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Nano CaO: Synthesis, characterization, and application as an efficient catalyst for the preparation of tetrazole analogues of protected amino acids

H.S. Lalithamba[a,*], M. Raghavendra[a], R. Bharath[a], H.K.E. Latha[b], and N. Bharath[a]

a. *Department of Chemistry, Siddaganga Institute of Technology, B.H. Road, Tumakuru-572 103, Karnataka, India.*
b. *Department of Electronics & Instrumentation Engineering, Siddaganga, Institute of Technology, B.H. Road, Tumakuru-572 103, Karnataka, India.*

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Black pepper seed extract.

Abstract. Nano CaO as a recyclable heterogeneous catalyst was synthesized via solution combustion method using *Black pepper* seed extract as a fuel and calcium nitrate as a source of calcium. It can also be characterized by various techniques such as X-Ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy (FT-IR), Energy Dispersive X-Ray (EDAX), and Scanning Electron Microscope (SEM) analyses. The proposed catalyst plays a superior catalytic role in the synthesis of tetrazole analogues of protected amino acids in good yield via cycloaddition reaction of sodium azides with protected nitriles in methanol and water system under reflux conditions. The synthesized *N*-protected tetrazoles were characterized by mass, FT-IR, ^{1}H NMR, and ^{13}C NMR. The experiment is simple, eco-friendly, takes shorter reaction time and low-cost catalyst.

1. Introduction

Tetrazole is an important five-membered heterocycle with poly-nitrogen electron-rich planar structural features. Structural alteration of amino acids and peptides is a traditional method used for synthesizing new conjugates as potential biologically active compounds. A promising reason behind the need to make such a modification is tetrazole fragment, which can be regarded as an analogous and stable substitute for carboxamide or carboxy group [1]. Being an interesting class of heterocyclic compounds, tetrazoles enjoy extensive applications as components of highly effective explosives, pyrotechnics, propellants [2], and drugs in pharmaceuticals and bioisosteres for carboxylic acids. They are important precursors in medicinal chemistry owing to their increased resistance to metabolic degradation pathways [3]. Tetrazoles have also been found as *cis*-peptide bond mimics and bioisosteres for carboxylic acids, and their applications extend to the synthesis of HIV-protease inhibitors and anti-inflammatory agents [4–7]. It was shown that these properties of tetrazolic rings might promote the substrate-receptor interaction [8]. Thus, synthetic strategies leading to the replacement of car-

*. *Corresponding author. Fax: 0816-2282994*
E-mail addresses: lalithambasit@yahoo.co.in and hslalithamba@gmail.com (H.S. Lalithamba); raghu1289@hotmail.com (M. Raghavendra); bharath0896@gmail.com (R. Bharath); lathahke@gmail.com (H.K.E. Latha); dhamaruga93@gmail.com (N. Bharath)

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boxylate groups by tetrazole moieties in biologically active molecules are of current interest. Heterocyclic chemistry is experiencing a dramatic change with the organometallic reaction approach to heterocycle creation and carbosubstitution. Heterocyclic compounds containing one or more heteroatoms like oxygen (O), nitrogen (N), or sulfur (S) in a ring are classified based on their electronic structure. The study of heterocyclic chemistry particularly focuses on unsaturated derivatives, and applications involve unstrained five and six membered rings. Tetrazoles under study include five-membered heterocyclic compounds characterized by polynitrogen electron-rich planar structural features and increasingly popular functionality [9]. This unique arrangement makes tetrazole derivatives valuable drugs such as antineoplastic, anti-inflammatory, antifungal, and antiviral [10,11]. Tetrazoles find a broad range of applications in the fields of agriculture, material science, biochemistry, and photography [12,13]. For these reasons, it is important to develop new synthetic protocols and to advance the classical routes for the preparation of tetrazole analogues.

The chemistry of such heterocyclic compounds has been a fascinating field of study. The growth of new protocols for the synthesis of tetrazoles and the advancement of known methods of their preparation have been achieved in the framework of two approaches: heterocyclization of readily available nitrogen containing substrates and functionalization of heterocycles with substituents. A relatively modest method was established in the early 1970s that allowed the synthesis of tetrazole, its 1-mono, and 1,5-disubstituted analogues using a three-component heterocyclization reaction of primary amines or their salts with ortho esters and sodium azide in the presence of acetic acid medium [14]. Himo et al. achieved a novel method for the synthesis of tetrazole derivatives with the addition of NaN_3 to nitriles employing stoichiometric amounts of salts in water [15,16]. Further, conventionally, 5-substituted tetrazoles were synthesized by [2 + 3] cycloaddition of an azide and a nitrile [17]. Tetrabutylammonium Fluoride (TBAF) is employed as a catalyst in the [3 + 2] cycloaddition reaction of nitriles with trimethylsilyl azide ($TMSN_3$), as presented by Amantini et al. [18]. Montmorillonite KSF is an efficient heterogeneous catalyst for the cycloaddition of NaN_3 with a variety of nitriles to afford 5-substituted 1*H*-tetrazoles in excellent yields [19]. In addition to Zn/Al, a variety of catalysts such as tributylmethylammonium chloride [20], $Pd(PPh_3)_4$ [21], nano ZnO anchored on the reduced graphene oxide [22], Metal triflate [23], graphene oxide-based solid acid carbocatalyst [24], phosphorazidates [25], and $Gd(III)/Fe_3O_4$ [26] were used for the synthesis of tetrazole analogues. Preparation of 5-substituted 1-*H*-tetrazole derivatives was achieved using heterogeneous Cu-based catalyst [27]. Tetrazole analogues of amino acids were also reported to be prepared starting from *N*-Fmoc amino acid in a three-step protocol [28]. MCM-41-SO_3H catalyzed synthesis of 5-substituted-1*H*-tetrazole derivative was described [29]. At present, the use of eco-friendly solid catalysts to reduce the quantity of toxic waste has received significance. The expansion of innovative procedures for the synthesis of heterogeneous catalysts is of immense importance in synthetic chemistry. Nano metaloxides have been efficiently used as catalysts for organic transformation. These reactivities result from high surface areas combined with unusually reactive morphologies. In this context, nanomaterials supporting catalytic systems have sparked much interest owing to their exceptional properties such as ease of chemical inertness, high surface area to volume ratio, and good thermal stability. Taghavi et al. achieved 5-substituted 1*H*-tetrazole derivatives using Cu(II) immobilized on Fe_3O_4@APTMS-DFX nanoparticle for the [2 + 3] cycloaddition of NaN_3 with nitriles [30]. Similarly, [3 + 2] cycloaddition reaction of aldoxime derivatives with sodium azide (NaN_3) was studied in the presence of nano-sized $Ni(OH)_2$ as an efficient recoverable catalyst [31]. Many catalysts in the literature exist that are still subject to a few limitations including expensive reagents, tedious separation, and prolonged reaction times. Due to the extensive applicability of tetrazole-embedded structures, convenient and economical synthetic routes are still worth exploring. Herein, a new, simple, convenient, and greener protocol is reported to synthetize tetrazoles from N^{α}-Fmoc/Cbz amino acids using nano CaO as a catalyst.

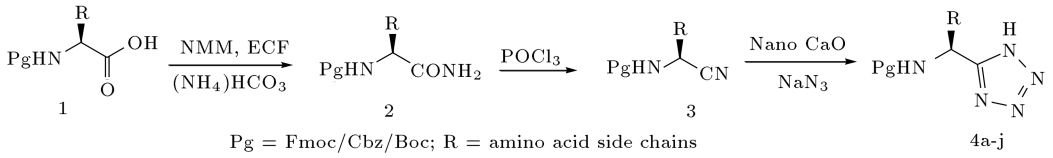
In the present work, the green synthesis of nano CaO via solution combustion synthesis is proposed using extract of *Black pepper* seeds. *Black pepper* is known as peppercorn or piper nigrum. *Black pepper* is composed of carbohydrate (37.4%), proteins (25.5%), fibre (23.6%), and fat (5.3%) acting as good fuels for the preparation of nanoparticles. Hence, this study attempts to develop the extract of *Black pepper* seeds as a reducing agent for the synthesis of nano CaO.

2. Results and discussion

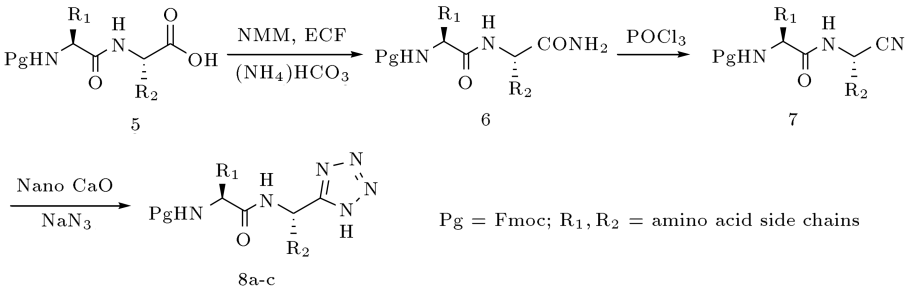
An effective, convenient approach to the preparation of nano CaO from the aqueous solution of $Ca(NO_3)_2.6H_2O$ using an aqueous extract of *Black pepper* seeds is presented. The prepared nano CaO exhibited strong catalytic activities for the synthesis of tetrazole derivatives of protected amino acids. Overall, the proposed green synthetic protocol is simple, efficient, and eco-friendly because it does not require extra surfactants or reductants.

2.1. Chemistry

To avoid drawbacks, like cost effectiveness, harsh



Scheme 1. Synthesis of N-protected tetrazole analogues of amino acids.



Scheme 2. Synthesis of N^{α}-Fmoc-protected tetrazole peptide hybrids.

reaction conditions, low yields, and long reaction times, this study attempted to develop well-organized routes with high yields for the synthesis of protected tetrazoles in the presence of nano CaO. Hence, the use of plants as a natural source for the preparation of nanomaterials remains our main interest. In continuation of our research on heterogeneous catalysts, we report a new protocol for the preparation of nano CaO by *Black pepper* seed extract and its catalytic applications as a catalyst for the synthesis of protected tetrazoles. The protocol is established based on a three-step strategy, involving a direct amidation of the acid group by ammonium bicarbonate with N-methylmorpholine (NMM) and ethyl chloroformate (ECF) followed by a reaction with $POCl_3$ leading to the formation of nitrile. Finally, this is coupled with sodium azide to get the desired tetrazole derivatives using nano CaO. In this respect, a solution of Fmoc/Cbz protected amino acid, N-methylmorpholine, and ethyl chloroformate in Tetrahydrofuran (THF) solvent was cooled to –10–15°C. $(NH_4)HCO_3$ was added after stirring for 20 min, and stirring continued for another 4 h. The solvent was removed and the residue was dissolved in EtOAc. After the simple workup and evaporation of the solvent, the pure amide was obtained in good yield. To the synthesized protected amino amide in THF, triethyl amine, $POCl_3$, and sodium carbonate were added and stirred for 5 h at room temperature. The solvent was removed *in-vacuo*, and the residue was dissolved in ethyl acetate and washed with dilute HCl solution, Na_2CO_3 solution, water, and brine. Finally, getting dried over anhydrous Na_2SO_4, the solvent was removed to obtain nitrile. N-protected-amino nitrile, NaN_3, and nano CaO were added to a mixture of methanol and water and stirred at reflux for 6 h. The reaction was monitored by TLC using chloroform: methanol (9:1) as eluent. After a simple workup, analytical-grade products of tetrazole derivatives of protected amino acids (**4a**-**4j**) were obtained (Table 1) (Scheme 1). The synthesized compounds were characterized by mass, ^{1}H NMR, ^{13}C NMR, and Fourier-Transform Infrared Spectroscopy (FT-IR) spectroscopy. The current protocol was examined for possible racemization through RP-HPLC analysis. To do so, a pair of epimeric tetrazoles ranging from Fmoc-L-Phe-OH to Fmoc-D-Phe-OH were prepared and analyzed through RP-HPLC. The RP-HPLC profiles of these two epimers had an exclusive major peak at R_t values of 16.7 and 16.9 min, respectively, while the equimolar mixture of these two epimers had two well-separated peaks at R_t 16.7 and 16.9 min (Spectrum 20). This confirmed that the reaction sequence was free from racemization.

Moreover, the protocol was extended to the preparation of N^{α}-Fmoc-protected tetrazole peptide hybrids (Table 2) starting from Fmoc-dipeptide acids via Scheme 2. N^{α}-Fmoc-peptide acid was prepared through the reaction of amino acid with trimethylsilyl chloride (TMS-Cl) and triethylamine (TEA) in DCM to get *O*,*N*-*bis*-trimethylsilyl amino acid. This was trapped with N^{α}-Fmoc-protected amino acid mixed anhydride. The dipeptides were converted into their respective amide derivatives using ammonium bicarbonate in the presence of NMM/ECF, followed by treatment with $POCl_3$ to yield nitrile and then, subjected to cycloaddition using sodium azide employing nano CaO to afford the tetrazole hybrids linked to peptidomimetics (**8a**-**8c**) in about 90% yield. The final tetrazole peptide hybrids were characterized using spectral techniques.

The reaction was optimized using different catalysts such as zinc bromide, tetrabulylammonium bromide (TBAB), and aluminium chloride as substitutes for nano CaO keeping the same solvent mixture. However, the yields in these cases were only up to 70–

Table 1. List of *N*-protected tetrazole analogues via Scheme 1.

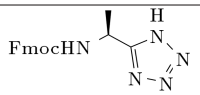
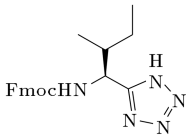
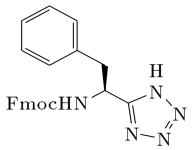
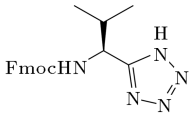
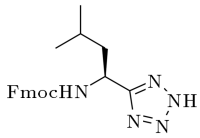
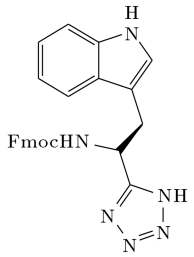
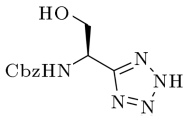
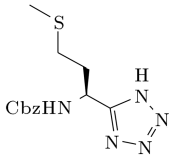
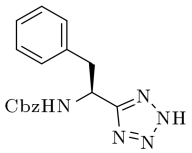
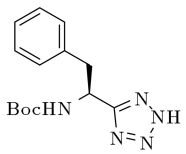
Entry	Tetrazoles	Yield (%)	M.p. (°C)	$[\alpha]_D^{25}$ (deg)
4a		89	190-192	−41.08
4b		91	202-204	−25.56
4c		91	198-200	−85.33
4d		90	188-190	−15.57
4e		88	169-171	−67.39
4f		86	198-200	−53.46
4g		85	Gum	−14.66
4h		80	Gum	−21.64
4i		88	Gum	−22.78
4j		70	146-148	−12.08

Table 2. List of N^{α}-Fmoc-protected tetrazole peptide hybrids via Scheme 2.

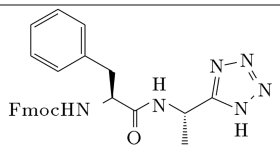
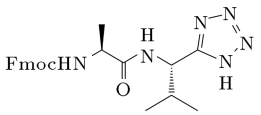
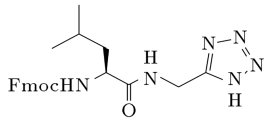
Entry	Tetrazoles	Yield (%)	M.p. (°C)
8a		88	Gum
8b		90	Gum
8c		89	Gum

Table 3. Comparison of the impact of various reaction parameters in the synthesis of tetrazole derivatives.

Solvent (ratio)	T (°C)	Catalyst	Reaction time (h)	Yield (%)
Water/methanol (2:1)	rt	$FeCl_3$	8	No reaction
Water/methanol (2:1)	60	$FeCl_3$	16	70–75
Water/methanol (2:1)	80	TBAB	16	74–78
Water/methanol (2:1)	rt	TBAB	8	No reaction
Water/methanol (2:1)	60	$AlCl_3$	16–18	70–74
Water/methanol (2:1)	80	$AlCl_3$	16–18	76–80
Water/methanol (2:1)	rt	Nano CaO	8	No reaction
Water/methanol (2:1)	60	Nano CaO	6	70–84
Water/methanol (2:1)	80	Nano CaO	6	86–94

82% even with higher equivalent. As demonstrated by the results (Table 3), the higher yields of protected tetrazoles in a shorter reaction time were achieved utilizing nano CaO. Thus, the development of an easy, safe, and fast route for the synthesis of tetrazole derivatives employing nano CaO was achieved. The overall course of nano CaO catalyzed reactions was most efficiently compared to other catalysts starting from the corresponding protected amino acid.

2.2. Morphological and structural characterization of nano CaO X-ray diffraction analysis

Nanoparticles were characterized using X-Ray Diffraction (XRD) and identified as crystalline. Figure 1 shows the XRD analysis of nano CaO to determine its crystalline structure. The sharp diffraction lines indicate high crystallinity of the sample. The existence of strong diffraction peaks corresponding to (111), (200), (220), (311), and (222) planes indicates the formation of CaO. CaO diffraction peaks were in compliance with the Powder Diffraction Standards set by the Joint Committee, JCPDS data file: 77- 2376].

Debye-Scherrer's equation was employed to calculate the average crystallite size of the prepared sample, i.e.:

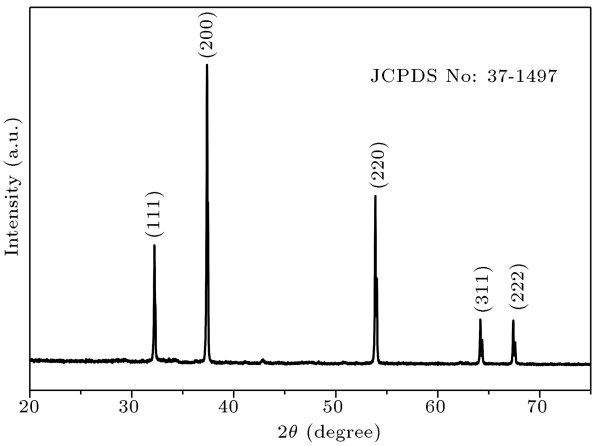


Figure 1. XRD pattern of nano CaO.

$$D = \frac{K\lambda}{\beta \cos\theta},$$

where D is crystalline size, K Scherrer constant, λ X-ray wavelength, β full-width at half-maximum, and θ Bragg's angle. Debye-Scherrer's calculations reveal

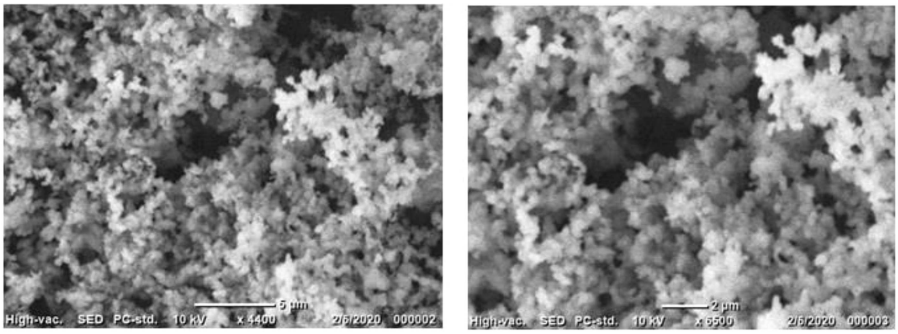


Figure 2. SEM images of nano CaO.

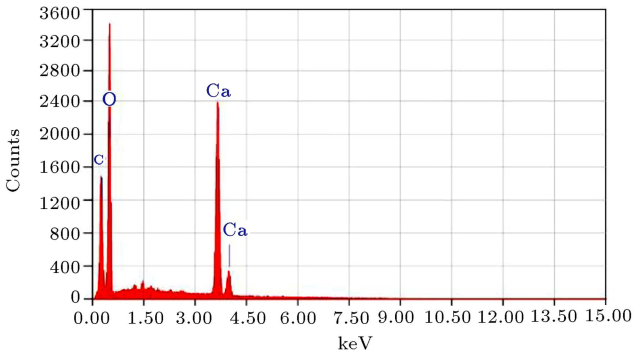


Figure 3. EDAX spectrum of nano CaO.

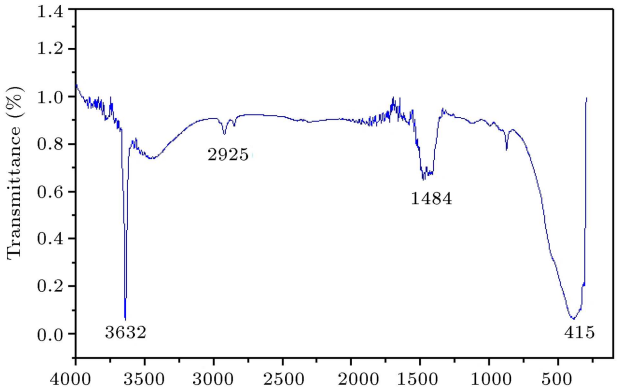


Figure 4. FT-IR pattern of nano CaO.

that the average crystallite size of the nano CaO was found to be 40 nm.

The morphology of the material can be recorded in the form of Scanning Electron Microscope (SEM) images. Figure 2 shows the images of nano CaO visualized by SEM. Analysis of the morphological aspect of nanoparticles based on their images indicates that the average size of the synthesized nano CaO is 40.0 nm. It can be demonstrated that the nanoparticles are agglomerated because of the highly hygroscopic nature of nano CaO.

The spectrum of Energy Dispersive X-ray (EDAX) analysis (Figure 3) shows the existence of Ca and O at appropriate concentrations. The atomic % of Ca and O elements in CaO is found to be 42.85 and 56.26, respectively, and it shows the stoichiometric relationship (1:1) between calcium and oxygen.

Metal oxide bond stretching frequencies were analyzed by FT-IR. A prominent peak at 415 cm^{-1} in the FT-IR spectrum (Figure 4) was attributed to the stretching vibrations of Ca-O bonds. The peak observed at 1484.0 cm^{-1} corresponds to the carbonyl group, and that at 3632.0 cm^{-1} corresponds to the presence of -OH stretching and deformation, respectively, due to water adsorption on the surface of metal.

3. Experimental

3.1. General

All chemicals were purchased from Sigma-Aldrich and used without purification. The solvents were distilled prior to use. The Black pepper seeds were collected from the Tumakuru market (India). Powder XRD data were recorded on Shimadzu X-ray diffractometer (PXRD-7000) using Cu-K∞ radiation of wavelength $\lambda = 1.541$ Å. Morphological features were studied by using Tescan Vega 3 LMU SEM. IR spectra were recorded on Bruker Alpha-T FT-IR Spectrometer (KBr windows, 2 cm^{-1} resolution). Melting points were taken in open capillaries and uncorrected. TLC analysis was carried out using precoated silica gel F_{254}. ^{1}H NMR and ^{13}C NMR spectra were done on a Bruker AMX 400 MHz spectrometer using Me4Si as an internal standard and DMSO/$CDCl_3$ as a solvent. Mass spectra were recorded on a Micromass Q-ToF micro mass spectrometer.

3.2. Synthesis of nano CaO using extract of Black pepper seeds

Nano CaO was prepared by combustion method using aqueous *Black pepper* seed extract and $Ca(NO_3)_2.6H_2O$. In the solution combustion method, the reaction mixture was prepared by treating *Black pepper* seed extract and $Ca(NO_3)_2.6H_2O$ as a source of calcium in a beaker and was stirred for few minutes until a uniform homogenous solution was formed. This reaction mixture was kept in a pre-heated muffle furnace maintained at 450°C. Within 30 min, nano CaO was formed and further calcined at 800°C. The

acquired particles were stored in a desiccator for further research.

3.3. General procedure for the synthesis of Fmoc/Cbz/Boc amino/peptide hybrid acid amides

A solution of Fmoc/Cbz/Boc protected amino/peptide acid (1 mmol), *N*-methylmorpholine (1.1 mmol), and ethyl chloroformate (1.1 mmol) in THF (5 mL) was cooled to –15°C. After stirring for 20 min at the same temperature, ammonium bicarbonate was added and stirring continued for another 4 h. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate. The organic layer was washed with dilute HCl solution, Na_2CO_3 solution, water, and brine and finally, it was dried over anhydrous Na_2SO_4. After the evaporation of the solvent, the amide was obtained as pure solid.

3.4. General procedure for the synthesis of Fmoc/Cbz/Boc amino/peptide hybrid nitriles

To a solution of Fmoc/Cbz/Boc protected amino amide (1 mmol) in THF (5 mL), triethyl amine (1.5 mmol), phosphorous oxychloride ($POCl_3$) (1.5 mmol), and sodium carbonate (1.5 mmol) were added and stirred for 5 h at room temperature. The solvent was removed in vacuo, and the residue was dissolved in ethyl acetate. The organic layer was washed with dilute HCl solution, Na_2CO_3 solution, water, and brine. Finally, dried over anhydrous Na_2SO_4, the solvent was removed to obtain the nitrile.

3.5. General procedure for the synthesis of Fmoc/Cbz/Boc amino/peptide hybrid tetrazoles (4a-j, 8a-c)

Fmoc/Cbz/Boc amino/peptide hybrid nitrile (1 mmol), sodium azide (2 mmol), and nano CaO (0.5 mmol) were added to a mixture of methanol (15 mL) and water (30 mL) and stirred at reflux for 4–5 h. Progress of the reaction was monitored by TLC using chloroform-methanol (9:1) as eluent. Following the completion of the reaction, dilute HCl solution and 20 mL of ethyl acetate were added and stirring continued until no solid remained. The aqueous layer was extracted twice with ethyl acetate. The combined organic layer was washed with water and dried over anhydrous Na_2SO_4. The solvent was removed in vacuo, and the residue was recrystallized from EtOAc-hexane (1:4).

3.6. Spectral data of the synthesized compounds (4a-4j, 8a-8c)

- (S)-(9H-fluoren-9-yl)methyl-1-(1H-tetrazol-5-yl) ethyl carbamate (**4a**):
  % Yield 89, Melting point 190–192°C. ^{1}H NMR (DMSO-d_6) δ 0.84–0.85 (*d*, J = 4.0 Hz, 3H), 4.18–4.41 (m, 3H), 5.10–5.60 (t, J = 8.0 Hz, 1H), 6.40 (br, 1H), 7.30–7.87 (m, 8H), 8.10 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 42.08, 43.13, 46.67, 46.71, 127.0, 127.54, 127.58, 143.76, 154.96, 155.51, 156.60; Calculated mass for $C_{18}H_{17}N_5O_2$ m/z 358.1 $[M+Na]^+$, observed mass: 358.2322.
- (S)-(9H-fluoren-9-yl)methyl-3-methyl-1-(1H-tetrazol-5-yl)butyl carbamate (**4b**):
  % Yield 91, Melting point 202–204°C. ^{1}H NMR (DMSO-d_6) δ 0.86–0.94 (m, 6H), 1.16–1.23 (m, 2H), 2.0 (m, 1H), 4.24–4.36 (m, 3H), 4.79 (br, 1H), 7.28–7.82 (m, 8H), 8.0 (br, 1H); ^{13}C NMR (DMSO-d_6) δ 31.31, 110.71, 126.88, 127.28, 128.24, 128.92, 129.10, 129.88, 130.06, 138.29, 140.28, 143.42, 152.15, 157.01, 171.40; Calculated mass for $C_{21}H_{23}N_5O_2$ m/z 400.1 $[M+Na]^+$, observed mass: 400.2525.
- (S)-(9H-fluoren-9-yl)methyl-2-phenyl-1-(1H-tetrazol-5-yl)ethyl carbamate (**4c**):
  % Yield 90, Melting point 198–200°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 3.31 (d, J 6.8 Hz, 2H), 4.12–4.21 (m, 3H), 5.07–5.11 (m, 2H), 5.15 (br, d, J 6.8 Hz, 1H), 7.22-7.87 (m, 13H), 8.0 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 40.00, 46.52, 50.06, 65.71, 127.02, 127.06, 127.61, 127.65, 128.22, 128.37, 129.19, 129.34, 143.66, 145.72, 155.60; Calculated mass for $C_{24}H_{21}N_5O_2$ m/z:434.1593 (M^++Na), observed mass: 434.1690.
- (S)-(9H-fluoren-9-yl)methyl-2-methyl-1-(1H-tetrazol-5-yl)propyl carbamate (**4d**):
  % Yield 90, Melting point 188–190°C. ^{1}H NMR ($CDCl_3$) δ 1.14–1.17 (6H, d, J = 12.0 Hz), 2.58-2.95 (1H, m), 4.0–4.36 (4H, m), 6.51 (1H, br), 7.30–7.88 (8H, m), 8.29 (1H, s); ^{13}C NMR ($CDCl_3$) δ 19.59, 39.67, 39.94, 40.22, 40.50, 120.06, 125.17, 126.98, 127.50, 140.70, 143.60, 143.80, 160.00; Calculated mass for $C_{20}H_{21}N_5O_2$ m/z 386.1 $[M+Na]^+$, observed mass: 386.1000.
- (S)-(9H-fluoren-9-yl)methyl-2-(4-hydroxyphenyl)-1(1Htetrazolyl)ethylcarbamate (**4e**):
  % Yield 86; Melting point 169-171°C; ^{1}H NMR ($CDCl_3$) δ 0.94–0.96 (t, J = 6.8 Hz, 6H), 1.67 (m, 1H), 1.97 (t, J = 8.4 Hz, 2H), 4.18 (t, J = 6.0 Hz, 1H), 4.52 (d, J = 5.9 Hz, 2H), 5.03 (m, 1H), 5.40 (br, d, J = 6.2 Hz, 1H), 7.22–7.62 (m, 8H), 7.82 (d, J = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 21.8, 24.0, 44.2, 46.6, 65.5, 120.0, 125.1, 126.9, 127.5, 127.8, 140.7, 143.6, 155.8, 158.1; Calculated mass for $C_{21}H_{23}N_5O_2$ m/z 400.1001 $[M+Na]^+$, observed mass: 400.2080.
- (R)-(9H-fluoren-9-yl)methyl-2-(1H-indol3yl) (1Htetrazol5yl)ethylcarbamate (**4f**):
  % Yield 86, Melting point 198–200°C. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 3.30-3.37 (m, 2H),

3.99–4.23 (m, 4H), 5.14–5.16 (br, d, J 6.8 Hz, 2H), 5.74 (s, 1H), 6.96–7.87 (m, 12H), 10.84 (s, 1H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 46.56, 54.10, 56.25, 65.74, 111.00, 111.42, 118.05, 118.44, 120.08, 120.97, 123.88, 125.20, 127.03, 127.61, 131.02, 140.66, 143.71, 155.69. Calculated mass for $C_{26}H_{22}N_6O_2$ m/z: 451.1882 (M^++H), observed mass: 451.1884.

- (R)-benzyl-2-hydroxy-1-(1H-tetrazol-5-yl) ethylcarbamate (**4g**):
% Yield 85, Gum. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.45 (s, 1H), 3.50 (s, 2H), 4.23–4.28 (t, J 6.6 Hz, 1H), 4.70 (s, 2H), 7.25–7.44 (m, 5H), 8.0 (s, 2H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 14.13, 67.39, 110.02, 132.31, 133.21, 133.52, 133.71, 142.07, 158.96, 161.00, 176.40. Calculated mass for $C_{11}H_{13}N_5O_3$ m/z: 264.10 (M^++H), observed mass: 263.0920.

- (S)-benzyl-3-(methylthio)-1-(1H-tetrazol-5-yl) propylcarbamate (**4h**):
% Yield 80, Gum. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 2.10 (s, 3H), 2.28–2.32 (m, 2H), 2.47–2.50 (t, J 6.8 Hz, 2H), 4.81–4.90 (t, J 6.8 Hz, 1H), 5.02 (s, 2H), 5.20 (s, 2H), 7.20–7.34 (m, 5H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 16.81, 29.00, 37.53, 52.18, 65.45, 127.0, 127.74, 129.00, 156.44. Calculated mass for $C_{13}H_{17}N_5O_2S$ m/z: 308.1182 (M^++H), observed mass: 308.2048.

- Benzyl (S)-(2-phenyl-1-(2H-tetrazol-5-yl)ethyl) carbamate (**4i**):
% Yield 88, Gum. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 3.13–3.31 (m, 2H), 4.82–5.13 (m, 2H), 5.14 (br, d, J 6.8 Hz, 2H), 7.21–7.73 (m, 10H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 40.08, 56.94, 61.95, 65.44, 126.56, 127.40, 127.52, 127.75, 128.00, 128.22, 128.28, 129.19, 136.78, 137.05, 155.64. Calculated mass for $C_{17}H_{17}N_5O_2$ m/z: 324.1460 (M^++H), observed mass: 324.1469.

- Tert-butyl (S)-(2-phenyl-1-(2H-tetrazol-5-yl)ethyl) carbamate (**4j**):
%Yield 70; Melting point 146–148°C. ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9H), 6.16 (d, J = 7.9 Hz, 1H), 7.24–7.42 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 36.09, 47.14, 65.59, 67.06, 127.11, 127.80, 128.28, 128.62, 171.66, 172.77; Calculated mass for $C_{13}H_{17}N_5O_2$ m/z 298.1000 $[M+Na]^+$, observed mass: 298.2001.

- (9H-fluoren-9-yl)methyl (S)-1-((S)-1-(1H-tetrazol-5-yl)ethylamino)-1-oxo-3-phenylpropan-2-yl carbamate (**8a**):
% Yield 88, Gum. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.38 (s, 2H), 3.20 (s, 2H), 3.70 (s, 3H), 4.37–4.50 (t, J 6.6 Hz, 2H), 4.62 (s, 3H), 4.76 (s, 2H), 5.20 (s, 2H). 7.25–7.68 (m, 10H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 47.0, 66.94, 68.20, 124.55, 124.76, 125.0, 127.06, 127.30, 128.22, 141.44, 156. Calculated mass for $C_{27}H_{26}N_6O_3$ m/z: 483.2104 (M^++H), observedv mass: 483.2120.

- (9H-fluoren-9-yl)methyl(S)-1((S)2methyl1 (1Htetrazol5yl)propylamino)-1-oxopropan-2-ylcarbamate (**8b**):
% Yield 90, Gum. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.04 (s, 6H), 2.40 (s, 2H), 3.75–3.78 (t, J 6.6 Hz, 2H), 3.90 (m, 2H), 4.68 (s, 2H), 5.0 (s, 2H), 7.20–7.80 (m, 10H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 47.12, 66.0, 68.18, 124.76, 124.76, 125.20, 127.30, 127.25, 128.20, 141.32, 156. Calculated mass for $C_{23}H_{26}N_6O_3$ m/z: 435.2145 (M^++H), observed mass: 435.1728.

- (S)-(9H-fluoren-9-yl)methyl-1-((1H-tetrazol-5-yl)methylamino)-4-methyl -1-oxopentan-2-ylcarbamate (**8c**):
% Yield 89, Gum. ^{1}H NMR (DMSO-d_6, 400 MHz): δ (ppm) 1.30 (s, 6H), 3.10 (s, 2H), 3.28–3.32 (t, J 6.6 Hz, 2H), 3.50 (s, 2H), 4.10 (s, 2H), 4.70 (s, 2H), 5.0 (s, 2H), 7.25–7.70 (m, 8H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ (ppm) 47.0, 66.94, 68.20, 124.74, 124.66, 125.10, 127.06, 127.27, 128.30, 141.34, 156.10. Calculated mass for $C_{23}H_26N6O_3$ m/z: 435.2112 (M^++H), observed mass: 435.2022.

3.7. Reusability and recyclability of nano CaO

Catalyst was reused and recycled without any loss of activity and product yield. Nano CaO can be recycled by a simple protocol after the completion of reaction. CaO was removed by filtration, washed with methanol, and dried. The recovered catalyst was reused for the second, third, and fourth consecutive cycles without any significant loss of catalytic activity.

4. Conclusion

In summary, this study established a simple, facile, and eco-friendly protocol for the synthesis of tetrazole analogues using nitrile and sodium azide employing nano CaO as an efficient catalyst. The synthesized nano CaO was characterized by X-Ray Diffraction (XRD), Energy Dispersive X-ray (EDAX), Scanning Electron Microscope (SEM), and Fourier-Transform Infrared Spectroscopy (FT-IR) techniques. This methodology may find widespread use in organic synthesis for the preparation of tetrazole derivatives. All the products were isolated after simple workup and were fully characterized by mass, IR, ^{1}H NMR, ^{13}C NMR spectroscopy.

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Biographies

Haraluru Shankaraiah Lalithamba was born in India in 1973. She received her BSc and MSc degrees in Chemistry and Organic Chemistry from Bangalore University, Bangalore, India in 1994 and 1996, respectively. Also, she received PhD degree in Peptides and Peptidomimetics from the same university in 2012. She is currently an Associate Professor at the Department of Chemistry, Siddaganga institute of Technology, Tumakuru, India. Her research interests include synthesis of biologically active peptides and peptidomimetics, biological activity, molecular docking, and nano metal oxides. She actively contributed to funded projects of VGST, Government of Karnataka on the synthesis of bioactive peptides and peptidomimetics research. She has published more than 35 scientific research articles in well-known journals.

Mahadevaiah Raghavendra was born in India in 1989 and is a PhD research scholar working under the supervision of Dr. Lalithamba. He received BSc and MSc degrees in Chemistry from Tumakuru University and Davanagere University in 2010 and 2012, respectively. His research interests include nano metal oxide, peptides, and peptidomimetics. He has published more than 10 scientific research articles in reputed journals. All the synthesized compounds presented in this research paper were prepared by him under the supervision of Dr. Lalithamba.

Rajanna Bharath was born in India in 1993 and is a PhD research scholar working under the supervision of Dr. Lalithamba. He received the BSc and MSc degrees in Chemistry from Tumakuru University and Mysore University in 2014 and 2016, respectively. His research interests include nano metal oxide, peptides, and peptidomimetics. All the synthesized compounds presented in this research paper were prepared by him under the supervision of Dr. Lalithamba.

Haraluru Kamala Eshwaraiah Latha was born in India in 1973. She received her BSc and MSc degrees in Instrumentation Technology and power Electronics from the Bangalore University, Bangalore, India in 1995 and 2000, respectively. Also, she received the PhD degree in Electrical and Electronics Engineering Sciences from the VTU in 2014. She is currently working as an Associate Professor at the Department of Electronics and Instrumentation Engg., Siddaganga Institute of Technology, Tumakuru, India. Her research interests include material science and development of thin film sensor. She actively contributes to funded projects of GTRE and VGST, Government of Karnataka on development of thin film sensors. She has published more than 15 scientific research articles in well-known journals

Nijalingappa Bharath was born in India in 1993 and is a PhD research scholar working under the supervision of Dr. Lalithamba. He received the BSc and MSc degrees in Chemistry from Tumakuru University and Davanagere University in 2014 and 2016, respectively. His research interests include nano metal oxide, peptides, and peptidomimetics. All the synthesized compounds presented in this research paper were prepared by him under the supervision of Dr. Lalithamba.