· Research report·

Synthesis and Bio-activity of Bivalent Imidazolinones

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Abstract: In biological system, bivalent ligands often possess increased functional affinity for their receptors compared with monovalent ligands. Based on the information of 3D structure of AHAS-imazaquin (IQ) complex, 8 symmetrical bivalent molecules of imazapic were designed and synthesized. Their structures were confirmed by IR, ^{1}H NMR and elementary analysis. The preliminary bioassay results showed that the inhibition rate to the acetohydroxyacid synthase from dimeric compounds were less potent than that of imazapic. And the inhibition rate to the growth of rape (Brassica campestris L.) root from bivalent compounds were very close to that of imazapic at concentration of 100μg/mL.

Key words: multivalent interactions; cluster effect; synthesis; bis-imazapic

[1] , [1] , [*1] , [*1] , [2]

(1. , , 100193;
2. , 300071)

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3D , 8 ,
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, 100μg/mL , Brassica campestris L.

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1 Introduction

Multivalent ligand-receptor interactions, known as the cluster effect, are defined as specific simultaneous associations of multiple ligands present on a molecular construct that bind to multiple receptors presented on a biological entity[1, 2]. In biological systems, multivalent ligands often possess increased functional affinity for their targets compared with that of monovalent ligands.

Laurence et al[3] deduced that symmetrical inhibitors might bridge two adjacent active sites via coupling two weakly binding benzamidine, which

demonstrated a remarkable distanced-defined structure-activity relationship against human tryptase with series possessing subnanomolar potencies. Kopytek et al [4] reported bis-methotrexate (MTX) binds simultaneously with two dihydrofolate reductase (DHFR) enzymes, thus forming a ternary complex. The bis-MTX shows about a 100-folds higher affinity than MTX (Fig. 1). Luedtke et al [5] found that the dimerization Kanamycin A increases its affinity to the HIV-1 Rev response element (RRE) by 2200-fold. Pang et al [6], Carlier et al [7], Hu et al [8] and Kryger et al [9] reported that inhibitors of acetylcholinesterase (AChE) were designed on the basis of a structure-based divalent approach. This approach leads to a dimeric inhibitor comprising two tethered inhibitors expected to bind simultaneously with two proximal sites, thereby achieving enhanced affinity and selectivity. There are many other successful medicinal examples of multivalent ligands that bind more strongly to their respective multivalent receptors[10 14]. Several different mechanisms may contribute to higher potency[15]. Up to now there is few reports on multivalent interaction in herbicide development. This highlight of cluster effect provided us a reference and guidance to design and synthesize multivalent pesticide molecules for the discovery of better agrochemicals.
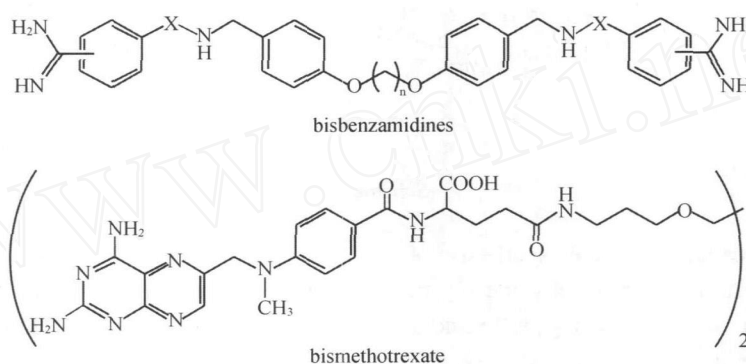

Fig. 1   Bisvalent compounds

Acetohydroxyacid synthase (AHAS) is the first common enzyme in the pathway for the biosynthesis of branched-chain amino acids. This enzyme may be inhibited by several families of compounds. Among these compounds, the sulfonylureas and imidazolinones, are developed extensively as commercial herbicides[16]. Recently Jennifer et al [17] reported the 3D structure of the Arabidopsis thaliana AHAS-imazaquin (IQ) complex. In this complex, there are two herbicide molecules bound to each subunit. One of these is within the channel leading to the active site, whereas a second is located 20 Å (2.0 nm) from the active site. According to multivalent interactions, multivalent ligands could be simultaneously associated with different subunits of AHAS and might show higher affinity with active sites. In this paper several bivalent imidazolinone compounds were designed and synthesized with expection that the synthesized bivalent compounds may bind more strongly to AHAS.

## 2 Materials and methods

2.1 Chemistry

Reagents and solvents were purchased from Beijing Chemical Reagents Company and were used without further purification. Column chromatography was performed on silica gel 200 300 mesh obtained from Qingdao Haiyang Chemical Co., Ltd. Analytical thin-layer chromatography was performed with silica gel plates, and the plots were visualized under UV light at 254 nm or iodine vapor. Elemental analysis (C, H, N) and ESI-MS were performed at Institute of Chemistry, Chinese Academy of Sciences. Nuclear magnetic resonance spectrum were recorded in CDCl$_3$ solution, using Brucker DPX 300 MHz spectrometer (performed by China Agricultural University). IR (KBr) (v_{max}, cm^{-1}) spectra were recorded on FT-IR IR200 spectrophotometer.

2.1.1   Procedure for the preparation of compound 2.

To a solution of dicyclohexylcarbodiimide (DCC) (5 g, 24 mmol) in dry methylene chloride (50 mL),

2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)-5-methylnicotinic acid (**1**) (6 g, 21 mmol) was added. After stirring at room temperature for 2. 5 hours, the mixture was filtered and concentrated to give a white solid. The crude product was recrystallized from methylene chloride to give 4. 3 g (80%) of the dione (**Scheme 1**).
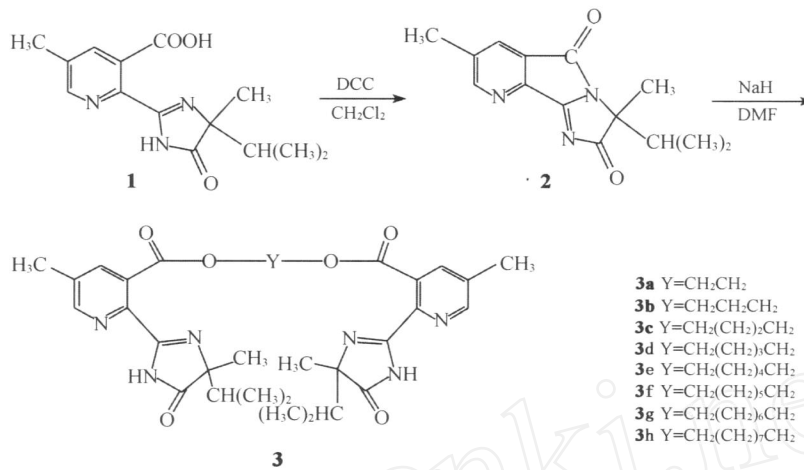
Scheme 1

2. 1. 2 General procedure for the synthesis of products 3a 3h To the mixture of ethylene glycol (0. 08 g, 1. 4 mmol) and compound **2** (1 g, 3. 9 mmol) in absolute DMF, sodium hydride (0. 09 g, 3. 9 mmol) was added with ice-cooling under nitrogen. Gas evolution was observed. After 4 hours, the reaction was neutralized with 0. 2 g (3. 9 mmol) aqueous ammonium chloride, stripped and partitioned with water and ethyl acetate. The organic layer was separated and dried over anhydrous magnesium sulfate. After filtered, the solution was concentrated in vacuo, and the resulting residue was purified by column chromatography with a mixture of ethyl acetate and petroleum ether (1 3, V/V) as eluent to give 0. 5 g (60%) of a white solid (**3a**). The same procedure was used for the synthesis of **3b 3h** (Scheme 1).

2. 2 Evaluation of biological activity in vitro **and** in vivo

2. 2. 1 AHAS inhibition in vitro. AHAS activity was measured using the colorimetric assay in 50 mmol/L potassium phosphate (pH 7. 0) containing 50 mmol/L pyruvate, 1 mmol/L thiamine diphosphate, 10 mmol/L $MgCl_2$ and 10 μmol/L FAD [18]. The synthesized compounds were emulsified firstly and then incubated at 37 for 30 min. The reaction was stopped with 25 μL of 10% H_2SO_4 and heated at 60 for 15 min to convert acetolactate into acetoin. The acetoin was

quantified by incubation with 0. 5% creatine and naphthol (5%, W/V) for 15 min at 60 and was measured at A 525. Imidazolinone was used as a control

2. 2. 2 Inhibition of the growth of rape (Brassica campestris L.) root in vivo. 20 rape seeds were soaked in distilled water for 4 h and then placed on filter paper in a 9-cm Petri plate, to which 3 mL of inhibitor solution had been added in advance. Duplicate was tested for each trial The plate was placed in climatic chambers and allowed to germinate for 72 h at (28 ± 1) . The length of roots was measured and the percentage inhibition was calculated.

3 Results and discussion

Intramolecular cyclization of 2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl) -5-methylnicotinic acid (**1**) using approximately an equimolar amount of the DCC in the presence of CH_2Cl_2 at 20 32 , gave compound **2** in 80% yield as a white solid. Esterification of compound **2** with different diol in the presence of sodium hydride in dimethylformamide gave bis-imazapic (**3a 3h**). The physical property, ESI-MS, yields and elemental analysis of compounds **3a 3h** are listed in Table 1, ^{1}H NMR and IR data are collected in Table 2.

Table 1   Experimental data for compounds **3a   3h**

Compd.	Formula	Appearance	m. p. /	ESI-MS [M+Na]$^+$	Yield (%)	Elemental analysis(%, Calcd.)		
						C	H	N
3a	$C_{30}H_{36}N_6O_6$	White solid	172.8 173.4	599	60.4	62.27(62.49)	6.40(6.29)	14.71(14.57)
3b	$C_{31}H_{38}N_6O_6$	Viscous white oil	-	613	49.8	62.89(63.04)	6.57(6.48)	14.34(14.23)
3c	$C_{32}H_{40}N_6O_6$	White solid	207.6 208.1	627	66.3	63.38(63.56)	6.82(6.67)	14.01(13.90)
3d	$C_{33}H_{42}N_6O_6$	White solid	212.3 213.0	641	63.4	63.79(64.06)	6.95(6.84)	13.69(13.58)
3e	$C_{34}H_{44}N_6O_6$	White solid	211.1 211.5	655	59.8	64.46(64.54)	7.22(7.01)	13.43(13.28)
3f	$C_{35}H_{46}N_6O_6$	Viscous yellow oil	-	669	50.2	64.79(65.00)	7.29(7.17)	13.18(12.99)
3g	$C_{36}H_{48}N_6O_6$	Viscous yellow oil	-	683	49.6	65.29(65.43)	7.49(7.32)	12.94(12.72)
3h	$C_{37}H_{50}N_6O_6$	Viscous yellow oil	-	697	44.9	65.71(65.85)	7.65(7.47)	12.68(12.45)

Table 2 ^{1}H NMR and IR data of compounds

Compd.	IR, v/cm^{-1}	^{1}H NMR (CDCl$_3$/TMS),
3a	2 968, 1 727, 1 627	0.78 0.81(m, 3H), 0.95 1.0(m, 3H), 1.28(d, 3H, J=3.3 Hz), 1.96 2.06(m, 1H), 2.43(d, 3H, J= 10.8 Hz), 4.55 4.66(m, 2H), 7.61 7.62(m, 1H), 8.50 8.51(m, 1H), 8.89(s, 1H)
3b	2 963, 1 732, 1 627	0.84 0.90(m, 3H), 0.97 1.04(m, 3H), 1.27(d, 3H, J=3.0 Hz), 1.97 2.06(m, 1H), 2.42(d, 3H, J= 3.0 Hz), 2.43 2.48(m, 1H), 4.42 4.62(m, 2H), 7.64 7.65(m, 1H), 8.50 8.51(m, 1H), 8.81(s, 1H)
3c	2 967, 1 723, 1 631	0.82 0.87(m, 3H), 0.99 1.05(m, 3H), 1.32(d, 3H, J=1.3 Hz), 1.82 1.86(m, 2H), 2.00 2.08(m, 1H), 2.43(d, 3H, J=2.3 Hz), 4.28 4.44(m, 2H), 7.60 7.63(m, 1H), 8.49 8.50(m, 1H), 8.73(s, 1H)
3d	2 931, 1 720, 1 628	0.83 0.87(m, 3H), 1.01 1.05(m, 3H), 1.34(d, 3H, J=7.4 Hz), 1.45 1.52(m, 1H), 1.74 1.80(m, 2H), 2.03 2.07(m, 1H), 2.45(d, 3H, J=12 Hz), 4.26 4.37(m, 2H), 7.60 7.61(m, 1H), 8.49 8.50(m, 1H), 8.81(s, 1H)
3e	2 963, 1 730, 1 631	0.83 0.90(m, 3H), 1.01 1.06(m, 3H), 1.33(d, 3H, J=1.9 Hz), 1.63 1.71(m, 2H), 1.73 1.76(m, 2H), 1.99 2.08(m, 1H), 2.45(d, 3H, J=12 Hz), 4.22 4.36(m, 2H), 7.61 7.62(m, 1H), 8.48 8.49(m, 1H), 8.70(s, 1H)
3f	2 931, 1 728, 1 628	0.84 0.87(m, 3H), 1.03 1.07(m, 3H), 1.25(d, 3H, J=3.0 Hz), 1.35 1.37(m, 1H), 1.53 1.58(m, 2H), 1.68 1.73(m, 2H), 2.03 2.07(m, 1H), 2.49(d, 3H, J=1.3 Hz), 4.26 4.35(m, 2H), 7.61 7.63(m, 1H), 8.49 8.50(m, 1H), 8.80(s, 1H)
3g	2 931, 1 729, 1 620	0.79 0.90(m, 3H), 1.02 1.07(m, 3H), 1.32(d, 3H, J=4.0 Hz), 1.40 1.43(m, 2H), 1.52 1.57(m, 2H), 1.67 1.74(m, 2H), 2.03 2.07(m, 1H), 2.45(d, 3H, J=10.5 Hz), 4.25 4.35(m, 2H), 7.62 7.64(m, 1H), 8.49 8.50(m, 1H), 8.76(s, 1H)
3h	2 929, 1 728, 1 629	0.96 0.98(m, 3H), 1.00 1.05(m, 3H), 1.35(d, 3H, J=4.1 Hz), 1.53 1.57(m, 1H), 1.67 1.74(m, 6H), 2.03 2.07(m, 1H), 2.43(d, 3H, J=1.2 Hz), 4.30 4.35(m, 2H), 7.62 7.63(m, 1H), 8.48 8.49(m, 1H), 8.79(s, 1H)

Inhibition of the plant AHAS was measured using the colorimetric assay. As shown in Table 3, the inhibition of the imazapic was 94.6% at the concentration of 100μg/mL, but the dimeric compounds were less potent than imazapic. The growth inhibition of rape root was undertaken in vivo to evaluate their herbicidal activity. As a whole, the potency of the bivalent compounds were very close to that of imazapic(Table 3). Compound **3b**(74%), **3f** (72.4%), **3g**(78%), **3h**(80%) expressed the same level of activity as monovalent compounds(75.3%).

It is obvious that bis-imazapic showed different activity in vivo and in vitro. First, maybe the length of linker was not much longer enough to bind simultaneously to the two active sites in one plant AHAS. Or the conformation and structure of the bivalent molecules was not perfectly fit to the active sites of bivalent receptors. Second, the bis-imazapic might express new physiological actions on weed, and there might be other mechanism of action on target enzyme. The accurate explanation to this problem needs further research.

Table 3   Biological activities of compounds

Compd	Inhibition of AHAS activities(%)			In vivo herbicidal activity of the compounds(% inhibition)
	1μg/mL	10μg/mL	100μg/mL	100μg/mL
1	-	-	94.6	75.3
3a	0	6.0	25.0	69.5
3b	7.1	12.6	21.6	74.0
3c	0	13.0	21.8	57.3
3d	20.4	24.8	30.2	67.1
3e	0	5.4	20.1	58.9
3f	7.4	17.1	38.0	72.4
3g	18.9	24.2	30.0	78.0
3h	13.1	20.8	28.2	80.0

In summary, symmetrical bivalent molecules via intramolecular cyclization and esterification with diol have been synthesized. All new compounds were confirmed by ^{1}H NMR, IR, ESI-MS and elemental analysis. Bis-imazapic compounds displayed potential herbicidal activity in vivo. However, the dimeric compounds expressed weak affinity to plant AHAS. The results suggested that these bivalent molecules maybe have different physiological actions on weeds and further research is needed.

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