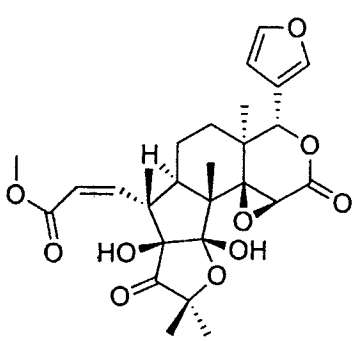
CHAPTER IV

CONCLUSION

The EtOAc extracts of fruits and roots of *H. perforata* (Blanco) Merr. were repeatedly isolated by chromatography techniques. Isolation of the EtOAc crude extract of the fruits provided a new highly rearranged limonoid, namely harperforatin (**4**), while that of the root extract yielded a new chromone, harperamone (**8**) and a new rearranged limonoid, harperfolide (**2**), along with six known compounds including harrisonin (**1**), obacunone (**3**), (+)-vouacapenic acid (**5**), harrisonol A (**6**), peucenin-7-methyl ether (**7**) and braylin I (**9**) as shown below. The structures of isolated compounds were elucidated by analysis of spectroscopic data, particulary 1D and 2D NMR, as well as single-crystal X-ray diffraction analysis, and by comparing with those previously reported in literature.



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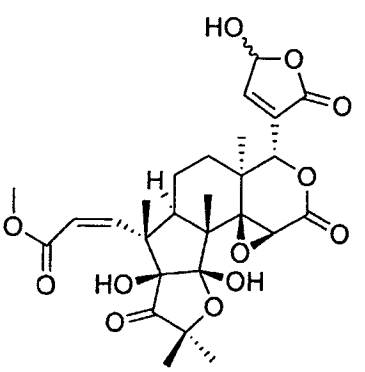

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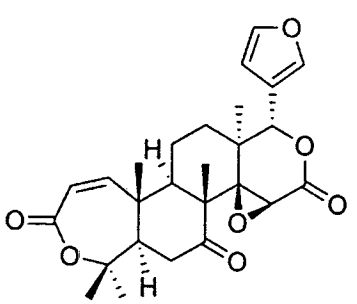

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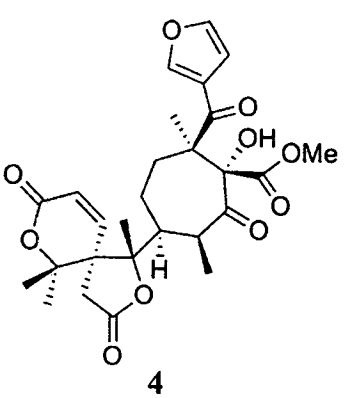

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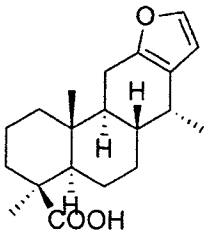

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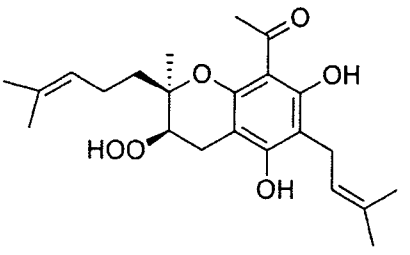

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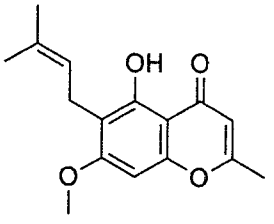

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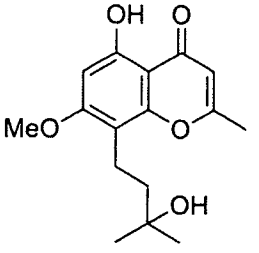

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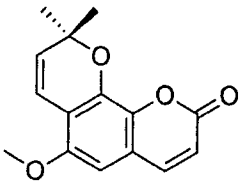

9

Further, compounds **1-8**, except for **4**, were tested for their anti-inflammatory activity using monitoring the inhibition of nitric oxide (NO) production in LPS-activated murine macrophage J774.A1 cells. Harperfolide (**2**) exhibited the most potent activity with IC_{50} value of 6.51 ± 2.10 μM. Its activity was around 20-fold greater than its analog harrisonin (**3**) (IC_{50} = 134.54 ± 5.66 μM), indicating that the presence of a γ-hydroxybutenolide group may significantly enhance the NO production inhibitory activity of this type limonoid. Furthermore, it was found that harperfolide (**2**) did not showed significant toxicity on macrophage J774.A1 cells, determined by the MTT colorimetric method. This result implied that **2** inhibited nitrite release without causing cell death. In addition, its anti-inflammatory effect was found to be mediated by the reduction of the iNOS protein expression as assessed by immunoblotting using a specific anti-iNOS antibody.