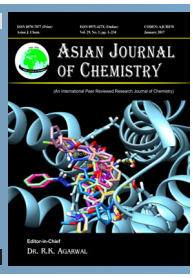
Asian Journal of Chemistry; Vol. 29, No. 4 (2017), 929-932

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20403



Pentyl Thiourea Derivatives as Potential Growth Inhibitor Towards *Oscillatoria* sp.

WAN M. KHAIRUL[1,*], YASMIN RIDWAN MORGAN[1], RAFIZAH RAHAMATHULLAH[1] and NOR AZMAN KASAN[2]

[1]School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[2]Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

*Corresponding author: Fax: +60 9 6683608; Tel: +60 9 6683315; E-mail: wmkhairul@umt.edu.my

Received: 10 November 2016; *Accepted*: 16 December 2016; *Published online*: 31 January 2017; AJC-18275

Nowadays, our aquatic environment is currently being polluted by harmful algal blooms, which affect water quality and eventually kill aquatic organisms. Many efforts have been carried out to overcome the problem but the methods are either money-consuming or non-environmental friendly. Therefore, this study was aimed to inhibit the growth of *Oscillatoria* sp. isolated from Kenyir lake in the state of Terengganu, Malaysia using the synthesized pentyl-thiourea derivatives (**1-4**). The series of compounds were successfully synthesized and spectroscopically characterized *via* infrared analysis and ^{1}H and ^{13}C NMR spectroscopy. The IR spectra of all the compounds showed five expected important stretching bands of interest which were ν(N-H), ν(C-H), ν(C=O), ν(C-N) and ν(C=S). In turn, they were introduced towards *Oscillatoria* sp. culture at different concentration of 2-10 µg/mL. All these compounds showed inhibition potential towards the *Oscillatoria* sp. growth in the range of 6 and 30 %. These types of molecules within thiourea family are indeed are potentially exhibit the growth of *Oscillatoria* sp.

Keywords: Thiourea, Spectroscopy, Algaecidal, *Oscillatoria* sp.

INTRODUCTION

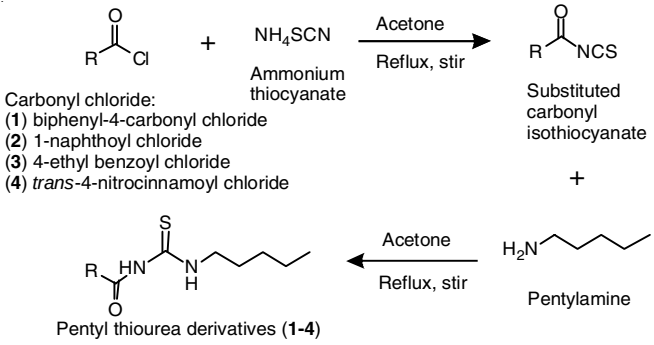
Water eutrophication is a critical issue for freshwater and marine ecosystems as it will result in widespread deterioration of the quality of surface water. Eutrophication occurs due to the over-enrichment of water by nutrients such as nitrates and phosphates, which eventually lead to the excessive growth of harmful algal blooms, degradation of biodiversity and depletion of oxygen concentration in water system [1,2]. Various factors can stimulate eutrophication for instance, the release of sewage effluents and fertilizer use on agricultural activities into water bodies [3,4]. This situation leads to the rapid increase of the growth of toxic blue-green algae (cyano-bacteria) and will cause harmful algal blue phenomenon. There is some toxic blue-green alga species that can produce harmful toxins once its blooming such as *Oscillatoria* sp. which releases microcystins and have been reported could give acute liver damage and liver cancer in laboratory animals [3,5]. Another example is *Lyngbya robusta* sp., which can produce hepatoxin and cyanotoxin [1]. At certain concentration level, these toxins can be very poisonous and significantly affect animal life and also human health [4,6]. In order to overcome this problem, several attempts have been made including physical (*e.g.*, trapping algae using nets), chemical (*e.g.*, use of copper sulphate) and biological treatments (*e.g.*, introduction other types of plankton) [7,8]. Although these methods can remove blue-green algae from surface water efficiently, however there is economic restriction in a long-term operation and also environmental effect due to the production by-products [3]. Our goal is to apply thiourea-based compounds to curb blue-green algae as in previous studies, thiourea derivatives were able to act as antimicrobial and antifungal agents [9-12]. Hence, this study was aimed to investigate the capability of pentyl-thiourea derivatives (**1-4**) to curb the growth of toxic blue-green algae, *Oscillatoria* sp., which was isolated from Kenyir lake in the state of Terengganu, Malaysia and perhaps in the near future could be further applied as algaecidal agents.

EXPERIMENTAL

Chemicals and reagents used were purchased from standard commercial suppliers and used as received without further purification. All reactions were carried out under an ambient atmosphere and no special procedures were taken to exclude air or moisture during work up. Infrared spectra of the synthesized compounds were recorded on Perkin Elmer Spectrum 100 Fourier Transform Infrared Spectroscopy in the spectral range of 4000-400 cm^{-1} using potassium bromide (KBr) pellet. Whilst, NMR spectra were recorded on Bruker Avance III 400 spectrometer using deuterated chloroform as a

solvent and internal standard within range δ_H 0-15 ppm for ^{1}H NMR and δ_C 0-200 ppm for ^{13}C NMR.

Synthesis of *N*-(pentyl)-*N'*-(biphenyl-4-carbonyl)thiourea (1): The general synthetic route to produce pentyl-thiourea derivatives (**1-4**) is presented in **Scheme-I**. The synthesis of **1** involved a reaction between an equimolar amount of biphenyl-4-carbonyl chloride (1.0 g, 4.61 mmol) and ammonium thiocyanate (0.7 g, 4.61 mmol) which produced biphenyl 4-carbonyl isothiocyanate in yellow solution and formation of ammonium chloride salt in 50 mL acetone and reflux for about 4 h. Then, an equimolar amount of pentylamine (1 mL, 4.61 mmol) was added dropwise to the reaction mixture and continued to reflux until the reaction has reached completion, determined using thin layer chromatography (TLC) technique, (hexane: dichloromethane; 4:1). Then, the reaction mixture was cooled to room temperature before filtration. The filtrate was added with several ice cubes to afford the crude product. Finally, the crude was then recrystallized from methanol to afford the title compound **1** as pale yellow crystalline solids (81 % yield). ^{1}H NMR (400.11 MHz, $CDCl_3$) (δ, ppm): 0.86 (t, J_{HH} = 7 Hz, 3H, CH_3); 1.32-3.66 (m, 8H, 4 × CH_2); 7.33-7.56 (m, 5H, C_6H_5); 7.64-7.86 (m, 4H, C_6H_4); 8.98, 10.70 (2 × s, 1H, NH); ^{13}C NMR (100.61 MHz, $CDCl_3$) (δ, ppm): 13.95 (CH_3); 22.34, 27.94, 29.11, 46.00 (4 × CH_2); 127.28, 127.72, 128.01, 128.56, 129.06, 130.32, 139.32, 146.41 (8 × C_6H_4); 166.56 (C=O); 179.69 (C=S).

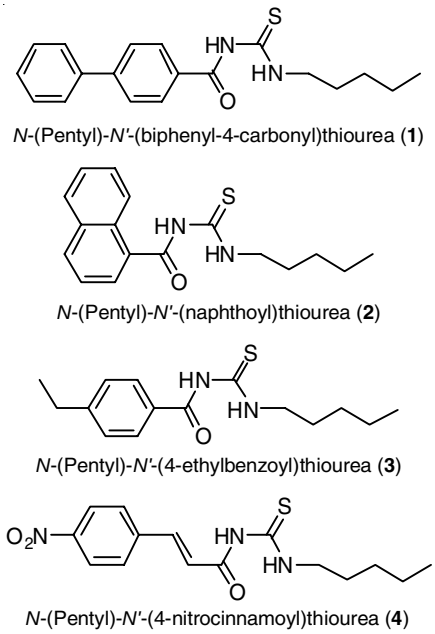


Scheme-I: Synthetic approach for the synthesis of pentyl-thiourea derivatives (**1-4**)

Synthesis of *N*-(pentyl)-*N'*-(naphthoyl)thiourea (2): Yielding white crystalline solid (75 % yield), **2** was prepared from 1-naphthoyl chloride (0.8 g, 4.20 mmol), ammonium thiocyanate (0.5 g, 4.20 mmol) and pentylamine (0.8 mL, 4.20 mmol) in the same manner as **1**. ^{1}H NMR (400.11 MHz, $CDCl_3$) (δ, ppm): 0.88 (t, J_{HH} = 7 Hz, 3H, CH_3); 1.29-3.65 (m, 8H, 4 × CH_2); 7.40-8.23 (m, 7H, naphthoyl); 9.00, 10.67 (2 × s, 1H, NH); ^{13}C NMR (100.61 MHz, $CDCl_3$) (δ, ppm): 13.98 (CH_3); 22.36, 27.95, 29.15, 45.98 (4 × CH_2); 124.52, 124.64, 126.23, 126.96, 128.04, 128.73, 129.81, 130.90, 132.88, 133.77 (10 × Ar-C); 169.07 (C=O); 179.76 (C=S).

Synthesis of *N*-(pentyl)-*N'*-(4-ethylbenzoyl)thiourea (3): The pale brown crystalline solid of **3** (72 % yield) was prepared from 4-ethylbenzoyl chloride (1.0 g, 5.93 mmol), ammonium thiocyanate (0.8 g, 5.93 mmol) and pentylamine (1.0 mL, 5.93 mmol) in the same manner as described above. ^{1}H NMR (400.11 MHz, $CDCl_3$) (δ, ppm): 0.93, 1.26 (t, J_{HH} = 8 Hz, 6H, 2 × CH_3); 1.35-3.70 (m, 10H, 5 × CH_2); 7.31-7.77 (m, 4H, C_6H_4); 9.05, 10.78 (2 × s, 1H, NH); ^{13}C NMR (100.61 MHz, $CDCl_3$) (δ, ppm): 13.94, 15.08 (2 × CH_3); 22.33, 27.93, 28.90, 29.09, 45.93 (5 × CH_2); 127.61, 128.62, 129.15, 150.64 (4 × C_6H_4); 166.52 (C=O); 179.79 (C=S).

Synthesis of *N*-(pentyl)-*N'*-(4-Nitrocinnamoyl)thiourea (4): The synthesis of **4** (83 % yield) was conducted by adding *trans*-4-nitrocinnamoyl chloride (0.8 g, 3.78 mmol), ammonium thiocyanate (0.5 g, 3.78 mmol) and pentylamine (1 mL, 3.78 mmol) to afford pale brown crystalline solid in the same manner as described above. ^{1}H NMR (400.11 MHz, $CDCl_3$) (δ, ppm): 0.87 (t, J_{HH} = 8 Hz, 3H, CH_3); 1.27-3.58 (m, 8H, 4 × CH_2); 7.42 (dd, 2H, HC=CH); 7.83-8.28 (m, 4H, C_6H_4); 10.75, 11.33 (2 × s, 1H, NH); ^{13}C NMR (100.61 MHz, $CDCl_3$) (δ, ppm): 13.78 (CH_3); 21.72, 27.21, 28.48, 44.50 (4 × CH_2); 124.22, 129.12, 141.35, 148.02 (4 × C_6H_4); 140.37, 141.17 (C=C); 165.12 (C=O); 179.69 (C=S).



Molecular structure of pentyl-thiourea derivatives (**1-4**)

Treatment of *Oscillatoria* sp. by pentyl thiourea derivatives: The pure culture of *Oscillatoria* sp. was obtained from culture collection in Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu and mass cultured using sterile BG-11 liquid media in 2 L conical flask. The cultivated culture was supplied with aeration under continuous illumination of cool fluorescent light at 20 ± 2 °C. After 6 days, the mass culture with optimum growth was transferred into 100 mL conical flask for treatment with thiourea derivatives.

All the compounds **1-4** were diluted by adding 1,000 µg of each compounds with 1 mL dimethyl sulfoxide to obtain total concentration of 1,000 µg/mL on five different concentrations; 2, 4, 6, 8 and 10 µg/mL. Each compound (2 mL) was added up into 30 mL of *Oscillatoria* sp. cultures in 100 mL conical flask. For controlled treatment, distilled water was used to replace the compound solution. All treatment cultures were incubated for 24 h under illumination at 25 °C. After that, the control and treated *Oscillatoria* sp. cultures were transferred into 50 mL centrifuge tube and centrifuged for 10 min at 5,000

rpm. Supernatant was removed and 5 mL of acetone was added for chlorophyll-*a* determination. The centrifuge tube were sealed with aluminium foil (dark condition) and left overnight at 4 °C. Then, the samples were centrifuged for 10 min at 3,000 rpm. The supernatant was taken out and measured at 665 nm (OD_{665}), 645 nm (OD_{645}) and 630 nm (OD_{630}) using UV-visible spectrophotometer. Eqn. 1 was used to determine the chlorophyll-a content:

$$\text{Chlorophyll-a } (\mu g/mg\ m^{-3}) = \frac{C_a \times V_a}{V_c} \quad (1)$$

where; $C_a = (11.6 \times OD_{665}) - (1.31 \times OD_{645}) - (0.14 \times OD_{630})$; V_a = Volume of acetone (mL) used for extraction; V_c = Volume of culture (mL).

The inhibition effects (in percentage) of pentyl thiourea derivatives (**1-4**) towards the growth of *Oscillatoria* sp. cultures were determined using eqn. 2:

$$\text{Inhibition effect } (\%) = \frac{C_c \times C_t}{C_c} \times 100 \quad (2)$$

where; C_c = Chlorophyll-a (mg m^{-3}) of control (untreated); C_t = Chlorophyll-a (mg m^{-3}) of treated culture.

RESULTS AND DISCUSSION

Infrared spectroscopy analysis: The IR spectra of the synthesized compounds (**1-4**) showed five absorption bands of interest namely, ν(N-H), ν(C-H), ν(C=O), ν(C-N) and ν(C=S) ranging from weak to strong intensities. The bands in the range of 3248-3213 cm^{-1} attributed to asymmetric and symmetric vibrations of ν(N-H) in the secondary thioamide moiety. The vibration mode of ν(N-H) can be observed in the range 3175-3300 cm^{-1} due to the existence of intramolecular hydrogen bonding [13,14]. Strong absorption band of ν(C=O) which occurred at 1680-1659 cm^{-1}, seemingly decreasing in frequency compared to ordinary carbonyl moiety at 1710 cm^{-1} [15]. This is due to the presence of resonance effect of conjugated phenyl ring and intramolecular hydrogen bonding towards N-H [16,17]. This statement is supported by the literature that ν(C=O) absorption band of thiourea moiety can be seen between 1800-1650 cm^{-1} [18]. Meanwhile, the assignment of absorption bands around 1343-1334 cm^{-1} as medium intensity peak corresponded to ν(C-N), where the result is in the same agreement with previous study which the assignments of ν(C-N) for thiourea compound can be found in the range 1400-1000 cm^{-1} [15]. The most important frequencies observed at 840-730 cm^{-1} corresponded to ν(C=S) and were assigned by comparison with other thiourea derivatives [13,14]. The medium intensity of ν(C=S) band designates double bond character, which can be observed at low frequency in the spectra. Table-1 shows the absorption data for all compounds.

Nuclear magnetic resonance (NMR) analysis: The ^1H NMR spectra for the series of compounds showed methyl resonances in the range of δ_H 0.86-1.26 ppm while protons for alkyl group were observed in the range δ_H 1.27-3.58 ppm. The aromatic protons were observed in the range δ_H 7.31-8.28 ppm as multiplet resonances due to the overlapped proton signals of the fused phenyl rings system. For compounds **2** and **3**, the resonances of aromatic protons were located in the higher chemical shift at δ_H 7.40-8.23 ppm and 7.83-8.28 ppm, respectively due to the existence of oxygen and nitro moieties attached to the phenyl ring. Meanwhile, for compound **4**, a set of doublet-of-doublet resonance can be seen at δ_H 7.43 ppm due to the HC=CH moiety in the compound. There were two singlet resonances for NH moiety which can be seen at two different environments, NH substituted to the carbonyl at δ_H 8.98-10.75 ppm and NH substituted to the thiocarbonyl at δ_H 10.67-11.33 ppm. The resonances for amines were different in location of chemical shift due to the presence of intramolecular hydrogen bonding and deshielding effect in the molecule [15,19].

Whilst, ^{13}C NMR spectra for these derivatives showed methyl group resonances at δ_C 13.78-15.08 ppm and carbon resonances for alkyl groups at δ_C 21.72-44.50 ppm. Aromatic ring resonances were observed at δ_C 124.22-129.81 ppm. For C=O and C=S, the signals were detected at δ_C 165.12-169.07 ppm and δ_C 179.69-179.79 ppm, respectively. Both resonances were deshielded to higher chemical shift due to the formation of intramolecular hydrogen bonding as well as electronegativity of oxygen and sulphur [20-23].

Growth inhibition effect of pentyl-thiourea derivatives (1-4): Four synthesized compounds of **1-4** were used as potential algaecidal agents to curb the growth of *Oscillatoria* sp. Overall, it was proven that the compounds **1-4** were able to act as growth inhibitor for toxic blue-green algae, *Oscillatoria* sp. at the concentration 2 μg/mL until 10 μg/mL. From the data obtained, compound **2** was the best candidate of pentyl-thiourea derivatives that can inhibit the growth of *Oscillatoria* sp. at concentration 10 μg/mL up to 30 % of inhibition percentage. Whereas, the least inhibition percentage (18 %) was obtained from treatment with compound **1** at concentration of 10 μg/mL. The trend of inhibition in this study showed that the percentage of inhibition will increase as the concentration increase.

Different substituent group that attached to pentyl-thiourea compounds showed different effect toward inhibition percentage. The naphthalene group attached in compound **2** showed the highest impact to inhibit the growth of *Oscillatoria* sp. As the results, the effectiveness of inhibition percentage towards the growth of *Oscillatoria* sp. depends in the substituent group of the compounds and their concentrations [5]. Table-2 shows the inhibition percentage of all the compounds from 2-10 μg/mL.

TABLE-1
KEY IR ABSORPTION BANDS (cm^{-1}) OF PENTYL-THIOUREA DERIVATIVES (**1-4**)

Compd.	ν(N-H)	ν(C-H)	ν(C=O)	ν(C-N)	ν(C=S)
1	3248 (m)	2929 (m)	1663 (s)	1335 (m)	746 (m)
2	3233 (m)	2927 (m)	1677 (s)	1340 (m)	787 (m)
3	3213 (m)	2929 (m)	1659 (s)	1334 (m)	730 (m)
4	3234 (m)	2942 (m)	1680 (s)	1343 (m)	840 (m)

TABLE-2
INHIBITION PERCENTAGE OF **1-4** TOWARDS THE GROWTH OF *Oscillatoria* sp.

Compound	Inhibition percentage (2-10 μg/mL) (%)
1	8.5-18.1
2	17.2-30.3
3	9.4-20.5
4	6.6-24.1

Conclusion

Four of pentyl-thiourea derivatives (**1-4**) have been introduced to the culture and it was proven that all the compounds showed potential effect as inhibitor towards toxic blue-green algae. Apparently, compound **2** containing naphthoyl motif can be used as potential candidate for *Oscillatoria* sp. growth inhibitor since it showed the highest inhibition percentage at low amount of concentration. Although the overall inhibition percentage for all compounds were less than 50 %, it still showed sign of inhibition that need to be more explored. In conclusion, this study showed that pentyl-thiourea derivatives have the ability to be developed as algaecidal agent. However, additional examination on this system especially at higher concentration level, the mechanism of inhibition and cytotoxic analysis should be further carried out.

ACKNOWLEDGEMENTS

The authors thank Ministry of Higher Education Malaysia (MOHE) for financial support (ERGS 55096), School of Fundamental Science and Institute of Tropical Aquaculture (AKUATROP), Universiti Malaysia Terengganu for their cooperation and support throughout the study.

REFERENCES

1. E. Rejmánková, J. Komárek, M. Dix, J. Komárková and N. Girón, *Limnologica*, **41**, 296 (2011); https://doi.org/10.1016/j.limno.2010.12.003.
2. M.D. Skogen, K. Eilola, J.L.S. Hansen, H.E.M. Meier, M.S. Molchanov and V.A. Ryabchenko, *J. Mar. Syst.*, **132**, 174 (2014); https://doi.org/10.1016/j.jmarsys.2014.02.004.
3. X. Wang, C. Hao, F. Zhang, C. Feng and Y. Yang, *Bioresour. Technol.*, **102**, 5742 (2011); https://doi.org/10.1016/j.biortech.2011.03.015.
4. C.C. Huang, X.L. Wang, H. Yang, Y.M. Li, Y.H. Wang, X. Chen and L.J. Xu, *Sci. Total Environ.*, **485-486**, 1 (2014); https://doi.org/10.1016/j.scitotenv.2014.03.031.
5. N.A. Kasan, S.Z.M. Yusof, N.B. Ramli, W.M. Khairul and H.A. Zakeri, *J. Environ. Sci. Eng.*, **4**, 389 (2015).
6. L. Du-Cuny, J. Huwyler, M. Wiese and M. Kansy, *Eur. J. Med. Chem.*, **43**, 501 (2008); https://doi.org/10.1016/j.ejmech.2007.04.009.
7. L. Heng, N. Jun, H. Wen-jie and L. Guibai, *Desalination*, **239**, 191 (2009); https://doi.org/10.1016/j.desal.2007.12.035.
8. S. Kotopoulis, A. Schommartz and M. Postema, *Appl. Acoust.*, **70**, 1306 (2009); https://doi.org/10.1016/j.apacoust.2009.02.003.
9. S. Saeed, N. Rashid, P.G. Jones, M. Ali and R. Hussain, *Eur. J. Med. Chem.*, **45**, 1323 (2010); https://doi.org/10.1016/j.ejmech.2009.12.016.
10. M. Eweis, S.S. Elkholy and M.Z. Elsabee, *Int. J. Biol. Macromol.*, **38**, 1 (2006); https://doi.org/10.1016/j.ijbiomac.2005.12.009.
11. E. Rodriguez-Fernandez, J.L. Manzano, J.J. Benito, R. Hermosa, E. Monte and J.J.J. Criado, *J. Inorg. Biochem.*, **99**, 1558 (2005); https://doi.org/10.1016/j.jinorgbio.2005.05.004.
12. S.Y. Abbas, M.A.M.Sh. El-Sharief, W.M. Basyouni, I.M.I. Fakhr and E.W. El-Gammal, *Eur. J. Med. Chem.*, **64**, 111 (2013); https://doi.org/10.1016/j.ejmech.2013.04.002.
13. S.M. Jasman, W.M. Khairul, T. Tagg, K. KuBulat, R. Rahamathullah, S. Arshad, I.A. Razak and M.I.M. Tahir, *J. Chem. Crystallogr.*, **45**, 338 (2015); https://doi.org/10.1007/s10870-015-0599-6.
14. A.I. Daud, W.M. Khairul, H. Mohamed Zuki and K. Kubulat, *J. Mol. Struct.*, **1093**, 172 (2015); https://doi.org/10.1016/j.molstruc.2015.03.065.
15. Z. Zhong, R. Xing, S. Liu, L. Wang, S. Cai and P. Li, *Carbohydr. Res.*, **343**, 566 (2008); https://doi.org/10.1016/j.carres.2007.11.024.
16. N.B. Arslan, C. Kazak and F. Aydin, *Spectrochim. Acta*, **89**, 30 (2012); https://doi.org/10.1016/j.saa.2011.12.040.
17. J. Pathak, V. Narayan, L. Sinha and O. Prasad, *J. Atomic Mol. Sci.*, **3**, 95 (2012); https://doi.org/10.4208/jams.051111.063011a.
18. W.M. Khairul, N.A. Roslan, R. Rahamathullah and H. Salleh, *Malaysian J. Microsc.*, **10**, 62 (2014).
19. W.M. Khairul, A.I. Daud, H. Mohamed Zuki and K. Kubulat, *Appl. Mech. Mater.*, **719-720**, 59 (2015); https://doi.org/10.4028/www.scientific.net/AMM.719-720.59.
20. Y.F. Yuan, J.T. Wang, M.C. Gimeno, A. Laguna and P.G. Jones, *Inorg. Chim. Acta*, **324**, 309 (2001); https://doi.org/10.1016/S0020-1693(01)00661-2.
21. M.S.M. Yusof, R.H. Jusoh, W.M. Khairul and B.M. Yamin, *J. Mol. Struct.*, **975**, 280 (2010); https://doi.org/10.1016/j.molstruc.2010.04.037.
22. W.M. Khairul, M.F. Yusof, R. Rahamathullah, A.I. Daud, S.M. Jasman, M.F.A. Hasan, H. Salleh, H.K. Adli and M.G. Tay, *Int. J. Electrochem. Sci.*, **8**, 8175 (2013).
23. W.M. Khairul, K.A. Mokthar, M.I.N. Isa, A.S. Samsudin, H.K. Adli, S.R. Ghazali and A.I. Daud, *Phosphorus Sulfur Silicon Rel. Elem.*, **189**, 640 (2014); https://doi.org/10.1080/10426507.2013.844137.