CHAPTER V
CONCLUSIONS

The two new series of anthracene, 9-10-dione derivatives were modified with both asymmetric and symmetric substituents at the 1- and/or 4-position of 1, 4-dihydroxy anthraquinone to investigate the effects of relationship between structure and cycotoxicity. Asymmetric substituents (3-7), benzylamine-containing anthraquinone (5) specifically shows inhibition of HPV16 Ca Ski with IC_{50} = 0.3 μM, 20 folds higher than cisplatin (IC_{50} = 8.0 μM), while the others were inactive in all the tested cancer cells. Interestingly, it also blocked colony formation at low concentration suggesting that compound 5 inhibited cell reproduction leading to cell death. This corresponds to our report with benzylamine-bearing planar molecule showed the highest cytotoxic activity.

After investigating molecular mechanism in the further step it has has been known that compound 5 induced G_2/M arrest. Moreover, it caused chromosome condensations in Ca Ski. This demonstrates that it may induce apoptosis. After compound 5 was determined for apoptosis study based on Western blotting, it inhibited anti-apoptosis Bcl-2 and activated Caspase-3 and p53 levels. Thus, this compound may induce apoptosis by down-regulation of Bcl-2 leading to up-regulation of p53. Our approach is to enhance of the therapeutic effect of the inhibitors of anti-apoptotic Bcl-2 family via p53-mediated members by using a drug that targets different Bcl-2 family members. This will determine the direction of future clinical development of the Bcl-2 inhibitors.

The data described here revealed that compound 5 is highly effective in inhibiting the growth of HPV positive cervical cancer cell line, Ca Ski (IC_{50} = 0.3 μM) via G_2/M arrest and targeting HPV16/E6 transcriptional level. It may induce apoptosis by down-regulation of anti-apoptotic protein (Bcl-2) via p53 dependent mechanism.

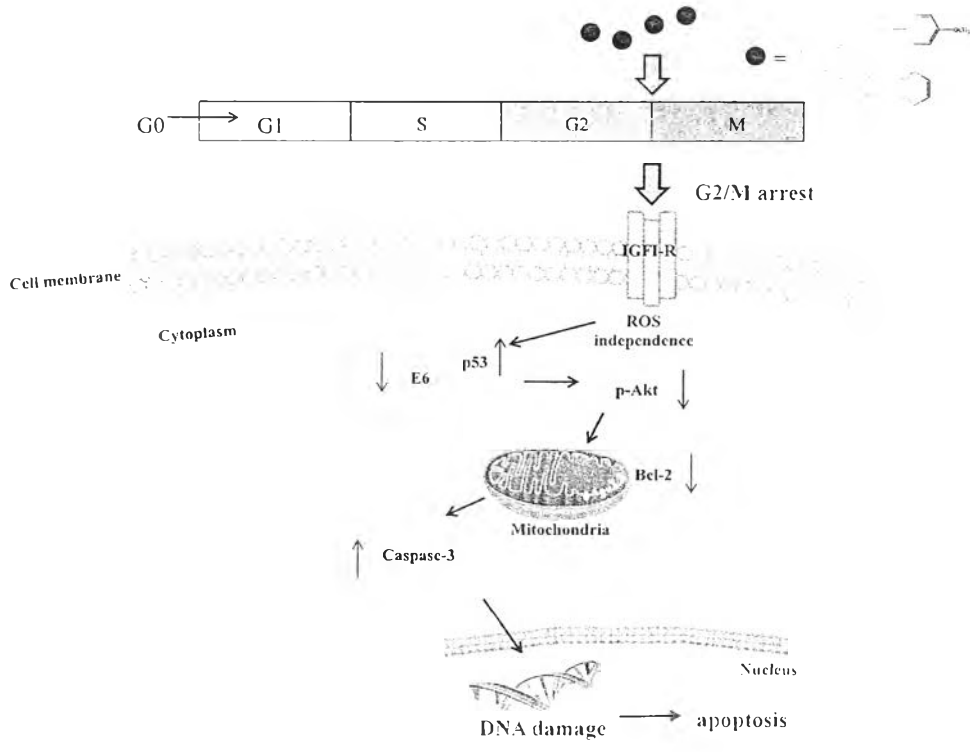

Figure 16. Schematic diagram showing the effects of anthraquinone to apoptosis in Ca Ski

Since drug resistance is a major impediment towards the successful treatment of various cancers, novel chemotherapeutics that can overcome chemoresistance such as doxorubicin is being developed. The best cytotoxicity (compound 5) was continually modified to study the effect of (i) electrophilic substituants group, 8-10 (-Cl, -NH_2, -OMe) on aromatic of benzylamine, (ii) long chain (11-13) and (iii) symmetric substituents (14-15) at the 1- and/or 4-position of anthraquinone for overcoming drug resistance. We have discovered a new class of anthraquinone derivatives that inhibit cancer cell proliferation in the low micromolar range. Compound 9 has shown to overcome drug resistance in NCI/ADR-RES, with IC_{50}'s 25 times greater than doxorubicin. Surprisingly, compound 9's cytotoxic effect does not appear to be ROS or p53-mediated suggesting its mechanism of action may be different from previously reported anthraquinones (Agbandje, 1992 and McKnight, 2004).

Suggestion for the future work

On the basic of our data, anthracene, 9-10-dione derivatives exhibit good activity and induce apoptosis. Further studies are required to further assess the precise mechanism of action. The efficacy of the new series in an *in vivo* model should be validated.