International Journal of Scientific Research in Science and Technology
Available online at : www.ijsrst.com
Print ISSN: 2395-6011 | Online ISSN: 2395-602X
doi : https://doi.org/10.32628/IJSRST25122212

Synthesis of Tetrazole Tethered Tetrahydrobenzo [B] Thiophene-3-Carbonitrile by Isocyanide-Based Condensation

Priyal Sukhwal, Akash Kalundha, Repale anil vithal, Dr. N.S Chundawat, Pathan Sultan Ismail, Dr. Girdhar Pal Singh

Department of Chemistry, Bhupal Nobles' University, Udaipur-313001, Rajasthan, India

ARTICLEINFO

Article History:

Accepted : 08 March 2025
Published: 10 March 2025

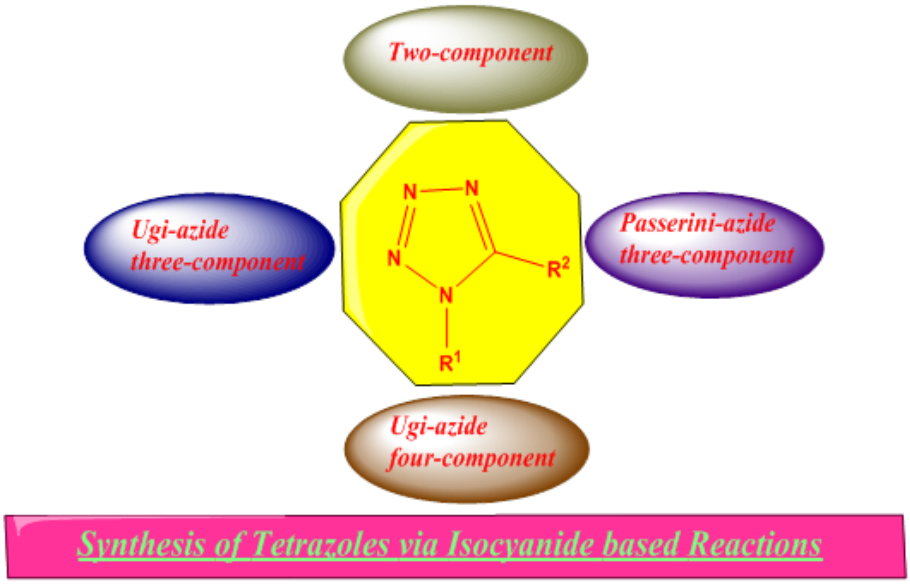
Publication Issue :
Volume 12, Issue 2
March-April-2025

Page Number :
234-243

ABSTRACT

Tetrazole-tethered tetrahydrobenzo[b]thiophene-3-carbonitrile derivatives have gained significant attention due to their diverse biological and pharmaceutical applications. In this study, we present an efficient synthesis of tetrazoles using an isocyanide-based multicomponent condensation strategy. The reaction involves a nitrile as precursor, isocyanide, aldehyde, and a sulfur source under optimized catalytic conditions, leading to the formation of highly functionalized frameworks. Furthermore, the cyclization of the nitrile with sodium azide facilitates the introduction of the tetrazole moiety, enhancing the molecular complexity. The developed methodology provides a facile, high-yielding, and sustainable approach to constructing novel heterocyclic scaffolds with potential medicinal relevance.

GRAPHICAL ABSTRACT



I. INTRODUCTION

Leishmania presents major challenges to public health, particularly in prevention, diagnosis, and treatment[1]. *Leishmania donovani*, the pathogen responsible for leishmaniasis[2], affects approximately 12 million people in nearly 90 countries, causing an estimated 51,000 deaths annually. This poses a significant health risk, especially in tropical and subtropical regions worldwide. The disease is primarily transmitted through the bites of infected phlebotomine sand flies[3]. Leishmaniasis presents in various clinical forms, ranging from mild cutaneous leishmaniasis (CL) to more severe manifestations such as mucocutaneous leishmaniasis (MCL) and [4]visceral leishmaniasis (VL), also known as kala-azar in India. If not treated promptly after the onset of clinical symptoms, active VL can be fatal. Caused by *Leishmania donovani* complex parasites, VL primarily affects tropical, subtropical, and temperate regions, with the highest number of cases reported in Bangladesh, Brazil, India, Nepal, and Sudan.

Symptoms of visceral leishmaniasis (VL) include hepatosplenomegaly, fever, anemia, immunosuppression, hypergammaglobulinemia, and weight loss. If not diagnosed and treated promptly, the disease can be fatal. *Leishmania* parasites employ various strategies to evade the immune response, particularly by interfering with host macrophage function. The outcome of the disease largely depends on the activation of parasite-protective molecules while simultaneously suppressing the host's protective microbicidal molecules and key cytokines, such as IFN-γ, IL-1, IL-12, and TNF.

Unfortunately[5], treatment options for leishmaniasis remain limited, with current drugs having been introduced over 50 years ago, each carrying significant drawbacks. First-line treatments like pentavalent antimony are associated with severe side effects, and resistance has rendered them ineffective in many regions of India. Second-line drugs, such as pentamidine and amphotericin B, are constrained by toxicity[6,7]. Liposomal amphotericin B is effective but costly, limiting its use in endemic areas. Miltefosine, the first oral treatment for visceral leishmaniasis (VL), presents reproductive toxicity risks for females of childbearing age[8,9]. Although clinical trials with injectable paromomycin show promise, there is a pressing need for a broader range of new drugs to combat resistance effectively.

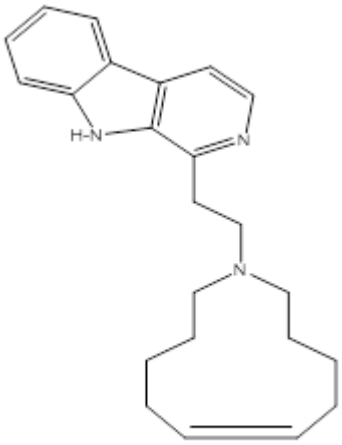
Leishmaniasis, recognized by the WHO as both a neglected and emerging disease, underscores the urgent need for novel drugs targeting specific parasite metabolic pathways. However, the limited understanding of leishmanial biology complicates the rational development of effective antileishmanial agents. Fragment-based molecular hybridization offers a promising strategy to design new compounds with improved bioactivity over existing therapies. Focusing research on innovative drug development and adopting advanced medicinal chemistry techniques are critical steps in building a pipeline of new therapeutics to combat leishmaniasis effectively.[10-12]

In this context, the exploration of natural products and their hybrids presents significant potential for discovering novel lead structures in chemical biology and medicinal chemistry research[13-15]. Natural products, with their inherent potency, selectivity, and favorable pharmacokinetic properties, are particularly valuable candidates for drug development[16]. By combining different natural products or drug fragments with natural products, a wide range of innovative combinations can be created, potentially enhancing biological activity and reducing toxicity. This strategy offers exciting opportunities for the discovery of diverse and effective therapeutic agents.

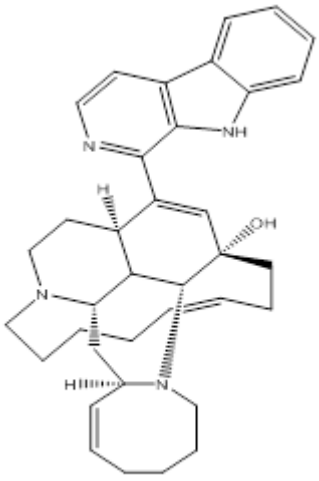
Natural products have long been a rich source of potential drug candidates for combating infectious diseases. One such structural motif, the β-carboline alkaloids, is commonly found among bioactive compounds. These alkaloids typically feature two essential components: the 9H-pyrido[3,4-b]indole core and a tail made up of heterocyclic structures. Examples of compounds with this motif include

Manzamine alkaloids[17], Quassidines A-D[18], Eudistomin I[19], Gesashidine A[20], and Annomontine[21].

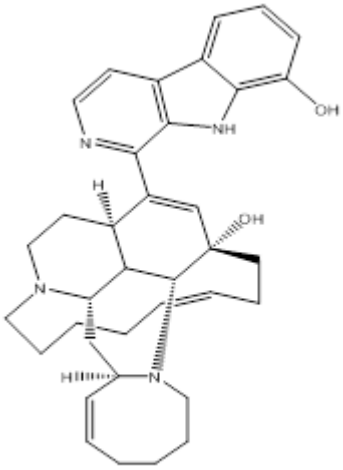
In 1998, researchers isolated Buchtienine, a tetrahydro-β-carboline alkaloid, from *Kopsia griffithii*, which demonstrated significant antileishmanial activity with IC50 values ranging from 0.30 to 1.56 µg/mL against *Leishmania donovani*. Later, Harmine, another β-carboline amine alkaloid derived from *Peganum harmala*, was also found to possess antileishmanial properties. Additionally, Pinheiro's group isolated Annomontine, a pyrimidine-β-carboline alkaloid, from the bark of *Annona foetida*, a Brazilian tree. This compound exhibited antileishmanial activity against *Leishmania braziliensis*, with an IC50 value of 34.8±1.5 µg/mL.



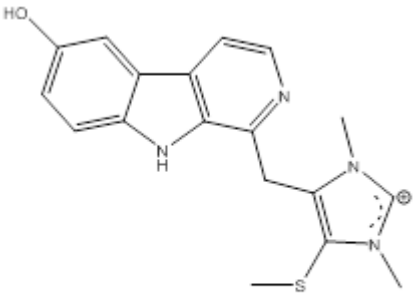
Manzamine C



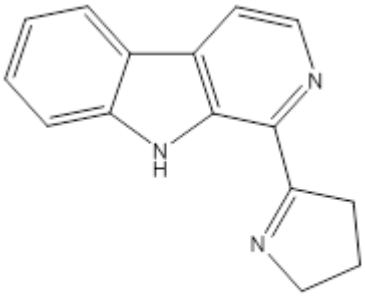
Manzamine A



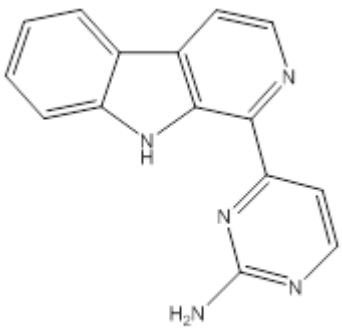
8- hydroxymanzamine A



Gasashidine A



Eudistomin I



Annomontine

Figure 1. Natural β-carboline alkaloids with different heterocyclic extension

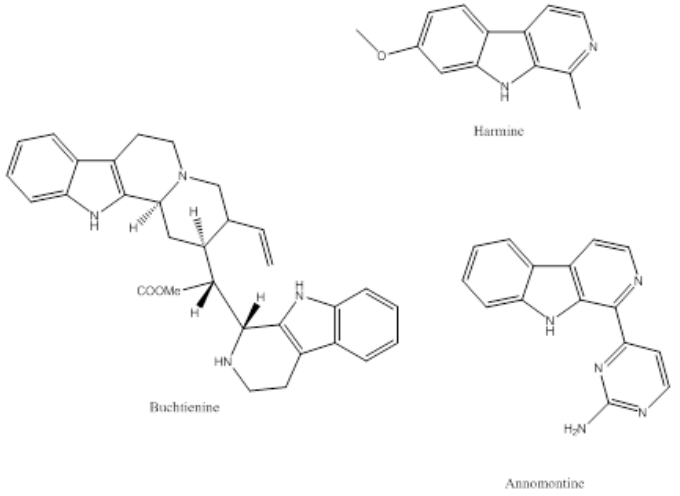


Figure 2. Natural β-carboline alkaloids with antileishmanial activity

Tetrazoles and their derivatives are commonly found in a wide variety of bioactive heterocyclic compounds, attracting significant interest due to their diverse biological, pharmaceutical, and clinical applications. One notable feature of tetrazoles is their similarity in pKa values to carboxylic acids, which often makes them suitable substitutes for carboxylic acids in pharmaceutical[22] development.

The medicinal activity of tetrazole functionality is largely attributed to its ability to act as a bioisostere of the carboxylic acid group. For example, 1,5-disubstituted tetrazoles can mimic the cis-amide bond found in peptides[23-25]. Additionally, tetrazoles can function as pharmacophores for the carboxylate group, further enhancing their versatility in drug design.

Several pharmaceuticals, including angiotensin II blockers such as Losartan and Candesartan, incorporate tetrazoles as part of their molecular structure. In addition, tetrazoles like MTT (dimethyl thiazolyl diphenyl tetrazolium salt) are widely used in the MTT assay to measure the respiratory activity of live cells in cell culture, although they exhibit cell-killing properties during the process[26-27].

The unique structure and broad range of applications of tetrazole and its derivatives, including their roles as antihypertensive, antiallergic, antibiotic, and anticonvulsant agents, have garnered significant attention in scientific research and pharmaceutical development[28-35].

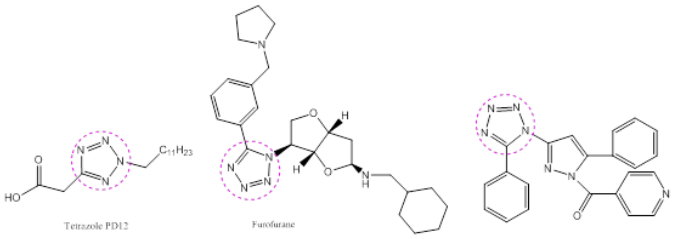


Figure 3. Tetrazole scaffold showing bioactivity

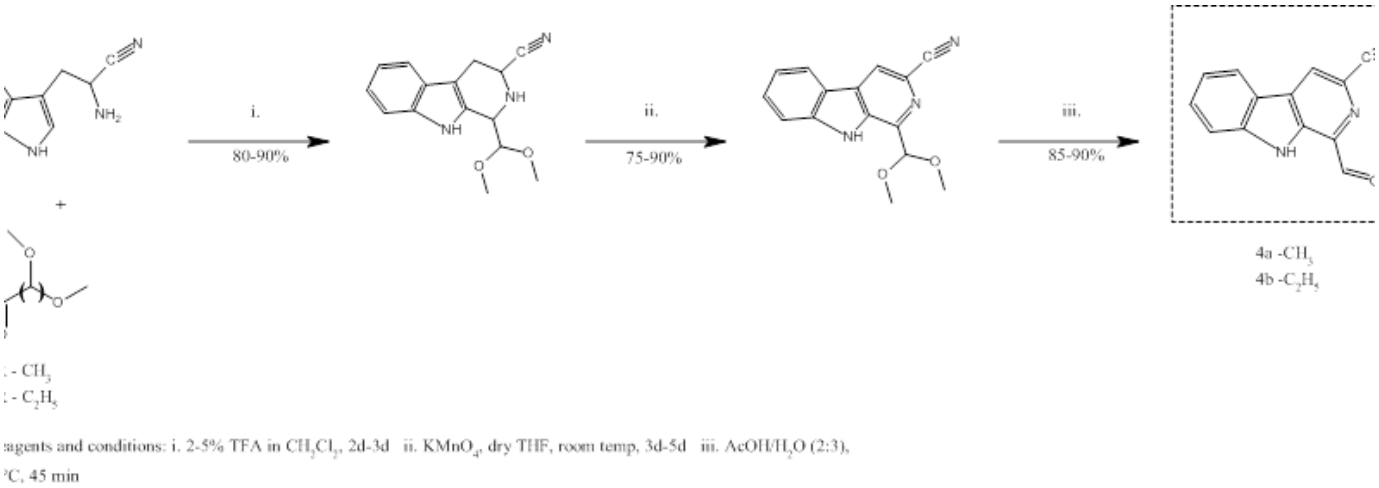
Multicomponent reaction (MCR) chemistry has emerged as a powerful method for the efficient and diverse synthesis of multiple bioactive scaffolds[36]. This technique has played a key role in the development of several biologically active compounds, some of which are currently in clinical evaluation or have already reached the market. As part of our ongoing efforts to discover novel and effective methods for accessing biologically relevant scaffolds, we have synthesized new β-carbolines tethered with tetrazole groups (as shown in Figure 4). Notably, this represents the first use of Ugi 4-component condensation (4CC) reactions for such a synthesis.

II. EXPERIMENTAL

The primary objective of this study was to synthesize methyl or ethyl 1-formyl-9H-β-carboline-3-carboxylate. We initiated the synthesis with the Pictet–Spengler reaction of tryptophan methyl or ethyl ester with dimethoxyglyoxal (60% solution in water), which resulted in the formation of the tetrahydro-β-carboline derivative 2 with a yield of 96% and good purity (Scheme 1). Oxidation of compound 2 with KMnO4 at room temperature overnight yielded acetal 3 in 90% yield. Deprotection of the formyl group with aqueous AcOH afforded the desired methyl 1-formyl-9H-β-carboline-3-carboxylate in 86% yield. Notably, this three-step synthesis did not require purification at any stage, and the reactions were scalable to produce up to 10 g of the aldehyde. [37]

The subsequent step involved an Ugi-MCR reaction,

where methyl or ethyl 1-formyl-9H-β-carboline-3-carboxylate 4 reacted with sodium azide, amines, and isocyanides, successfully synthesizing β-carbolines tethered with tetrazole 8 in good to excellent yields.

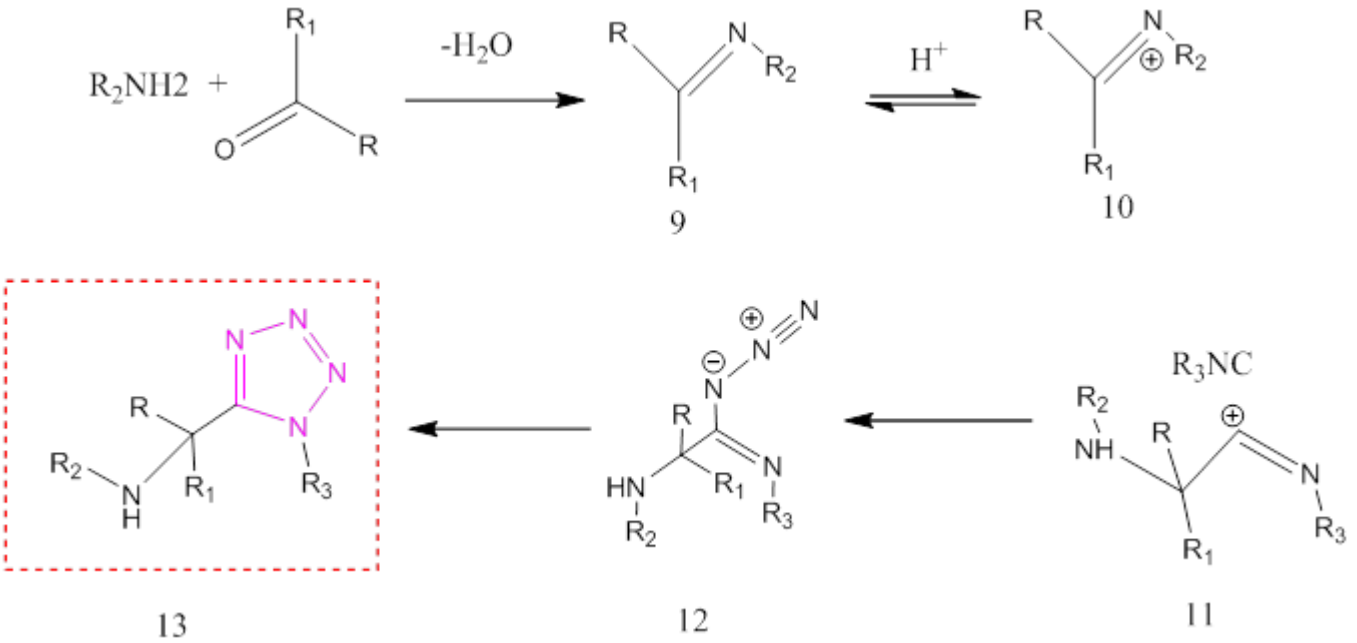


Scheme 1. Synthesis of the 1-formyl-9H-b-carbolines-3-Carboxylate

The mechanistic pathway for tetrazole formation is outlined in Scheme 2. The process begins with the formation of imine 9, which is produced by the reaction of an amine with an aldehyde. Imine 9 then undergoes conversion into iminium ion 10, which undergoes nucleophilic addition with an isocyanide, resulting in the formation of intermediate 11. After the insertion of azide, intermediate 11 leads to the formation of the final tetrazole product.

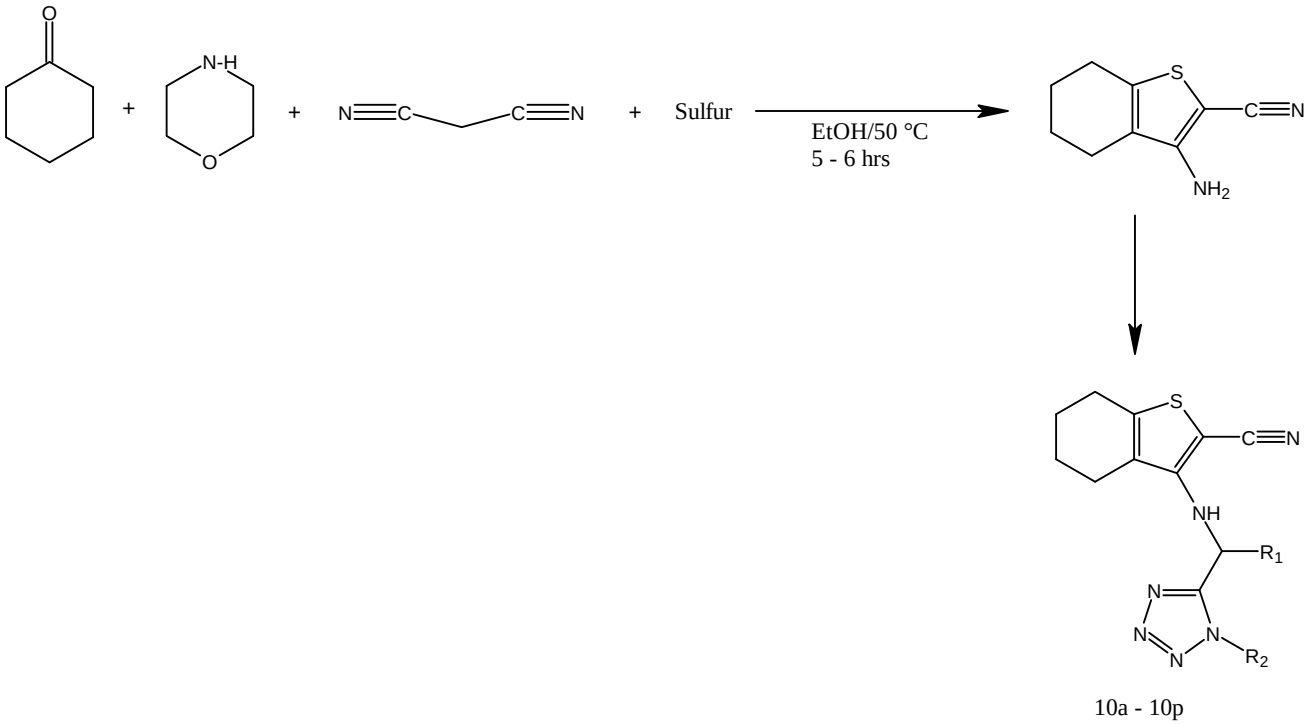
The initial aim of this study was to synthesize methyl or ethyl 1-formyl-9H-β-carboline-3-carboxylate. We started with the Pictet–Spengler reaction of tryptophan methyl or ethyl ester with dimethoxyglyoxal (60% solution in water), resulting in the tetrahydro-β-carboline derivative 2 with a yield of 96% and good purity (Scheme 1). Oxidation of compound 2 with $KMnO_4$ at room temperature overnight produced acetal 3 in 90% yield. Deprotection of the formyl group with aqueous AcOH gave the desired methyl 1-formyl-9H-β-carboline-3-carboxylate in 86% yield. Notably, this three-step synthesis required no purification at any stage, and the reactions could be scaled up to obtain 10 g of the aldehyde. [38] The next step involved an Ugi-MCR reaction using methyl or ethyl 1-formyl-9H-β-carboline-3-carboxylate 4 with sodium azide, amines, and isocyanides to synthesize β-carbolines with tethered tetrazole 8 in good to excellent yields.

The mechanistic pathway for tetrazole formation is illustrated in Scheme 2. The process begins with the formation of imine 9 through the reaction of an amine and an aldehyde. Imine 9 is then converted into iminium ion 10, which undergoes nucleophilic addition with an isocyanide to form intermediate 11. Following the insertion of azide, intermediate 11 yields the tetrazole.



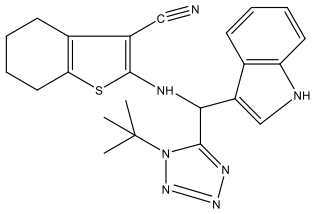
Scheme 2. Mechanism of tetrazole synthesis

A mixture of cyclohexanone (1 mmol), malononitrile (1 eq.), morpholine (1 eq.), and elemental sulfur (1 eq.) in 5 mL of ethanol was stirred at 40-50 ºC for 5-6 hours. After completion of the reaction, the solvent was evaporated under reduced pressure, and the crude product was extracted with ethyl acetate three times. The combined organic layers were treated with anhydrous sodium sulfate and evaporated once more. The resulting crude product was then used directly in the next step without any further purification.



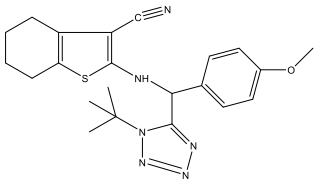
Scheme 3. Synthesis of tetrazole tethered tetrahydro benzo[b]thiophene-3-carbonitrile by Isocyanide Based Condensation

Characterization of Synthesized Compounds



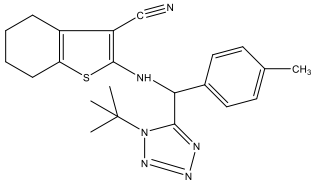
Synthesis of **2-(((1-(tert-butyl)-1H-tetrazol-5-yl)(1H-indol-3-yl)methyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile**

^{1}H NMR (500 MHz, CHLOROFORM-d, 27°C) δ ppm 1.21 - 1.39 (1 H, m), 1.66 - 1.71 (3 H, m), 1.85 - 1.93 (9 H, m), 2.65 - 2.74 (9 H, m), 7.31 - 7.37 (6 H, m), 7.39 - 7.50 (3 H, m), 7.71 (2 H, d, J=2.84 Hz), 7.88 (1 H, d, J=2.84 Hz), 8.30 - 8.37 (1 H, m), 8.59 - 8.66 (6 H, m), 8.94 (2 H, br s), 9.15 (1 H, br s), 10.06 (1 H, s), ^{13}C NMR (126 MHz, CHLOROFORM-d, 27°C) δ ppm 29.78 (1 C, s)



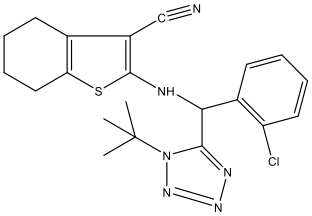
Synthesis of **2-(((1-(tert-butyl)-1H-tetrazol-5-yl)(4-methoxyphenyl)methyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile**

^{1}H NMR (500 MHz, CHLOROFORM-d, 27°C) δ ppm 1.56 - 1.70 (1 H, m), 1.83 - 1.92 (4 H, m), 2.64 - 2.75 (4 H, m), 3.90 (3 H, s), 6.99 (2 H, m, J=8.51 Hz), 7.91 (2 H, m, J=8.51 Hz), 8.37 (1 H, s), ^{13}C NMR (126 MHz, *CHLOROFORM-d*, 27°C) δ ppm 22.06 (1 C, s), 23.14 (1 C, s), 24.33 (1 C, s), 25.19 (1 C, s), 29.79 (1 C, s), 55.52 (1 C, s), 105.88 (1 C, s), 114.41 (1 C, s), 114.73 (1 C, s), 128.07 (1 C, s), 131.41 (1 C, s), 131.49 (1 C, s), 134.91 (1 C, s), 158.44 (1 C, s), 160.63 (1 C, s), 163.17 (1 C, s)



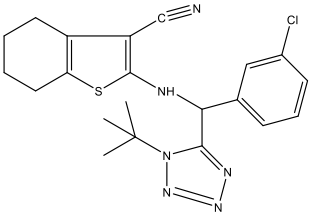
Synthesis of **2-(((1-(tert-butyl)-1H-tetrazol-5-yl)(p-tolyl)methyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile**

^{1}H NMR (500 MHz, *CHLOROFORM-d*, 27°C) δ ppm 1.84 - 1.92 (4 H, m), 2.44 (3 H, s), 2.66 - 2.74 (4 H, m), 7.29 (2 H, d, *J*=7.88 Hz), 7.85 (2 H, d, *J*=7.88 Hz), 8.40 (1 H, s), ^{13}C NMR (126 MHz, *CHLOROFORM-d*, 27°C) δ ppm 21.81 (1 C, s), 22.04 (1 C, s), 23.11 (1 C, s), 24.33 (1 C, s), 25.22 (1 C, s), 29.79 (1 C, s), 106.52 (1 C, s), 114.62 (1 C, s), 129.52 (1 C, s), 129.68 (1 C, s), 132.07 (1 C, s), 132.53 (1 C, s), 135.04 (1 C, s), 143.20 (1 C, s), 159.00 (1 C, s), 160.24 (1 C, s)



Synthesis of **2-(((1-(tert-butyl)-1H-tetrazol-5-yl)(2-chlorophenyl)methyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile**

^{1}H NMR (500 MHz, *CHLOROFORM-d*, 27°C) δ ppm 0.79 - 0.96 (16 H, m), 1.00 - 1.14 (4 H, m), 1.24 - 1.33 (13 H, m), 1.35 - 1.41 (3 H, m), 1.45 (1 H, s), 1.49 - 1.54 (1 H, m), 1.78 - 1.93 (5 H, m), 2.49 - 2.55 (3 H, m), 2.73 (2 H, dt, *J*=19.15, 5.40 Hz), 3.55 (1 H, br s), 4.59 (1 H, br s), 7.36 - 7.46 (2 H, m), 7.51 (1 H, d, *J*=6.65 Hz), 7.85 - 7.89 (1 H, m), 8.38 (1 H, d, *J*=7.57 Hz), 8.90 (1 H, s), ^{13}C NMR (126 MHz, *CHLOROFORM-d*, 27°C) δ ppm 22.11 (1 C, s), 50.66 (1 C, s), 53.08 (1 C, s), 71.85 (1 C, s), 74.24 (1 C, s), 95.44 (1 C, s), 125.81 (1 C, s), 127.87 (1 C, s), 158.95 (1 C, s), 179.88 (1 C, s), 188.50 (1 C, s), 188.92 (1 C, s), 216.05 (1 C, s)



Synthesis of **2-(((1-(tert-butyl)-1H-tetrazol-5-yl)(3-chlorophenyl)methyl)amino)-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carbonitrile**

^{1}H NMR (500 MHz, *CHLOROFORM-d*, 27°C) δ ppm 1.85 - 1.93 (4 H, m), 2.64 - 2.77 (4 H, m), 7.45 (2 H, m, *J*=8.20 Hz), 7.88 (2 H, m, *J*=8.20 Hz), 8.38 (1 H, s), ^{13}C NMR (126 MHz, *CHLOROFORM-d*, 27°C) δ ppm

21.98 (1 C, s), 23.06 (1 C, s), 24.31 (1 C, s), 25.25 (1 C, s), 29.78 (1 C, s), 107.45 (1 C, s), 114.37 (1 C, s), 129.24 (1 C, s), 130.51 (1 C, s), 132.98 (1 C, s), 133.58 (1 C, s), 135.33 (1 C, s), 138.40 (1 C, s), 157.27 (1 C, s), 159.26 (1 C, s)

III.RESULT & DISCUSSION

The initial step in the synthesis involved a Pictet–Spengler reaction, employing tryptophan methyl or ethyl ester and dimethoxyglyoxal (a 60% aqueous solution), which notably yielded the tetrahydro-β-carboline derivatized Tetrazoles. The reaction's efficacy was underscored by a high yield of 96% and commendable purity of the synthesized product. The high yield and purity observed in our reaction highlights the potential of this approach for the streamlined synthesis of complex heterocyclic scaffolds, offering a foundation for further diversification and functionalization

IV. CONCLUSION

The synthesis of coumarin derivatives is often plagued by issues such as the use of toxic catalysts, extended reaction times, low yields, and the formation of by-products, necessitating the development of milder, more efficient, and environmentally conscious protocols. In this context, the Pictet–Spengler reaction stands out as a powerful tool for generating resultant product in high yield. The high yield and purity highlight the potential of this approach. The ability to easily synthesize and decorate the coumarin nucleus allows for the design of new coumarin-based tetrazoles for treating various diseases. We believe that further modifications to the tetrazole moiety will uncover even more valuable biological activities. We hope future research will reveal additional applications of tetrazole in medical science.

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