Benzylidene 2-Aminoimidazolones Derivatives: Synthesis and *in Vitro* Evaluation of Anti-tumor Carcinoma Activity

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A series of benzylidene 2-aminoimidazolones derivatives were synthesized. Most compounds displayed strong inhibitory activity on the proliferation of human HepG2 cells *in vitro*. The active compounds were further evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against five human cancer cell lines *in vitro*. Compound 2b exhibited the strongest antitumor activities with IC_{50} values ranging from 12.87–17.10 μM which were nearly 1–3.5 fold less than that of 5-FU (IC_{50}=18.39–56.12 μM) *in vitro*. Furthermore, compound 2b could induce SMMC-7721 cell apoptosis in a dose-dependent manner. Therefore, our novel findings may provide a new framework for the design of new benzylidene 2-aminoimidazolones derivatives for the treatment of cancer.

Key words synthesis; 2-aminoimidazolone derivative; anti-tumor agent; inhibitory activity; cell apoptosis

Cancer has been reported to become the leading cause of mortality worldwide in 2010 and cancer cases are expected to double by 2020.[1)] Resourceful novel and structurally diverse metabolites from marine organisms, in particular those coming from sponges, have proved to display a wide variety of biological activities including antihistamine activity,[2)] anti-inflammation,[3)] neuroprotective activity,[4,5)] antitumor,[6)] antioxidant,[7)] *etc.* For example (see Fig. 1), marine natural product Dispacamide, which was isolated from *Carriban Agelas* sponges, shows a potent antihistamine activity,[2)] and Leucettamine B, from the sponge *Leucetta microraphis* Haeckel (alcarea class) of the Argulpelu Reef in Palau, exhibits to play a role as mediator of inflammation.[3)] Polyandrocarpamines A, extracted from a Fijian ascidian,[8)] has been shown to have diverse pharmacological activities.[6,9,10)] Many recent reports and reviews have also highlighted that Polyandrocarpamines A exhibit potent antitumor activity.[6,11,12)] Phorbatopsins A which was isolated from the Mediterranean marine sponge *Phorbas topsent* exhibited antioxidant activity.[7)] Interestingly, these marine natural products share a common skeletal structure that is 2-aminoimidazolone core. Both natural and synthetic 2-aminoimidazolone-containing compounds were identified to possess anticancer activities.[13,14)] However, their antitumor activities are limited. Therefore, development of new benzylidene 2-aminoimidazolones derivatives with potent antitumor activity should be of great significance.

For the further improvement of their antitumor activities, different substituted alkane amines and alcohol amines were selected to make further modifications on the 2-aminoimidazolone core by ammonification. Furthermore, with the consideration whether the methoxy group on benzene ring could confer to pharmacological activity, benzylidene 2-aminoimidazolones derivatives were designed *via* the conjugation of 2-aminoimidazolone core with vanillin or 4-hydroxybenzaldehyde. Herein, 22 target compounds **1a**–**k**, **2a**–**k** were synthesized and their biological evaluation for inhibitory activities on the proliferation of human cancer cell lines and cell apoptosis effect were carried out *in vitro* with expectation to find better antitumor agents.

Results and Discussion

Chemistry The synthetic route of 2-aminoimidazolone derivatives **1a**–**k**, **2a**–**k** was outlined in Chart 1. The starting material glycine **3** was treated with NH_4SCN at the present of acetic anhydride (Ac_2O) to give 1-acetyl-2-thioxoimidazolidin-4-one **4** by cyclization under microwave condition. The intermediate **4** was reacted with other commercially available material 4-hydroxybenzaldehyde **5a** or vanillin **5b** to form **6a** or **6b** by condensation according to the literature.[6,8)] Compounds **6a**,**b** was oxidized by *tert*-butylhydroperoxide (TBHP) to obtain the important intermediate **7a**,**b**. Then corresponding amine, alkane amines or alcohol amines were introduced onto the sulfonic group of compounds **7a**,**b** to obtain the targeted compounds **1a**–**k**, **2a**–**k**. The structures of the target compounds were shown in Chart 1 and confirmed by of MS, IR, ^{1}H-NMR spectra and elemental analysis (EA).

***In Vitro* Biological Assessment** The inhibitory activity of target compounds **1a**–**k**, **2a**–**k** on Hep G2 which was incubated with 50 μM of each test compound for 48 h, was primarily screened and evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-

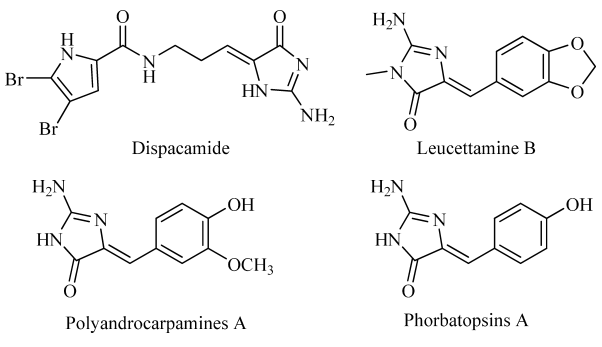

Fig. 1. The Structures of Marine Natural Products

The authors declare no conflict of interest.

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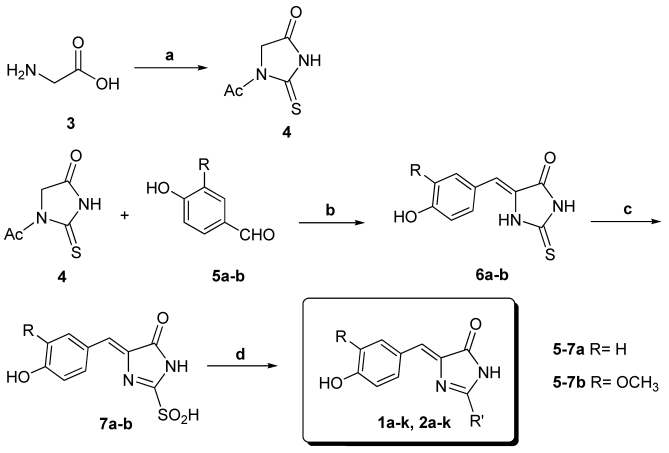

1a R= H, R'= NH_2
1b R= H, R'= $NHCH_3$
1c R= H, R'= $NHCH_2CH_3$
1d R= H, R'= $NHCH_2CH_2CH_3$
1e R= H, R'= $NHCH_2CH_2CH_2CH_3$
1f R= H, R'= $NHCH_2CH_2OH$
1g R= H, R'= $NHCH_2CH_2CH_2OH$
1h R= H, R'= $NHCH_2CH(OH)CH_3$
1i R= H, R'= $N(CH_2CH_2OH)_2$
1j R= H, R'= $NHCH_2CH(OH)CH_2OH$
1k R= H, R'= $N(CH_3)CH_2CH(OH)CH_2OH$

2a R= OCH_3, R'= NH_2
2b R= OCH_3, R'= $NHCH_3$
2c R= OCH_3, R'= $NHCH_2CH_3$
2d R= OCH_3, R'= $NHCH_2CH_2CH_3$
2e R= OCH_3, R'= $NHCH_2CH_2CH_2CH_3$
2f R= OCH_3, R'= $NHCH_2CH_2OH$
2g R= OCH_3, R'= $NHCH_2CH_2CH_2OH$
2h R= OCH_3, R'= $NHCH_2CH(OH)CH_3$
2i R= OCH_3, R'= $N(CH_2CH_2OH)_2$
2j R= OCH_3, R'= $NHCH_2CH(OH)CH_2OH$
2k R= OCH_3, R'= $N(CH_3)CH_2CH(OH)CH_2OH$

Reagents and conditions: (a) NH_4SCN, Ac_2O, AcOH, reflux, 2 h. (b) NaOAc, AcOH, reflux, 5 h. (c) TBHP, MeOH, rt, 2 h. (d) Alkane amines or alcohol amines, MeOH, rt, 10 h.

Chart 1

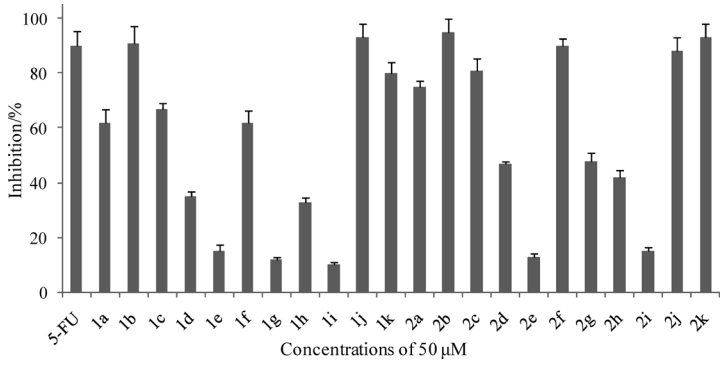

Fig. 2. The Inhibitory Activity of Target Compounds **1a k**, **2a**–**k** (50 μM) on Proliferation of Hep G2 Cells

diphenyltetrazolium bromide (MTT) assay *in vitro*, and using 5-fluorouracil (5-FU) as positive control. The results showed in Fig. 2 illustrated the inhibitory effects of target compounds on the proliferation of Hep G2. Twelve out of the twenty two tested compounds exhibited inhibitory effects that were similar or superior to 5-FU. Especially, compounds **1b**, **1j**, **2b** and **2k** (percent inhibition over 90%) displayed more potent inhibition than 5-FU. Therefore, ten compounds (list in Table 1) possessing strong inhibition on the proliferation of human Hep G2 cells were selected for the further investigation of the inhibitory activity against five human cancer cell lines *in vitro*, such as SMMC-7721, HepG2, MCF-7, SW480 and SGC-7901. Their IC_{50} values are presented in Table 1. All the tested compounds showed remarkable antitumor activities in a low micromolar range (except **2a**) with IC_{50} 12.87–39.51 μM. In addition, the antitumor activities of each active compound against SMMC-7721 and SGC-7901 cells showed much more potent compared to that against the other tumor cells. Especially, compounds **2b** exhibited the strongest antitumor activities with IC_{50} values ranging from 12.87–17.10 μM which was 1–3.5 fold less than that of 5-FU (IC_{50}=18.39–56.12 μM).

For determining whether the most potent compound **2b** was due to cell apoptosis, apoptosis assay employing human hepatocellular carcinoma cells (SMMC-7721) was further assessed to determine the effect of **2b** on cell apoptosis. The SMMC-7721 cells were incubated with vehicle alone or with **2b** at 6.25, 12.5 or 25 μM final concentrations. The percentages of apoptotic cells were determined by flow cytometry analysis. As shown in Fig. 3, in the untreated group, the frequency of SMMC-7721 cell apoptosis was unobvious. In contrast, the frequency of the cell apoptosis showed an upward trend in **2b**-treated SMMC-7721 cell with the dose increased. Low concentration (6.25 μM) of **2b** only induced 25.9% apoptosis activity on SMMC-7721 cells. Following treatment with 25 μM

Table 1. The Inhibitory Activity (IC_{50}, μM) of **1a–v** against Five Human Cancer Cell Lines

Compound	*In vitro* inhibitory activity (IC_{50}, μM)				
	SMMC-7721	MCF-7	Hep G2	SW480	SGC-7901
5-FU	37.84	18.39	35.67	23.11	56.12
1b	17.76	25.03	21.52	22.87	20.97
1j	19.62	22.18	19.87	23.79	27.29
1k	28.06	35.02	25.96	33.05	29.65
2a	33.48	>50	39.51	34.75	35.61
2b	12.87	15.16	14.93	17.10	15.53
2c	27.46	30.24	32.39	29.65	35.13
2f	25.02	19.98	20.53	26.75	22.87
2j	16.65	24.86	20.25	27.02	17.84
2k	14.12	20.74	18.05	21.17	16.92

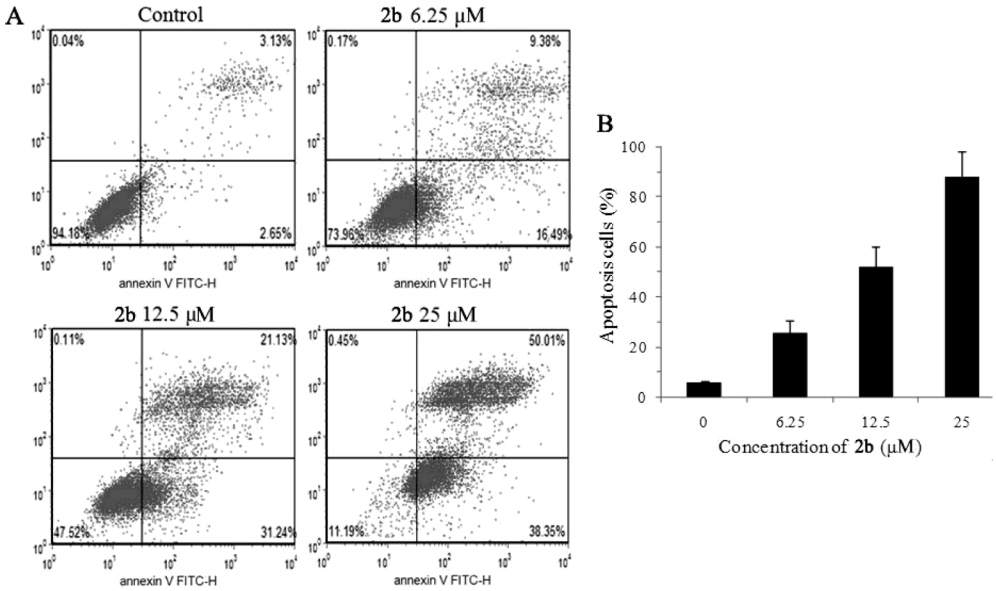

Fig. 3. Analysis of **2b**-Induced Cell Apoptosis in SMMC-7721 Cells

The SMMC-7721 cells were treated with 6.25, 12.5 or 25 μM **2b** for 48 h. (A) Apoptosis was determined by Annexin V-FITC/PI staining in the SMMC-7721 cells by flow cytometry. (B) Quantitative analysis of apoptotic cells. Data are representative flow cytometry charts that are illustrated as a mean±S.E.M. of the percentages of apoptotic cells from three duplicate experiments.

of **2b** induced over 80% of SMMC-7721 cell apoptosis, which was significantly higher frequency of the cell apoptosis compared to the control cells, and it revealed that the antitumor activity of **2b** appeared to be concentration-dependent. However, the precise mechanisms underlying the apoptosis effect of these compounds selectively to tumor cells remain to be further investigated.

Structure–Activity Relationships (SARs) Analysis of structure–activity relationships revealed that in series of target compounds **1a–e** or **2a–e** with different unsubstituted or substituted alkane amines, the compound **1b** or **2b** linked with methylamine possessed better anticancer activity compared to compound **1a** or **2a** linked with unsubstituted amine. However, increasing the length of alkane did not contribute to their activities *in vitro* while **1b** or **2b** showed the strongest anticancer activity among their respective series *in vitro*. In the other hand, the compounds **1f–k** or **2f–k** linked with alcohol amine substituents displayed slightly stronger anticancer activity compared to that with alkane amine substituents, and the anticancer activity was similarly decreased with increasing the chain length of alcohol amines *in vitro*. Interestingly, the compounds **2j** and **2k** linked with propylene glycol side chain displayed strong anticancer activity. In addition, the active compounds displayed more potent inhibitory activity on SMMC-7721 cells than the other four cancer cells. However, the precise SAR of these compounds with potent anticancer activity should be further investigated.

Conclusion

In summary, 20 benzylidene 2-aminoimidazolones derivatives with various substituted alkane amines or alcohol amines were synthesized. Their inhibitory activity against tumor cell lines of synthetic compounds was evaluated *in vitro*. Most compounds displayed strong anticancer activity. Particularly compounds **2b** and **2k** had a great potency superior to 5-FU in these cancer cells. Furthermore, compound **2b** with the strongest antitumor activity could induce cancer cell apoptosis in a dose-dependent manner. We are interested in further investigating the mechanisms of these 2-aminoimidazolone derivatives underlying the action of these compounds in inhibition of human tumorigenesis.

Experimental

General chemistry methods, synthesis procedures, spectral data, and bioassay methods are given in Supplemental information.

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