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Design, synthesis and structure–activity relationship of indoxacarb analogs as voltage-gated sodium channel blocker

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ABSTRACT

Indoxacarb, the first commercialized pyrazoline-type sodium-channel blocker, is a commonly used insecticide because of high selectivity. To discover sodium-channel blocker with high insecticidal activity, a series of novel indoxacarb analogs were designed and synthesized by judicious structural modifications of the substituent group of C_5, C_6 in indenone and C'_4 in benzene ring. Some analogs exhibited significant insecticidal activities against *Spodoptera litura* F. and excellent BgNav1-1a channel inhibitory activity. The structure–activity analysis indicated that the presence of strong electron-withdrawing group and decreased steric hindrance of indenone ring (R^1, R^2) in 5- and 6-position could enhance larvicidal activity and BgNav1-1a channel inhibitory activity.

Highly efficacious insecticides with novel modes of action which act on unique biochemical target sites are becoming increasingly important in agriculture.[1] There is great demand for safer and more selective insecticides that spare natural enemies and non-target organisms. The modes of action of the major insecticide classes and biochemical sites related to insecticide action have been reviewed.[2] Sodium channel is an important target for a variety of neurotoxic insecticides, including pyrethroids, DDT, isobutylamides, and dihydropyrazoles.[3] Indoxacarb, a sodium channel blocker insecticide (SCBI), was introduced by the E.I. DuPont Company as a pro-insecticide because of its outstanding field insecticidal activity, environmental compatibility, and safety to non-target organisms.[4] It was found to be hydrolyzed by an esterase or amidase into a much more potent *N*-decarbomethoxyllated (DCJW) metabolite[5] (Fig. 1) that paralyzes insects by blocking action potential initiation in nerve cells. However, higher animals primarily degrade indoxacarb to inactive metabolites via alternative route. Therefore, indoxacarb has a selective toxicity toward insect.[6]

In order to clarify the insecticidal molecular mechanisms and selective toxicity of indoxacarb, a dock of DCJW in open bacterial sodium channel NavAb has been built based on the X-ray structure of the closed bacterial sodium channel NavAb (PDB code 3RVY) and the open potassium channel Kv1.2 (PDB code 2A79).[7] Substituent R^1 or R^2 of C_5- or C_6-position of the indenone ring, 5- or 6-Cl, Br, OCH_2CF_3, and CF_3 groups render compounds the highest activity.[8] However, R^1 or R^2 substituents such as methyl, methoxy or large steric hindrance and strong electron-donating groups, are unfavorable for the sodium channel blocker insecticides (SCBIs) (data not shown). Probably compound cannot pass through the open bacterial sodium channel NavAb smoothly to reach the target site on the receptor protein because of high electronic density and large steric hindrance of the indenone ring. Therefore, a series of novel indoxacarb analogs were designed by modifying the substituent group of C_5 and C_6 in indenone, C'_4 in benzene ring (Scheme 1) to reduce the steric hindrance and electronic density of the benzo ring. The goal is to develop new pyrazoline type sodium channel blocker insecticides that are active against indoxacarb resistant strains, and safe to non-target organisms.

It is worth noting that Claisen condensation (**step i**) and cyclization reaction (**step ii**) are crucial procedures in the total synthetic routes of the title compounds **7a**–**n**, which is outlined in Scheme 1. The intermediates **2a**–**g** were synthesized in high yields by stirring indenone analogs **1a**–**g** with NaH and potassium *tert*-butylate in dry dimethyl carbonate (DMC) for 1 h.[9] Potassium *tert*-butylate played an important role for boosting the reaction. The crude products (**2a**–**g**) need to be added in the next step reaction immediately after preparation because they are oxidized easily when exposed to air. The intermediates (**4a**–**g**) are instable in column chromatography with silica gel. After many attempts, we successfully synthesized the target compounds (**7a**–**n**) by utilizing phosphorus

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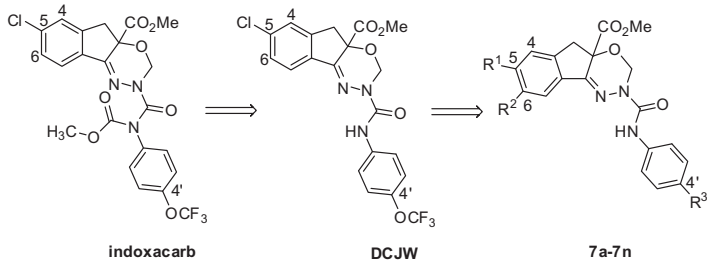
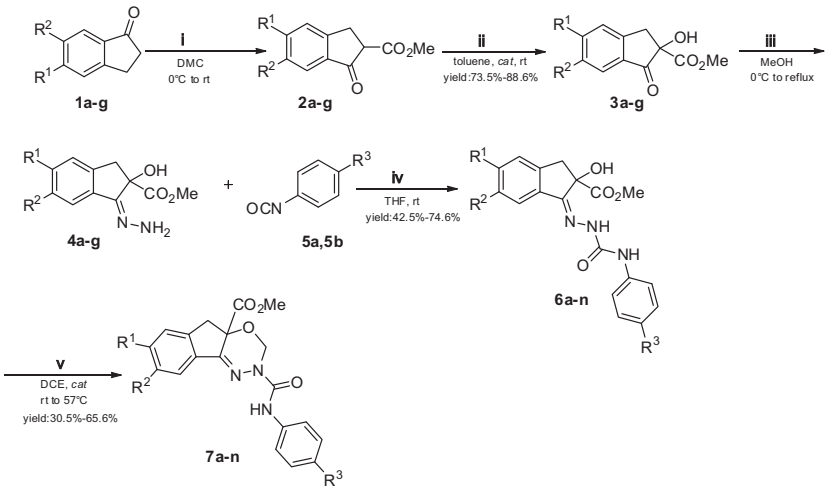


Figure 1. Conceptual relationship of indoxacarb and its analogs.



Compd.	R^1	R^2	R^3
7a	F	H	CF_3
7b	F	H	OCF_3
7c	Cl	H	CF_3
7d	Cl	H	OCF_3
7e	Br	H	CF_3
7f	Br	H	OCF_3
7g	H	H	CF_3
7h	H	H	OCF_3
7i	CH_3	H	CF_3
7j	CH_3	H	OCF_3
7k	OCH_3	H	CF_3
7l	OCH_3	H	OCF_3
7m	OCH_3	OCH_3	CF_3
7n	OCH_3	OCH_3	OCF_3

Reagents and conditions: (i) DMC, NaH, *t*-BuOK; (ii) Cinchonine, *t*-BuOOH, PhMe; (iii) NH_2-NH_2·H_2O, CH_3COOH, MeOH; (iv) THF; (v) $(MeO)_2CH_2$, P_2O_5, $ClCH_2CH_2Cl$.

Scheme 1. Synthesis of **7a**–**n**. Reagents: (i) DMC, NaH, *t*-BuOK; (ii) Cinchonine, *t*-BuOOH, PhMe; (iii) NH_2–NH_2·H_2O, CH_3COOH, MeOH; (iv) THF; (v) $(MeO)_2CH_2$, P_2O_5, $ClCH_2CH_2Cl$.

pentoxide (P_2O_5) as catalyst, dimethoxymethane as donor of '–CH_2–' and solvent, Celite as dispersant and 1,2-dichloroethane as solvent at 57 °C. Nevertheless, the group 'C═N' in indenone ring are likely to hydrolyze to 'C═O' (i.e., intermediates **6a**–**n** change to **3a**–**g**) under acid condition. And the use of strong oxidizing P_2O_5 gave less than 10% yields of the compounds.[10] By increasing the volume ratio of dimethoxymethane/1,2-dichloroethane, supplementing Celite, phosphorus pentoxide and dimethoxymethane successively, the yields could be increased to 30–60%.

The structures of the synthetic compounds were confirmed by melting points, ^{1}H NMR, ^{13}C NMR and the structures of the title compounds **7a**–**n** were confirmed by HRMS spectroscopic data additionally.

The preliminary insecticidal activities of indoxacarb and its analogs were determined by the artificial diet dipping methods at a concentration of 50 μg·mL^{-1}. The mortalities of 3rd-instar larvae of *Spodoptera litura* F. were shown in Table 1. Indoxacarb and six analogs **7a**, **7b**, **7c**, **7d**, **7e** and **7f** showed potent toxicity (93.33–100%). Larvicidal activities of other eight analogs were very low (<13.3%).

To gain further insight into potent toxicity of these compounds, indoxacarb and the six analogs **7a**–**f** were investigated further at serial concentration gradient to determine their LC_{50}s. The LC_{50} values of indoxacarb and compounds **7a**–**f** were 6.38, 3.58, 6.52, 6.25, 6.97, 11.91, and 13.27 μg·mL^{-1}, respectively.

It is well known that indoxacarb preferably block slow inactivated states of sodium channels.[11] Thus, in order to compare the status-dependent inhibition of BgNav1-1a channels by indoxacarb and its analogs, we performed experiments with indoxacarb and its analogs at −55 mV of depolarized holding potentials, to kept consistent with the states of slow inactivation of BgNav1-1a channels.[7] The time course of inhibition of BgNav1-1a channels of 1 μM indoxacarb and analogs were shown in Figure 2.