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Research Article

SYNTHESIS AND ANALGETIC ACTIVITY EVALUATION OF 4-[N-(4-HYDROXYPHENYL)CARBOXYMIDOYL]-2-METHOXYPHENOL

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ABSTRACT

Paracetamol is an analgesic-antipyretic compound derived from *p*-aminophenol. Though paracetamol has good efficacy and safety on consumption, parasetamol has hepatotoxic effect as its adverse drug reaction. 4-[*N*-(4-hydroxyphenyl)carboxymidoyl]-2-methoxyphenol is one of *p*-aminophenol derivative that was already been determined *in silico* using molecular docking PLANTS method, and it was known that 4-[*N*-(4-hydroxyphenyl) carboxymidoyl]-2-methoxyphenol has analgesic effects more potent and has hepatotoxic adverse effect lower than paracetamol. 4-[*N*-(4-hydroxyphenyl)carboxymidoyl]-2-methoxyphenol can be synthesized through reaction of *p*-aminophenol with vanillin under acid condition. The synthesized products were recrytalized, dried, and the purity was determined with melting point determination and Thin Layer Chromatography. The structure of pure crystals were elucidated using IR, ^{1}H-NMR, C-NMR, and Mass Spectroscopy. The analgesic evaluation was carried *in vivo* using writhing test method. The synthesized compound were divided into three dosage variations, 0,5; 1; and 2mol equivalent to 100mg/kgBB of paracetamol (reference drug). 4-[*N*-(4-hydroxyphenyl)carboxymidoyl]-2-methoxyphenol with 1mol dosage has analgesic activity better than paracetamol but the difference was not significant.

Keywords: 4-[*N*-(4-hydroxyphenyl)carboxymidoyl]-2-methoxyphenol, *p*-aminophenol, analgesic, writhing test

INTRODUCTION

Paracetamol is a non-opiate synthetic compound derived from *p*-aminophenol and it has analgesic-antipyretic effects (McEvoy, 2002). Parasetamol acted as selective inhibitor of cyclo-oxygenase-2 (COX-2) enzyme, worked by inhibit inflammatory reaction (Anderson, 2008; Hinz and Brune, 2012; Hinz *et al*.., 2009).

Paracetamol was metabolized by CYP450 enzyme through oxidation, and it produced a *N*-acetyl-*p*-benzoquionimin, NAPQI (Aripin and Choonara, 2009; Vale, 2007). NAPQI was a hepatotoxic compound (Mutschler, 1986). The level of hepar damage caused by paracetamol was depended on dosage consumption (Vale, 2007). On theraphy dose, paracetamol was relatively safe and non-toxic. The toxicity of paracetamol appeared on acute consumption of more than 10 g and on chronic consumption of 3-4g/day. New *p*-aminophenol derivative that has more potent analgesic activity dan has lower hepatotoxic adverse effect can be found through modification of *p*-aminophenol structure. 4-[*N*-(4-hydroxyphenyl) carboxymidoyl]-2-methoxyphenol, the is one of *p*-aminophenol derivative and it was already been test *in silico* using molecular docking PLANTS method. 4-[*N*-(4-hydroxyphenyl)-carboxymidoyl]-2-methoxyphenol (the authors shorted the name as PAPVN) has -75,0088 of docking score and paracetamol has -67,3820 of docking score. The smallest or the bigger negative value of docking score, the more stable the binding mode of drug molecules with its receptor, so the more potent the effect of molecule acted as drug (Purnomo, 2011). Based on *in silico* evaluation it can be said that PAPVN has more potent analgesic activity than paracetamol, and based on structure-activity relationship it can be said that PAPVN has lower hepatotoxic adverse effect than paracetamol.

PAPVN can be synthesized through reaction of *p*-aminophenol with vanillin under acid condition. The analgesic evaluation was carried *in vivo* using writhing test as

method, against Balb/c strain mice induced acetic acid.

MATERIALS AND METHODS

Materials needed for PAPVN synthesis were *p*-aminophenol for synthesis (Merck), vanillin, ethanol (Merck), methanol, chloroform, HCl 37%, silica gel GF_{254} plate (Merck), spectroscopies : IR (Perkin Elmer FTIR 100); ^{1}H-NMR and C-NMR (Jeol JNMECA500); LC-MS.

Materials needed for analgesic activity evaluation of PAPVN using writhing test method were Balb/c strain mice (2-3 months); PAPVN (synthesized products); CMC-Na 0.5% (b/v) suspensions; paracetamol 0.5% (b/v) suspensions in CMC-Na; acetic acid 3% (b/v) solutions.

Synthesis

Fit a 100mL round –bottom flask to a reflux condenser, and mix *p*-aminophenol (2.2g) with 40mL water and then HCl 37% was added drop by drop until all of *p*-aminophenol was dissolved in aquadest. Vanillin (3.04g) was added into round bottom boiling flask and shaked lightly. The mixture was refluxed using Liebig as condensor, for 2h. The solution obtanined was moved into 250mL Erlenmeyer flask, then it was cooled using ice; the purpose is to build the synthesized crystals. The obtanined wet crystals were recrystalized using ethanol as the solvent. The obtained pure crystals were dried in an oven at 50°C for 1 day. The purity of crystals were determined its melting point and Thin Layer Chromatography using silica gel GF_{254} plate with chlorofom:methanol (7:3) as eluent. The structure elucidation was carried using IR, ^{1}H-NMR, C-NMR, dan LC-MS spectroscopy.

Writhing Test

The animal subjects used in this test were Balb/c strain mice, with 20-35g range in weight and 2-3 months in age, and it was obtained form Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. This experiment was performed following the writhing test method of Turner, 1965. Thirty mices were fasten for 12h, and then allocated into 5 groups of 6 mices each. Group I acted as negative control, in this group the mices received 0.5mL CMC-Na 0.5% oral administration (p.o). Group II acted as reference group, in this group the mices received 0.5mL paracetamol 100mg/kg p.o. Group III-IV were the test groups, the mices received 0.5mL PAPVN p.o with the dosage of each group were 81mg/kg; 162mg/kg and 324mg/kg paracetamol.

The 0.3mL acetic acid 200mg/kg was given intraperitonial to each group after oral administration of the test compound. The number of writhes induced in each mice was counted every 5min for 60min. The percent (%) of analgesic protection was calculated and statistically analyzed with non-paramteric test of ANOVA (level of confidence 95%) using SPSS version 22.1 for windows software.

RESULTS AND DISCUSSION

PAPVN was synthesized from the reaction of *p*-aminophenol and vanillin in aquadest, and it was catalyzed by HCl. The occurred reaction was nucleophilic addition of amine, forming the imine group (Figure 1). The free aromatic amine group in *p*-aminophenol reacted to carbonyl group in vanillin, resulted in addition reaction and releasing of water (H_2O), formed imine group in PAPVN structure.

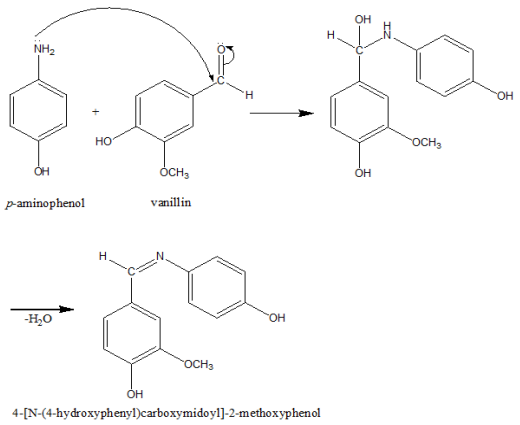


Figure 1. Nucleophilic addition of amine in *p*-aminophenol and vanillin, forming imine group in PAPVN structure

In the LC-MS results, there was only one peak of compound at 2.41 min of the retention time, so it can be said that the synthesized compound was pure, free of residue and by product. From the determination of melting point, PAPVN has a melting point 165-168°C.

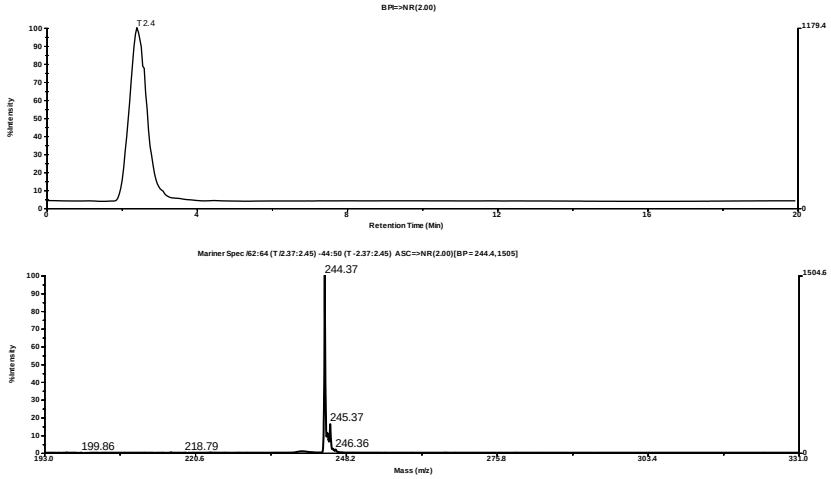


Figure 2. Chromatogram and spectra of LC-MS

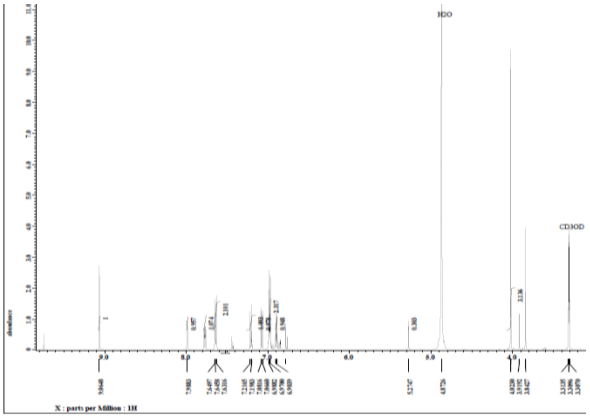
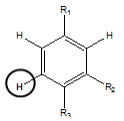
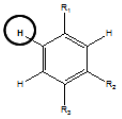
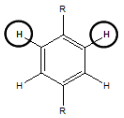
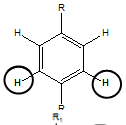
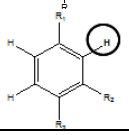


Figure 3. Spectra of ^{1}H-NMR

Table I. The interpretation of ^{1}H-NMR spectra

δ (ppm)	Integration (H)	Multiplicity	Group Structure Possibility	δ (ppm)	Integration (H)	Multiplicity	Group Structure Possibility
9.0642	1	Singlet	**H**-C=N	7.6497	0.978 ≈ 1	Doublet	
7.7652	1.074 ≈ 1	Doublet of doublet		6.9791	2.317 ≈ 2	Doublet	
7.6497	2.101 ≈ 2	Doublet		4.0230	3.024 ≈ 3	Singlet	-O-C**H**$_3$
7.2165	1.072 ≈ 1	Doublet					

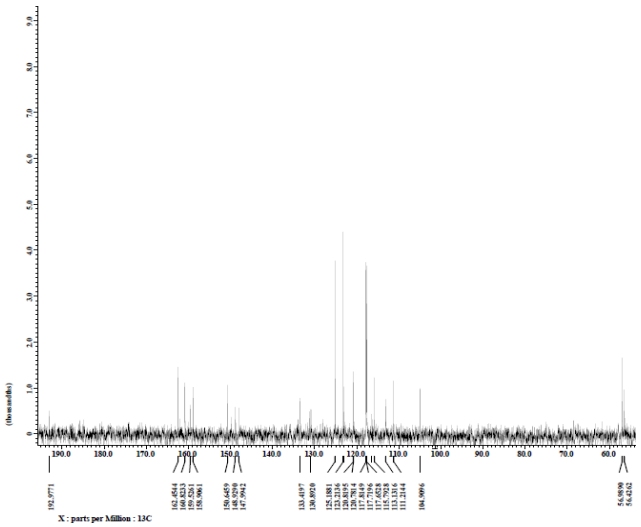


Figure 4. Spectra of C-NMR

Table IV. Results of statistical analysis of % analgesic protection using one-way ANOVA

	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	**Reference Group**	Negative Control	-51.00000*	12.66943	.000	-77.1484	-24.8516
		Dose 81mg/kg	-4.83333	12.66943	.706	-30.9817	21.3151
		Dose 162.5mg/kg	-7.76667	13.28781	.564	-35.1914	19.6580
		Dose 324.5mg/kg	-4.00000	12.66943	.755	-30.1484	22.1484
	Negative Control	Reference Group	51.00000*	12.66943	.000	24.8516	77.1484
		Dose 81mg/kg	46.16667*	12.66943	.001	20.0183	72.3151
		Dose 162.5mg/kg	43.23333*	13.28781	.003	15.8086	70.6580
		Dose 324.5mg/kg	47.00000*	12.66943	.001	20.8516	73.1484
	Dose 81 mg/kg	Reference Group	4.83333	12.66943	.706	-21.3151	30.9817
		Negative Control	-46.16667*	12.66943	.001	-72.3151	-20.0183
		Dose 162.5mg/kg	-2.93333	13.28781	.827	-30.3580	24.4914
		Dose 324.5mg/kg	.83333	12.66943	.948	-25.3151	26.9817
	Dose 162.5 mg/kg	Reference Group	7.76667	13.28781	.564	-19.6580	35.1914
		Negative Control	-43.23333*	13.28781	.003	-70.6580	-15.8086
		Dose 81mg/kg	2.93333	13.28781	.827	-24.4914	30.3580
		Dose 324.5mg/kg	3.76667	13.28781	.779	-23.6580	31.1914
	Dose 324.5 mg/kg	Reference Group	4.00000	12.66943	.755	-22.1484	30.1484
		Negative Control	-47.00000*	12.66943	.001	-73.1484	-20.8516
		Dose 81mg/kg	-.83333	12.66943	.948	-26.9817	25.3151
		Dose 162.5mg/kg	-3.76667	13.28781	.779	-31.1914	23.6580

*. The mean difference is significant at the 0.05 level

From the LC-MS results, the molecular weight of synthesized compound was obtained; it was 244.37 (m/z). This molecular weight is suitable with the theoritic molecular weight of PAPVN (243.26g/mol).

Spectra data of IR (cm^{-1}, KBr) were: 1376 (m, C-O str); 1595 (s, -C_6H_5 str.); 1652(m, C=N str); 1891(w-trisubstituted benzene); 3077-3558(s, OH).

Based on the analysis of ^{1}H-NMR and C-NMR spectra, it can be interpreted that the carbon framework and compound structure is suitable with the theoritic structure of PAPVN. This C-NMR interpretation results is being a support to the ^{1}H-NMR interpretation.

From the interpretation results of fourth spectra it can be summarize that the synthesized compound is a correct PAPVN compound. The analgesic activity evaluation was conducted using writhing test method. Cumulative writhes of the mices were statistically analyzed using one-way ANOVA with level confidence of 95%. The data were distributed normally and homogenized.

The results table above show that the 3 doses of PAPVN (81; 162.5; 324.5mg/kg) have bigger value in mean difference of writhes than that of reference group, but it do not have significant difference between the two. So it can be said that PAPVN compound has analgesic activity but it is not different than that of paracetamol. The 3 doses of PAPVN also do not have significant difference of mean whrites among each other. The 3 doses of PAPVN do not have analgesic activity differ from each other, so it can be said that rising up the dose is not going to rising up the analgesic response. Reference group and negative control have a significant difference in mean whrites, it means that the method was performed accordingly.

CONCLUSION

4-[*N*-(4-hydroxyphenyl)carboxymidoyl]-2-methoxyphenol can be synthesized through reaction of *p*-aminophenol with vanillin under acid condition. The compound has analgesic activity but its not better than paracetamol. Further research on toxicity of 4-[*N*-(4-hydroxyphenyl)-carboxymi-doyl]-2-methoxy-phenol was interesting to investigate.

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