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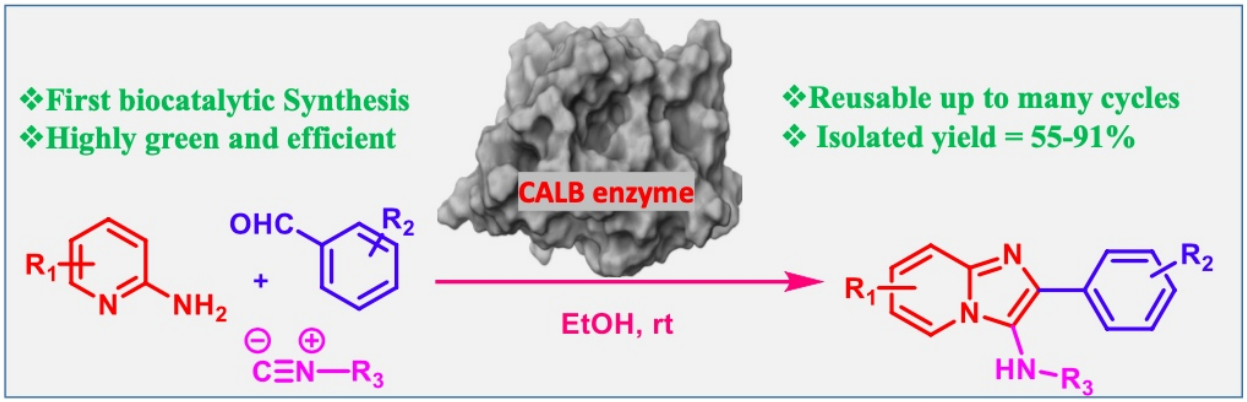

First biocatalytic Groebke-Blackburn-Bienaymé reaction to synthesize imidazo[1,2-a]pyridine derivatives using lipase enzyme

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Abstract: In this study, first biocatalytic synthesis of clinically important imidazo[1,2-a]pyridine based compounds has been achieved. The *Candida antarctica* lipase B (CALB) enzyme was found suitable to catalyze the Groebke-Blackburn-Bienaymé (GBB) multicomponent reaction of substituted 2-aminopyridine, aryl benzaldehyde and isocyanides to synthesize imidazo[1,2-a]pyridine derivatives in very good yields. Further, CALB enzyme was immobilized on mesoporous silica and characterized using FT-IR, XRD, SEM-EDS and HR-TEM to use as a reusable catalyst in this transformation. The immobilized catalyst CALB@SiO_2 displayed high catalytic efficiency up-to many cycles. In addition, preliminary mechanistic studies such as molecular docking and molecular dynamics (MD) simulation were performed which suggested that Thr40 and Ser105 residues are playing an important role in catalyzing the GBB-reaction.

Key words: Imidazo[1,2-a]pyridine, Groebke-Blackburn-Bienaymé, lipase, immobilization, SiO_2, molecular dynamics

Introduction: Imidazo[1,2-a]pyridine is a valuable framework found in a number of nitrogen-bridgehead fused heterocycles having diverse biological activities such as antiviral, antifungal, antiparasitic, anti-inflammatory, and anti-cancer.[1] Also, a number of drugs containing imidazo[1,2-a]pyridine framework have been commercialized such as hypnotic drug zolpidem, the anxiolytic drugs alpidem, saridipem or nicopedem, the antiulcer agent zolmidine and PDE-3 inhibitor drug olprione for the treatment of heart and circulatory failures (Figure 1).[2] Besides that, they have various applications in material and organometallic chemistry.[3]The most common method for the synthesis of imidazo[1,2- a]pyridine at the laboratory as well as at industrial level is the condensation reaction of 2- aminopyridines with mono-α-halogenocarbonyl compounds.[3c, 4] However, this method is not very suitable for the synthesis of diverse imidazo[1,2-a]pyridine derivatives due to the shortage of commercially available monohalogenated compounds. To overcome the shortcomings of conventional method a number of synthetic strategies have been developed over the years (Scheme 1).[5] In this context, Groebke-Blackburn-Bienaymé (GBB) reaction, which takes place between an aldehyde, 2-aminoazine, and an isocyanide to synthesize imidazo[1,2-a]pyridine framework remains the most efficient and common method (Scheme 1f).[6] In the last decades, numerous catalyst including Brønsted acids, Lewis acids, solid-supported acids, organic bases and ionic liquids have been reported to catalyze this reaction.[7] Besides, Song et al. reported microwave assisted GBB-multicomponent reaction for the synthesis of benzimidazole derivatives.[8] Recently, Gámez-Montaño's group reported green synthesis of carbazolylimidazo[1,2-a]pyridines via GBB- reaction using ultrasound.[9]

On the other hand, the use of biomolecules to catalyze non-natural organic reactions at laboratory as well as industrial level have seen exponential growth in last few years.[10] Further, lipase enzymes have grabbed lot of attention to catalyze new to nature organic reactions like multicomponent reactions due to their high operational stability in the reaction conditions and easy availability in the market.[11] In this context, Berlozecki and co-workers reported a lipase catalyzed Ugi-reaction to synthesize dipeptides and this method had numerous advantages over the previous procedures.[12] Further, Wu et al. reported a lipase catalyzed five component synthesis of spirooxazino derivatives using olefins, aldehydes, acetamides and cyclohexanone.[13] This group further reported the synthesis of spiroheterocycles using isatin, 1,3-cyclohexanedione and pyrazoles.[14] Still, there is no biocatalyst reported to catalyze the GBB-multicomponent reaction to synthesize imidazo[1,2-a]pyridine framework. Encouraged by the biological importance of imidazo[1,2-a]pyridines and our recent work in the area of biocatalysis[15] herein, we are reporting first biocatalytic Groebke-Blackburn-Bienaymé multicomponent reaction to synthesize imidazo[1,2- a]pyridine derivatives using lipase enzyme as a catalyst.

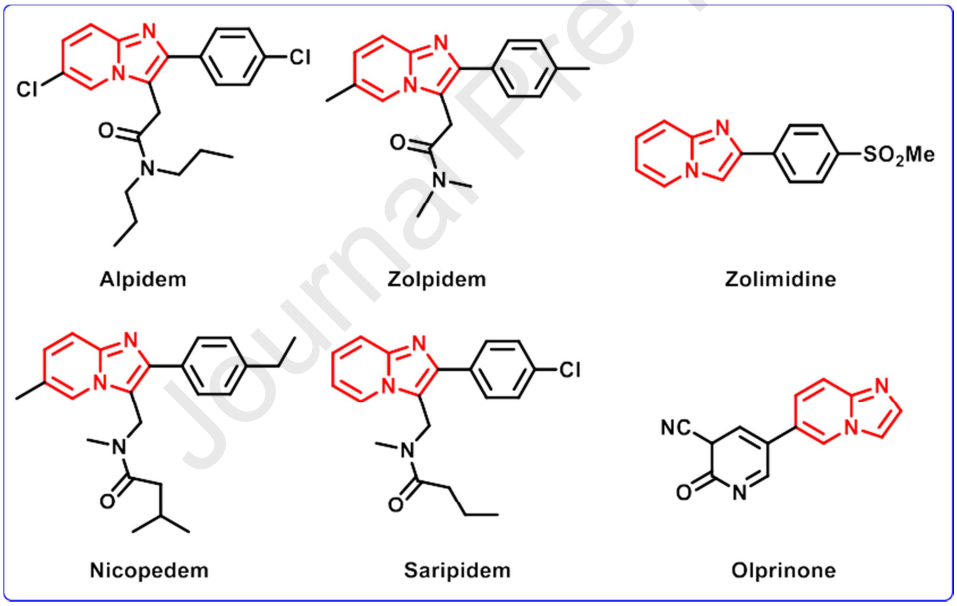
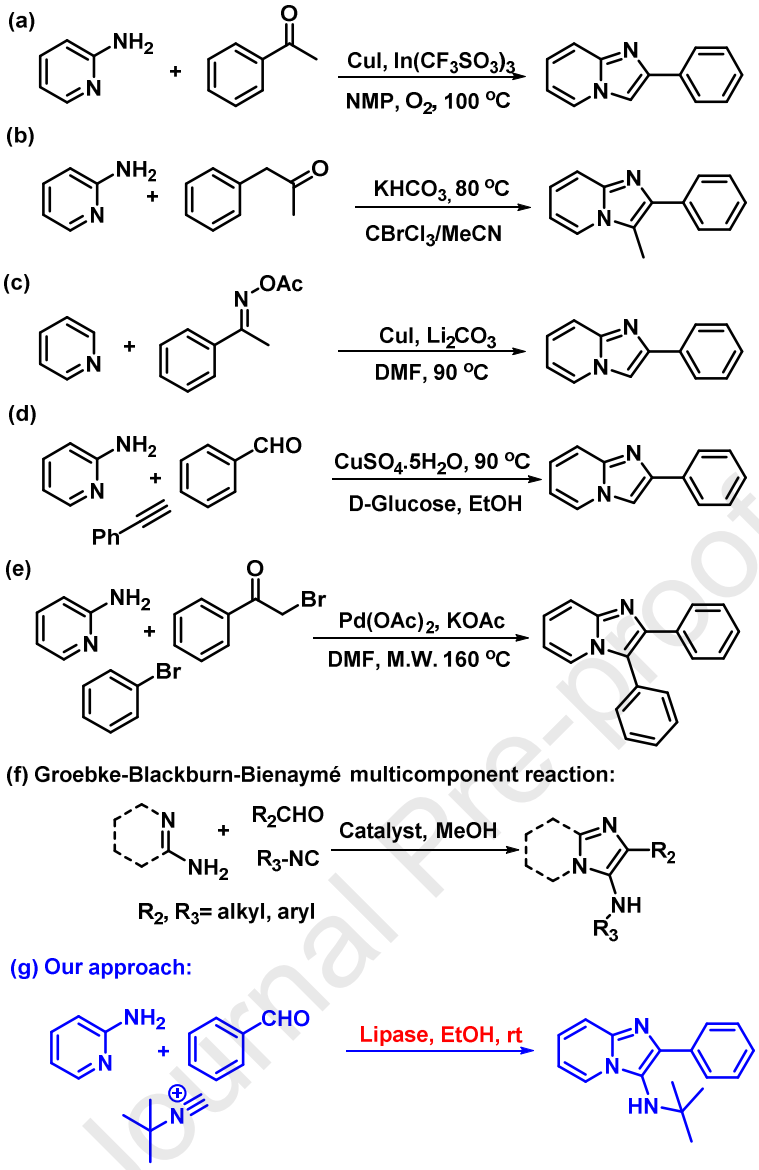


Figure 1. Examples of imidazo[1,2-a]pyridine framework containing drugs

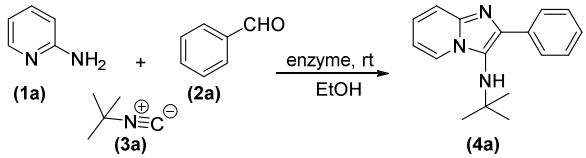


Scheme 1. Representative approaches for the synthesis of imidazo[1,2-a]pyridines framework

Results and Discussion

We started our investigation by screening of various lipase enzymes to catalyze the GBB-multicomponent reaction of model substrates 2-aminopyridine, benzaldehyde and tert-butyl isocyanide (Table 1). Interestingly, the model reaction gave product (4a) in 29% yield in the absence of any catalyst (entry 1, Table 1), however, addition of different lipases increased the yield of the reaction significantly (entries 2-5, Table 1). Among different lipase enzymes, *Candida antarctica* lipase B (CALB) *and Aspergillus niger* lipase were found best and provided product (4a) in 63% and 64% isolated yield respectively (entry 3-4, Table 1). Although, we choose CALB enzyme for further investigation in this study due to low cost and easy availability of CALB in comparison of *Aspergillus niger* lipase enzyme.

Table 1. Screening of different lipases to catalyse the reaction of model substrates[a]

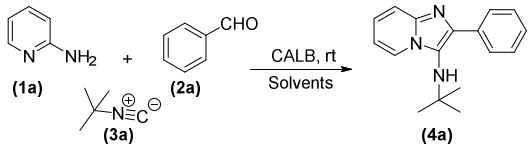


entry	lipases	% yield[b]
1	No enzyme	29
2	*Rhizomucor mehei* lipase (RML)	40
3	*Candida antarctica* lipase B (CALB)	63
4	*Aspergillus niger* lipase	64
5	*Candida rugosa* lipase	50

[a]Reaction conditions: 2-aminopyridine (1a) (50 mg, 0.563 mmol), benzaldehyde (2a) (67.6 mg, 0.637 mmol, 1.2equiv.), tert-butyl isocyanide(3a) (72.5 mg, 0.637 mmol, 1.2 equiv.) and enzyme (20 mg) in 2 ml of ethanol were taken in a glass tube with a teflon cap and stirred the reaction mixture at room temperature for overnight, [b]isolated yield.

Next, we tested different solvents along with the combination of solvents to improve the yield of the model reaction (entries 1-10, Table 2). In case of H_2O and DMSO the product (4a) formed in trace amount only however, no product formation was observed in the case of DMF, THF and hexane (entries 1-5, Table 2). Further, reaction gave product (4a) in 62% yield when MeOH was used as a solvent (entry 6, Table 2). When we used phosphate buffer (pH=7) as a solvent, product (4a) was obtained in 48% yield (entry 10, Table 2). Besides, reaction gave no product when we used the mixture of water-ethanol (1:1 v/v) or water-DMSO (1:1 v/v) as a solvent (entries 7 & 9, Table 2). As a result, ethanol remained best solvent to attain the maximum conversion in the model reaction (entry 8, Table 2).

Table 2. Screening of different solvents for CALB catalyze the reaction of model substrates[a]



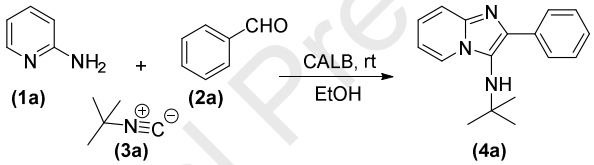
entry	solvent	%yield[b]
1	H_2O	trace
2	DMSO	trace
3	DMF	NR
4	THF	NR
5	Hexane	NR
6	Methanol	62%
7	EtOH + H_2O (1:1 v/v)	NR
8	Ethanol	63%
9	DMSO + H_2O (1:1 v/v)	NR
10	Phosphate buffer	48%

[a]Reaction conditions: 2-aminopyridine (1a) (50 mg, 0.563 mmol) , benzaldehyde (2a) (67.6 mg, 0.637 mmol, 1.2equiv.), tert-butyl isocyanide(3a) (72.5 mg, 0.637 mmol, 1.2 equiv.) and enzyme (20 mg) in 2 ml of solvent were taken in a glass tube with a teflon cap and stirred the reaction mixture at room temperature for overnight, [b]isolated yield.

After choosing best biocatalyst (CALB) and solvent (EtOH), we investigated the effect

of the enzyme loading and substrate ratio on the conversion of the model reaction. As shown in table 3, when loading of enzyme was decreased from 20 mg to 10 mg, the reaction gave product (4a) in slightly lower yield (entries 1-2, Table 3). Next, we increased the enzyme loading up-to 50 mg, however, maximum yield was observed in the case of 40 mg of CALB enzyme (entries 2-5, Table 3). Further, the substrates ratios were changed from 1:1.2:1.2 to 1:1:1 for model substrates, however, 1:1.2:1.2 remained the best substrate ratio in comparison to 1a:2a:3a to obtain the highest conversion (entries 4 & 6, Table 3). After having the optimized reaction conditions in hand, the effect of different substitutions including electron withdrawing and donating groups on the aromatic ring of 2-aminopyridines and benzaldehydes was explored (Table 4).The reaction of unsubstituted 2-aminopyridine, benzaldehyde and *tert*-butyl isocyanide furnished the product (4a) in 91% yield under the optimized reaction conditions (entry 1, Table 4).Subsequently, we examined the effect of different substitutions such as NO_2, -Cl, -Br and -OMe on the *ortho*- or *para*-position of benzaldehyde.

Table 3. Optimization of the ratio of substrates and enzyme loading[a]



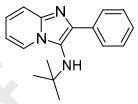
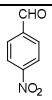
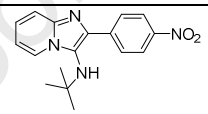
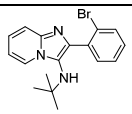
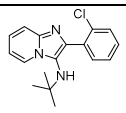
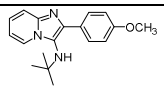
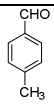
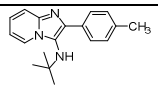
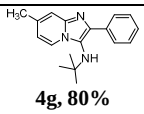
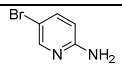
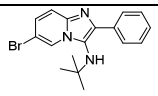
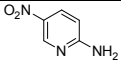
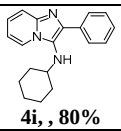
entry	enzyme loading	ratio of 1a:2a:3a	%yield[b] (4a)
1	10	1:1.2:1.2	55
2	20	1:1.2:1.2	63
3	30	1:1.2:1.2	80
4	40	1:1.2:1.2	91
5	50	1:1.2:1.2	80
6	40	1:1:1	58

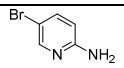
[a]Reaction conditions: 2-aminopyridine **(1a)** (50 mg, 0.563 mmol) , benzaldehyde **(2a)** (67.6 mg, 0.637 mmol, 1.2equiv.), tert-butyl isocyanide**(3a)** (72.5 mg, 0.637 mmol, 1.2equiv.) and enzyme in 2 ml of ethanol were taken in a glass tube with a teflon cap and stirred the reaction mixture at room temperature for overnight, [b]isolated yield.

Unexpectedly, in case of strong electron withdrawing group such as NO_2, reaction gave product (4b) only in 55% yield (entry 2, Table 4), however, good yield was observed in case of halides such as –Br, -Cl at the ortho-position of benzaldehyde (entries 3,4, Table 4). Moreover, when benzaldehyde having donating group such as 4- OMe and 4-Me, reaction gave product (4e) and (4f) in slightly inferior yield i.e 61% and 63% respectively (entries 5 and 6, Table 4). Next, we investigated the effect of different substitutions such as $–CH_3$, -Br and $-NO_2$ at different positions of 2-amino pyridine. In case of 4-CH_3 substituted 2-aminopyridine, reaction gave products (4g) in 80% yield (entries 7, Table 4), whereas, in case of 5-bromo substitution reaction provided product (4h) in slightly inferior yield (entry 8, Table 4). Further, we observed no product formation in the case of 5-NO_2 at the aromatic ring of 2-aminopyridine and it might be due to the strong decrement in the

nucleophilicity of 2-aminopyridine (entry 9, Table 4). Interestingly, when tert-butyl isocyanide was replaced with cyclohexyl isocyanide, a slightly lower yield of product (4i) was observed (entry 10, Table 4). Surprisingly, on taking –Br substitution on pyridine as well as on benzaldehyde no product formation was observed (entry 11, Table 4).

Table 4. Scope of substrates for the CALB-catalyzed GBB-reaction[a]

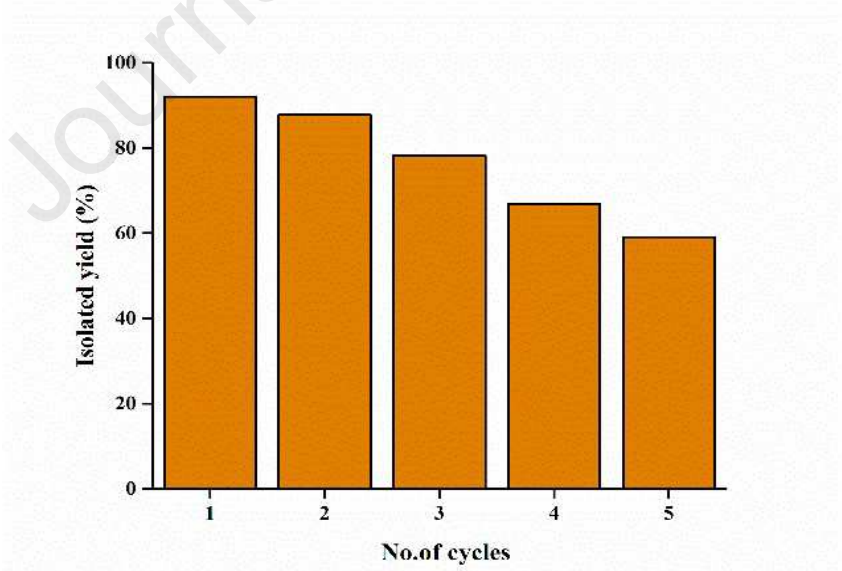
entry	Pyridine (1)	Aldehyde (2)	Isocyanide (3)	Product (4), yield[b]
1	 **1a**	 **2a**	 **3a**	 **4a, 91%**
2	**1a**	 **2b**	**3a**	 **4b, 55%**
3	**1a**	 **2c**	**3a**	 **4c, 72%**
4	**1a**	 **2d**	**3a**	 **4d, 70%**
5	**1a**	 **2e**	**3a**	 **4e,61%**
6	**1a**	 **2f**	**3a**	 **4f, 62%**
7		**2a**	**3a**	 **4g, 80%**
8		**2a**	**3a**	 **4h, 70.4%**
9		**2a**	**3a**	**NR**
10	**1a**	**2a**	 **3b**	 **4i, , 80%**

11			**3a**	**NR**

[a]Reaction conditions: 2-aminopyridine (1 equiv.), benzaldehyde **(**1.2equiv.), isocyanide**(**1.2equiv.) and enzyme (40 mg) in 2 ml of ethanol were taken in a glass tube with a teflon cap and stirred the reaction mixture at room temperature for overnight, [b]isolated yield.

In the next phase of our endeavor, we envisioned to reuse CALB enzyme after completion of first catalytic cycle, however, it could not be possible with pure enzyme. Previously, immobilization technique of confining naturally existing free enzyme on
/in different support has been useful to make enzyme reusable.[15] There are plenty of ways for immobilization of enzyme for example covalent binding, H-bonding, adsorption, entrapment, cross linking etc.[17] In present work, we have used entrapment method for immobilizing CALB on silica particles to make it reusable under the optimized reaction conditions which could not be possible in the case of pure CALB enzyme (The details of synthetic procedure, characterization and catalyst loading optimization is given in supporting information). Next, we tested CALB@SiO_2 for the reusability using the GBB-multicomponent reaction of 2-aminopyridine, benzaldehyde and tert-butyl isocyanide and compiled the results in Figure 2. These results revealed that the immobilized enzyme i.e. CALB@SiO_2 was reusable up-to many catalytic cycles with high catalytic efficiency.

Figure 2: Reusability test of CALB@SiO2 for the reaction of 2-aminopyridine, benzaldehyde and tert-butyl isocyanide



[a]Reaction conditions: 2-aminopyridine (1a) (100 mg, 1.06mmol), benzaldehyde (2a) (135.2 mg, 1.27mmol, 1.2equiv.), tert-butyl isocyanide(3a) (145 mg, 1.27 mmol,1.2equiv.) and CALB@SiO2 (50 mg) in 2 ml of ethanol were taken in a glass tube with a teflon cap and stirred the reaction mixture at room temperature for overnight, [b]isolated yield.

Further, the synthetic utility of CALB catalyzed GBB-multicomponent reaction was explored by increasing the concentration of model substrates up-to seven folds such as 2-amino pyridine (0.35 g, 3.71 mmol, 1 equiv.), benzaldehyde (0.45 g, 4.46 mmol,1.2

equiv.) and *tert*-butyl isocyanide (0.51 g, 4.45 mmol,1.2 equiv.) under the optimized reaction conditions. The successful isolation of 0.903 g of product (4a) in 91.6% yield from this reaction proved the synthetic utility and opens the door for the synthesis of various drug precursors using lipase catalyzed GBB-multicomponent reaction.

Active site prediction and proposed mechanism: The molecular docking approach was carried out for the prediction of model substrates orientation inside the active site of the enzyme and to investigate the role of the active site residues in catalyzing the Groebke-Blackburn-Bienaymé reaction. Previous studies have been revealed that residues Thr40, Ser105, Gln106, Asp187, and His224 constitute the active site of *Candida antarctica* lipase B (CALB) enzyme.[18]Hence, we started our molecular docking studies with the X-ray crystal structure of CALB enzyme (PDB.ID: 4K6G). The GLIDE[19]tool of Schrodinger software with the SP method was used to find the positioning of 2-aminopyridine (1a) and benzaldehyde (2a) substrates inside the active site of the enzyme (Figure 3a). Further, the general reaction mechanism of GBB- reaction reported previously in literature suggested that 2-aminopyridine (1a) and benzaldehyde (2a) substrates reacts under the catalytic conditions to form imine intermediate (i) which further gives addition reaction with tert-butyl isocyanide to produce subsequent intermediate (ii) which furnished the final product via [4+1] cycloaddition reaction followed by 1,3-proton transfer (Scheme S1).[7f, 7i, 7j] Encouraged by previous reports, intermediates such as (Z)-N-benzylidenepyridin-2-aminium (i) formed by the reaction of 2-aminopyridine (1a) and benzaldehyde (2a) substrates and 2-methyl-N-(2-phenyl-2-(pyridin-2ylamino)ethylidyne) propan-2-aminium (ii) formed by the addition of tert-butyl isocyanide on imine intermediate during the GBB-reaction were also docked in the binding site of the protein using the aforementioned method (Figure 3b & 3d). Further, the Molecular Dynamics (MD) simulations were performed on the initially docked complexes of the enzyme with substrates and intermediates for predicting the preferable orientation using GPU based DESMOND[20]software.

The enzyme complex with substrates and intermediates were prepared by neutralizing the system through adding ions, salt and the SPC solvent. The prepared system was minimized with a 10ns time scale using OPLS2005[21]force field. Later relaxation procedure NVT and NPT, each with 10ns time scale were carried out on the minimized system. Finally, the MD simulations were performed with 50ns time scale. (The detailed procedure of molecular docking and MD simulations has discussed in the supporting information). From the computational studies, it was observed that the 2-aminopyridine substrate forming a hydrogen bond with Thr40 (2.51 Å) and Ser105 (2.59 Å) residues as a result getting a better orientation and increasing nucleophilicity to react as a nucleophile in the protein environment (Figure 3a). The benzaldehyde substrate interacts with Ser105 (1.61 Å) residue through a strong hydrogen bond and became a better electron accepter (Figure 3a) whereas tert-butyl isocyanide did not show any hydrogen bonding (Figure 3c),

however, there is a possibility of forming π- cation interaction, which has a long bond length than the threshold, therefore ignored. Interestingly, we found that the imine intermediate (i) showed strong interaction with Thr40 (2.52 Å) and Ser105 (2.59 Å) residues so becoming a good electrophile for the addition of tert-butyl isocyanide (Figure 3b). Further, the addition of tert- butyl isocyanide on the imine intermediate (i) altered the orientation of intermediate (ii), as a result, no interaction remained between intermediate (ii) and Ser105 residue (Figure 3d). Based on these results, we proposed a mechanism for the CALB catalyzed GBB- reaction (Scheme 3). The first step is formation of imine intermediate (i) by the reaction of 2-aminopyridine and benzaldehyde which was facilitated via Ser105 residue through a strong hydrogen bond with carbonyl oxygen and nitrogen of 2- aminopyridine as a result making the benzaldehyde a better electrophile and increasing

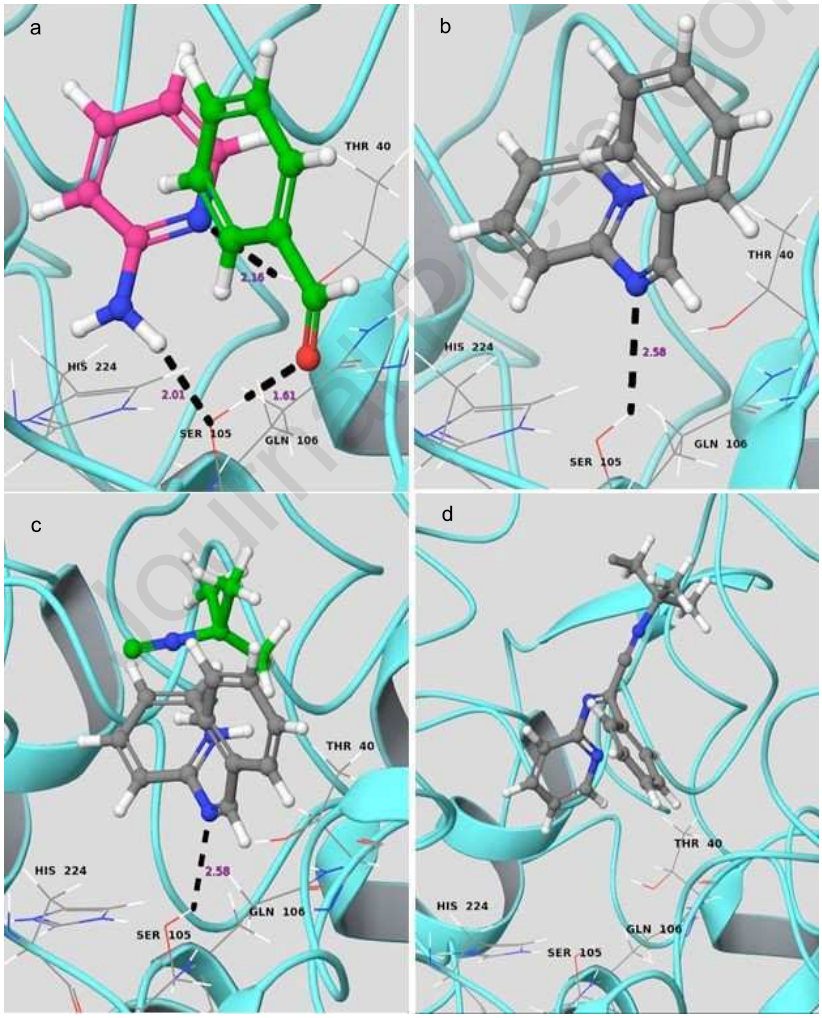
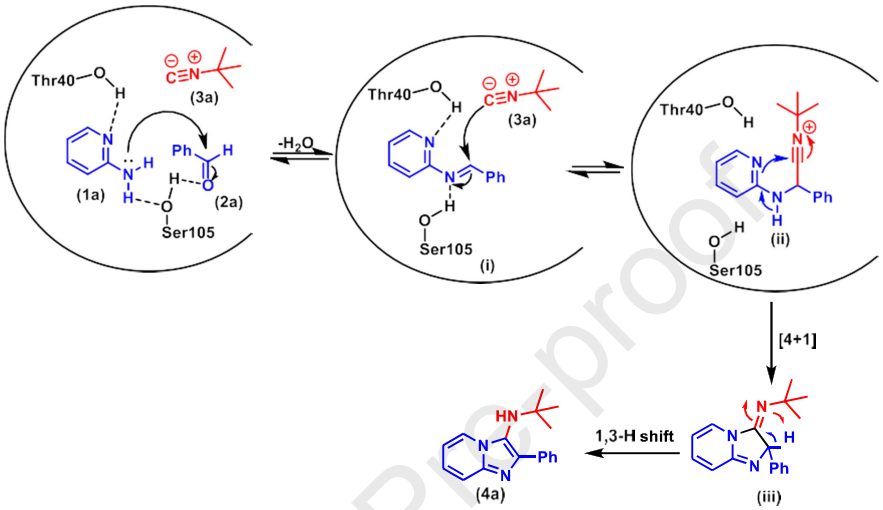


Figure 3: Orientation and interactions of substrates and intermediates in the binding site of CALB enzyme (a) 2-aminopyridine & benzaldehyde substrates (**b)** imine intermediate (i) formed by the reaction of 2-aminopyridine & benzaldehyde (c) Orientation of isocyanide (**d)** 2-methyl-N-(2-phenyl-2-(pyridinylamino)ethylidyne)propan-2-aminium (ii) formed by the addition of *tert*-butyl isocyanide on imine intermediate.

the nucleophilicity of 2-aminopyridine in protein environment. In the next step imine intermediate (i) was attacked by tert-butyl isocyanide, this nucleophilic addition was also assisted through Ser105 residues by making a strong hydrogen bond with the imine nitrogen. The subsequent intermediate (ii) did not show any interaction with the residues in the active site and provides the final product (4a) via [4+1] cycloaddition followed by 1, 3-proton transfer. Moreover, the formation of final product (4a) from intermediate (ii) may take place either in the active site of the enzyme or outside of the active site.



Scheme 3: Plausible mechanism of CALB-catalyzed GBB-multicomponent reaction

To get more evidence about the role of Thr40 and Ser105 residues in this transformation, we also investigated the substrates 5-bromo-2-aminopyridine and 2-bromobenzaldehyde (Figure 4a) those did not show any reaction in previous experiment under the optimized reaction conditions (entry 12, Table 4). We also docked the imine intermediate formed by the reaction of these substrates (Figure 4b). Interestingly, the imine intermediate formed by the reaction of 5-bromo-2-aminopyridine and 2-bromobenzaldehyde substrates did not show any interactions with Thr40 sand Ser105 residues as a result no GBB-reaction was occurred (Figure 4b). This gave evidence that Thr40 and Ser105 residues play a crucial role in the activation of imine intermediate to facilitate the addition of isocyanide.

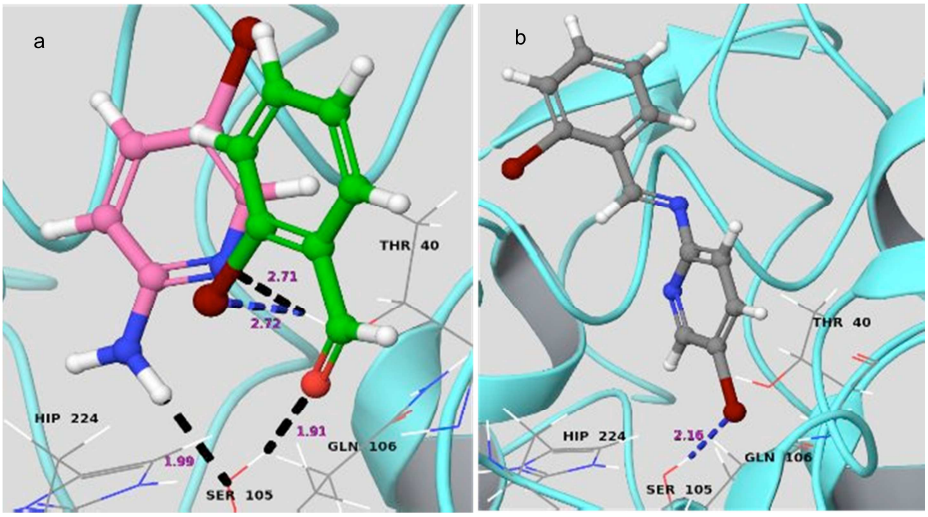


Figure 4. Orientation and interactions of substrates and intermediates in the binding site of CALB enzyme (a) Orientation of 5-bromo-2-aminopyridine and 2-bromobenzaldehyde (b)orientation of imine intermediate formed by the reaction of 5-bromo-2-aminopyridine & 2- bromo benzaldehyde**.**

Conclusion

In summary, we have developed first biocatalytic Groebke-Blackburn-Bienaymé (GBB) multicomponent reaction to synthesize fused imidazo[1,2-*a*]pyridine derivatives using *Candida antarctica* lipase B (CALB) enzyme. Also, various substitutions were tolerated well during the enzymatic synthesis and provided imidazo[1,2-*a*]pyridine derivatives in good to excellent yield. Further, CALB enzyme was immobilized on mesoporous silica and used as reusable catalyst which displayed high catalytic efficiency up-to many cycles. A preliminary mechanistic studies including molecular docking and molecular dynamics (MD) simulation revealed that Thr40 and Ser105 residues played a crucial role in catalyzing the GBB-multicomponent reaction, however further studies such as Quantum Mechanical/ Molecular Mechanical (QM/MM) approach to get more insights about the mechanism of this transformation are under progress in our lab. Finally, this work contributes to expand the number of enzymatic transformations to synthesize clinically important heterocycles.

Experimental Section

General information: All lipases, chemicals and solvents used in this project were commercially purchased and used without extra purification. Lipases (EC 3.1.1.3) like *Candida antarctica* lipase B (bought from Sigma-Aldrich, product number: L3170), *Rhizomucor miehei, Aspergillus niger, Candida rugosa,* APTES (3-aminopropyl triethoxysilane) and TEOS (tetraethoxyorthosilicate) were purchased from various suppliers. The reaction progress was monitored using thin layer chromatography (TLC: thin silica layer coated on glass slide). The compounds were purified by column chromatography using silica of mesh size 60-120 and ethyl acetate in hexane as mobile phase. A JEOL NMR spectrometer was used to study proton and carbon NMR spectrum of products at frequency 400 and 100 MHz using $CDCl_3$ with TMS as internal reference. The Coupling constant (J) is expressed in Hertz (Hz) and Chemical shift (δ) is expressed in parts per million (ppm). Multiplicities are abbreviated as s: singlet, d: doublet, dd: doublet of doublet, t: triplet, brs: broad singlet. The scanning electron microscopy- electron diffraction X-ray analysis (SEM-EDS) of catalyst was done using JEOL microscope. The X-ray analysis was done using X-ray Diffractometer (PanAlytical). The Fourier transform infrared (FT-IR) spectrum was collected using Perkin Elmer Spectrum version 10.4.00. High resolution Transmission electron Microscope (HR-TEM) was done using JEOL microscope.

General procedure for the CALB-catalyzed GBB-reaction:

In a glass tube having a teflon cap and a stirrer bar added 2-aminopyridine **(1a)** (50 mg, 0.531 mmol, 1.0 equiv.), benzaldehyde **(2a)** (67.61 mg, 0.637 mmol, 1.2 equiv.)

and tert-butyl isocyanide **(3a)** (72.5 mg, 0.637 mmol, 1.2 equiv.) in 2 ml of ethanol as a solvent. Thereafter, 40 mg of enzyme (CALB) was added and the reaction mixture was stirred on a magnetic stirrer at room temperature for overnight and the progress of reaction was monitored using TLC. After completion of the reaction as indicated by TLC, the resulting mixture was filtered through a small pad of cellite followed by washing of the cellite pad with ethyl acetate (2x5 ml). The solvent was evaporated under reduced pressure and the residue was purified by column chromatography using ethyl acetate in hexane as eluents affording the corresponding products **4a-4i** in 55-91% yields.

Procedure for the CALB@SiO_2 catalyzed GBB-reaction:

In a glass tube having a teflon cap and a stirrer bar added 2-aminopyridine **(1a)** (100 mg, 1.06 mmol, 1.0 equiv.), benzaldehyde **(2a)** (129.7 mg, 1.27 mmol, 1.2 equiv.) and tert-butyl isocyanide **(3a)** (144.8 mg, 1.27 mmol, 1.2 equiv.) in 2 ml of ethanol as a solvent. Thereafter, 50 mg of CALB@SiO_2 was added and the reaction mixture was stirred on a magnetic stirrer at room temperature for overnight and the progress of reaction was monitored using TLC. After completion of the reaction as indicated by TLC, the resulting mixtures was transferred to the 10 ml tube and centrifuge for 20 min. @ 10,000 RPM, the CALB@SiO_2 catalyst precipitate as a pellet which was reused for further catalytic cycle and the supernatant was used to extract crude product using ethyl acetate. Next, the solvent was evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (eluent: hexane/ EtOAc) affording the corresponding product 4a in 90 % conversion yield (*same procedure was followed for reusability experiments*).

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Conflicts of interest:

The authors declare no conflicts.

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Highlights:

- First biocatalytic synthesis of clinically important imidazo[1,2-a]pyridine
- CALB catalyzed Groebke-Blackburn-Bienaymé multicomponent reaction
- Immobilization of CALB@SiO_2 as reusable catalyst
- Molecular docking and molecular dynamics (MD) simulation studies suggested Thr40 and Ser105 residues play an important role in catalysis.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: