

# Microwave Induced Facile One-pot Access to Diverse 2-cyanobenzothiazole-A Key Intermediate for the Synthesis of Firefly Luciferin

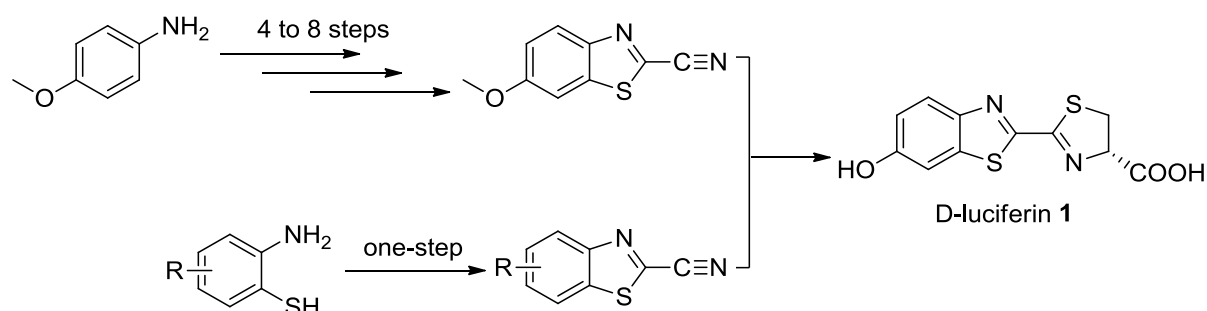
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## ABSTRACT

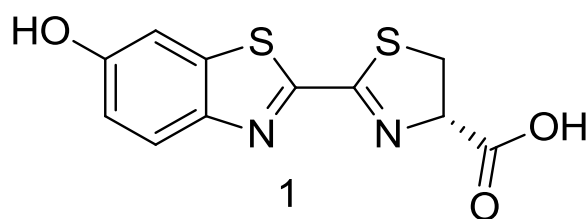
An eco-friendly one-step preparation of various substituted 2-cyanobenzothiazole by condensation of corresponding substituted *ortho*-aminothiophenol with ethyl cyanoformate, employing an effective amount of Lawesson's reagent, under microwave irradiation (MWI) and solvent free conditions is presented. The structures of the compounds were elucidated with the aid of elemental analysis, IR, <sup>1</sup>H-NMR and mass spectral data. The targeted various substituted 2-cyanobenzothiazole are obtained in good yields and high purity.



**Keywords:** Benzothiazoles; Lawesson's reagent; microwave; one-step reaction; D-luciferin

## 1. INTRODUCTION

D-luciferin [(*S*)-2-(6'-hydroxy-2'-benzothiazolyl)thiazoline-4-carboxylic acid] is the natural substrate of the enzyme luciferase (Luc), that catalyzes the production of the typical yellow-green light of fireflies [1]. Fireflies are only a part of the big family of bioluminescent insects that can emit light with wavelengths going from yellow-green (560 nm) to red (620 nm) [2]. All the enzymes use the same substrate, i.e. D-luciferin 1.



**Figure 1.** Structure of D-luciferin.

Firefly luciferases can modulate the proportion of green and red bioluminescence through a pH-sensitive mechanism [3]. The luciferase from the North American firefly *Photinus pyralis* (PpyLuc) is a well characterized enzyme that finds a large number of applications in mammalian cells. PpyLuc has been used in living animals as a transgenic marker or as a tag for cells that have been transplanted [4]. Due to its bioluminescence characteristics, PpyLuc is at present the most used enzyme in optical molecular imaging [5] and is the most studied enzyme of the luciferase family.

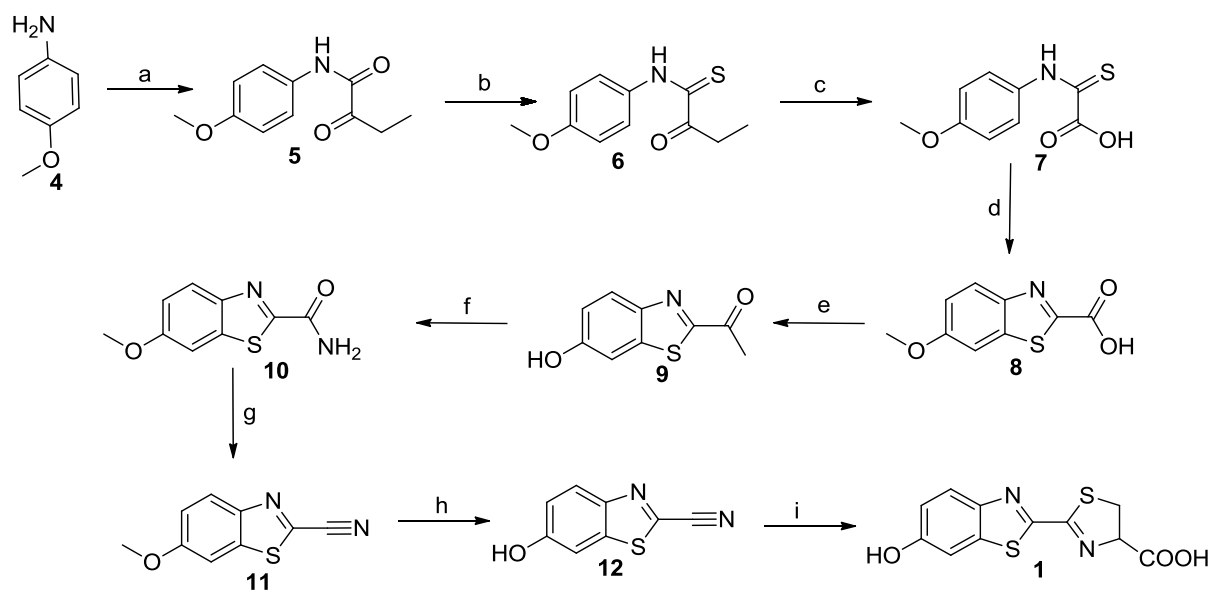
The importance of green chemistry in organic synthesis has encouraged scientists to explore the use of microwave irradiation for the organic synthesis. Over the last few years, microwave irradiation (MWI) has emerged a great energy source for the wide range of organic transformation with short reaction time and high yield of the products with high purity [6-19]. Hence, a study has been undertaken using microwave irradiation for the condensation of *ortho*-aminothiophenol with ethyl cyanofornate using Lawesson's reagent under solvent and catalyst free condition (**Scheme 4**).

During the literature search, we have found out that there are only a few numbers of synthetic path-ways available for the synthesis of D-luciferin. These all possible synthetic pathways for the synthesis of D-luciferin are described below.

### 1. 1. Synthesis of D-luciferin from *p*-anisidine 4

The chemical structure of D-luciferin, isolated from firefly tails, was proposed in 1961 [20] and later confirmed by synthesis (**Scheme 1**) [21]. The overall yield of D-luciferin **1** from *p*-anisidine **4** is 9% through nine steps. 2-cyano-6- hydroxybenzothiazole **12** is the key intermediate for the synthesis of D-luciferin **1**.

In this procedure, *p*-anisidine **4** is the starting material that, through intermediates **5** and **6**, is transformed into the thioacid **7**, in turn cyclized to 6- methoxybenzothiazole-2- carboxylic acid **8**. From this benzothiazole derivative, 2-cyano-6- hydroxybenzothiazole **12** is prepared in four steps. Compound **12** is the key intermediate for the synthesis of **1** that can be obtained almost quantitatively by reaction with D-cysteine, *in situ* produced by reduction of D-cystine.



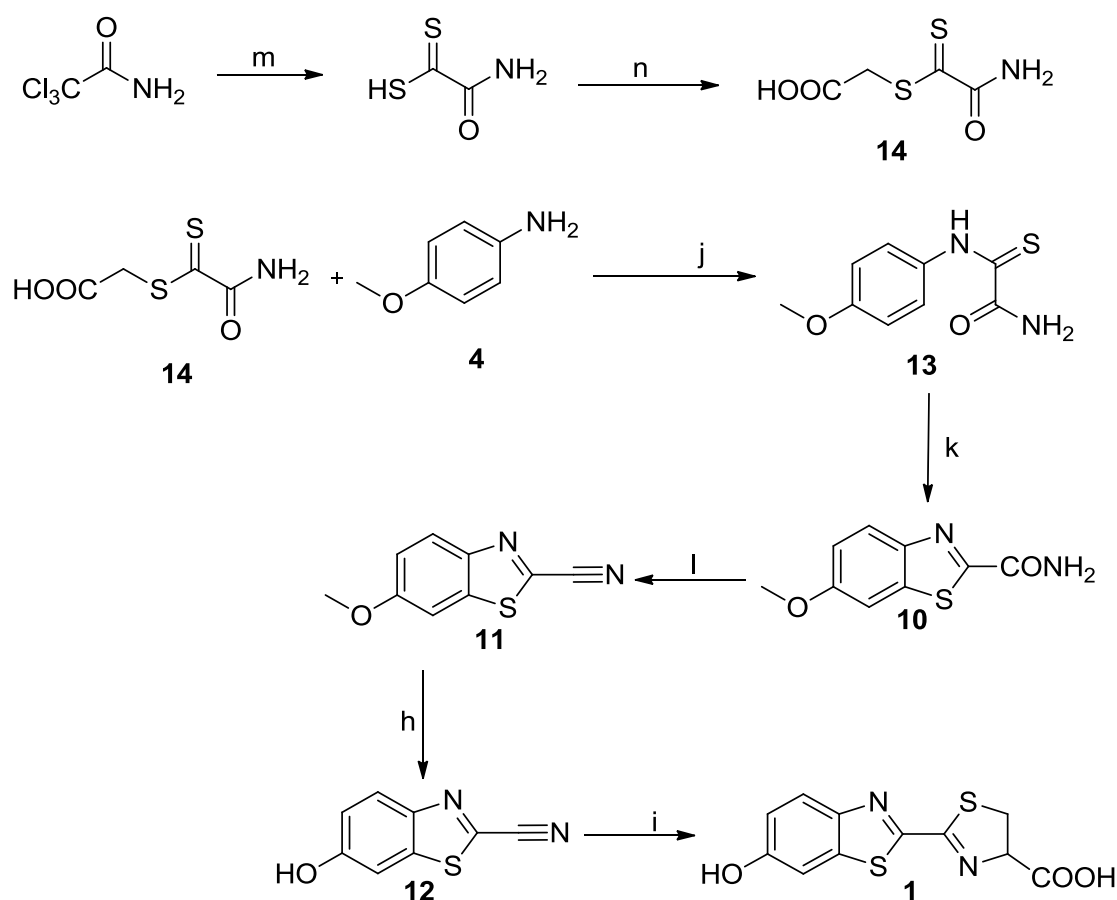
Scheme 1

**Reagents and Conditions:** (a) ethyl oxalate, 180 °C, 5 min, 58%; (b) P<sub>2</sub>S<sub>5</sub>, reflux, 40 min; (c) NaOH, 0 °C then HCl; (d) K<sub>3</sub>Fe(CN)<sub>6</sub>/OH<sup>-</sup>, <10 °C, 15 min; (76% crude, no isolation in steps b-d); (e) CH<sub>2</sub>N<sub>2</sub>, 0 °C, 15 min, 40%; (f) anhydrous NH<sub>3</sub>/MeOH, heat, 30 min, 100%; (g) POCl<sub>3</sub>, reflux, 15 min, 56%; (h) PyHCl, 200 °C, 1 h, 62%; (i) D-cysteine (*in situ* from D-cysteine/liquid NH<sub>3</sub>/Na, r.t., 10 min) and **12** in H<sub>2</sub>O/MeOH, r.t., 0.5 h, 94%.

### 1.1. Synthesis of D-luciferin from 4-methoxythioxanilamide **13**

This experimental protocol has been applied to the preparation of 5-10 g of D-luciferin **1** [23]. According to Seto *et al.*, [22] 6-methoxybenzothiazole-2-carboxamide **10** is prepared from the 4-methoxythioxanilamide **13** by oxidative cyclization with alkaline K<sub>3</sub>[Fe(CN)<sub>6</sub>] (**Scheme 2**). The transformation of compound **10** into the nitrile **11** has been carried out essentially as reported by White *et al.* [21]

The preparation of 4-methoxythioxanilamide **13**, according to Seto *et al.* can be carried out in good yields from *p*-anisidine **4** and carbamoylthiocarbonylthioacetic acid **14**. However, this compound is unstable and has to be prepared *in situ*, as described in detail by Bowie [23]. The experimental procedure allows to prepare compound **11** from *p*-anisidine **4** with an overall 39% yield.



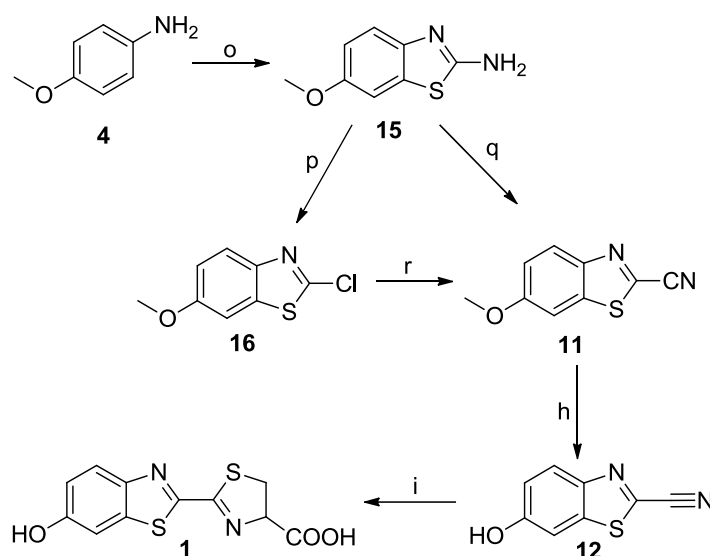
Scheme 2

**Reagents and Conditions:** (j) aq. MeOH; (k)  $K_3[Fe(CN)_6]/OH^-$ , r.t., 1 h, 60%; (l)  $POCl_3$ , reflux, 1.5 h, 67% (m) aq. KOH,  $H_2S$ ; (n)  $ClCH_2COOH$ .

## 1. 2. Synthesis of D-luciferin by following a Sand-Meyer approach from 2-amino-6-methoxybenzothiazole 15

According to another synthetic approach, 2-amino-6-methoxybenzothiazole **15** can be prepared from *p*-anisidine **4** [24] and different routes can lead to 2-cyano-6-hydroxybenzothiazole **11** using a classical Sand-Meyer reaction.

In a first synthesis, [24,25] 2-chloro-6-methoxybenzothiazole **16** was prepared by reaction of compound **15** with nitrous acid and HCl. Reaction of compound **16** with KCN in DMSO afforded the nitrile **11** (Scheme 4). Conditions of formation of 2-chloro derivative **16** were improved using isoamyl nitrite and copper (II) chloride in polyethylene glycol 200 [26] as solvent and yields were improved to 56%. More recently, the Sand-Meyer reaction was carried out by direct introduction of cyanide with  $CuCN/KCN$  [27] and following this approach a 41% yield was obtained.

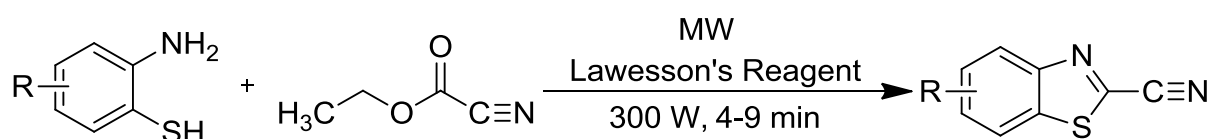


Scheme 3

**Reagents and Conditions:** (o) KSCN, Br<sub>2</sub>/AcOH, 35 °C, 10 h, 87%; (p) HNO<sub>2</sub>, HCl, 0-60 °C, 2 h, 35-45%; (q) HNO<sub>2</sub>, CuCN/KCN, 0 °C, 1 h, 41%; (r) KCN/DMSO, 140 °C, 1 h, 40%.

## 2. GENERAL REACTION SCHEME (PRESENT WORK)

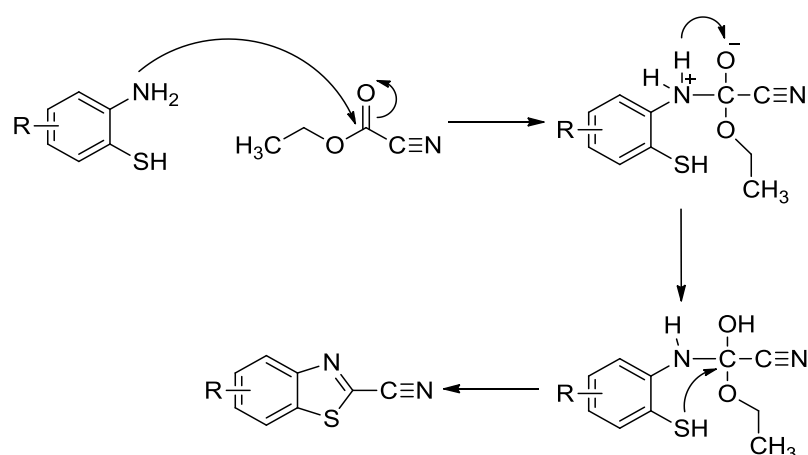
We here in present an eco-friendly one-step preparation of various substituted 2-cyanobenzothiazole by condensation of corresponding substituted *ortho*-aminothiophenol with ethyl cyanoformate, employing an effective amount of Lawesson's Reagent, under microwave irradiation (MWI) and solvent free conditions.



Scheme 4. Synthetic route for 2-cyanobenzothiazole derivatives.

## 3. RESULT AND DISCUSSION

Some mechanistic aspects involved in this reaction are still under study (**Figure 2**). It was supposed that *ortho*-aminothiophenol and carboxylic ester were activated by Lawesson's reagent (LR) and formed adduct. Finally the ethanol molecule was eliminated from adduct and produced 2-cyanobenzothiazole derivatives in excellent yield. The title compounds, 2-cyanobenzothiazole derivatives (**Table 1**) were synthesized via cyanobenzothiazole by condensation of substituted *ortho*-aminothiophenols with ethyl cyanoformate, employing the catalytic amount of Lawesson's reagent, under microwave irradiation (MWI) and solvent free conditions.



**Figure 2.** Proposed reaction mechanism.

Here, *ortho*-aminothiophenols undergoes condensation reaction with ethyl cyanoformate, employing an effective amount of LR under microwave irradiation (MWI), afforded title compounds in excellent yield within short period of time (4-9 min.). In a typical example we have reacted 1.0 equiv. of *ortho*-aminothiophenols with 1.0 equiv. of ethyl cyanoformate and 0.35 equiv. of Lawesson's reagent under microwave irradiation (300 W) at 190°C. It was noted that 0.35 equiv. Lawesson's reagent was sufficient for this transformation as compared to lower amount of Lawesson's reagent. It was interesting to mention that at 300 W (microwave irradiation power), the reaction was completed in shorter time as compared to lower irradiation power. In addition, it was also observed that, high power (Up to 300 W) of microwave irradiation does not increase the yield of product. Both the electron-withdrawing group and electron-releasing groups on the *ortho*-aminothiophenols showed equally efficiency. The structures of products were characterized unambiguously by elemental and spectroscopic (IR, Mass, <sup>1</sup>H-NMR) analysis.

**Table 1.** Synthesis of benzo[d]thiazole-2-carbonitrile derivatives using *ortho*-aminothiophenols (1 equiv.), ethyl cyanoformate (1 equiv.) and 0.35 equiv. of Lawesson's reagent.

No.	2-aminothiophenol	Product	Time (min)	Yield <sup>a</sup> (%)	M.P. (°C)
1			4	90	129
2			4	83	105
3			6	71	134
4			4	62	156
5			9	67	263

<sup>a</sup> Yields refer to isolated pure products

#### 4. MATERIALS AND METHOD

Chemicals used in this reaction such as Ethyl cyanoformate were of analytical grade and purchased from Sigma-Aldrich, USA. The various substituted *ortho*-aminothiophenols were synthesised in the laboratory using the previously reported method [28]. Lawesson's Reagent was purchased from Spectrochem Pvt. Ltd., Mumbai, India.

The reactions were assayed by thin layer chromatography (TLC) and terminated as judged by the consumption of starting material. Analytical thin-layer chromatography (TLC) was performed on silica gel G 60 F254 (Merck) plates and eluted with the appropriate solvent ratios 10% Hexane-CH<sub>2</sub>Cl<sub>2</sub> (v/v). The melting points were recorded in Optimelt Automated Melting point System and were uncorrected. IR spectra were recorded on a Perkin-Elmer 377 spectrophotometer, <sup>1</sup>H NMR spectra was measured in Bruker AV 400 MHz using CDCl<sub>3</sub> as a solvent and TMS as an internal standard. Mass spectra was recorded on Advion Expression CMS, USA. Elemental analysis was performed on the Vario MICRO cube, elementary CHN analyzer serial no.: 15084053. Here we have used the CEM Discover microwave system for synthesis. Its model no.: 908010 and made by CEM Matthews. Inc, USA.

##### General procedure for the synthesis of Benzo[d]thiazole-2-carbonitrile derivatives:

A mixture of Ethyl cyanoformate (136 mg, 1 mmol), 2-amino-5-methoxybenzenethiol (109 mg, 1 mmol) and Lawesson's Reagent (141 mg, 0.35 mmol) was irradiated in an open vessel with microwaves in a monomode oven (Discover CEM, 300 W and temperature control set at 190 °C measured with an IR sensor) for 4 min. The crude was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) and washed with 10% aq NaOH (2 × 20 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated. The residual red-brown solid was purified by silica-gel TLC with 10% Hexane-CH<sub>2</sub>Cl<sub>2</sub> to give the nitrile (188 mg, 90%) as pale yellow needles, which was used for the next step without further purification. An analytical sample was obtained by recrystallizing the product from CH<sub>2</sub>Cl<sub>2</sub>-Hexane; mp 129-130 °C.

The other four derivatives of substituted 2-cyano benzothiazole were also synthesised using this method described above. They are conformed by spectral as well as analytical analysis.

##### 6-Methoxybenzo[d]thiazole-2-carbonitrile: (1)

Pale yellow needles; M.P. 129-130 °C; Yield: 90%; IR (ν<sub>max</sub>): 2231, 1325, 1252, 1132, 1029, 624 cm<sup>-1</sup>; Mass (*m/z*): 191.2 (M+1)<sup>+</sup> (Base peak), 192.4 (M+2)<sup>+</sup>; <sup>1</sup>H-NMR (ppm): (CDCl<sub>3</sub>) δ 8.690 (1H, d, *J*=9.0), 8.269 (1H, dd, *J*=9.0), 8.050 (1H, d, *J*=2.4), 3.349 (3H, s); Elemental Analysis: Calculated: C, 56.83; H, 3.18; N, 14.73; S, 16.86%; Found: C, 56.55; H, 3.22; N, 14.97; S, 16.88%.

##### Benzo[d]thiazole-2-carbonitrile: (2)

M.P. 105 °C; Yield: 83%; IR (ν<sub>max</sub>): 2250, 1331, 1258, 617 cm<sup>-1</sup>; Mass (*m/z*): 161.1 (M+1)<sup>+</sup> (Base peak), 162.1 (M+2)<sup>+</sup>; <sup>1</sup>H-NMR (ppm): (CDCl<sub>3</sub>) δ 8.28 (1H, d, *J*=9.2), 8.08 (1H, d, *J*=8.7), 7.63 (2H, m); Elemental Analysis: Calculated: C, 59.98; H, 2.52; N, 17.49; S, 20.02%; Found: C, 59.76; H, 2.68; N, 17.34; S, 20.15%

##### 6-Hydroxybenzo[d]thiazole-2-carbonitrile: (3)

M.P. 134 °C; Yield: 71%; IR (ν<sub>max</sub>): 3315, 2231, 1325, 1252, 1132, 1029, 624 cm<sup>-1</sup>; Mass (*m/z*): 177.2 (M+1)<sup>+</sup> (Base peak), 178 (M+2)<sup>+</sup>; <sup>1</sup>H-NMR (ppm): (CDCl<sub>3</sub>) δ 7.57 (1H, d, *J*=8.73), 7.17 (1H, s), 6.68 (1H, d, *J*=2.43), 5.14 (1H, s); Elemental Analysis: Calculated: C, 54.53; H, 2.29; N, 15.90; S, 18.20%; Found: C, 54.87; H, 2.02; N, 16.05; S, 17.98%.

##### 5-Chlorobenzo[d]thiazole-2-carbonitrile: (4)

M.P. 156 °C; Yield: 62%; IR (ν<sub>max</sub>): 2231, 1325, 1252, 680 cm<sup>-1</sup>; Mass (*m/z*): 195.1 (M+1)<sup>+</sup> (Base peak), 196.1 (M+2)<sup>+</sup>; <sup>1</sup>H-NMR (ppm): (CDCl<sub>3</sub>) δ 8.30 (1H, d, *J*= 9.25), 7.98 (1H, d,

$J=9.0$ ), 7.52 (1H, dd,  $J=9.4$ ); Elemental Analysis: Calculated: C, 49.37%; H, 1.55%; N, 14.39%; S, 16.47%; Found: C, 49.62%; H, 1.45%; N, 14.21%; S, 16.54%.

#### ***N*-(2-cyanobenzo[d]thiazol-6-yl)acetamide (5)**

M.P. 263 °C; Yield: 67%; IR ( $\nu_{\max}$ ): 2231, 1645, 1325, 1252, 1132, 1029, 624  $\text{cm}^{-1}$ ; Mass ( $m/z$ ): 218.2 (M+1)<sup>+</sup> (Base peak), 219.1 (M+2)<sup>+</sup>; <sup>1</sup>H-NMR (ppm): ( $\text{CDCl}_3$ )  $\delta$  8.45 (1H, d  $J=9.1$ ), 7.75 (2H, m), 7.17 (1H, s), 2.19 (3H, s); Elemental Analysis: Calculated: C, 55.29%; H, 3.25%; N, 19.34%; S, 14.73%; Found: C, 55.09%; H, 3.33%; N, 19.12%; S, 14.70%.

## 5. CONCLUSIONS

In this article, we have elaborated the initial efforts made toward the sighting of highly important Benzo[d]thiazole-2-carbonitrile derivatives which were synthesized by green and efficient method. It is interesting to note that the conventional procedure have six to ten steps for the preparation of Benzo[d]thiazole-2-carbonitrile derivatives, while in present work it is prepared in two steps only. The present procedure offers various advantages such as a very short reaction time, microwave assisted synthesis, simple work-up procedure, excellent yield of products and step-down synthesis by green chemistry protocol.

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## References

- [1] E. H. White, E. Rapaport, H. H. Seliger, T. A. Hopkins, *Bioorg. Chem.* 1 (1971) 92-122.
- [2] V. R. Viviani, *Cell. Mol. Life. Sci.* 59 (2002) 1833-1850.
- [3] H. H. Seliger, W. D. McElroy, *Proc. Natl. Acad. Sci. USA.* 52 (1964) 75-81.
- [4] C. H. Contag, M. H. Bachmann, *Annu. Rev. Biomed. Eng.* 4 (2002) 235-260.
- [5] G. D. Lüker, K. E. Lüker, *J. Nucl. Med.* 49 (2008) 1-4.
- [6] R. A. Irgashev, A. A. Karmatsky, P. A. Slepukhin, G. L. Rusinov, V. N. Charushin, *Tetrahedron Lett.* 54 (2013) 5734-5738.
- [7] T. M. Potewar, K. T. Petrova, M. T. Barros, *Carbohydr. Res.* 379 (2013) 60-67.
- [8] R. C. Lian, M. H. Lin, M. H. Liao, J. J. Fu, Y. C. Wu, F. R. Chang, C. C. Wu, M. J. Wu, P. S. Pan, *Tetrahedron.* 70 (2014) 1800-1804.
- [9] M. Y. Mentese, H. Bayrak, Y. Uygun, A. Mermer, S. Ulker, S. A. Karaoglu, N. Demirbas, *Eur. J. Med. Chem.* 67 (2013) 230-242.
- [10] F. Messina, O. Rosati, *Curr. Org. Chem.* 17 (2013) 1158-1178.
- [11] P. Appukkuttan, V.P. Mehta, E. V. Van der Eycken, *Chem.Soc.Rev.* 39 (2010) 1467-1477.
- [12] X. Zhang, H. Jiang, D. Ye, H. Sun, H. Liu, *Green Chem.* 11 (2009) 1881-1888.



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- [13] B. Maiti, K. Chanda, M. Selvaraju, C. C. Tseng, C. M. Sun, *ACS Comb. Sci.* 15 (2013) 291-297.
- [14] A. Walia, S. Kang, R. B. Silverman, *J. Org. Chem.* 78 (2013) 10931-10937.
- [15] E.F. Dimauro, J. M. Kennedy, *J. Org. Chem.* 72 (2007) 1013-1016.
- [16] R. B. Sparks, A. P. Combs, *Org. Lett.*, 6 (2004) 2473-2475.
- [17] H. D. Patel, S. M. Divatia, E. D. Clercq, *Indian J. Chem.*, 52B (2013) 535-545.
- [18] S. M. Prajapati, R. H. Vekariya, K. D. Patel, S. N. Panchal, H. D. Patel, D. P. Rajani, S. Rajani, *Int. Lett. Chem. Phys. Astron.* 20 (2014) 195-210.
- [19] R. H. Vekariya, S. N. Panchal, K. D. Patel, H. D. Patel, *Curr. Microwave Chem.* (2015) DOI: 10.2174/2213335601666141023223657.
- [20] E. H. White, F. McCapra; G. F. Field, W. D. McElroy, *J. Am. Chem. Soc.* 83 (1961) 2402-2403.
- [21] E. H. White, F. McCapra, G. F. Field, *J. Am. Chem. Soc.* 85 (1963) 337-343.
- [22] S. Seto, K. Ogura, Y. Nishiyama, *Bull. Chem. Soc. Jpn.* 36 (1963) 331-332.
- [23] L. Bowie, *J. Method Enzymol.* 57 (1978) 15-28.
- [24] G. C. Stuckwisch, *J. Am. Chem. Soc.* 71 (1949) 3417-3417.
- [25] E. H. White, H. Woelther, G. F. Field, W. D. McElroy, *J. Org. Chem.* 30 (1965) 2344-2348.
- [26] N. Suzuki, T. Nomoto, N. Kanamori, B. Yoda, A. Saeki, *Biosci. Biotech. Biochem.* 57 (1993) 1561-1562.
- [27] Y. Toya, M. Takagi, H. Nakata, N. Suzuki, M. Isobe, T. Goto, *Bull. Chem. Soc. Jpn.* 65 (1992) 392-395.
- [28] P. K. Sharma, B. S. Rathore, M. Kumar, A. Fogla, *Res Chem Intermed* 37 (2011) 1103-1111.

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