′-((4-Chlorophenyl)methylene)bis(1H-indole) (Ji et al. 2003) CAS NO. 178946-89-9; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 10.87 (s, 2H), 7.37 (d, $J = 8.0$ Hz, 3H), 7.31 (t, $J = 7.2$, 15.2, 8.0 Hz, 4H), 7.05 (t, $J = 7.6$, 14.8, 7.2 Hz, 2H), 6.88 (m, 4H), 5.89 (s, 1H); IR (neat): 3,411, 1,613, 1,594, 1,474, 1,456, 1,353, 1,095, 743.

3,3′-((3-Chlorophenyl)methylene)bis(1H-indole) (Krishnammagari et al. 2012) CAS NO. 865717-24-4; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.83 (s, 2H), 7.34 (m, 4H), 7.24 (m, 3H), 7.54 (t, $J = 15.2$, 7.6 Hz, 3H), 7.02 (t, $J = 15.2$, 7.6 Hz, 2H), 6.58 (s, 2H), 5.85 (s, 1H); IR (neat): 3,408, 1,617, 1,596, 1,487, 1,455, 1,352, 1,089, 742.

Table 5 Comparison of the catalytic efficiency of PPL with some reported catalysts

Entry	Catalyst	Time/h	Yield/%	Reference
1	PPL	72	87	This work
2	TiO_2 (nano)	12	64	Kantam et al. (2006)
3	Cp_2ZrCl_2	36	79	Kantam et al. (2004)
4	Zeolites	2	80	Karthik et al. (2004)
5	Acidic IL immobilized on Silica	5.5	97	Hagiwara et al. (2007)

3,3′-((2-Chlorophenyl)methylene)bis(1H-indole) (Ji et al. 2003) CAS NO. 74944-40-4; ^1H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 2H), 7.43 (t, $J = 8.4, 16.0, 7.6$ Hz, 3H), 7.37 (d, $J = 8.0$ Hz, 2H), 7.24–7.16 (m, 4H), 7.12 (t, $J = 6.4, 14.4, 8.0$ Hz, 1H), 7.04 (t, $J = 6.8, 14.4, 7.6$ Hz, 2H), 6.62 (d, $J = 2.0$ Hz, 2H), 6.36 (s, 1H); IR (neat): 3,412, 1,618, 1,589, 1,487, 1,456, 1,337, 1,093, 741.

3,3′-Phenylmethylenebis(1H-indole) (Yadav et al. 2001) CAS NO. 35173-74-1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 2H), 7.41 (d, $J = 8.0$ Hz, 2H), 7.37–7.25 (m, 7H), 7.19 (t, $J = 7.6, 17.2, 9.6$ Hz, 2H), 7.02 (t, $J = 7.2, 14.8, 7.6$ Hz, 2H), 6.59 (d, $J = 1.2$ Hz, 2H), 5.90 (s, 1H); IR (neat): 3,395, 1,618, 1,599, 1,492, 1,455, 1,336, 1,092, 746.

3,3′-((4-Hydroxylphenyl)methylene)bis(1H-indole) (Yang et al. 2011) CAS NO. 151358-47-3; ^1H NMR (400 MHz, DMSO-d_6) δ 10.78 (s, 2H), 9.23 (s, 1H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.26 (d, $J = 8.4$ Hz, 2H), 7.14 (d, $J = 8.8$ Hz, 2H), 7.02 (t, $J = 7.2, 14.8, 7.6$ Hz, 2H), 6.85 (t, $J = 7.6, 14.8, 7.2$ Hz, 2H), 6.78 (s, 2H), 6.66 (d, $J = 8.0$ Hz, 2H), 5.71 (s, 1H); IR (neat): 3,446, 3,426, 3,405, 1,614, 1,597, 1,512 1,485, 1,456, 1,353, 1,089, 743.

3,3′-((3-Hydroxylphenyl)methylene)bis(1H-indole) (Kidwai et al. 2011) CAS NO. 96060-62-7; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 2H), 7.38 (d, $J = 8.0$ Hz, 2H), 7.34 (d, $J = 8.0$ Hz, 2H), 7.25–7.13 (m, 4H), 7.00 (t, $J = 7.2, 15.2, 8.0$ Hz, 1H), 6.93 (d, $J = 8.0$ Hz, 1H), 6.76 (s, 1H), 6.68 (d, $J = 7.6$ Hz, 1H), 6.64 (s, 2H), 5.82 (s, 1H), 5.01 (s, 1H); IR (neat): 3,410, 1,618, 1,597, 1,486, 1,455, 1,338, 1,093, 744.

3,3′-((2-Hydroxylphenyl)methylene)bis(1H-indole) (Yang et al. 2011) CAS NO. 33948-97-9; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (s, 2H), 7.42 (d, $J = 7.6$ Hz, 2H), 7.36 (d, J = 8.0 Hz, 2H), 7.21 (t, $J = 8.8, 15.6, 6.8$ Hz, 4H), 7.05 (t, $J = 14.4, 7.2$ Hz, 4H), 6.90 (t, $J = 8.0, 15.6, 7.6$ Hz, 2H), 6.71 (s, 2H), 6.02 (s, 1H), 5.52 (s, 1H); IR (neat): 3,502, 3,419, 1,618, 1,592, 1,487, 1,454, 1,336, 1,090, 741.

3,3′-((4-Methylphenyl)methylene)bis (1H-indole) (Yadav et al. 2001) CAS NO. 223753-09-1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 2H), 7.41 (d, $J = 7.6$ Hz, 2H), 7.33 (d, $J = 8.0$ Hz, 2H), 7.24 (d, $J = 8.0$ Hz, 2H), 7.18 (t, $J = 7.2, 14.8, 7.6$ Hz, 2H), 7.10 (d, $J = 8.4$ Hz, 2H), 7.02 (t, $J = 7.2, 15.2, 8.0$ Hz, 2H), 6.60 (d, $J = 1.6$ Hz, 2H), 5.86 (s, 1H), 2.34 (s, 3H); IR(neat): 3,417, 1,618, 1,510, 1,487, 1,456, 1,338, 1,091, 744.

3,3′-((4-Methoxylphenyl)methylene)bis(1H-indole) (Yadav et al. 2001) CAS NO. 33985-68-1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 2H), 7.39 (d, $J = 8.0$ Hz, 2H), 7.33 (d, $J = 7.6$ Hz, 2H), 7.25 (d, $J = 8.4$ Hz, 2H), 7.17 (t, $J = 7.6, 15.2, 7.6$ Hz, 2H), 7.01 (t, $J = 7.6, 14.8, 7.2$ Hz, 2H), 6.82 (d, $J = 8.0$ Hz, 2H), 6.60 (s, 2H), 5.84 (s, 1H), 3.78 (s, 3H); IR (neat): 3,395, 1,610, 1,587, 1,509, 1,487, 1,455, 1,336,1,093, 741.

5-(Di(1H-indol-3-yl)methyl-2-methoxyphenol (Yang et al. 2011) CAS NO. 354786-65-5; ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, 2H), 7.50 (d, $J = 8.0$ Hz, 2H), 7.30 (d, $J = 8.0$ Hz, 2H), 7.19 (t, $J = 7.2, 14.8, 7.6$ Hz, 2H), 7.05 (t, $J = 14.4, 7.2$ Hz, 2H), 6.75-6.83 (m, 3H), 6.62 (s, 2H), 6.34 (s, 1H), 5.91 (s, 1H), 3.90 (s, 3H); IR (neat): 3,502, 3,413, 1,615, 1,592, 1,478, 1,455, 1,350, 1,086, 741.

4-Bromo-2-(di(1H-indol-3-yl)methyl)phenol ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 2H), 7.42 (d, $J = 8.0$ Hz, 2H), 7.35 (t, $J = 8.0, 18.4, 10.4$ Hz, 3H), 7.21–7.30 (m, 4H), 7.07 (t, $J = 7.6, 14.8, 7.2$ Hz, 2H), 6.75 (d, $J = 8.4$ Hz, 1H), 6.67 (s, 2H), 5.99 (s, 1H), 5.66 (s, 1H); IR (neat): 3,411, 1,618, 1,593, 1,487, 1,456, 1,338, 1,098, 743; ^{13}C NMR ($CDCl_3$; 100 MHz): δ 60.51 (Ar_3CH), 153.67, 136.82, 132.40, 131.56, 112.90 (all Ar–C), 130.80, 126.59, 123.67, 122.45, 119.65, 118.42, 116.48, 111.33 (all Ar–CH); HRMS: Mass 416.0518 for $C_{23}H_{17}N_2OBr$, Calc. Mass 416.0524.

2-(1-(1H-Indol-3-yl) ethyl)-1H-indole (Xu et al. 2013) CAS NO. 5030-91-1; ^1H NMR (400 MHz, $CDCl_3$) δ 7.81 (s, 2H), 7.48 (d, $J = 8.0$ Hz. 2H), 7.23 (d, $J = 8.0$ Hz, 2H), 7.06 (t, $J = 7.2, 14.8, 7.6$ Hz, 2H), 6.94 (t, $J = 7.6, 14.8, 7.2$ Hz, 2H), 6.79 (s, 2H), 4.58 (m, 1H), 1.71 (d, $J = 7.2$ Hz, 3H); IR (neat): 3,408, 1,617, 1,596, 1,455, 1,352, 1,094, 743; GC–MS: m/z = 260.

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Conflict of interest We declare that we have no competing financial interests.

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