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SYNTHESIS AND BIOLOGICAL ACTIVITY EVALUATION OF SOME DERIVATIVES SYNTHESIZED FROM CURCUMIN AND CURCUMIN ANALOG

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Abstract: The acetohydrazides **A5** containing an isoxazole ring and **B5** containing an indazole ring were synthesized from the corresponding acetohydrazide derivatives with acetic anhydride in about 80% yield. Also, two acetohydrazones **B6** and **B7** were driven from acetohydrazide **B4** by condensation reaction. Bioactivity tests showed that only acetohydrazone **B7** were active against KB cancer cell line at IC_{50} = 57 µg/L.

Keywords: characterization, acetohydrazide, indazole, curcumin, curcumin analog, acetohydrazone

1 Introduction

In our previous work, the modification of curcumin and monocarbonyl curcumin analog with heterocyclic bridges – oxazole or indazole rings and the pharmacophore groups did not give any improvement of bioactivity for these types of derivatives, Figure 1 [1,2].

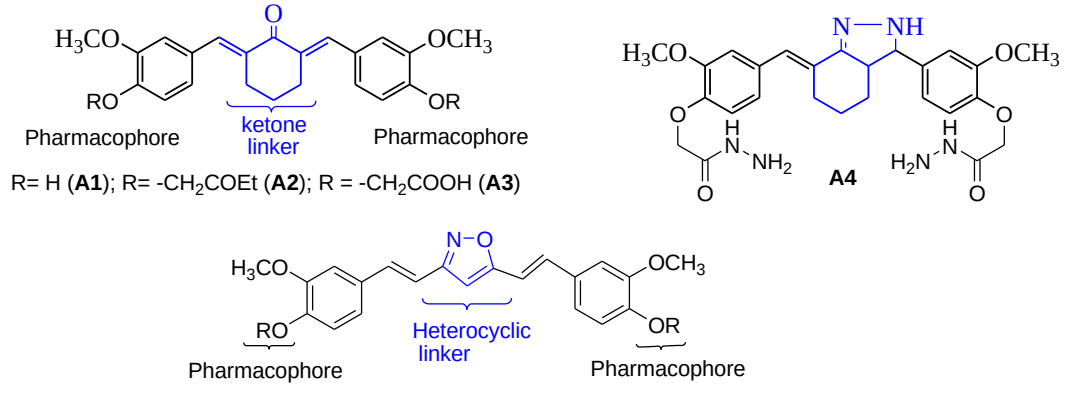

Fig. 1. Modification of curcumin and curcumin analog

Interestingly, according to literature, an acetohydrazone contains a hydrogen bonding domain (HBD); acetyl acetohydrazide contains two hydrogen bonding domains [3] that can

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excess DNA easily giving higher potencies for bioactivities. For example, hydrazone derivatives **I**, **II** and **III** worked as an anticonvulsant, antimicrobial and antimycrobacterial compounds, Figure 2 [4,5]. More examples can be found in review article of Sevim Rollas and Ş. Güniz Küçükgüzel [6].

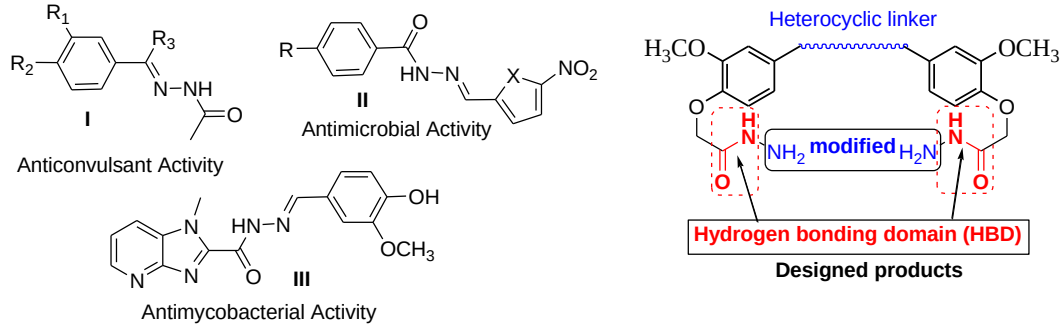

Fig. 2. Some examples of hydrazones and designed target structures

Based on the literature, therefore, in this work, the target compound structures were designed as shown in the Figure 2. The amide group was kept to work as an HBD and the -NH_2 groups were acetylated or condensed with aldehydes to improve bioactivities.

2 Experimental section

2.1 General

Solvents and other chemicals were purchased from Sigma-Aldrich, Merck and used as received, unless indicated. The ^{1}H NMR and ^{13}C NMR spectra were recorded on the Bruker Avance 500 NMR spectrometer in deuterated solvents. Chemical-shift data for each signal was reported in ppm units. IR spectra were recorded on the Mattson 4020 GALAXY Series FT-IR. Mass spectra were obtained from Mass Spectrometry Facility of Vietnam Academy of Science and Technology on LC-MSD-Trap-SL spectrometer.

2.2 Synthesis procedure

General procedure of acetylation [8]: To a solution of acetohydrazide **A4** or **B4** in DMF was added triethylamine and acetic anhydride. The resulting solution was stirred at room temperature for 1 h. The progress of reaction was monitored with TLC (MeOH/ DCM = 1/19). Then the mixture was diluted with water to form solid. Re-crystallization in DMSO/ water (1/2) gave pure products.

Synthesis of (E)-N'-acetyl-2-(4-((2-acetyl-3-(4-(2-(2-acetylhydrazinyl)-2-oxoethoxy)-3-methoxy phenyl)-3a,4,5,6-tetrahydro-2H-indazol-7(3H)-ylidene)methyl)-2-methoxyphenoxy) acetohydrazide **(A5)**

Following the general procedure, using (*E*)-2-(4-(7-(4-(2-hydrazinyl-2-oxoethoxy)-3-methoxybe--nzylidene)-3,3a,4,5,6,7-hexahydro-2H-indazol-3-yl)-2-methoxyphenoxy) acetohydrazide (**A4**) (262 mg, 0.5 mmol, 524 g/mol), triethylamine (0.34 mL, 2.5 mmol, d = 0.7255 g/mL, 101 g/mol)

and acetic anhydride (0.18 mL, 1.75 mmol, d = 1.08 g/mL, 102 g/mol) gave compound **A5** as a milky powder (260 mg, 650 g/mol, 80%). IR (KBr), ν (cm^{-1}): 3433, 3241, 2930, 2855, 1733, 1654, 1601, 1513, 1420, 1363, 1220, 1117; ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 9.95 (s, 1H), 9.92 (s,1H), 9.87 (s, 1H), 9.86 (s, 1H), 7.07 (d, *J* = 9.0 Hz, 2H), 6.98 (s, 1H), 6.96 (s, 1H), 6.92 (d, *J* = 8.5 Hz; 1H), 6.84 (1H), 6.71 (d, *J* = 8.0 Hz; 1H) 4.84 (d, *J* = 9.5 Hz; 1H), 4.60 (s, 2H), 4.54 (s, 2H), 3.80 (s, 3H), 3.78 (s, 3H), 2.92 (d, *J* = 12.0 Hz, 1H), 2.91 (m, 1H), 2.50 (d, *J* = 12.0 Hz, 1H), 2.27 (s, 3H), 2.06 (s, 2H), 1.86 (s, 6H), 1.68 (d, *J* = 10.5 Hz, 1H), 1.46 (d, *J* = 12.5 Hz, 1H); ^{13}C NMR (DMSO, 125 MHz) δ (ppm): 169.0, 167.88, 167.87, 166.59, 166.39, 158.20, 149.28, 148.78, 146.99, 146.34, 136.24, 129.68, 129.34, 126.75, 122.04, 117.48, 114.76, 113.96, 113.83, 110.87, 72.03, 67.22, 66.88, 55.65, 56.42, 55.59, 29.03, 28.49, 23.53, 22.05, 20.41, 19.22.

*Synthesis of 2,2′-((((1E,1′E)-isoxazole-3,5-diylbis(ethene-2,1-diyl))bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N′-acetylacetohydrazide) (**B5**)*

Following the general procedure, using 2,2'-((((1*E*,1'*E*)-isoxazole-3,5-diylbis(ethene-2,1-diyl))bis(2-methoxy-4,1-phenylene))bis(oxy))di(acetohydrazide) (**B4**) (276 mg, 0.5 mmol, 552 g/mol), triethylamine (0.34 mL, 2.5 mmol, d = 0.7255 g/mL, 101 g/mol) and acetic anhydride (0.18 mL, d = 1.08 g/mL, 102 g/mol) gave compound **B5** as a milky powder (267 mg, 593 g/mol, 90 %). IR (KBr), ν (cm^{-1}): 3412, 3224, 3030, 2918, 2874, 1714, 1650, 1508, 1420, 1264, 1136, 1026; ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 10 (s, 1H), 9.9 (s, 1H), 7.55 (d; *J* = 16.5 Hz, 1H), 7.4 (d; *J* = 18.5 Hz, 1H), 7.37 (s, 1H), 7.21 (d; *J* = 13.5 Hz, 1H), 7.19 (d; *J* = 18.0 Hz, 1H), 7.17 (d; *J* = 6.0 Hz, 1H), 7.14 (d; *J* = 8.0 Hz, 1H), 6.96 (d; *J* = 8.0 Hz; 1H), 6.91 (s; 1H), 4.62 (s, 2H), 3.86 (s; 3H), 1.87 (s, 3H); ^{13}C NMR (DMSO, 125 MHz) δ (ppm): 168.2, 168.0, 168.0, 166.4, 166.4, 162.2, 149.3, 149.3, 148.4, 148.1, 136.0, 134.3, 129.8, 129.4, 121.1, 120.7, 114.1, 114.0, 114.0, 111.7, 110.1, 98.5, 66.9, 66.7, 55.74, 55.70, 20.4, 20.4. ESI-MS *m*/*z*: 594 $[C_{29}H_{32}N_5O_9]^+$ and 592 $[C_{29}H_{30}N_5O_9]^-$.

General procedure of hydrazone derivatives[3]: To a solution of **B4** (0.5 mmol) in DMSO (5 mL) was added aldehydes (0.25 mmol). The resulting mixture was refluxed for 5 h. Then it was diluted with water to form solid. The solid was filtrated and washed with 3% HCl solution then washed with ethanol several times to rinse out the unreacted andehydes and **B4**. Re-crystallization in DMSO/ water (1/2) gave pure products as powder.

*Synthesis of (N′E,N′′′E)-2,2′-((((1E,1′E)-isoxazole-3,5-diylbis(ethene-2,1-diyl))bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N′-(4-nitrobenzylidene)acetohydrazide) (**B6**)*

Following the general procedure, using **B4** (254 mg, 0.5 mmol, 509 g/mol) and *p*-nitro benzaldehyde (151 mg, 1 mmol, 151 g/mol) gave compound **B6** as a milky powder (260 mg, 775 g/mol, 67 %). IR (KBr), ν (cm^{-1}): 3440, 3320, 3107, 2930, 2845, 1693, 1596, 1518, 1345, 1264, 1145, 1019; ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 11.89 (s, 2H), 8.40 (s, 0.76H), 8.27 (d, *J* = 9.0, 2H), 8.11 (s, 1.4 H), 7.97 (d, *J* = 8.0 Hz, 2H), 7.37 (d, *J* = 14.0 Hz, 2H), 7.32 (d, *J* = 5.5 Hz, 2H), 7.18 (d, *J* = 14.0 Hz, 2H), 7.14 (d, *J* = 6.5 Hz, 2H), 6.98 (s, 1H), 6.91 (d, *J* = 7.0 Hz, 2H), 5.24 (s, 1.38 H), 4.73 (s, 0.7 H), 3.88 (s, 6H); ^{13}C NMR (DMSO, 125 MHz) δ (ppm): 169.2, 149.0, 148.7, 148.4, 147.7, 145.3, 141.4, 140.2, 128.0, 127.8, 123.9, 121.1, 120.7, 113.0, 110.0, 109.9, 98.2, 65.1, 55.6, 55.6. ESI-MS *m*/*z*: 776 $[C_{39}H_{34}N_7O_{11}]^+$ and 774 $[C_{39}H_{32}N_7O_{11}]^-$.

*Synthesis of (N′E,N′′′E)-2,2′-((((1E,1′E)-isoxazole-3,5-diylbis(ethene-2,1-diyl))bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N′-(4-hydroxy-3-methoxy-5-nitrobenzylidene)acetohydrazide) (**B7**)*

Following the general procedure, using **B4** (254 mg, 0.5 mmol, 509 g/mol) and *5*-nitrovanillin (200 mg, 1 mmol, 197 g/mol) gave compound **B7** as a milky powder (303 mg, 867 g/mol, 70 %). IR (KBr), ν (cm^{-1}): 3436, 3340, 3164, 2935, 2855, 1698, 1539, 1511, 1427, 1261, 1139, 1019; ^{1}H NMR (DMSO, 500 MHz) δ (ppm): 11.60 (s, 2H), 8.20 (s, 0.78H), 7.94 (s, 1.12 H), 7.72 (s, 1H), 7.50 (s, 1H), 7.37 (d, J = 15.5 Hz, 2H), 7.36 (d, J = 6.5 Hz, 1H), 7.33 (d, J = 6.5 Hz, 2H), 7.17 (d, J = 15.5 Hz, 2H), 6.97 (s, 2H), 6.87 (s, 1H), 5.21 (s, 1.2 H), 4.69 (s, 0.8 H), 3.89 (s, 6H), 3.81 (s, 3H); ^{13}C NMR (DMSO, 125 MHz) δ (ppm): 168.79, 168.7, 168.2, 164.0, 163.9, 150.5, 149.3, 149.0, 148.9, 148.6, 148.4, 148.1, 146.6, 142.4, 136.8, 136.0, 134.4, 129.8, 128.9, 121.1, 120.7, 117.1, 114.1, 110.2, 109.9, 98.4, 67.4, 65.2, 56.4, 55.6.

2.3 Bioactivity test

Bioactivity tests were followed by the Broth dilution method [7]. **A5** and **B7** were selected for bacterial test including Gram (+) (Staphylococcus aureus, Bacillus subtilis, Lactobacillus fermentum) and Gram (-) (Salmonella enteric, Escherichia coli, Pseudomonas aeruginosa) and fungal test (Candida albican). **B7** was also selected for anticancer test with KB cancer cell line. All tests were screened in the Laboratory of Applied Biochemistry of Vietnam Academy of Science and Technology.

3 Results and discussion

Acetylation of **A4** was not only occurred at -NH_2 of the hydrazide groups but also at nitrogen of the indazole ring to form **A5** with three acetyl amide groups. Meanwhile, the acetylation of acetohydrazide using **B4** gave compound **B5** with two acetyl amide groups in high yield expectedly, Fig. 3.

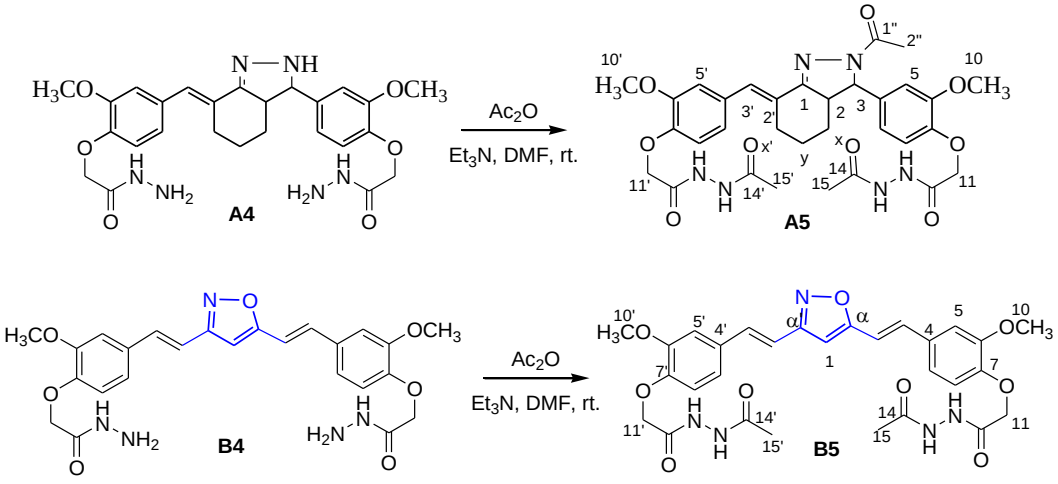

Fig. 3. Acetylation of acetohydrazides A4 and B4

Classic method was used to make hydrazones **B6** and **B7** when acetohydrazide **B4** was condensed with some substituted aldehydes. Unfortunately, only substituted aldehydes which contained a nitro group drove the expected products **B6** and **B7**. That might be explained by the

increasing reactivity of these aldehydes bearing strong withdrawing electron groups as nitro group. Many other aldehydes such as *p*-methoxybenzaldehyde; *p*-hydroxylbenzaldehyde … did not give the designed products.

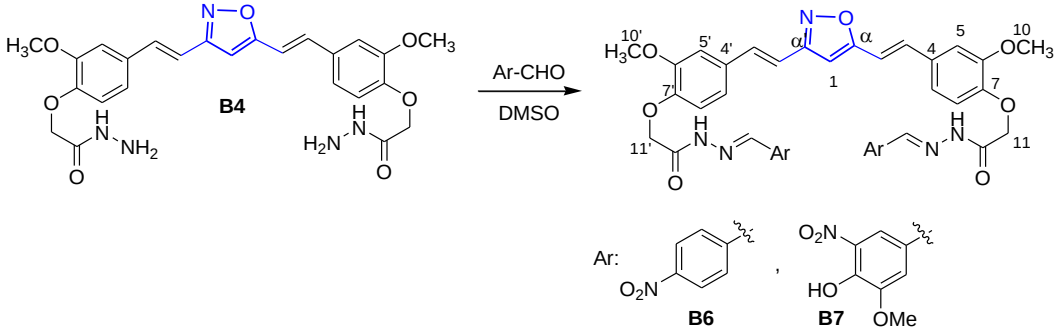

Fig. 4. Synthesis of some hydrazones of acetohydrazides B6 and B7

Structures of **A5**, **B5**, **B6** and **B7** were first determined with IR spectroscopy method. IR spectra of all showed the vibration of N-H bond. Since each compound had at least two N-H bonds therefore, their IR spectra indicated many peaks in range of 3200-3500 cm^{-1}. Comparing with results published in our previous paper [1], the vibration of >C=O (CH_3C=O) was larger than that of O=C-NH-NH_2 group. So **A5** and **B5** showed two vibrations of two types of >C=O bonds. For example, IR spectrum of **A5** showed vibrations at 1733 cm^{-1} and at 1654 cm^{-1}. IR spectra of **B6** and **B7** had one vibration of two >C=O groups. The formation of hydrazonyl groups increased wavelength number about 10 cm^{-1}. Other vibrations agreed with the expected structures (see experimental section). **A5**, **B5**, **B6** and **B7** were studied MS spectra, **B5** and **B6** showed the peaks of pseudo molecular ions, unfortunately, **A5** and **B7** did not. This method indicated molecular weight of **B5** was 593 g/mol and molecular weight of **B6** was 775 g/mol. ^{1}H NMR spectra of **A5** showed all protons on its structure. In comparison to ^{1}H NMR spectrum of **A4** whose structure was confirmed with 1D, 2D NMR and MS spectra [1], in this case, assignment of all peaks was expressed in the Figure 3. There were three signals assigned for three methyl groups of three acetyl ones. The peak at δ 2.24 ppm indicated the acetyl group on the nitrogen atom in the indazole ring. Next, the signals at δ 1.86 ppm belonged to 6 protons of H14 and H14′ since they were far from unsymmetric center-indazole ring. This observation agreed with ^{13}C NMR spectra that showed 6 peaks in high field at δ 29.0, 28.4, 23.5, 22.0, 20.4, 19.2 ppm that included Cx, Cx′, Cy, C2′′, C14 and C14′. It also showed 4 peaks at δ 169.0, 167.8, 166.5 and 166.3 ppm according to four carbonyl groups C12, C12′ C13, C13′and C1′′.

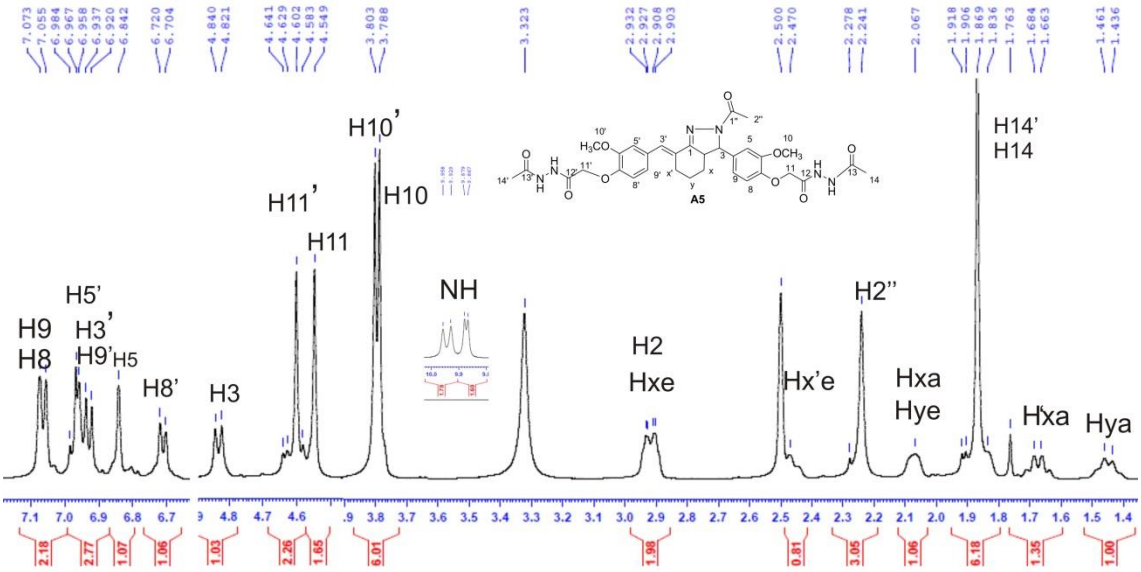

Fig. 5. 1H **NMR analysis of** A5

Similar to **A5**, ^{1}H NMR spectrum of **B5** indicated the presence of two acetyl groups at δ 1.87 ppm that supported the ^{13}C NMR peak at δ 20.4 ppm of methyl group (see experimental section). All indicated the right expected products.

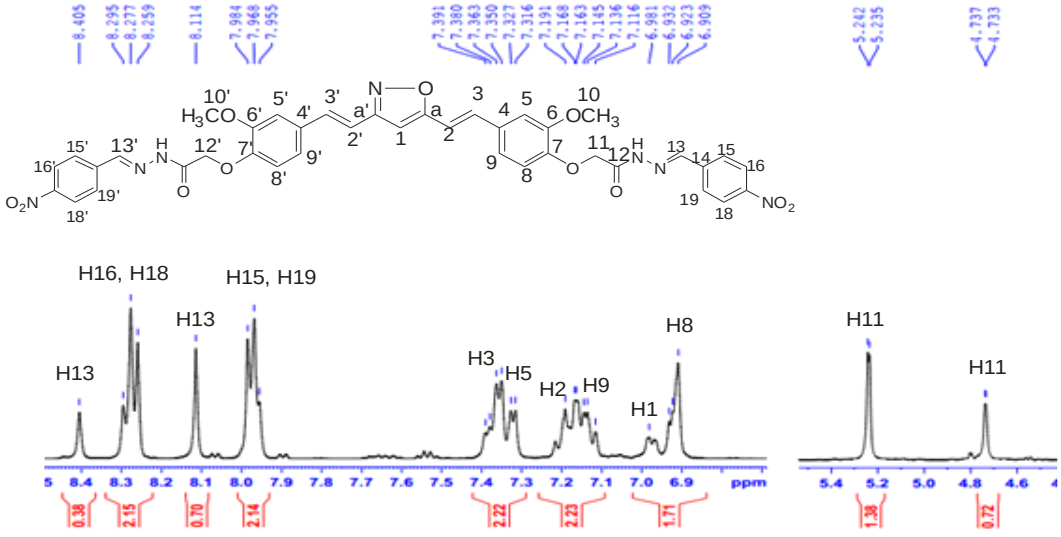

Fig. 6. 1H **NMR analysis of** B6

^{1}H NMR spectra of compounds **B6** and **B7** gave lot of peaks than expected due to they had two isomeric forms of E and Z around the >C=N bonds with ratio about 1/2. To be simple, label from H1 to H19 used instead of accompanying with "prime number" was shown in the Figure 4, ^{1}H NMR of compound **B6**. H13 was at δ 8.40 ppm and δ 8.11 ppm with total intensity about 2H. An extra observation of the existence of two isomers was H11 at δ 5.23 ppm and at δ

4.73 ppm. In comparison with our previous work, other protons were assigned as shown in Figure 4. **B7** was screened anticancer activity. It was against on KB cancer cell line with IC_{50} = 57.6 μg/mL. However, neither of **A5** and **B7** showed positive results on bacterial tests.

4 Conclusion

The modification of curcumin and curcumin analog based on heterocyclic linker and pharmacophore groups gave 4 new derivatives **A5**, **B5**, **B6** and **B7**. Acetylation gave compound **A5** with three acetyl groups and **B5** with two acetyl ones. **B6** and **B7** were a mixture of E and Z isomers. **A5** and **B7** did not show any activities against on bacteria or fungi, but **B7** was against on KB at IC_{50} = 57.6 μg/mL.

References

1. Duong Quoc Hoan, Nguyen Thi Thanh Xuan, Truong Minh Luong, Tran Thi Trang (2016), Characterization and bioactivity evaluation of two new acetohydrazides synthesized from curcumin and monocarbonyl curcumin analog, *J. Sci. HNUE, Chemical and Biological Sci.*, 61(9), 11-20.
2. Duong Quoc Hoan, Dam Thi Uyen, Pham Thi Yen, Nguyen Hien (2015), Synthesis and structure of some phenoxy acetic acid derivatives from curcumin and monocarbonyl curcumin analogs, *Vietnam J. Chem.*, 53 (6e1,2), 348-353.
3. Gökçe, M., Geciken, A. E., Yıldırım, E., Tosun, A. U., (2008), Synthesis and Anticonvulsant Activity of 5-Chloro-2(3H)-benzoxazolinone-3-acetyl-2-(*o*/*p*-substituted benzal) hydrazone Derivatives, *Arzneimittel-Forschung (Drug Research),* 58 (11), 537–542.
4. Küçükgüzel, Ş.G.; Oruç E.E.; Rollas S.; Şahin, F.; Özbek, A. (2002), Synthesis, Characterization and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds. *Eur. J. Med. Chem*., *37*, 197-206.
5. Kaymakçıoğlu K.B.; Oruç, E.E.; Unsalan, S.; Kandemirli, F.; Shvets, N.; Rollas, S.; Anatholy, D. (2006), Synthesis and characterization of novel hydrazide-hydrazones and the study of their structureantituberculosis activity. *Eur. J. Med. Chem*., *41*, 1253-1261.
6. Rollas, S. and Küçükgüzel, Ş. G., (2007). Biological Activities of Hydrazone Derivatives *Molecules*, 12, 1910-1939.
7. Ericsson, J. M.; Sherris, J. C., (1971), Antibiotic sensitivity testing: report of an international collaborative study, *Acta Pathol Microbiol Scand*, 217, 1–90.
8. Saeed, A., Hussain, M., Qasim, M., (2014) Novel *N*-acyl/aroyl-2-(5-phenyl-2*H*-tetrazol-2-yl)acetohydrazides: synthesis and characterization, *Turkish Journal of Chemistry*, 38, 436-442.