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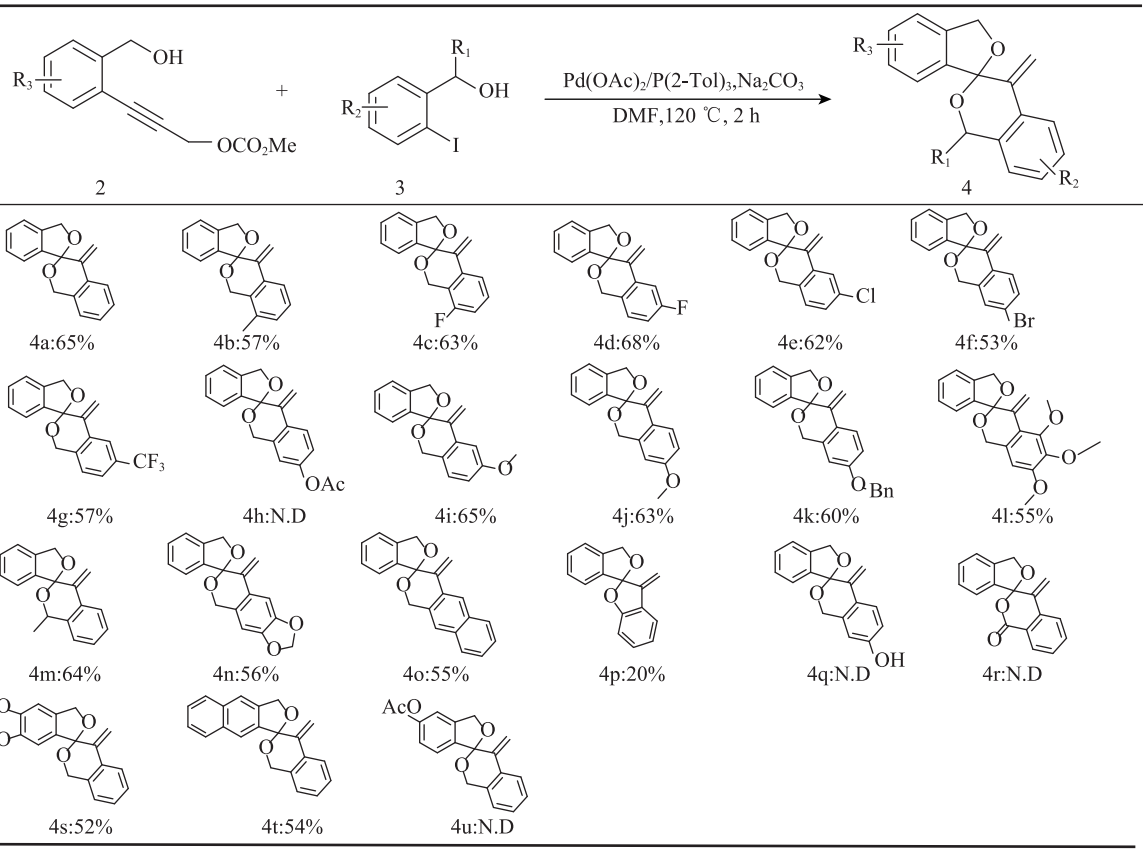
4 - 45 Retrospect and Prospect of Several Interesting Work of Heavy-ion Irradiation Drug

Liang Jianping, Wang Liang, Xin Zhijun, Li Xuehu, Du Wenyue, Lu Xihong and Zhou Xiang

The world's pharmaceutical industry is facing the shrink of new drug R & D investment, reduction of the number of new product approval and listed and patent expiration, and the drug production patterns are changing. After years of development, China has a very good accumulation on the drug production. Facing the opportunities for change, how to use and play our advantage, breaking the barriers of large foreign pharmaceutical companies, become the key to the future development of China's pharmaceutical industry. Heavy ion irradiation drug production as a new method plays an important role both in the discovery of the structure of new drugs, high-quality traditional Chinese medicine and Resources of medicinal microorganisms.

In terms of drug molecular skeleton, eleganketal A 1, isolated from the fungus Spicaria elegans KLA03, is the first naturally occurring aromatic polyketide with a rare 3H-spiro[isobenzofuran-1,3′-isochroman] ring system. Its permethylated analogue 1a demonstrates good bioactivity against the influenza A H1N1 virus. However, only a few synthetic methods for the construction of this [6-5-6-6] tetracyclic skeleton have been reported, including strong acid/base induced spiroketalization, transition-metal catalyzed tandem cyclization, and double Friedel-Crafts cyclization. In addition, the reported methods have certain limitations: 1) the starting materials are restricted by complex multi-step syntheses; 2) the starting materials is limited to alkynediols or equivalents; 3) strong acidic/basic conditions do not tolerate sensitive functionality[1−4]. A consequence as showed in Table 1, the development of novel and efficient strategies for the synthesis of 3H-spiro[isobenzofuran-1,3′-isochroman] scaffolds from readily accessible starting materials is of great significance.

Table 1 Palladium-catalyzed tandem cyclization reaction between propargylic carbonates 2 and various 2-iodobenzyl alcohols 3[a].



[a]All reactions were carried out under the optimal conditions reported in the text. b Isolated yield N.D = no detected

We investigated the scope and generality of this tandem reaction with various substituted propargylic substrates and 2-iodobenzyl alcohols under the optimal conditions. To explore the potential and robustness of this protocol, a facile route to synthesis of 1a was then conducted. As summarized Fig. 1, the investigations show that the 1a could be acquired smoothly within 7 synthetic steps. We have described a palladium-catalyzed highly regioselectivity intermolecular tandem cyclization of propargylic compounds with 2-iodobenzyl alcohols. This tandem cyclization protocol furnishes functionalized 3H-spiro[isobenzofuran-1,3′-isochroman] scaffolds with a broad substrate scope. Notably, the reaction proceeds efficiently, with sequential C-O, C-C and C-O bonds formation in a one-pot procedure. Currently, further investigations involving the mechanism of this reaction and their applications in other organic reactions are ongoing in our laboratory.

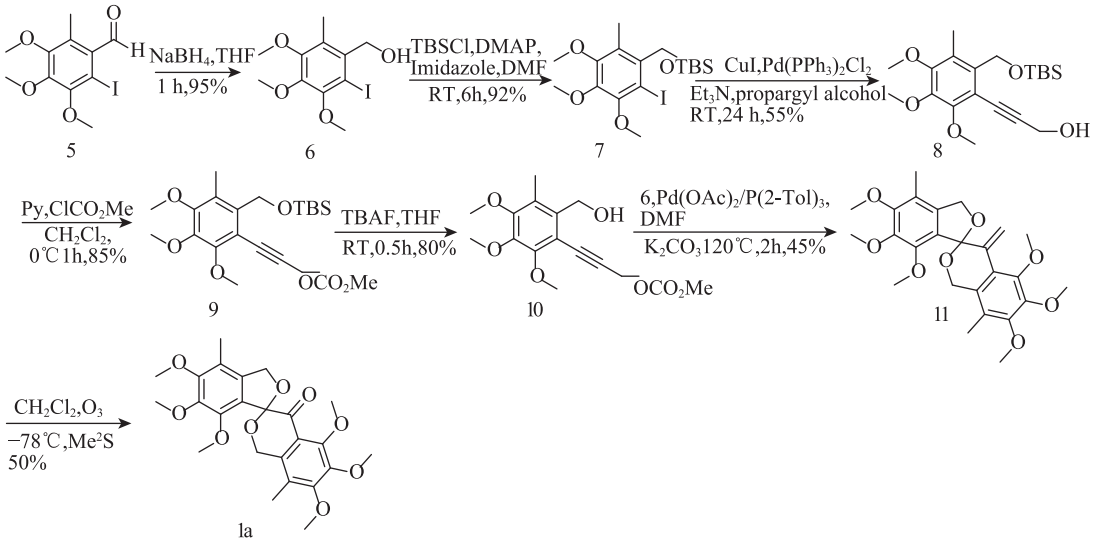


Fig. 1 A facile route to synthesis of 1a.

In terms of Chinese herbal breeding, however, both the output of *Isatis indigotica* and the level of its active ingredients vary greatly according to different varieties of the plant and the atmospheric conditions under which it is produced, so its quality can be unstable. Traditional hybridization based breeding is inefficient because of the lack of genetic resources and a long breeding cycle. The use of Heavy-ion irradiation is an effective method to generate plant mutants. Therefore, we chose SRAP-PCR amplification system to screen and primary identification of variants in *folium isatidis from*[5]. Taim was to establish SRAP-PCR amplification system which was suitable for *Isatis indigotica* Fort DNA. The optimized SRAP-PCR amplification system for *Isatis indigotica* Fort was established by the orthogonal design (Fig. 2). The results suggested that the order of factors which effect on the result of SRAP-PCR were Mg^{2+}, dNTPs, primers, template DNA andTaq DNA polymerase (Fig. 3) A suitable SRAP-PCR system for Chinese bayberry was that total 25 μL reaction system containing 1.5 mmol/LMg^{2+}, 0.15 mmol/L dNTPs, 0.60 μmol/L primers, 2.0 U Taq DNA polymerase and 50 ng template DNA could be able to be amplified the richest polymorphism and clear bands (Fig. 4). This reaction system has been experimentally validatied and and primers selected, and should be a suitable system for the genetic diversity analysis of *Isatis indigotica* Fort.

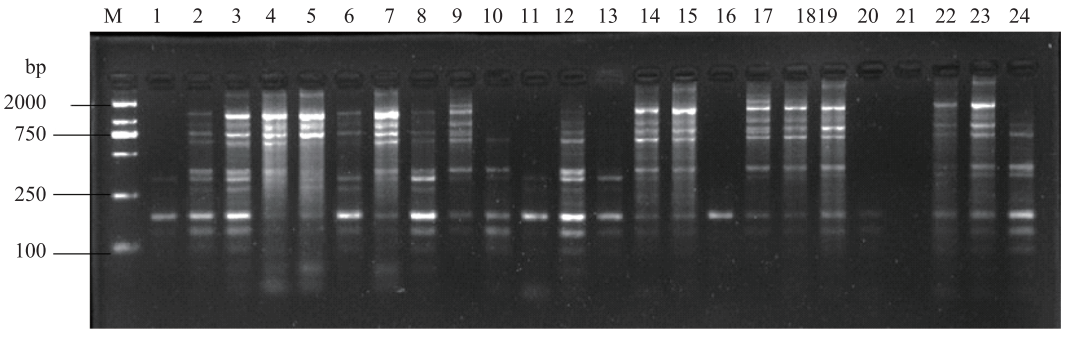


Fig. 2 Amplification of SRAP-PCR by orthogonal design.

In terms of terpenoid antibiotic produced by *Clitopilus* fungi, Pleuromutilin is terpenoid antibiotic produced by *Clitopilus* fungi, and its derivatives Tiamulin and valnemulin have an efficient antibiotic activity for treating pig

diseases like swine dysentery and endemic pneumonia[6,7]. Although pleuromutilin antibiotics have the advantages in high bioavailability, low toxicity and less residues, its industrial production in China hits the bottleneck of low pleuromutilin producing strain titer, long fermentation period and the unideal fermentation conditions. Heavy ion beam which has the characters of the high LET value, the sharp Bragg peak of dose distribution, and the higher relative biological effect, leads to a large number of stable mutants. Therefore, applying heavy ion irradiation induced mutation for breeding and screening pleuromutilin producing strains has a considerable economic benefit. *Clitopilus pinsitus* protoplast was irradiated by $^{12}C^{6+}$ion beam at varied absorbed doses for mutagenesis, and high-yielding pleuromutilin mutant strains were screened using high-throughput method and solid fermentation. The results showed that the optimum $^{12}C^{6+}$ ion beam absorbed dose is 1.5 Gy, and under this condition the positive mutation rate is 37.58%(Figs. 5 and 6).

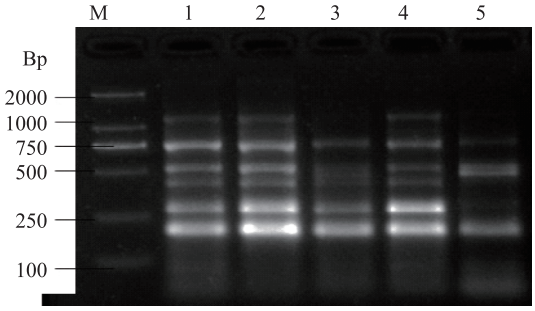


Fig. 3 SRAP-PCR amplication profiles of 5 *Isatis indigotica* with Me6 and Em2 primer combination.

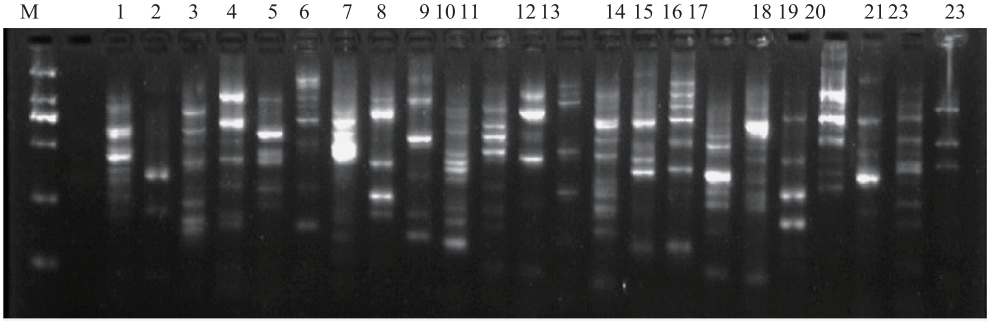


Fig. 4 SRAP profiles amplified results using some primer combination.

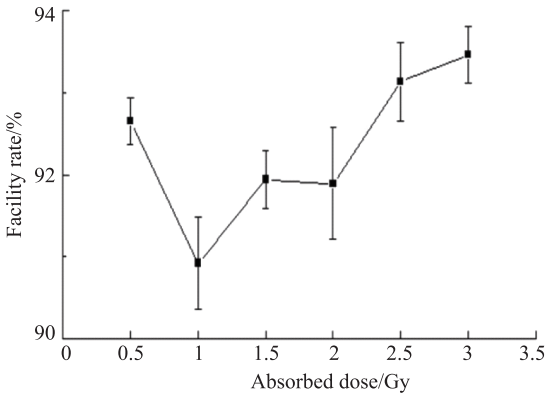


Fig. 5 Fatality rate of $^{12}C^{6+}$ion beam irradiated *Clitopilus pinsitus* protoplast at different absorbed doses.

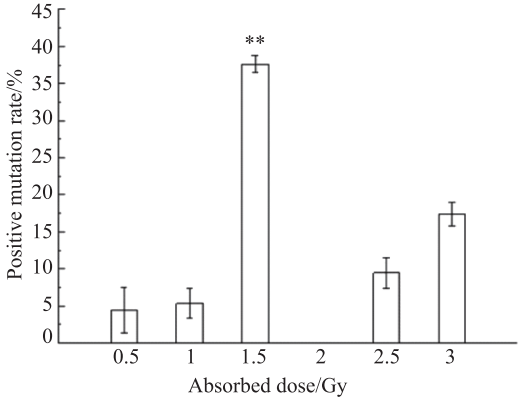


Fig. 6 Positive mutation rate of $^{12}C^{6+}$ ion beam irradiated *Clitopilus pinsitus* protoplast at different absorbed doses (**P <0.01).

Table 2 Pleuromutilin production of the mutant strains.

Strain No.	Sample size	Average pleuromutiln production /(μg/mL)	Production promotive rate / %
C.pin0	3	284.4±30.3	0.00
C.pin15I5E	3	330.4±16.4	16.17
C.pin15II6B	3	328.4±44.8	15.47

By agar column prescreening and 96-plate solid fermentation, the mutant strains, C.pin15II6B and C.pin15I5E, were selected. The yields of the strain increased by 16.17% and 15.47%, respectively, compared with that of the

original strain. The research suggests that applying heavy ion irritation to protoplast is a possible method to breed mutant strains.

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4 - 46 An Experimental Design for Optimization in Some Data Matrices for the Mutant Metabolism Process*

Zhou Xiang, Jiang Tingting, Liang Jianping, Lu Xihong, Xin Zhijun, Li Xuehu and Wang Liang

Before applying the Response surface methodology (RSM) methodology, it is first necessary to choose an experimental design that will define which experiments should be carried out in the experimental region being studied. There are some experimental matrices for this purpose. Experimental designs for first-order models can be used when the data set does not present curvature. However, to approximate a response function to experimental data that cannot be described by linear functions, experimental designs for quadratic response surfaces should be used, such as the approach to the applications of its more frequently used second-order experimental designs is broached, as well as the optimization of procedures that generate multiple responses.

The simplest model which can be used in RSM is based on a linear function. For its application, it is necessary that the responses obtained are well fitted to the following equation[1]:

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \varepsilon, \tag{1}$$

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon, \tag{2}$$

$$y = \beta_0 \sum_{i=1}^{k} \beta_i x_i + \sum_{j}^{k} \beta_{ii} x_i^2 \sum_{1 \le i \le j}^{k} \beta_{ij} x_i x_j + \varepsilon, \tag{3}$$

where k is the number of variables, β is the constant term, β_i represents the coefficients of the linear parameters, x_i represents the variables, β_{ij} represents the coefficients of the interaction parameters, β_{ii} represents the coefficients of the quadratic parameter and ε is the residual associated to the experiments. To estimate the parameters in Eq. (3), the experimental design has to assure that all studied variables are carried out at in at least three factor levels.

Codification of the levels of the variable consists of transforming each studied real value into coordinates inside a scale with dimensionless values, which must be proportional at its localization in the experimental space. The following Eq. (4) can be applied to transform a real value(z_i) into a coded value (x_i) according to a determinate experimental design[2]:

$$x_i = \left(\frac{z_i - z_i^0}{\Delta z_i} \right) \cdot \beta_{\mathrm{d}}, \tag{4}$$

where z_i is the distance between the real value in the central point and the real value in the superior or inferior level of a variable, β_{d} is the major coded limit value in the matrix for each variable, and z is the real value in the central point. There must be estimates of the b parameters of Eqs. (1)∼(3). In matrix notation, Eqs. (1)∼(3) can be represented as Eq. (5). After mathematical transformations of Eq. (5), a vector b containing the parameters can be obtained by the following Eq. (6). The variance estimate to each component of vector b is commonly obtained by authentic repetitions of the central point according to Eq. (7):

$$y_m x_i = X_{mX_n} b_{nX_1}, \tag{5}$$

$$b_{n\cdot 1} = \left(X_{n\cdot m}^T X_{m\cdot n} \right)^{-1} \cdot \left(X_{n\cdot m}^T y_{m\cdot i} \right), \tag{6}$$

$$\hat{V}(b)_{n\cdot n} = \left(X_{n\cdot m}^T X_{m\cdot n} \right)^{-1} \cdot s^2, \tag{7}$$