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Mosquitocidal activity of penfluridol as juvenile hormone antagonist

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ABSTRACT

Juvenile hormone antagonists (JHANs) are known to interfere with the formation of juvenile hormone (JH) receptor complex. JHANs might be effective for control of target pests in larval stages at which stages high level of endogenous JH titer is maintained. In order to identify novel insecticidal compounds, 2352 compounds were surveyed on their JHAN activities using the yeast-two hybrid system. Among 53 compounds with JHAN activities, penfluridol showed high level of insecticidal activity against larvae of *Aedes albopictus*. JHAN activity was increased in proportion to the concentration of penfluridol. Larvicidal activity of penfluridol was 1.3–2.0 folds higher than that of pyriproxyfen. These results suggested that penfluridol could be useful for control of mosquito larvae.

Introduction

Mosquitoes are medically important insect pests that transmit various diseases when they feed on humans. The Asian tiger mosquito, *Aedes albopictus*, is one of the most invasive vector of various diseases including dengue fever (Sang et al., 2015), chikungunya (Reiter et al., 2006), and zika virus (Grard et al., 2014). Although *A. albopictus* is originated from Asia, they have spread to almost all countries over the past several decades (Bonizzoni et al., 2013). Chemical insecticides such as *N,N*-diethyl-*m*-toluamide and temephos have been commonly used to control of mosquitoes (George et al., 2015). However, due to their toxicity to environments and development of insect resistance, the demands for environmentally benign insecticides is on the rise (Briassoulis et al., 2001; Polson et al., 2011; Vontas et al., 2012).

Insect growth regulators (IGRs) could become an effective alternative to control mosquitoes and other vector transmitted diseases because they are specific to target insects and relatively low toxic to environment (Pener and Dhadialla, 2012). IGRs are insecticides that disrupt the normal development of target insects by inducing symptoms such as abnormal development, premature molting or supernumerary larval stages (Beckage et al., 2000). IGRs have been divided into juvenile hormone (JH) analogues, ecdysone agonists, and chitin synthesis inhibitors based on their mode of action (Pener and Dhadialla, 2012).

As JH is important for molting, metamorphosis, reproduction, polyphenism, caste differentiation, and various physiological functions in insects (Hartfelder and Emlen, 2012; Nijhout, 1998; Raikhel et al., 2005; Riddiford, 1994), JH-based IGRs including JH agonists (JHAs) and antagonists (JHANs) fatally affect the physiological regulations in insects and are effective for control the target insects (Lee et al., 2015; Slama, 1971). Recently, we have developed effective in vitro JHAN screening system using yeast cells transformed with the *A. aegypti* JH receptors, Met and FISC (Lee et al., 2015). In this study, novel JHAN compounds were identified from chemical library and their larvicidal activities against *A. albopictus* were investigated.

Materials and methods

Insect

The *A. albopictus* was provided by the Korea National Institute of Health (Cheongwon, Korea). The mosquitoes were reared at 28 °C and 70% relative humidity with a 12 h dark/12 h light cycles in aged tap water. Larvae were fed on a diet of Tetramin fish flakes, and adults were reared using 10% sucrose solution.

Yeast two-hybrid β-galactosidase assays

The yeast two-hybrid binding test using quantitative β-galactosidase

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assay was carried out using Y-187 yeast cells transformed with JH receptor and its partner, Met-FISC, of *A. aegypti* as previously described (Lee et al., 2015). The transformed Y187 cells were incubated at 30 °C in DDO (SD -Leu/-Trp) media until OD_{600} values reached 0.3–0.4. After harvest, the cells were suspended in the fresh media at a concentration of 2.0×10^6 cells/ml and 100 µl of the cells was distributed in 96-well plates.

To determine JHAN activity, 100 µl of yeast cells (2.0×10^6 cells/ml) distributed in 96-well plates was treated with corresponding concentrations (0.1, 1, or 10 ppm) of compound and 0.033 ppm of pyriproxyfen which was identified as the JHA that mediated binding of Met-FISC of *A. aegypti* in the yeast two-hybrid (Lee et al., 2015). A negative control treated with 0.033 ppm of pyriproxyfen and control solvent (dimethyl sulfoxide, DMSO) was placed in each tested plate.

For JHA activity, 100 µl of yeast cells (2.0×10^6 cells/ml) distributed in 96-well plates was treated with corresponding concentrations (0.1, 1, or 10 ppm) of compound. A positive control treated with 0.033 ppm of pyriproxyfen and a negative control treated with control solvent was placed in each tested plate.

The cells treated chemical compounds for JHAN and JHA activity were incubated for further 3 h and subjected to the β-galactosidase assays using the Yeast β-galactosidase Assay Kit (Thermo Scientific. USA). The obtained OD_{420} values were converted to an arbitrary unit representing JHAN activity and JHA activity (Lee et al., 2015).

Larval toxicity tests

Thirty 3rd instar larvae of *A. albopictus* were transferred to 5 ml tap water which was treated with a final concentration of 10 ppm of each chemical compounds and food mixtures. The number of dead larvae was counted at 24 h after treatment for 3 days. To determine the median lethal dose (LC_{50}), thirty larvae of 2nd and 3rd instar were treated with serial dilutions of each compound, respectively. The number of dead larvae was counted at every 24 h for 3 days. Experiments for determine the LC_{50} were performed in triplicates and the IRMA QCal program was used to calculate LC_{50} via linear regression.

Results and discussion

To isolate novel insecticidal compounds with JHAN activity, 2352 chemical compounds (Korea Chemical Bank, Daejeon, Korea) were tested on the pyriproxyfen-mediated binding of *A. aegypti* Met-FISC could be disrupted in the yeast two-hybrid β-galactosidase assays. Fifty three compounds were found to interfere with the pyriproxyfen-mediated binding of *A. aegypti* Met-FISC (Fig. 1). This result demonstrated that these 53 chemical compounds have high level of JHAN activity (JHAN activity > 0.6). Growth inhibition of the yeast transformed with *A. aegypti* Met-FISC was conducted to eliminate the possibility of false signals originating from anti-yeast activity of the chemical compounds without the action of the agonist pyriproxyfen. The addition of chemical compounds with high JHAN activity resulted in normal growth of yeast cells transformed with Met-FISC in non-selective double dropout minimal media, suggesting that these chemical compounds directly disrupt the formation of JH receptor complex. To investigate the mosquitocidal activity of chemical compounds with high JHAN activity, 3rd instar larvae of *A. albopictus* were treated with a 10 ppm concentration of each chemical compound. Among 53 chemical compounds, penfluridol (K265674) showed the highest mosquitocidal activity with 100% of mortality (Fig. 2).

Penfluridol interfered with pyriproxyfen-mediated Met-FISC binding in the β-galactosidase assay using yeast cells transformed with the *A. aegypti* Met-FISC in a dose-dependent manner (Fig. 3A). However, penfluridol showed no JHA activity even at high concentrations (Fig. 3B). To further investigate mosquitocidal activity of the penfluridol, median lethal concentration (LC_{50}) against 2nd and 3rd instar larvae of *A. albopictus* was determined (Table 1). Penfluridol exhibited a significant mosquitocidal activity against 2nd and 3rd instar larvae of *A. albopictus*, with 1.3–2.0 times lower LC_{50} values compared to those of pyriproxyfen.

In animals, Penfluridol has been known as a long-acting, oral neuroleptic drug belonging to the diphenylbutylpiperidines (Janssen et al., 1970). The pharmacological profile of penfluridol is a typical antipsychotic and a dopamine receptor blocking agent (Jackson et al., 1975). In insects, dopamine has been reported as an important

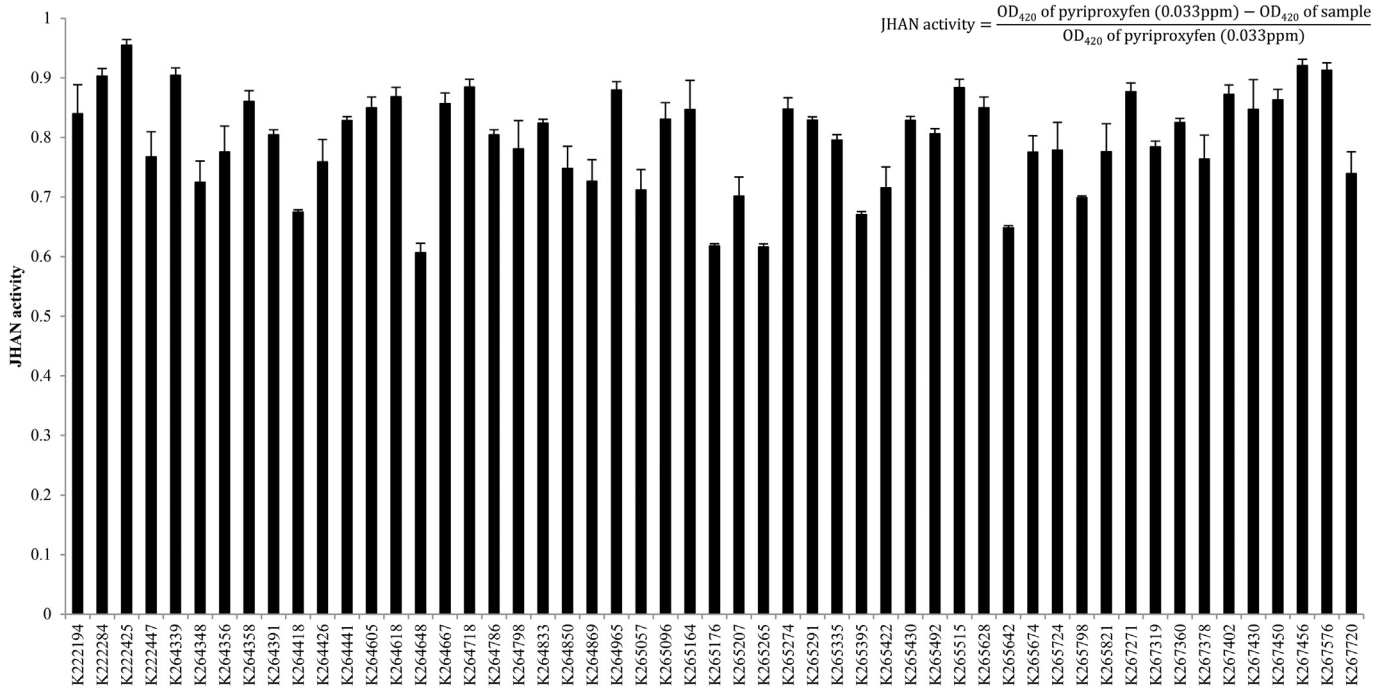

Fig. 1. Screening of the chemical compounds with JHAN activity. The Met-FISC binding triggered by 0.033 ppm of pyriproxyfen was simulated by β-galactosidase activity in the yeast two-hybrid system. Each chemical compounds was added to the yeast culture at a concentration of 10 ppm.