

Full Length Research Paper

Synthesis and characterization of 2-mercapto-N-methyl imidazole substituted benzimidazole derivatives and investigation of their effect on production of plantlets in *Oncidium Gower Ramsey*

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A series of benzimidazole derivatives (5a-c) were synthesized by coupling 5-substituted 2-chloromethyl benzimidazole (3a-c) with 2-mercapto-N-methyl imidazole. The synthesized compounds were characterized by infra-red (IR), nuclear magnetic resonance (NMR) and elemental analyses. Further, the synthesized compounds were tested on plantlet production from protocorm like bodies (PLBs) sections of *Oncidium*. PLBs sections were cultured on half strength modified Murashige and Skoogs (MS) medium alone and also modified MS medium supplemented with the synthesized chloro, methyl and nitro derivatives individually at 2 or 5 μM concentrations. Among these three compounds, PLBs sections cultured on medium supplemented with nitro compound (5c) at 5 μM concentration produced maximum number (95) of plantlets.

Key words: Benzimidazole derivatives, protocorm like bodies, plant growth regulators, 2-mercapto-N-methyl imidazole.

INTRODUCTION

The presence of imidazole and benzimidazole moieties in biological systems such as histidine, vitamin-B₁₂ etc. has proven their biological relevance (Sorrell, 1989). Thus, these compounds have been extensively studied for anticancer (Swiatkiewicz et al., 2014), antibacterial

(Zhang et al., 2009), anti-trypanosomatid (Boiani et al., 2009), antimicrobial (Jardosh et al., 2013), antitumor (Hranjec et al., 2010), fungicide (Wena et al., 2013) and other biological activities. Reports are available where imidazole and benzimidazole derivatives have been

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Abbreviations: PLBs, Protocorm like bodies; CMNP, 5-chloro-3-methyl-4-nitro-1H-pyrazole; ZnAAC, zinc-amino acid complexes; TIBA, 2,3,5-triiodobenzoic acid; PCIB, 2-(p-chlorophenoxy)-2-methylpropionic acid; IR, infra-red; NMR, nuclear magnetic resonance.

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tested even for plant growth regulation (Lacova et al., 1993; Cavender, 1986). PLBs of orchids are the versatile system to study the effect of plant growth regulators on plant morphogenesis. *Oncidium* Gower Ramsey is commercially an important ornamental orchid. *In vitro* production of somatic embryos/PLBs from explants of *Oncidium* are controlled by several factors including nature of explants, sugar source, macro and micro nutrients, vitamins, amino acids, and plant growth regulators (Chen and Chang, 2001; 2002; 2003a; 2003b; 2004; Su et al., 2006). Apart from auxins, cytokinins, gibberellin, abscisic acid, ethylene, jasmonic acid, Brassinosteroids, salicylic acid and polyamines, there are several reports on the synthesis of other novel compounds, which are regulating the plant growth and development. Phenylurea derivatives [N,N-bis-(2,3-methylenedioxyphenyl)urea (2,3-MDPU)] have been found to enhance production of root system in *Capparis spinosa* (Carra et al., 2012). Cytokinin-like activity was exhibited by the urea derivatives but their activity was less compared to kinetin and N-phenyl-N'-(4-pyridyl) urea (Yonova and Stoilkova, 2004). The compound, in which the un-substituted phenylcarbamoyl group was directly attached to the piperazine ring, also showed cytokinin-like activity and significantly stimulated betacyanin synthesis in *Amaranthus caudatus* (Stoilkova et al., 2014). Adventitious shoot proliferation was enhanced in *Spathiphyllum floribundum* with the addition of imidazole and paclobutrazol individually to medium containing cytokinin (Werbrouck and Debergh, 1996). *In vitro* shoot and root growth in wheat and sorghum was inhibited by 3-aryl-1H-indazoles (Chattha et al., 2012).

The 5-chloro-3-methyl-4-nitro-1H-pyrazole (CMNP), a pyrazole-derived plant growth regulator promoted starch degradation and senescence-related symptoms in *Arabidopsis* (Alferez et al., 2007). Zinc-amino acid complexes (ZnAAC) have been shown to stimulate the root and shoot growth in *Lactuca sativa* (Ghasemi et al., 2013). A derivative of benzimidazole like 5-hydroxybenzimidazole (Hoyle and Robin, 2009) helps in increasing shoots growth, root growth, leaf area. Literature survey indicates that very little attention has been paid to the investigation of the benzimidazole derivatives (5a-c) for their effect on production of plantlets in *Oncidium* Gower Ramsey. In the present investigation, 2-mercapto-N-methyl imidazole substituted benzimidazole derivatives were synthesized, characterized and studied for their effects on production of plantlets from PLBs sections of *Oncidium*.

MATERIALS AND METHODS

All the chemicals used in the present study were procured from Sigma-Aldrich, USA. The synthesized compounds were confirmed by elemental and spectral analyses. ¹H NMR spectra were recorded on a Bruker 400 MHz multinuclear spectrometer with TMS as internal standard (chemical shift in δ ppm) and are given in Figures 5 to 7. Mass spectra were recorded on a LC/MSD-Trip-XCT. C, H,

N, S analyses were carried out using a Carlo-Erba analyzer. IR spectra were recorded on a Bruker IR spectrometer after grinding the sample with KBr.

Plant growth regulation study

Oncidium Gower Ramsey plants were procured from Indo-American Seeds Pvt. Ltd. Bangalore, India and maintained in Biotechnology department, R V College of Engineering, Bangalore. The PLBs were produced from the nodal explants of inflorescence on Murashige and Skoog (MS, 1962) medium with modification [$\frac{1}{2}$ strength MS salts, MS vitamins, 1 g/l tryptone, 20 g/l sucrose, 1 g/L charcoal, 65 g/L potato tubers, 8 g/l agar, and 5 μ M of thidiazuron (TDZ)]. The *in vitro* produced PLBs were maintained on modified MS medium free from TDZ. The pH of media was adjusted to 5.8 before solidifying with agar (Himedia, India). Media were dispensed to culture bottles and sterilized at 121°C for 20 min. The thermolabile compounds such as TDZ, vitamins and glycine were filter sterilized using 0.45 μ m membrane filters (Sartorius, Germany) and added to autoclaved media. The sections of PLBs were cultured on modified MS medium supplemented with chloro, methyl and nitro derivatives individually at 2 or 5 μ M concentrations. The cultures were maintained under controlled environmental conditions (22 \pm 2°C temperature with 16/8 h photoperiod). Five PLBs sections were placed in each culture bottle, three replicates were maintained for each concentration, and each experiment repeated twice. Cultures were observed and morphological changes such as number of explants responded and number of shoots developed from explants were recorded. One-way analysis of variance (ANOVA) was performed using SPSS software and means were compared with Duncun's multiple range test (Duncan, 1955).

Experimental

Synthesis of the compounds (3a-c)

To a solution of 4-substituted benzene-1,2-diamine (1.0 mmol) in 4N HCl (10 ml), chloroacetic acid (2.0 mmol) in 4 N HCl (7 ml) was added. The reaction mixture was refluxed for 4 h. The completion of reaction was monitored by TLC. The reaction mixture was cooled to room temperature then it is basified with Sodium bicarbonate. The precipitate so obtained was filtered, dried and recrystallized with ethanol. Characterization of the compound was done by NMR and liquid chromatography mass spectrometry (LCMS). Compounds were directly taken to next step without purification. NMR and LCMS data complies with literature data (Satyanarayana and Nagasundara, 2007).

Synthesis of the compounds (5a-c)

The compounds were synthesized by the following common procedure. A mixture of 2-mercapto-N-methyl imidazole (1 mmol) and sodium hydroxide (1 mmol) in methanol (10 mL) was stirred for about 30 min. A methanol solution (10 mL) of 5(6)-substituted 2-chloromethyl benzimidazole (1 mmol) was added slowly with stirring and the mixture was refluxed for about 8 h. The progress of the reaction was monitored by TLC and after completion; reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with water and brine, finally dried with anhydrous sodium sulphate. The organic layer was concentrated and the resulting crude compound was purified by column chromatography over silica gel (60 to 120 mesh) using ethyl acetate (100%). Synthesis of above benzimidazole derivatives 5(a-c) is given in Figure 1. Synthesis of 6(5)-Chloro-2-((1-methyl-1H-

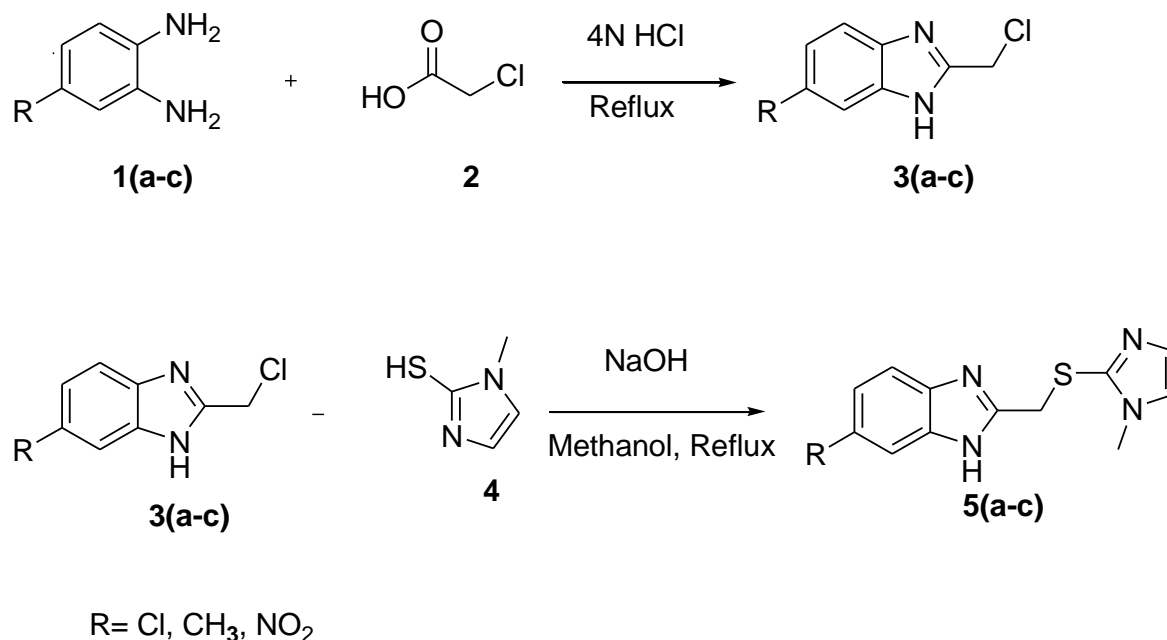


Figure 1. Synthesis of 2-mercapto-N-methyl imidazole substituted benzimidazole derivatives (5a-c).

imidazol-2-ylthio)methyl)-1*H*-benzimidazole (5a) Compound (5a) was synthesized from 2-mercapto-N-methyl imidazole (0.114 g, 1 mmol) and 6-chloro-2-(chloromethyl)-1*H*-benzimidazole (0.201 g, 1 mmol). Physical data for 5a: Yield: 85%, m.p: 130 to 132°C, Analysis for C₁₂H₁₁ClN₄S: found: C (51.28), H (3.98), N (20.73), S (10.86). Calculated % C (51.70), H (3.98), N (20.10), S (11.50). ¹H NMR (CDCl₃, 400 MHz): 3.49 (s,3H,N-CH₃), 4.39 (s,2H, -CH₂), 6.98 (s,1H,Ar-H), 7.41 to 7.17 (m,1H,Ar-H), 7.26 (s,1H,Ar-H), 7.50 (d,1H, *J* = 12.0Hz, Ar-H), 7.56 (d,1H, *J* = 4.0 Hz Ar-H). ¹³C NMR δ(ppm): 31.53, 32.90, 121.96, 123.79, 126.08, 128.77, 138.84, 152.53, MS (ESI) m/z: 279.06, 281.06 (M+2), IR (KBr, cm⁻¹): 3657, 3383, 1625, 1341, 1229, 853.

Synthesis of 6(5)-Methyl-2-((1-methyl-1*H*-imidazol-2-ylthio)methyl)-1*H*-benzimidazole (5b)

Compound (5b) was synthesized from 2-mercapto-N-methyl imidazole (0.114 g, 1 mmol) and 2-(chloromethyl)-6-methyl-1*H*-benzimidazole (0.181 g, 1 mmol). Physical data for 5b: Yield: 62%, m.p: 62 to 64°C, Analysis for C₁₃H₁₄N₄S: found % C (60.19), H (5.05), N (21.88), S (11.98); calculated % C (60.44), H(5.46) N (21.69), S (12.41); ¹H NMR (DMSO-d₆, 400 MHz): 3.59 (s,3H,N-CH₃), 4.41 (s,2H,-CH₂), 2.45 (s, 3H, Ar-CH₃), 6.96 (s,1H,Ar-H), 7.03 (d,1H, *J*=8.08Hz, Ar-H), 7.15 (s,1H, Ar-H), 7.35 (s,1H,Ar-H), 7.45 (d,1H, *J*=8.2Hz, Ar-H). ¹³C NMR δ(ppm): 29.66, 31.00, 33.48, 122.60, 123.97, 128.73, 132.34, 142.54, 151.97. MS (ESI) m/z: 259.10; IR (KBr, cm⁻¹): 3664, 3144, 1704, 1314, 1265, 865.

Synthesis of 2-((1-Methyl-1*H*-imidazol-2-ylthio)methyl)-6-nitro-1*H*-benzimidazole (5c)

Compound (5c) was synthesized from 2-mercapto-N-methyl imidazole (0.114 g, 1 mmol) and 2-(chloromethyl)-6-nitro-1*H*-benzimidazole (0.211 g, 1 mmol). Physical data for 5c: Yield: 55%, m.p: 134 to 136°C, Analysis for C₁₂H₁₁N₅O₂S: found %: C (49.11),

H (3.35), N (23.92), S (10.75); calculated % C (49.82), H (3.83), N (24.21), S (11.08); ¹H NMR (DMSO-d₆, 400 MHz): 3.50 (s,3H,N-CH₃), 4.46 (s,2H,-CH₂), 6.96 (s,1H,Ar-H), 7.25 (s,1H,Ar-H), 7.67 (d,1H, *J*=12Hz, Ar-H), 8.09 to 8.06 (m,1H,Ar-H), 8.41 (s,1H,Ar-H). ¹³C NMR δ(ppm): 31.45, 32.93, 123.87, 128.85, 138.61, 142.49. MS (ESI) m/z: 290; IR (KBr, cm⁻¹): 3603, 3244, 1712, 1322, 1277, 866. The structural and spectroscopic assignments were made according to the reported literature (Shivakumaraiah et al., 2003; Sahin et al., 2002).

RESULTS AND DISCUSSION

IR spectra

The compounds 5(a-c) exhibit a broad band around 3244 to 3383 due to ν(NH) of the Benzimidazole ring and band in the region at 1625 to 1704 assignable to ν(C=C) and ν(C=N). All the three compounds exhibit characteristic band around 1400 cm⁻¹ due to N-CH₃ stretching vibration.

NMR Spectra

The ¹H NMR spectra of the heterocycles (5a-c) exhibit a singlet in the range of δ 4.39 to 4.46 due to -CH₂-protons. The N-CH₃ proton signals were observed in the range of δ 3.49 to 3.60. Compound 5b exhibited signals due to aromatic protons in the range δ 7.15 to 7.47, whereas, in the corresponding chloro and nitro derivatives (5a and 5c), these signals were observed in the range δ 7.26 to 8.41. The downfield shift in the aromatic

Table 1. Effects of compounds 5(a-c) on plantlet regeneration from sections of *Oncidium* PLBs*.

Treatment	Concentration (μM)	No. of plantlets per culture bottle*
Control		41 ^f
5a	2	56 ^e
	5	84 ^b
5b	2	69 ^d
	5	84 ^b
5c	2	76 ^c
	5	95 ^a

Control: modified MS medium (1/2 strength MS salts, MS vitamins, 1 g/l tryptone, 20 g/l sucrose, 1g/l charcoal, 65 g/l potato tubers, 8 g/l agar).

*Fifteen (15) PLBs sections were cultured for each treatment (five PLBs sections per culture bottle). *Means followed by same letters are not significantly different according to DMRT at P= 0.05 (Duncan, 1955)

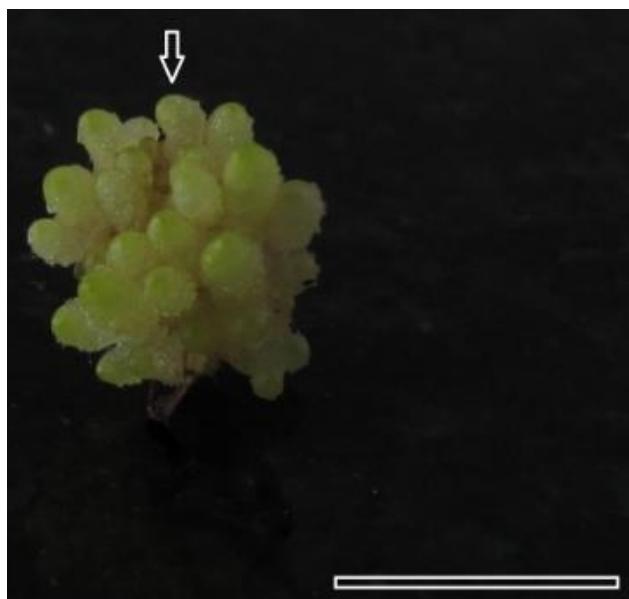


Figure 2. Induction of globular embryos from PLBs sections of *Oncidium* on modified MS medium supplemented with 5 μM compound 5c (bar = 0.5 cm).

proton signals in the case of 5a and 5c is attributed to the deshielding effect of -Cl and -NO₂ groups on the ring. The number of protons calculated from the integration of ¹H NMR spectra is in accordance with C, H, N, S analysis of the above compounds.

Effect of 2-mercapto-N-methyl imidazole substituted benzimidazole derivatives on plantlet production

PLBs sections of *Oncidium* cultured on modified MS medium alone and also on modified MS containing chloro,



Figure 3. Development of cotyledonary stage embryos of *Oncidium* on modified MS medium containing 5 μM compound 5c (bar = 1.4 cm).



Figure 4. Development of plantlets on modified MS medium containing 5 μM compound 5c (bar = 3 cm).

methyl and nitro derivatives at 2 or 5 μM concentrations showed significant difference in production of plantlets (Table 1). After two-weeks of culture, PLBs sections were swollen, and in another two weeks it produced globular-embryo-like structures (Figure 2). Globular embryo-like-structures were developed into cotyledonary-embryo-like structures (Figure 3). On the same medium, these cotyledonary-embryo-like-structures developed into plantlets (Figure 4). PLBs explants produced average

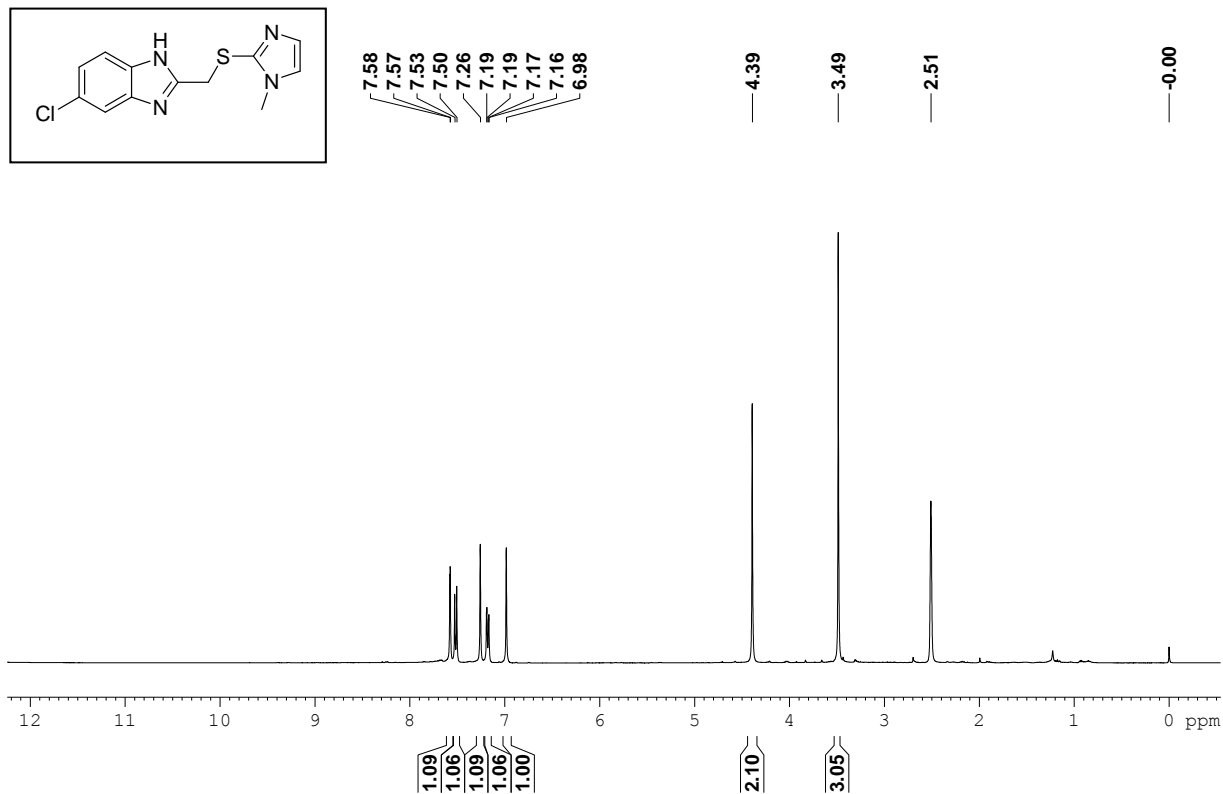


Figure 5. ¹H NMR spectrum of compound 5a.

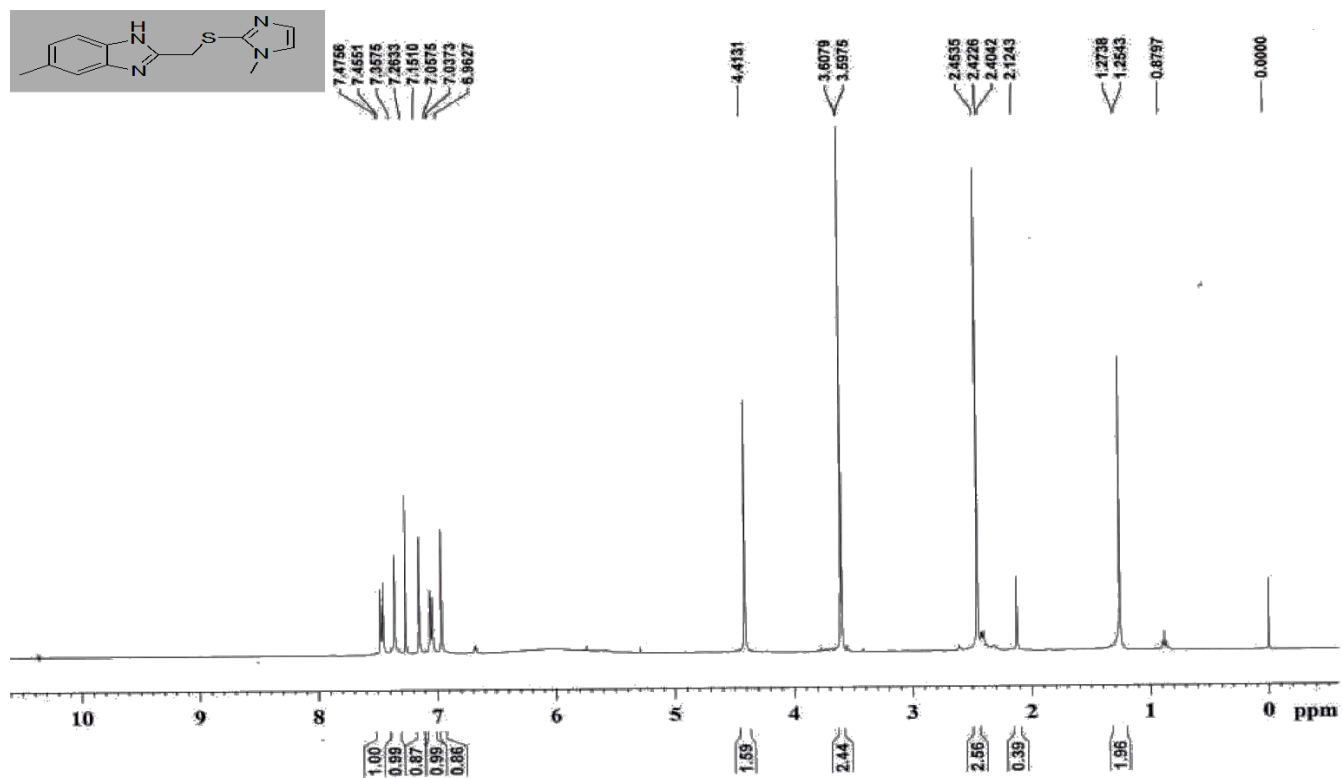


Figure 6. ¹H NMR spectrum of compound 5b.

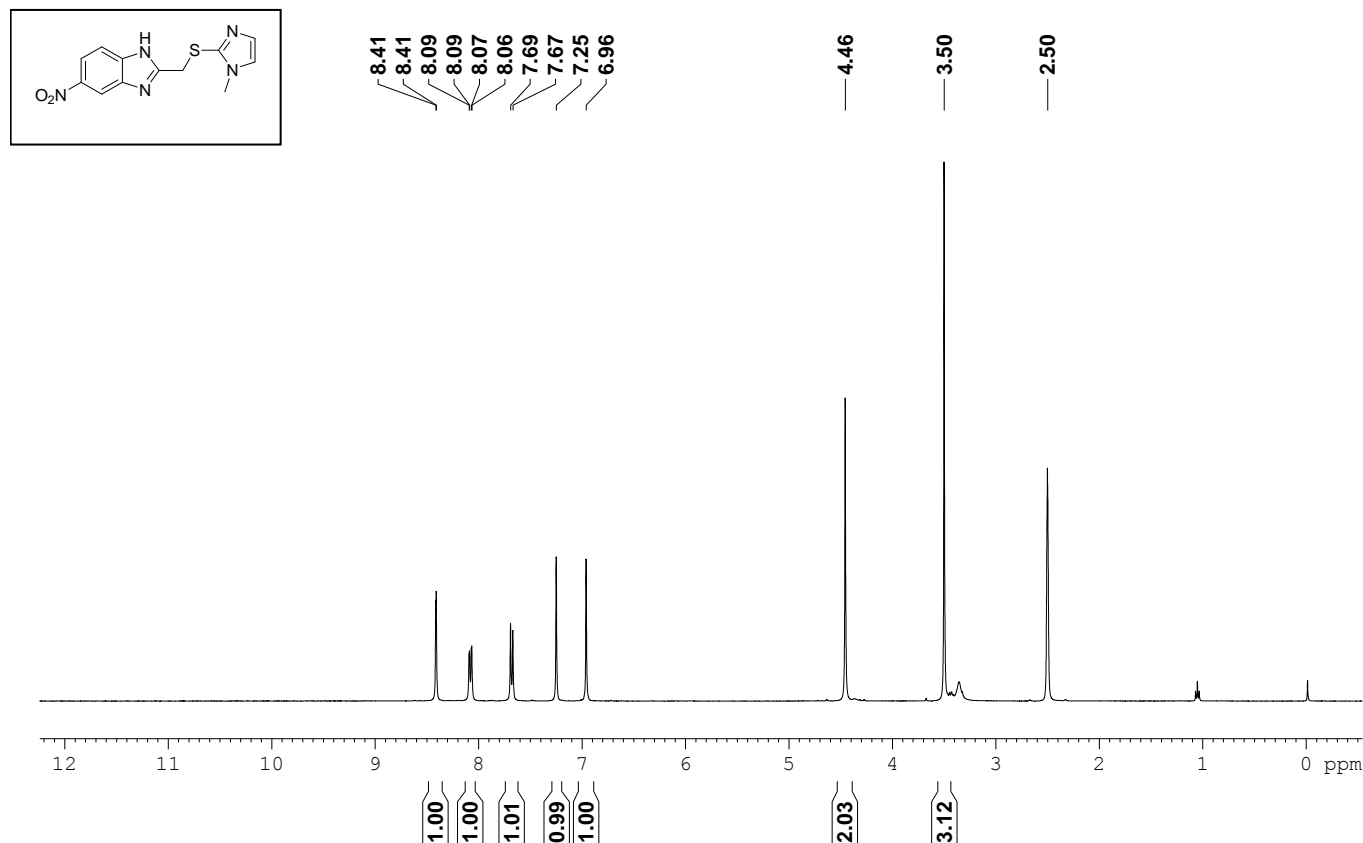


Figure 7. ¹H NMR spectrum of compound 5c.

number (41) of plantlets on modified MS medium alone. The production plantlets from PLBs explants were enhanced significantly with the addition of chloro, methyl or nitro derivatives to the modified MS medium. Chen and Chang (2001; 2003a; 2003b; 2004) studied the effects of plant growth regulators such as auxins, cytokinins, gibberellins (GA₃), ancymidol, cycocel, paclobutrazol, 2,3,5-triiodobenzoic acid (TIBA), 2-(p-chlorophenoxy)-2-methylpropionic acid (PCIB) on production of somatic embryos and subsequent development of plantlets in *Oncidium*. In present study, PLBs sections produced 56, 69 and 76 numbers of plantlets on medium supplemented with 2 μM of chloro, methyl and nitro derivatives, respectively (Table 1).

Direct somatic embryogenesis was reported in *Oncidium* Gower Ramsey from leaf explants (Chen and Chang, 2001). Similarly, in present investigation, plantlets were produced directly from the PLBs sections through somatic embryogenesis without intervening callus phase. Among three compounds studied in the present investigation, explants cultured on medium supplemented with 5 μM nitro derivatives produced maximum number (95) of plantlets (Table 1). The involvement of nitro radical in the nitro derivative in a wide range of plant functions such as growth senescence, fruit ripening and

responses to adverse environmental conditions (Sanchez B-Calvo et al., 2013) is expected to be the reason for its higher activity when compared to chloro and methyl derivatives

Conclusions

The analytical data and spectral data confirm the formation of the compounds (5a-c). Production of plantlets from PLBs section of *Oncidium* was improved with the addition of chloro or methyl or nitro derivatives at 2 or 5 μM concentrations to the induction medium. Medium supplemented with 5 μM nitro derivative exhibited maximum number of plantlets.

Conflict of interests

The authors did not declare any conflict of interest.

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