CHAPTER V

CONCLUSION

The Thai marine sponge, *Pachastrissa nux*, was collected from Sichang Island, Chonburi Province, Thailand. The crude EtOAc extract of this sponge showed potent antifungal activity against *C. albicans*. Directed by antifungal activity against *C. albicans*, two known trisoxazole macrolides, kabiramides C (KabC, 1.07×10^{-2} %w/w, wet weight) and D (KabD, 2.54×10^{-4} %w/w, wet weight), and one new compound, kabiramide F (KabF, 0.91×10^{-4} %w/w, wet weight), were isolated from the extract of the sponge. Previous studies showed that the actin targeted trisoxazole macrolides, the family of compounds typified by KabC, functioned as unregulated biomimetics of actin (+)-end-capping proteins. The optical and chemical probes of this group of compounds could provide new information on the regulation of actin filament dynamics in living cells. The major compound, kabiramide C, was modified to five fluorescent derivatives. First, kabiramide C was converted to 7-azidokabiramide C *via* Mitsunobu reaction and then coupled to 3-(fluoren-9-yl-methoxycarbonyl)aminopropyne using 1,3-dipolar cycloaddition reaction to give 7-[4-*N*-(9*H*-fluoren-9-yl-methoxycarbonyl)aminomethyl-1,2,3-triazol-1-yl]kabiramide C. Finally, this compound was deprotected using piperidine to create the key intermediate, 7-(4-aminomethyl-1*H*-1,2,3-triazol-1-yl) kabiramide C (AMT-KabC). The chemical structures of these compounds were elucidated by spectroscopic techniques, mainly NMR spectra and mass spectra.

The terminal amino group of AMT-KabC facilitated the synthesis of a diverse range of fluorescence probes using commercially available succinimidyl ester, including tetrametylrhodamine (TMR), rhodol green (RG), IC5, dapoxyl (DAP), and fluorescein diester (FDE). The fluorescent kabiramide C probes were synthesized and characterized by their molecular weights and UV absorptions.

All isolated and synthesized compounds formed highly stable complexes with G-actin in 1:1 stoichiometry. AMT-KabC exhibited cytotoxicity on human cervix

carcinoma (HeLa) cells. Cells treated with AMT-KabC at a concentration of 1 μM died within 16 hours whereas at lower concentrations (10 nM and 100 nM) the compound caused defects in cytokinesis and loss of cell-cell contacts. The confocal images of TMR-KabC and FDE-KabC treated live NIH 3T3 cells showed that the probes readily crossed plasma membrane of living cells and displayed strong labeling at the leading edge of motile cells and other dynamic cytoskeleton structures.

The fluorescence properties of each KabC probe were largely unaffected following the polymerization of G-actin to F-actin. The extremely tight molecular complex between the fluorescent KabC and G-actin together with the fact that this complex can only associate with actin filaments at the barbed (+)-end of the actin filament in an unregulated manner fulfills a specific information on molecular events at the (+)-end of the filament. The family of fluorescent, membrane permeable unregulated (+)-end KabC based capping probes provided the necessary contrast to map molecular events occurring at the (+)-end of actin filaments in motile cells and should be useful to advance the understanding of the regulation of actin filament dynamics in cell protrusion. The fluorescent KabC probes can be part of a new approach for sensitive and high throughput (HT) quantitative analysis of G-actin for drug and diagnosis development.