SYNTHESIS AND BIOASSAY STUDIES OF BENZOXAZIN-4-ONE AND QUINAZOLIN-4-IMINE DERIVATIVES

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Abstract

The 4*H*-3,1-benzoxazin-4-one and quinazolin-4-imine derivatives have been synthesized in simple and one step reaction. The reaction of anthranilic acid and benzoyl chloride, terephtaloyl dichloride and 4-chlorobenzoyl chloride in pyridine yielded 2-phenyl-4*H*-3,1-benzoxazin-4-one (78.91%), 1,4-(di-4*H*-3,1-benzoxazin-4-one)benzene (55.23%) and 2-(*p*-chlorophenyl)-4*H*-3,1,-benzoxazine-4-one (33.20%) respectively. The benzoxazin-4-one derivatives were then treated with hydrazine hydrate in absolute ethanol to form 2-phenylquinazolin-4-imine (70.90%), 1,4-(diquinazolin-4-imine)benzene (43.70%) and 2-(*p*-chlorophenyl)quinazolin-4-imine (35.81%). The resulting compounds were characterized using ATR and ^{1}H NMR using $CDCl_3$ as solvent. From both spectra it showed that the synthesis of targeted compound, 4*H*-3,1-quinzolin-4-one was unsuccessful. The resulting compounds after treated with hydrazine hydrate were proposed to be quinazolin-4-imine compounds based on the data analyzed from ATR and ^{1}H NMR spectrum. Antioxidant test using DPPH free radical scavenging has been carried out on the six compounds synthesized. The results showed that 4*H*-3,1-benzoxazin-4-one derivatives did not show antioxidant activity while the compounds of quinazolin-4-imine derivatives showed good antioxidant activity as the IC_{50} value obtained are lower than positive control of ascorbic acid except for 1,4-(diquinazolin-4-imine)benzene. Among the quinazolin-4-imine derivatives, 2-phenylquinazolin-4-imine showed the highest antioxidant activity at IC_{50} value at 2.66ppm. The introducing of electron withdrawing group at phenyl substituent was found to reduce ability of compounds in antioxidant activity.

Keywords: Heterocycle, synthesis, 4*H*-3,1-benzoxazin-4-one, quinazolin-4-imine, antioxidant

INTRODUCTION

Benzoxazinone belongs to the group of heterocyclic compounds that consist of unsaturated six-membered rings with two heteroatoms of oxygen and nitrogen. While the quinazoline consist of unsaturated six-membered rings with two heteroatoms of nitrogen. Heterocyclic compounds are commonly become an interest in pharmaceuticals and agrochemical industries due to their natural occurrence [1]. Numerous additives and dyes used in industrial application such as cosmetic are heterocyclic in nature. The common biological activities possessed by synthetic heterocyclic compounds are antibacterial [2], antifungal [3], anti-inflammatory [4] and antioxidant [5].The wide range of biological activities possessed by heterocycles is mainly due to the extraordinary wide range of reaction of the compounds. Heterocyclic can behave as either acid or base to form anion or cation depending on the pH value of the medium. Besides, some heterocyclic easily interact with electrophilic reagent while some with nucleophiles, or both. Some can be easily oxidized but reduction resistant, or vice versa. Furthermore, there are heterocyclic compounds which simultaneously demonstrate all of the mentioned properties.

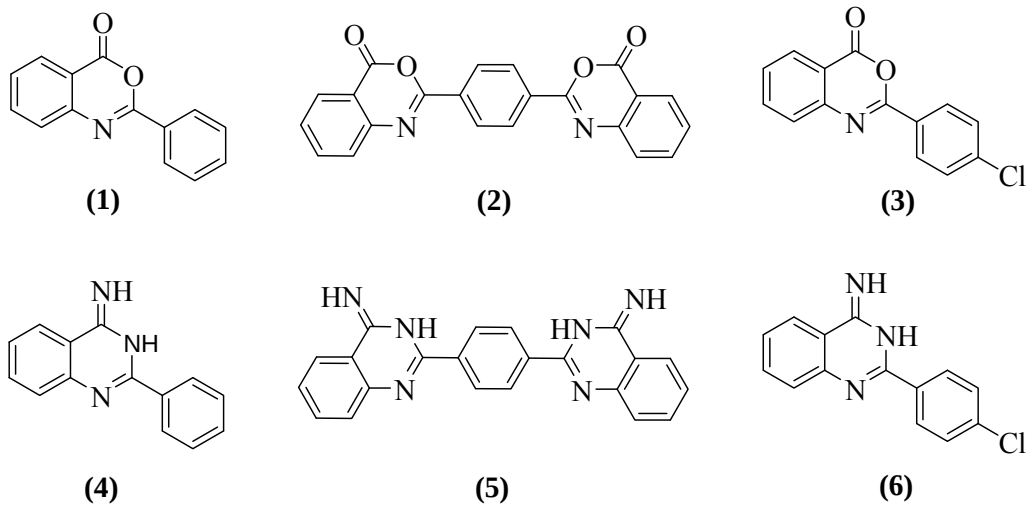
Benzoxazinone can exist in various types depends on the position of keto group. The keto group may occur ateither position two, four or both [6].The keto group also may occurat position three of the structure such as natural occurring of benzoxazinone in maize and wheat [7]. Among all the types of benzoxazinone and quinaozoline, 4(3*H*)-benzoxazinone and quinazolinone are more prevalent to be used as intermediates of drugs synthesis or as natural products in biosynthetic pathway. This is partly because of its structure possess wide range of reaction and being derived from anthranilates with various esters, isotoicanhydride and anthranilamide.In this paper, we report the synthesis and characterization of 4*H*-3,1-benzoxazin-4-one and quinazolin-4-imine compounds **(1-6)**. In addition, all the compounds were evaluated for their antioxidant activity using DPPH radical scavenging.

EXPERIMENTAL

Thin layer chromatography (TLC) analysis was conducted by using thin aluminium plate of Merck Silica gel 60F254 of 0.2 mm thickness. The spots on TLC were visualized using Ultraviolet at 254nm and 365nm. The purification of compound was carried out by column chromatography using Merck Silica gel and the eluent used was mixture of hexane acetone in the ratio of 40:60. The products were characterized by using ATR and ^{1}H NMR. Sample product was measured on Attenuated Total Reflectance (ATR) with 20 scans for each sample at a resolution of 4cm^{-1} per measure. The IR spectra was recorded on PerkinElmer FT-IR Spectrometer Frontier. The ^{1}H spectra were obtained using 300MHz spectrometer for benzoxazinone and 400MHz spectrometer for

 ISBN 978-967-0194-52-3

quinazoline and the chemical shift were reported in ppm using $CDCl_3$ as solvent. The ^1H NMR spectra was recorded at 300 MHz and 400 MHz using Bruker Avance Spectrophotometer.



(1) **(2)** **(3)**

(4) **(5)** **(6)**

ORGANIC MATERIALS

Starting material used was anthranilic acid which was purchased from Sigma-Aldrich. The derivatives of acyl chloride of benzoyl chloride, terephtaloyl dichloride and 4-chlorobenzoyl chloride were purchased from Tokyo Chemical Industry CO., LTD in December 2014.

SYNTHESIS AND CHARACTERIZATION

Benzoyl chloride (10.00mmol) was added into anthrannilic acid (1.03g, 7.50 mmol) and pyridine (30 mL) separately. The mixture was then refluxed for 3 hours and the reaction was monitored under thin layer chromatography (TLC). The spots on TLC were visualized under UV light. Upon the reaction was completed, the reaction mixture was cooled and then poured into cooled diluted hydrochloric acid (15mL). The solid was filtered and recrystallized from ethanol. The synthesis method is repeated by replacing terephtaloyl dichloride (1.00g, 7.3mmol) and 4-chlorobenzoyl chloride (1.00g, 7.3mmol). The compound synthesized from terephtaloyl dichloride**(2)** is further purified by silica gel-60 column chromatography and mixture of hexane-acetone in 40:60 ratio.The compound **(1)** was obtained is a white solid (1.33 g, 78.91%). Compound **(2)** obtained as yellow solid (0.74 g, 55.23%), compound**(3)** appeared as white solid (0.62g, 33.20%). The 4*H*-3,1-benzoxazin-4-one derivatives synthesized were subjected to characterize by using ATR and ^1H NMR. The confirmed compounds were used for the synthesis of the corresponding quinazolin-4-imine derivatives.

A mixture of 2-phenyl-4*H*-3,1-benzoxazin-4-one (0.30g, 1.34mmol)**(1)** and hydrazine hydrate (64%, 2.00mmol) was refluxed in ethanol (30 mL) for 4 hours. The reaction was monitored under thin TLC. The spots on TLC were visualized under UV light. Upon the reaction was completed, the reaction mixture was cooled and poured into cooled distilled water (20 mL). The mixture was then concentrated and filtered off the solid. The separated solid was recrystallized from ethanol. The synthesis method was then repeated by replacing 2-phenyl-4*H*-3,1-benzoxazin-4-one with 1,4-(Di-4*H*-3,1-benzoxazin-4-one)benzene (0.10g, 0.39mmol)**(2)** and 2-(*p*-chlorophenyl)-phenyl-4*H*-3,1-benzoxazin-4-one (0.10g, 0.35mmol)**(3)**.The compound **(4)** was obtained as a white solid (0.41 g, 70.90%). Compound **(5)** obtained as yellow solid (0.34 g, 43.70%), compound **(6)** appeared as white solid (0.03 g, 35.81%).

The six compounds synthesized were used to carry out antioxidant testing. The method in determination of antioxidant activity was referring to the method used by Fu. R. *et al* in their previous research [8]. Each sample was dissolved in methanol to prepare 1000 ppm of sample solution. The stock solution was then diluted to 800ppm, 600ppm, 400ppm, 200ppm, 100ppm, 80ppm, 60ppm, 40ppm, 20ppm, 10ppm, 5ppm and 1ppm. The purple solution of 2,2-diphenyl-1-picrylhydrazyl, DPPH was prepared by dissolve DPPH (3.94 mg) in methanol (100mL) in a dark container covered with aluminium foil. Ascorbic acid was used as the positive control. Each sample solution of different concentration (100µL) was added into the sample well together with the DPPH

 ISBN 978-967-0194-52-3

solution (100µL) and methanol (100µL) for blank sample separately. Triplicate samples were used for each sample with different concentration. The mixtures were then incubated at 25°C for 30min, and the absorbance at 517nm was measured using microplate reader.The radical scavenging activity was calculated using equation as follow:

$$radicalscavengingactivity(\%) = \left[1 - \frac{(A_i - A_s)}{A_c}\right] x\,100$$

Where A_c is the absorption of the negative control, A_i is the absorbance of the experimental group and A_s represents the background absorption.

RESULTS AND DISCUSSION

Compound **(1)** was obtained from the recrystallization appeared as a white solid (1.33 g, 78.91%). The ATR spectrum of compound **(1)**is inagreement with the research conducted before [8].The ATR spectrumshowed a stretching peak for C-H bond of aromatic is observed above 3000cm^{-1} and was typically showed as a multiplicity of weak band due to the present of more than one benzene system in the structure. The stretching ofC=O bondwas observed as a strong band at 1762 cm^{-1} which is slightly higher than the theoretical value of normal ester compound.The increase in intensity and wavenumber were caused by the conjugation with the ester single bonded-oxygen. The lone pair electron on oxygen atom donated to form a temporary C=O bond causes the oxygen atom to be more positive. This causes the electron deficiency in oxygen atom and results in the pulling of electron from C=O ester by inductive effect.The C=N stretching mode is observed at wavenumber of 1610 cm^{-1} as studied by previous research [9]. The stretching of C=N bondat lower wavenumber is due to the conjugate effect of C=C from the phenyl group attached at position two of the benzoxazinone skeleton. The C=C aromatic absorption peaks are observed at 1572 cm^{-1} and 1474 cm^{-1}.Analysis of the ^{1}H NMR spectrum showed that all proton of benzoxazinone exhibited a low field signal started from 7.5ppm. The most low field signal δ 8.33(*J*=7.2Hz) belongs to hydrogen located at *ortho*-position of the phenyl substituent, H_O which was subjected to the electrostatic field effect from lone pair electron of nitrogen [10]. The proton in fifth position, H_5 showed signal at δ 8.26 (*J*=7.8Hz, 0.9Hz) which is low field than H_6 to H_8 due to the electron deshielded by an anisotropic effect of the *peri*-carbonyl at the fused ring.H_7 and H_8 were observed at δ 7.85 (*J*=7.8Hz, 0.9Hz) and δ 7.71 (*J*=7.8Hz). The remaining protons were observed at δ 7.49-7.61 (4H).

Compound **(2)** obtained was appeared as yellow solid (0.74g, 55.23%) after recrystallization and purification using silica gel column. The ATR spectrum showed absorption peak similar to compound **(1)** where3040 cm^{-1} belongs to C-H aromatic, 1763 cm^{-1} was C=O ester, 1694 cm^{-1} was C=N, 1612 and 1474 cm^{-1}for C=C aromatic. From the ^{1}H NMR spectrum, the low field peak was the peak of ortho-position proton, $H_O$at δ 8.50. This peak represented four protons from the phenyl substituent without splitting as the protons are identical to each other by symmetry [11]. These protons would give only a single NMR peak since they have the same chemical shift. The other protons at the fused ring exhibited similar chemical shift and splitting with the compounds**(1)**, which peaks at δ 8.31for H_5(*J*= 7.4Hz), H_7 atδ 7.90 (*J* = 7.4Hz), H_8 at δ 7.78 (*J* = 7.4Hz) and H_6 at δ 7.60 (*J* = 7.4Hz). Compound**(3)** obtained is a white solid (0.62 g, 33.20%). Both ATR and ^{1}H NMR spectrum showed similar peak as observed in compound **(1)**except for the absence of H_p in ^{1}H NMR since the $H_p$in **(3)**had been replaced by the chlorine atom. The ATR spectrum of **(3)** showed absorption peak at 1769 cm^{-1} for C=O ester, 1622 cm^{-1} for C=N, 1604 cm^{-1} and 1489 cm^{-1} for C=C aromatic. ^{1}H NMR spectrum showed peaks at δ 8.27 for both H_O and H_5, δ7.85 for H_7(*J* =7.8 Hz, 1.2 Hz), δ7.70 H_8(*J* =7.8 Hz) and δ7.54 for both H_m and H_6.

For the condensation process of 4*H*-3,1-benzoxazin-4-one with hydrazine hydrate, the targeted compound synthesis was the 3-amino-2-substituted-4*H*-3,1-quinazolin-4-one derivatives as reported previously [12]. However, from the ATR and ^{1}H NMR obtained, the proposed structure of compounds synthesized was quinazolin-4-imine derivatives. Compound **(4)** obtained as a white solid (0.21g, 70.90%).From the ATR, the C=O stretching did not observe in compound **(4)** and a peak was observed at 1649 cm^{-1} was believed to belongs to C=N. Besides, the N-H stretching peak at 3318 cm^{-1} also observed in the ATR.Other than that, two peaks which were 1603 cm^{-1} and 1449 cm^{-1} also observed for C=C aromatic. From the ^{1}H NMR, a N-H peak was observed at δ 11.88and it was believed to come from N-H at position 3 that resembled to the compound of 2-phenyl-4H-3,1-quinazolin-4-one as reported in other research [14]. Therefore it is further proven that the solid formed was a quinazolin-4-imine derivative. The proton at the fuse ring, H_5 was found to shift to lower field at δ 8.83 (*J* =7.7 Hz) as compare to the H_5 in **(1)**. The H_O was observed atδ 8.06 (*J* =6.8 Hz), H_6 at δ 7.12 (*J* =7.7 Hz) and the remaining six proton were observed in the δ 7.63 to 7.12.

 ISBN 978-967-0194-52-3

Compound **(5)** was obtained as a yellow compound (0.24g, 43.70%). From the ATR, the N-H stretching was observed at 3351 cm^{-1}, followed by C=N stretching at 1666 cm^{-1}. The C=C aromatic stretching were observed at 1587 cm^{-1} and 1446 cm^{-1}. The ^1H NMR spectrum was similar to compound **(1)**, where the N-H peak was observed at δ 12.44, followed by H_5 at δ 8.83 (*J* =7.6 Hz). The *ortho*position proton, H_O was observed at δ 8.25 (*J* =7.0 Hz), H_8 at δ 7.92 (*J*=7.6 Hz), H_7 at δ 7.61 (*J*=7.6 Hz) and H_6 at δ 7.22 (*J*=7.6 Hz). The N-H from imine was observed at δ 8.16 (*J*=7.0 Hz).Compound **(6)** appearedas a white (0.23g, 35.81%). From the ATR, the N-H stretching was observed at 3319 cm^{-1}, followed by C=N stretching at 1668 cm^{-1}. The C=C aromatic stretching were observed at 1600 cm^{-1} and 1451 cm^{-1}. The ^1H NMR spectrum showed the peak of N-H at δ 11.97, followed by H_5 at δ 8.82 (*J*=7.7 Hz). The *ortho*position proton, H_O was observed at δ 8.00 (*J*=8.4 Hz), H_8, H_7, H_m, and imine N-Hwere observed as multiplet peak at δ 7.46 - 7.61. The H_6 was observed at δ 7.15 (*J* =7.7 Hz).

The antioxidant activity of benzoxazzinone and quinaozolin-4-imine was tested using 2,2'-diphenyl-1-picrylhydrazyl (DPPH). From the results obtained, it clearly shows that the compounds of 4*H*-3,1-benzoxazin-4-one derivatives do not possess any antioxidants activities after incubation of 30 mins with DPPH. The negative results of the antioxidant activities from 4*H*-3,1-benzoxazin-4-one can be explained by the lack of free hydrogen atom to be donate and stabilize the free radical DPPH. The compounds of all quinazolin-4-imine synthesized possess good antioxidant activities even at low concentration of 100ppm. The capability in antioxidant is due to the active hydrogen at the nitrogen atom of the quinazolin-4-imine skeleton. The IC_{50} of compound **(4)**, **(5)** and **(6)** were 2.66ppm, 28.40ppm and 8.04 ppm respectively. From the IC_{50} values, it was concluded that the introducing of chlorine atom at *p*-position of phenyl group slightly reduced the ability of the compound due to the inductive effect.

CONCLUSION

A series of 2-substituted-4*H*-3,1-benzoxazin-4-one and quinazolin-4-imine compounds had been synthesized with a simple and single step method by using anthranilic acid as starting material. The benzoxazin-4-one compound derivatives synthesized are 2-phenyl-4*H*-3,1-benzoxazin-4-one **(1)** (78.91%), 1,4-(di-4*H*-3,1-benzoxazin-4-one)benzene **(2)** (55.23%) and 2-(*p*-chlorophenyl)-4*H*-3,1,-benzoxazine-4-one **(3)** (33.20%). In the synthesis of the quinazolin-4-one derivatives, the desired product is not obtained. Instead of quinazolin-4-one is synthesized, the unexpected products synthesized were proposed to be the quinazolin-4-imine derivatives based on the data analysis from ATR and ^1H NMR spectrum obtained. Therefore in the study, the quinazolin-4-imine derivatives obtained are 2-phenylquinazolin-4-imine **(4)** (70.90%), 1,4-(diquinazolin-4-imine)benzene **(5)** (43.70%) and 2-(*p*-chlrophenyl)quinazolin-4-imine **(6)** (35.81%).

The compounds synthesized were characterized and the bioactivity of anti-oxidant of each compounds were tested by using DPPH radicals scavenging method. From the bioassay, all the 4*H*-3,1-benzoxazin-4-one derivatives compounds do not shows antioxidant as there is no free active hydrogen to stabilize the radical of DPPH. While all the quinazolin-4-imine derivative compounds synthesized shows good antioxidant ability as the IC_{50} is lower compare to ascorbic acid except for compound **(5)**. Among the quinazolin-4-imine derivatives, 2-phenylquinazolin-4-imine shows the lowest IC_{50} value at 2.66ppm while the highest IC_{50} value belongs to 1,4-(diquinazolin-4-imine)benzene **(5)**, which is 25.40ppm. From the IC_{50} values it can conclude that the present of electron withdrawing group at the phenyl substituent causes inductive effect to quinazolin-4-imine compounds and decreases its ability to donate hydrogen for antioxidant purpose.

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