Heuristic Approaches to Finding New Herbicides Active in the Chloroplast

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Herbicides inhibiting the photosynthetic electron transport (PET) system may be an important research target to introduce a biorational approach, since accumulated knowledge of the biological system has already reached a satisfactory level for detail explanation of their behaviors.

Besides structural modification of the known herbicides, an effective molecular design of potent PET inhibitors can be arrived at through consideration of the function of PQ at D1 protein. New PET-inhibitory structures may also be discovered in natural products. Those compounds should be modified for the installation of herbicidal potency, which might require chemical parameters additional to those of the optimized structures at the binding site.

Binding studies of the PET inhibitors are essential in this type of research since it is very difficult to understand their structure-activity correlation in cases when a minor structural modification causes a dramatic change in their attacking sites.

It is also necessary to devise quick and reliable assay methods for herbicide screening in order to get accurate information about the structural effects and with this strategy the bioassay using cultured cells has been proven to be a useful tool of structure-activity studies to detect the herbicidal requisites in the structures of PET inhibitors.

Introduction

Extensive usage of agrochemicals during the last few decades has generated many environmental and social problems due to its unexpected side effects, so that wordings related to "pesticide" have become notorious in mass media as a symbol of nasty cultures. Even though application of herbicides have been heavily supporting current agriculture in a global view, most people may not trust benefits from those chemicals any longer. There are many arguments for reducing fears of herbicides in use, however the most important point may concern with a logical explanation on modes of actions of herbicidal chemicals in nature.

The history of herbicides demonstrates effectiveness of random screenings using plant entities in order to discover herbicidal structures without attention to the principle of biological mechanisms which requires large effort to be revealed. The random screenings may be still a convenient method for research and development of new commercial herbicides, however we should introduce an alternative way to design new herbicides considering about interaction of active molecules with biological mechanisms. In fact, medicinal researches have widely applied biochemical *(in vitro)* tests as the first screenings for new drugs. Why can't we try such a heuristic approach for the herbicide research by the use of *in vitro* screenings?

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Research Target

Herbicides inhibiting the photosynthetic electron transport (PET) system may be an ideal research target to try the heuristic approach, since accumulated knowledge of the biological system has already reached a satisfactory level for an explanation of its physiology. For instance, the chloroplast genome including the gene expression of the herbicide binding protein (D1) has been known in detail [1] so that image of the binding niche is deducible from X-ray crystallographic data of *Rhodopseudomonas* considering evolutional correlation between chloroplast and the photosynthetic bacteria [2–4]. This image must be an useful templet to design new PET inhibitors with information about herbicide-resistant mutation [5–10] which is ready to provide a new way of analysis for the molecular action. Further, special plant cell lines showing photoautotrophic characters have been created in the suspension culture, and those were suggested to have specific response to herbicides inhibiting the PET [11]. Consequent-

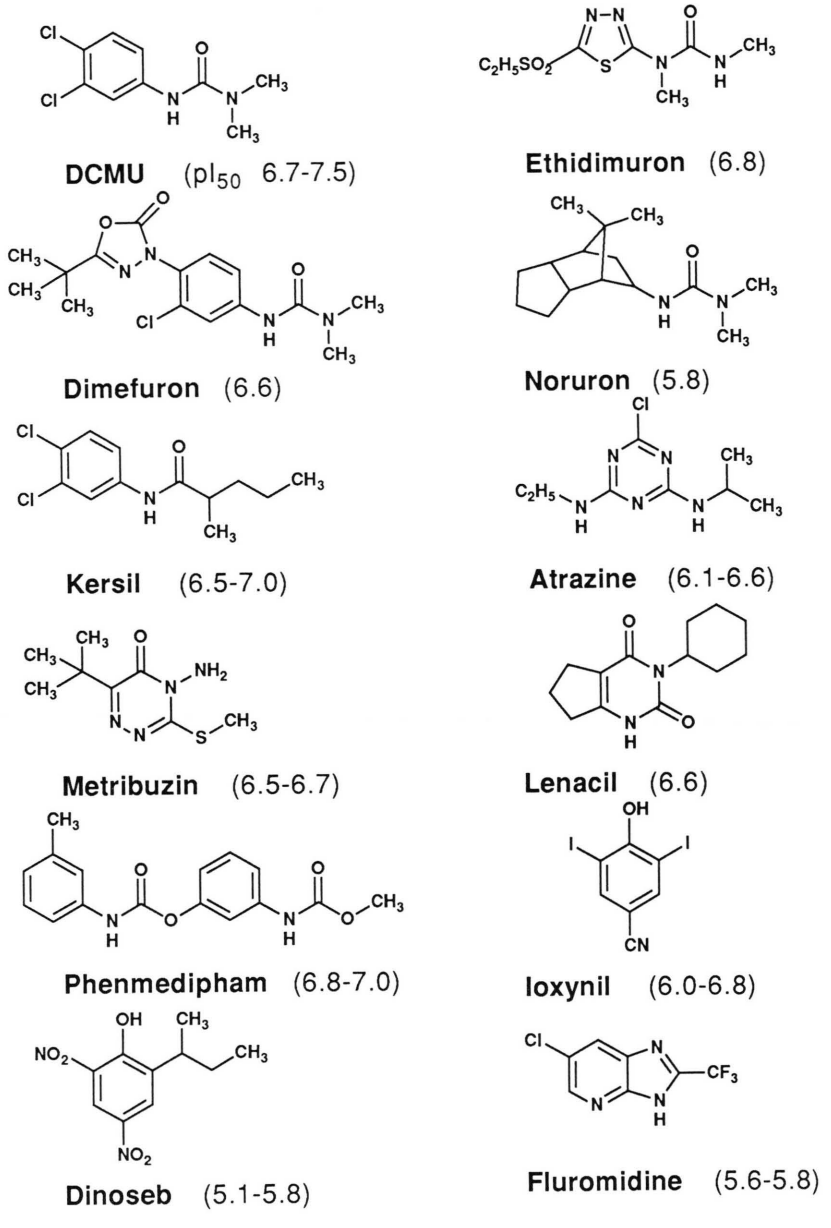
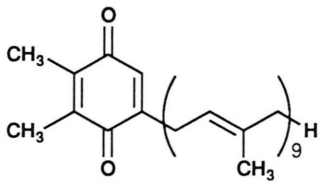


Fig. 1. Typical herbicides interfering the PS II function.

ly we are now able to analyze the action of those particular herbicides at various levels of plant materials such as thylakoid membranes, chloroplasts, cells and whole plants *etc.*, and this wide availability in bioassays is likely the most important point to start the heuristic research.

Designing new inhibitors using Hill reaction

There are many herbicides [12, 13] inhibiting D 1 protein as partly shown in Fig. 1, however it is rather difficult to understand the common principle for the mode of action from a structural view point. Since plastoquinone (**1**) is a substrate play-



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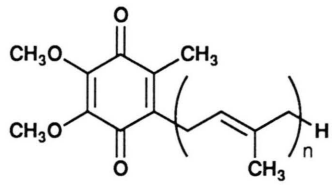
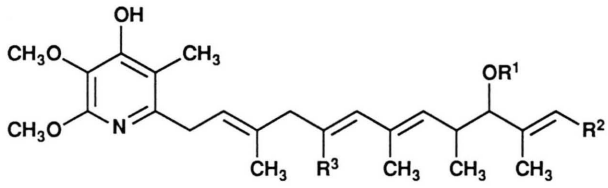


2 n=9 or 10 in higher plants



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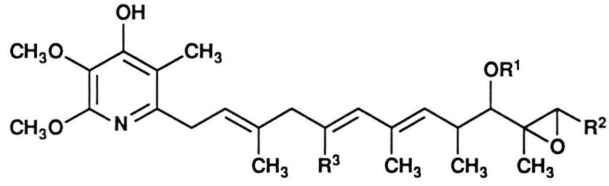

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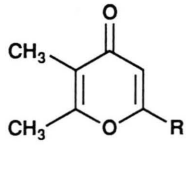

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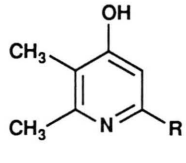

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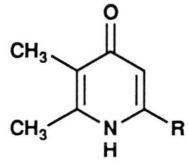

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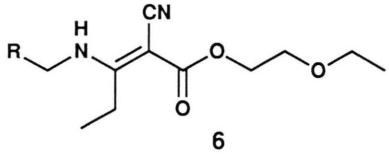

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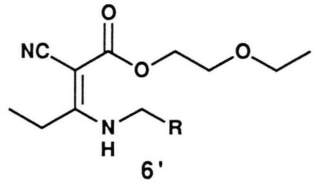

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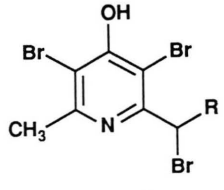

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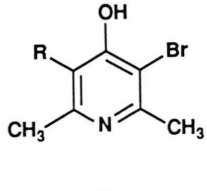

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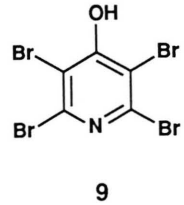

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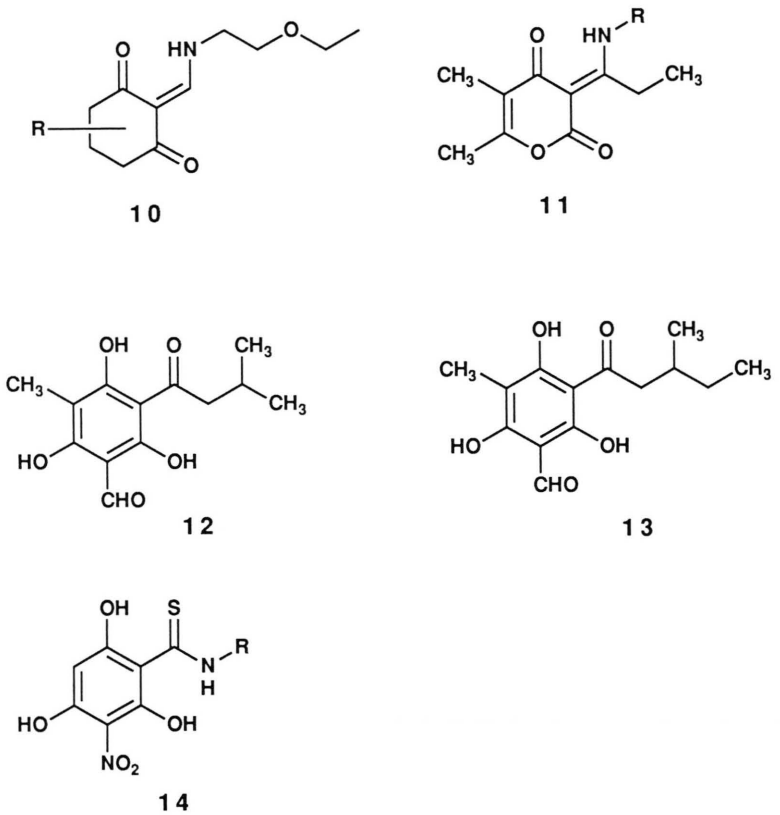

Fig. 2.

ing as an electron carrier at D 1 protein, it is suggested that compounds with antagonistic functions to **1** should inhibit the photosynthetic electron transport. In fact, there is a similar case in mitochondrial respiration, namely the action of ubiquinone (**2**) is specifically blocked by piericidins (**3**) in the respiratory electron transport system [14, 15]. If the structural similarity between benzoquinones and heterocyclic compounds as well as that between **2** and **3** is the working principle of electron transport inhibitors, pyrones (**4**) or pyridinols (**5**) should be expected as a new class of inhibitors for the plastoquinone functions. This speculation was partially correct, because those compounds clearly inhibited the PET at rather high concentration [16]. This fact was analyzed by the flush light experiment where **4** and **5** were suggested to be retardants for the turnover rate of **1** in the electron flow [17]. It should be noted that the above findings could be only done by the thylakoidal bioassay, because neither **4** or **5** affects on plant entities. Regardless of functional change in side chain of those compounds, their activity reached to the certain level depending on lipophilicity. Discovery of powerful Hill inhibitors in cyanoacrylate derivatives (**6**) [18, 19] encouraged investigation for more active pyridinol derivatives by installing a functional pattern of **6** in **5** as shown in the relationship between **5′** and **6′**. It was also evocative to compare structures of known PS II inhibitors which commonly carry strong electron withdrawing groups. Those ideas was amalgamated to design new pyridone derivatives carrying electron withdrawing substituents. Among various functionalities, bromine was the most effective enhancer of the activity for this type of compounds (**7, 8**) [16]. This result was comparable with the activity of tetrabromopyridinol (**9**) [20], a very strong PS II inhibitor which showed well structural coincidence with **7** and **8,** however positions of bro-

mines in the pyridine system might affect on the activity to a large extent. Analysis on the meaning of functionalities and structures in **6–9** gave a hint to design highly active molecules, conjugated enamine derivatives (**10, 11**) [21–24]. This process indicates that there may be a way different from the classical herbicide researches in order to design new PET inhibitors (proto-herbicides).

Another source of new PET inhibitors might be found in natural products. For instance, unique phloroglucinol derivatives, grandinol (**12**) and homo-grandinol (**13**) were discovered as the PET inhibitors in the extracts of *Eucalyptus grandis* leaves [25]. The strucure-activity study on these compounds [26, 27] gave advantageous information for design of extremely potent inhibitors (**14**). The Hill assay was again proven to be a highly efficient tool for those studies.

The PET inhibitors are generally classified into three types (urea, triazine and phenol) due to their binding modes of action at D1 protein [28], and this suggests that there should be at least three kinds of binding domains for inhibitors in the protein. An accurate structure-activity study of proto-herbicides requires this binding problem to evaluate the effect of individual functionality, therefore analysis of their binding modes is definitely important for this research. Although classic methods to differentiate binding modes were rather complicated, two convenient techniques are available today. One is the thermoluminescence glow curve analysis [29, 30] and the other is evaluation of resistance using thylakoids of D1 mutants [31–34].

Evaluation of herbicidal parameters in PET inhibitors

Since the good proto-herbicides found at the level of thylakoidal bioassay (Hill reaction) are irrelevant to potent herbicides, an innovative manner is necessary for installation of chemical parameters onto those inhibitors to generate the *in vivo* effect. Either immobility or degradation of the proto-herbicides in a plant body is presumably a main factor for irrelevance of the activities between *in vitro* and *in vivo* levels, therefore if there is a suitable bioassay system simply reflecting those factors, valuable information will be afforded to install the *in vivo* activity to proto-herbicides. The mobility of herbicides may be related to their permeability and/or stability at various barriers between surface of a plant entity and the receptor in thylakoids. Following this idea, the response of cells to the proto-herbicides is worth studying because it may show an intermediate character somewhere between thylakoid and whole body. Photoautotrophic (PA) cells are especially suitable for bioassay of proto-herbicides inhibiting photosynthesis due to their growth mechanisms [35]. Although photomixotrophic (PM) and heterotrophic (H) cells are prepared from the common source, PA cells are the most sensitive against herbicides interfering PET [11]. Recently the structure-activity study of proto-herbicides using the cell assays indicated that responses of PA and PM cells are highly correlated with those of intact plants [36]. In other words, highly active compounds in those cell bioassays are really worthy to apply herbicide tests. Although PA cells have been obtained from only a few species of plants [37–39], utilization of those cells may provide a new screening system to estimate herbicidal parameters in specific inhibitors of PET.

In addition to applicability of the cell system for the herbicide screening, it has been proven to be a good tool for selection of herbicide resistant mutants which are generated under the drug stress. The D1 protein of atrazine resistant cells thus obtained showed a new type mutation at Ser 264, which was replaced with Thr 264 [40].

Biorational approaches for herbicides

Herbicides inhibiting PET could provide an ideal situation for challenging to biorational approaches but those interfering other biological mechanisms may need further progress of researches in the level of molecular biology. For instance, acetolactate is a very attractive site to create new herbicides as indicated by sulfonylureas, imidazolinones and triazolo pyrimidines *etc.*, however it is still difficult to image out binding niche(s) or mode(s) of those chemicals at the level of enzyme where should be fundamental to design new compounds. Thus challenge biorational approaches would be a model case in herbicide researches, and this will lead us to new phases of bioscience with assists of computer technology and gene engineering (*cf.* [41]).

Conclusion

Besides structural modification of the known herbicides, an effective molecular design of potent PET inhibitors can be obtained by consideration of the function of plastoquinones at D1 protein. New PET-inhibitory structures may also be discovered in natural products. Our several series of the new compounds originated with these concepts, and within a short time their optimal activities at the binding site were determined by the thylakoidal Hill assay. Those compounds should be modified for the installation of herbicidal potency, which might require chemical parameters additional to those of the optimized structures in the Hill assay. We found that photoautotrophic cultured cells are suitable materials of the bioassay for this purpose, because those cells show specific responses to herbicidal compounds inhibiting PET, in other words active molecules in the cell assay are worthy of applying the pot tests for development of new herbicides. Thus we are now feeling the touch of a new methodology for herbicide research with the biorational idea.

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