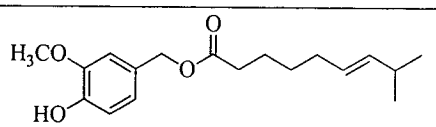
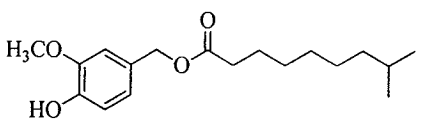
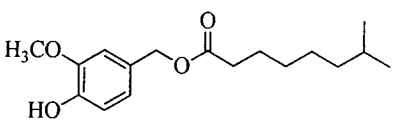
CHAPTER I

INTRODUCTION

Rationale for the study

In 1998, capsinoids was first isolated by Kobata and co-workers [1] from a sweet pepper named "CH-19 sweet" with an exceptional physical property that was totally different from capsaicinoids. Capsinoids, an analogue of capsaicinoids, were composed of capsiate (**1**), dihydrocapsiate (**2**) and norhydrocapsiate (**3**) (Table 1). Usually, capsaicinoids are high pungency and exhibit a significant burning sensation; the strong pungency of these substances and possibility for neurotoxicity limit their use as food additives, nutritional supplements and pharmaceuticals. However, capsinoids show less pungency and no sign of burning sensation when exposure to skin or during oral administration. It was proposed that the diverse property of capsinoids might contribute from the minuscule different in molecular structure.

Table 1 Chemical structure of capsinoids

Capsinoids	Chemical Structure
Capsiate (**1**)	
Dihydrocapsiate (**2**)	
Nordihydrocapsiate (**3**)	

Structurally, capsinoids and capsaicinoids are almost identical. Both of capsinoids and capsaicinoids are composed of an aromatic ring which well known as

vanilloid region (A-region) and a lipophilic carbon chain (C-region) [2, 3, 4, 5]. The only difference is that capsinoids employ an ester bond linkage (B-region) to connect A-region and C-region together while capsaicinoids connected A and B region with an amide bond. (Figure 1)

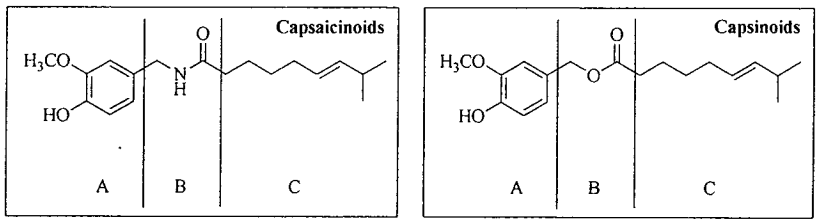


Figure 1 Structure comparison between capsaicinoids and capsinoids dividing into 3 parts; aromatic region (A-region), bond linkage region (B-region) and a lipophilic carbon chain (C-region)

With interesting physical properties mentioned above, several researches were concentrated on what activity of capsinoids would be as well as how to implement capsinoids for pharmaceutical applications. Since then, several biological activities were disclosed. For example, capsinoids has a wide range of biological effects on the cardiovascular, nervous, and respiratory systems and anti-obesity [6, 7, 8]. Some capsinoid analogues were useful as an analgesic drug, anti-inflammatory, anti-diabetic and anti-obesity [9, 10, 11, 12]. The potential clinical uses of capsinoids are diverse such as supplement for energy boost, improvement oxygen consumption, blood circulation and immunity [13, 14, 15]. It has also been proven to aid with weight loss which has attracted much attention and prompted investigations into the relationship between the structure of capsinoid analogues and their increasing metabolism activities [16, 17].

Although, capsinoids has played an important role of beneficial effects similarly to capsaicinoids without the associated irritating properties in term of non-pungent and non-toxicity; on the other hand, they were highly unstable and easily decomposed when exposed under the light and high temperature over a short periods of time [18, 19]. Consequently, it would be very difficult to apply capsinoids in pharmaceutical applications due to capsinoids would rapidly decompose after

exposure to water and all protic solvents which make capsinoids become less practical for using as drugs.

Propose of the study

The instability of capsinoids was possibly originating from the destruction of ester bond linkage *via* *p*-quinone methide pathways. From figure 2, it was clearly shown that OH group and benzylic proton (CH_2 group between benzene ring and ester bond) could contribute on their instability.

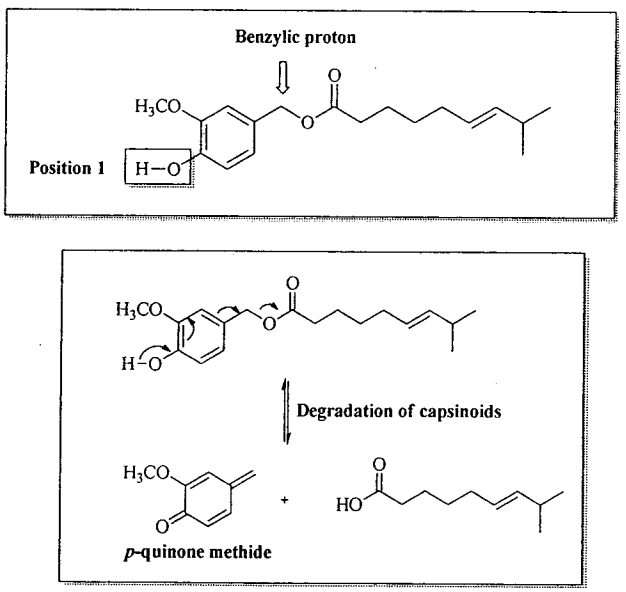


Figure 2 Degradation of capsinoids *via* *p*-quinone methide pathways

From the degradation mechanism, two approaches are considered in order to render the decomposition process. First approach is permanent modification of OH group (position-1) on benzene ring and this should significantly hinder degrading of capsinoids; however, this process will drastically affect biological activities because hydrogen bonding (position-1) between OH group and receptor such as TRPV1 is obstructed. Second approach is an inversion of carbonyl ester bond position and this will inhibit the degradation of capsinoids due to the leaving group generating from *p*-quinone methide under an inversion approach would be less stable than those in first approach making the degradation process unfavorable; consequently higher stability.

Additionally, an inversion approach should provide less interference on biological activities because of hydrogen bonding interaction between OH group on aromatic ring and receptor still remain unchanged and electrostatic interaction between "inverse carbonyl ester bond" and receptors might be minimal effect (Figure 3 and 4).

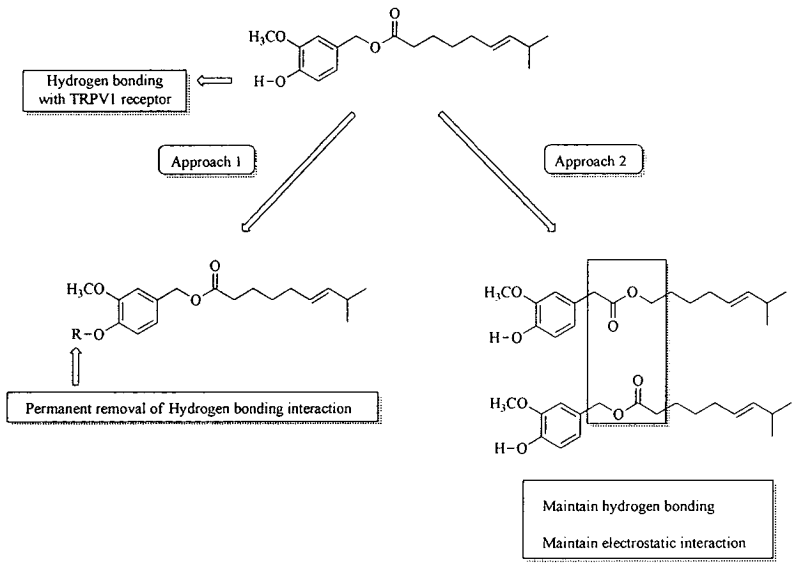


Figure 3 Conceptual modification of capsinoid analogues

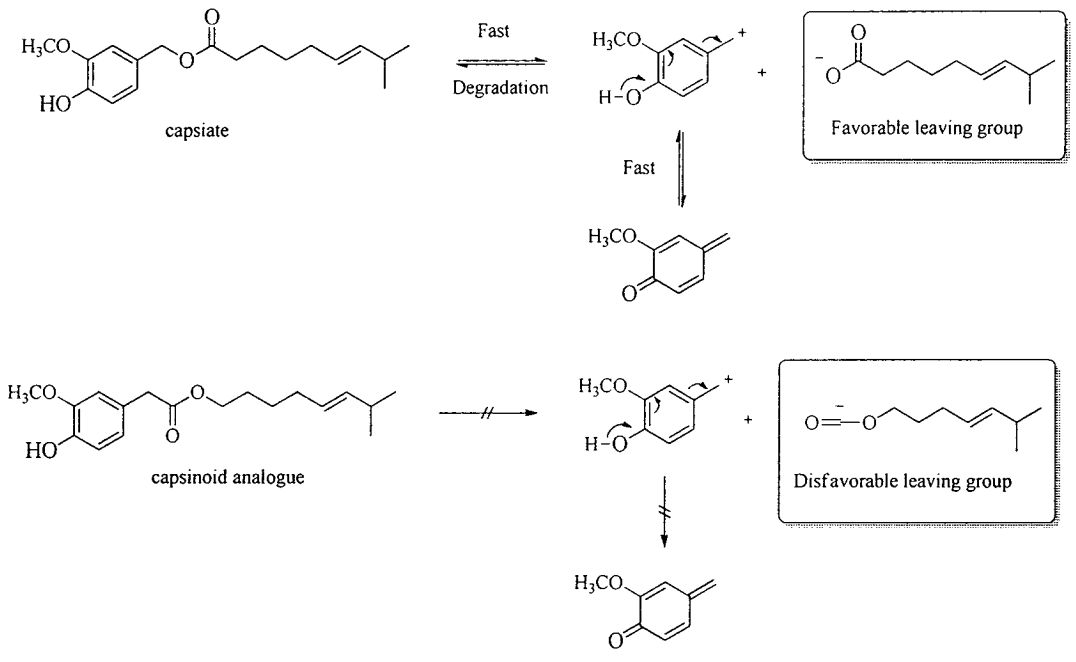


Figure 4 Proposed decomposition mechanism of capsinoid analogue through *p*-quinonemethide comparing with capsiate

Interestingly, the inversion position of ester bond in capsinoid analogues is also partially similar with a natural occurring resiniferatoxin (RTX) on the aromatic region (Figure 5). It is very well known that Resiniferatoxin (RTX) possess an excellence anti-inflammatory with excellent binding with TRPV1 receptor (similar binding receptor for capsaicinoids) and quite stable in both protic and non protic solvent [20]. Additionally, Ritesh K. Baboota and co-worker investigated the dose of RTX and capsaicin dependent effect on the differentiation of 3T3-L1 pre-adipocyte cells into adipocytes. It was provided evidence that the dual effect of RTX and capsaicin could inhibit adipogenesis at lower dose 200 nM- 1 μM and 0.1–1 μM, respectively and stimulated adipogenesis at higher dose 10–100 μM. The anti-adipogenic effect of capsaicin and RTX were accompanied with induction of brown-adipose tissue in 3T3-L1 adipocytes [21]. With this crucial data, it is promising that the modification of capsinoids with inversion approach would offer a higher stability and still sustain the biological activities.

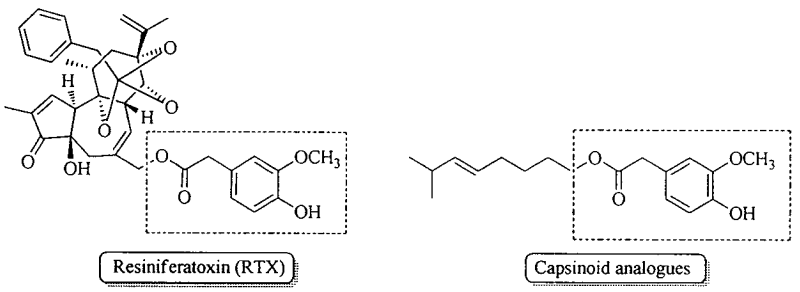


Figure 5 Comparison of Resiniferatoxin (RTX) and capsinoid analogues on aromatic region and ester linkage

In this investigation, we will prepare capsinoid analogues with novel modification: inversion of ester bond position, a new possible approach to enhance stability when exposed to high temperature and light in cell solution condition as well as still maintain biological activities as previously reported for feasible application use in the future.

Significance of the study

Capsinoid analogues which contain inverse position of ester bond should improve their stability and should significantly hinder the destruction from *p*-quinone methide pathway. Additionally, the biological activities should remain unchanged and this study will give a new view of stabilization approach of capsinoid analogues for promising applications of capsinoid analogues in order to utilize them in the future.

Scope of the study

To design and synthesize capsinoid analogues by inverse position of ester bond *via* esterification reaction (Figure 6), then investigate their stability by comparing with capsiate under ambient environment by using HPLC, and evaluate their biological property using cytotoxicity MTT Assays.

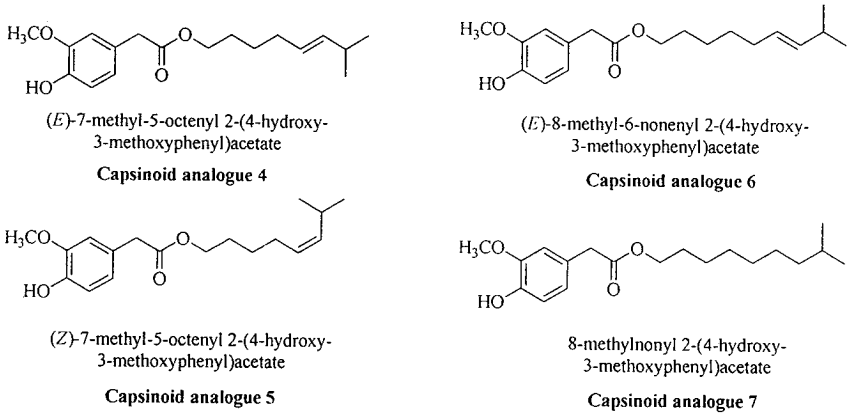


Figure 6 Structure of capsinoid analogues (4-7)