Short Communication

A New Method for Fast Isolation of GLI Inhibitory Compounds

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ABSTRACT

Bioassay-guided isolation of bioactive natural products from complex plant extracts is usually a very long process. Numerous samples or chromatographic fractions are evaluated against a target protein, in a sequence of bioassay. This is an expensive process, especially with Glioma (GLI) inhibitory assay. We report here a new method, based on the principle of nano-magnetic dynabeads, for a quick isolation of GLI inhibitor compounds from natural resources. GLI-GST were cultured, immobilized on carboxylic acid magnetic dynabeads, and mixed with extract of plants. Hit Plants were selected if mixed suspension of GLI-magnetic beads and the plant extracts formed pellet. Supernatant, which consisted of non-specific compounds, was removed whereas pellet was extracted with methanol and separated by Ultra flash chromatography column. This method allows for a very fast and efficient isolation of GLI-associated cancer inhibitors, and results are presented here for a model plant, *Piper nigrum* (Piperaceae). Using this novel approach, four known lignans were isolated from *Piper nigrum* and quickly identified as; (8*R**,8'*R**)-9-hydroxy-3,4-dimethoxy-3',4'-methylene dioxy-9,9' epoxy lignan, kusunokinin, haplomyrfolol and dihydroclusin. The structure elucidation was on the basis of spectral data of 1D NMR compared to literatures.

Keywords : Anticancer, glioma, hedgehog signaling, magnetic beads, piper nigrum

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INTRODUCTION

The Hedgehog signaling is a key regulator of tissue regeneration, cancer growth, and stem cell renewal. Damage to essential core components of the Hh pathway often results in congenital birth defects [1] whereas an aberrant activated Hh pathway leads to cancer. In the absence of a Hh ligand, the patched 1 (PTCH) transmembrane receptor inhibits the activity of transmembrane protein Smoothened (Smo). Smo in turn promotes the expression of Hh target genes by GLIs transcription factors [2]. The Hh/GLI signaling is constitutively activated in several types of human tumors such as basal cell carcinoma and medullablastoma due to mutations in Ptch or Smo. These mutations activate GLI-mediated transcription hence cause tumor formation and progression [3]. We previously reported Hedgehog/GLI inhibitors from plants and myxomycete [4-7] isolated by using *Bioassay-guided Isolation* (BGI). To provide a substantial reduction in cost, time, and labor in BGI, we have established a new method for a quick isolation of compounds-bound to a specific target protein that is immobilized on magnetic beads (**Fig.1**). Here we report four GLI-associated cancer inhibitors isolated from *Piper nigrum* fruits using this novel approach.

MATERIALS AND METHODS

Instrumentation

The ^{1}H and ^{13}C NMR spectra were recorded on JEOL AX 500 and JEOL JNM ECP 600 spectrometers. The δ-values were reported as ppm relative to TMS in suitable solvents. UV spectra were recorded on a Shimazu UV mini-1240 spectrometer. Optical rotation was obtained on a JASCO P-1020 polarimeter. IR spectra were measured on

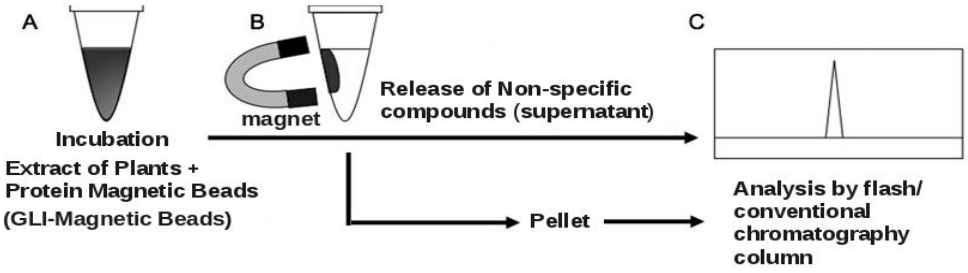

Fig 1: Application of nano-magnetic dynabeads to quickly isolate GLI inhibitory compounds from *Piper ningrum*

ATR on a JASCO FT-IR 230 spectrophotometer.

Plant material

The fruits of *Piper nigrum* (**Fig. 2**) were collected in June 2012 from UPT Materia Medica, Kota Batu, Indonesia. Plant identification was conducted in Health Discipline of East Java, Indonesia.

***Target-oriented Isolation* (TOI) approach using GLI-bound magnetic beads:** GST-GLI was bound to magnetic beads according to the manual procedure (Invitrogen). MeOH extract of *Piper nigrum* fruits in EtOH solution (370 mg in EtOH, 50 mL) was added to GLI-bound magnetic beads (2 mL). The mixture was gently kept for 2 h at -4 ^{o}C prior to centrifugation for 10 min (12,000 rpm, -4 ^{o}C). The supernatant solution was removed and the pellet was analyzed by HPLC to yield compounds **1**–**4**.

Fig 2: *Piper nigrum*

RESULTS

A new method for a quick isolation of GLI inhibitory constituents from *Piper nigrum* led to the separation of 4 known lignans such as (8*R**,8'*R**)-9-hydroxy-3,4-dimethoxy-3',4'-methylene dioxy-9,9' epoxy lignan (**1**) [8], kusunokinin (**2**) [9], Haplomyrfolol (**3**) [10], Dihydroclusin (**4**) [11] The structure of known compounds, was elucidated on the basis of comparison with reported spectral data, were shown in (**Fig.3**).

DISCUSSION

The screening, isolation, and purification of active compounds are often expensive and long processes. We have successfully adapted the concept of nano magnetic beads to the isolation of GLI inhibitory compounds, using GLI-GST culture which is immobilized on the beads and plant extracts. The isolation of *Piper nigrum* using this method only need one fractionation and one purification steps.

CONCLUSION

In conclusion, four lignan type compounds were obtained from the separation of *Piper nigrum* fruits guided by Hh/GLI signaling inhibition. This is the first report of lignan type Hh signaling inhibitors from natural resources. The core structure of isolated compounds this time would be untried in candidate, which might be worth to be explored as Hh inhibitors for clinical use.

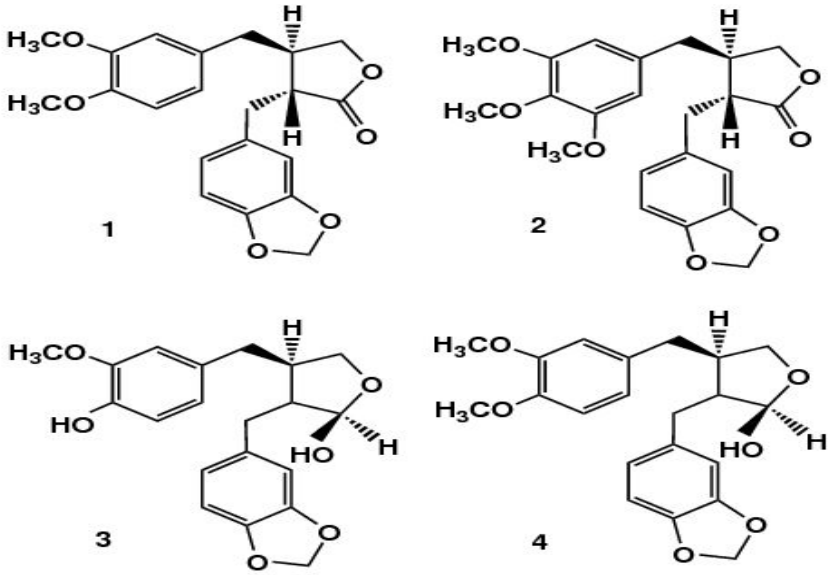

Fig 3: Chemical structures of compounds 1-4 from *Piper ningrum*

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