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Quantitative structure–activity relationship of substituted imidazothiadiazoles for their binding against the ecdysone receptor of Sf-9 cells[†]

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[†]Dedicated to Professor Emeritus Toshio Fujita, who passed away on August 22, 2017.

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Keywords

Ecdysone, imidazothiadiazole, larvicide, Lepidoptera, QSAR

Abbreviations

20E, 20-hydroxyecdysone; DAH, diacylhydrazine; DBU, 1,8-diazobicyclo[5.4.0]undec-7-ene; DMF, *N,N*-dimethylformamide; EcR, ecdysone receptor; ITD, imidazothiadiazole; PonA, ponasterone A; QSAR, quantitative structure–activity relationship; USP, ultraspiracle

Abstract

Imidazothiadiazoles (ITDs) are a class of potent nonsteroidal ecdysone agonists with larvicidal activity. Previously, we performed the Hansch–Fujita type of quantitative structure–activity relationship (QSAR) analysis for ITD analogs (Yokoi et al., *Pestic. Biochem. Physiol.* **2015**, *120*, 40–50). The activity was reasonably explained by hydrophobicity and electronegativity of substituents on the imidazothiadiazole ring system. However, the limited data points (n = 8) hampered the examination of other physicochemical parameters. In the present study, we expanded the library of ITD congeners and evaluated their receptor-binding affinity using intact Sf-9 cells. The QSAR analysis for the expanded set revealed the significance of the third physicochemical parameter, the negative steric effect for long substituents. We also evaluated the larvicidal activity of the synthesized compounds against *Spodoptera litura*; however, it was not correlated to the binding affinity. The results obtained here suggests that the pharmacokinetic properties must be improved to enhance the larvicidal activity of ITDs.

Insects grow by shedding off their old exoskeleton and replacing it with a new one. This process, known as molting or ecdysis, is regulated by 20-hydroxyecdysone (20E; Figure 1). The molecular action of 20E is mediated by the heterodimeric complex of nuclear receptors: ecdysone receptor (EcR) and ultraspiracle (USP). In the presence of 20E, the ternary complex of 20E/EcR/USP activates the transcription of its target genes to trigger molting.[1] Since the 20E-dependent molting is crucial to insects, disruptors of this process are good candidates for insecticides that are harmless to vertebrates. However, 20E and the related steroids (collectively called ecdysteroids) share the highly hydroxylated, complex molecular framework with multiple chiral centers, thereby hampering their practical use as insecticides.

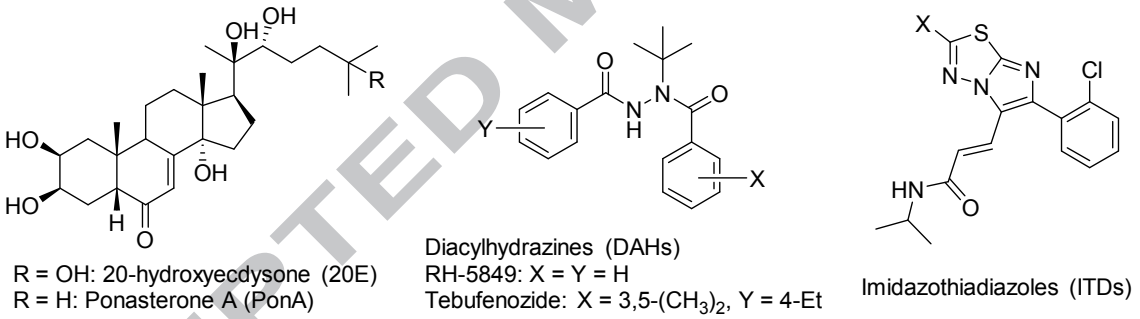


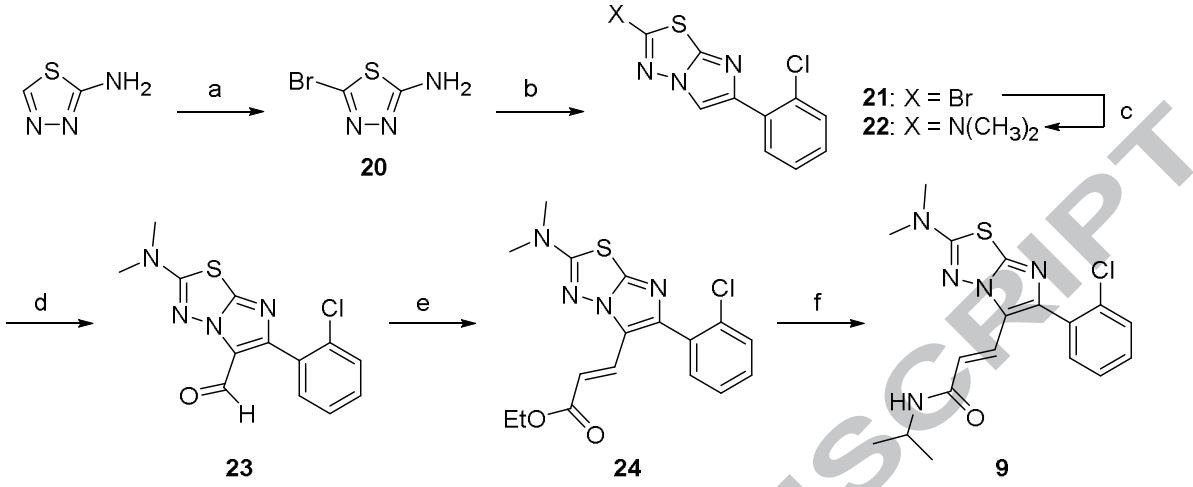
Figure 1. Chemical structures of ecdysone agonists.

In 1988, diacylhydrazines (DAHs; Figure 1) were serendipitously identified as nonsteroidal ecdysone agonists with larvicidal activity.[2,3] This discovery made agricultural chemists realize that nonsteroidal ecdysone agonists are druggable, and the subsequent lead optimization efforts resulted in the development of 5 DAH congeners as agricultural insecticides.[4] Intriguingly, DAHs are highly toxic to Lepidoptera, but less toxic to Diptera and

Coleoptera.[5] This specific toxicity results from the higher binding affinity of DAHs against lepidopteran EcRs than those from other insect orders.[6] X-ray crystal structures of EcR/USP complexes imply that the structural plasticity of lepidopteran EcRs could be essential for the perception of DAHs with high affinity.[7]

The discovery of DAHs also stimulated the exploration of completely different chemical class of ecdysone agonists. Among various nonsteroidal chemotypes screened to date,[5] imidazothiadiazoles (ITDs; Figure 1) are remarkable in that they achieved nanomolar potency in an ecdysone-inducible gene expression system.[8] These authors also provided the possible binding mode of ITDs against EcR, which is somewhat different from that of DAHs. In their report, however, any experimental procedures and target insect species were not described. The synthesized compounds were not chemically characterized, either.

Previously, we synthesized ITD congeners to confirm their potency as ecdysone agonists.[9] As is the case with DAHs, ITDs are highly specific to lepidopteran EcRs, and the binding affinity of ITDs with fluoroalkyl group as substituent X reached nanomolar level in Sf-9 cell. The Hansch–Fujita type of quantitative structure–activity relationship (QSAR) analysis[10] disclosed that hydrophobicity and electronegativity of substituent X are important to exhibit high binding affinity. However, the number of the compounds used for the analysis was limited (n = 8), and was insufficient for revealing the involvement of other physicochemical properties. Herein, we report the updated results of our QSAR study for ITDs.



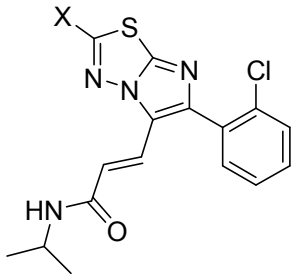
Scheme 1. Synthetic route to compound **9**. Reagents and conditions: (a) Br_2, NaOAc, AcOH, RT, 18 h, 87%; (b) 2-chlorophenacyl bromide, EtOH, reflux, overnight, 73%; (c) Me_2NH, MeOH/H_2O, microwave, 100°C, 10 min, 87%; (d) $POCl_3$, DMF, 70°C, 1 h, 97%; (e) $(EtO)_2P(O)CH_2CO_2Et$, LiCl, DBU, MeCN, RT, 2 h, 85%; (f) isopropylamine, $AlMe_3$, CH_2Cl_2/hexane, 35°C, 1 day, 81%.

To expand the compound set for QSAR analyses, we newly prepared a congeneric series of ITDs with various substituents on the imidazothiadiazole ring (**3**–**9**). Of these, compounds **3**–**8** were synthesized according to the conventional method.[9] Compound **9** was synthesized following the procedure illustrated in Scheme 1. Treatment of 2-amino-1,3,4-thiadiazole with bromine gave compound **20**, which was condensed with 2-chlorophenacyl bromide to afford compound **21**. This was subjected to a nucleophilic aromatic substitution reaction with dimethylamine under microwave heating to give compound **22**. Vilsmeier–Haack formylation of **22** cleanly furnished aldehyde **23**, which was transformed to α,β-unsaturated ester **24** via Horner–Wadsworth–Emmons reaction. Finally,

aminolysis of ester **24** with the aid of trimethylaluminum[11] gave *N*-isopropylamide **9**. The authenticity of the synthesized compounds was confirmed by spectroscopic analyses (see Supplementary Materials).

The synthesized compounds were then subjected to a competitive binding assay in lepidopteran Sf-9 cells, wherein binding affinity of each compound was evaluated as the 50% inhibition concentration [IC_{50} (M)] for binding of the reference ligand, [^{3}H]ponasterone A.[12,13] The results are summarized in terms of the reciprocal logarithmic values (pIC_{50}; Table 1). All compounds synthesized in the present study (**3**–**9**) exhibited higher binding affinity than the unsubstituted one (**1**), and some compounds (**4**, **7**, **8**) were more potent than the natural insect molting hormone, 20E (**18**). Across compounds with linear alkyl substituents, stepwise enhancement in the activity was observed for CH_3, Et, and *n*-Pr groups (**2**–**4**), whereas *n*-Bu group (**6**) decreased the activity. Among compounds with branched alkyl chains, hydrophobic, bulky *i*-Bu (**7**) and *t*-Bu (**8**) groups are more favorable than *i*-Pr (**5**) group. Compound with $N(CH_3)_2$ group (**9**) are about 5 times less potent than that with *i*-Pr group (**5**) despite the similar shapes between these two substituents. Compounds with fluoroalkyl groups (**13**–**15**) were tens to hundreds of times more potent than those with the corresponding non-fluorinated alkyl groups (**2**–**4**), demonstrating the importance of electronegative fluorine atoms.

Table 1. Biological activity and physicochemical parameters of synthesized ITDs.



Compounds		Physicochemical parameters			Binding activity [pIC_{50} (M)]		Larvicidal activity
No.	Substituent (X)	Clog P[a]	σ_I[b]	ΔL[c]	Obsd.[d]	Calcd.[e]	[pLD_{50} (mmol/larva)][d,f]
1	H	3.74	0.00	0.00	4.79[g]	5.15	< 4.00 (0%)
2	CH_3	4.24	–0.04	0.88	5.44[g]	5.49	< 4.30 (0%)[g]
3	Et	4.77	–0.01	2.07	6.23 ± 0.12 (2)	6.12	< 4.00 (0%)
4	*n*-Pr	5.30	–0.01	3.00	7.15 ± 0.01 (2)	6.69	< 4.00 (0%)
5	*i*-Pr	5.17	0.01	2.02	6.78 ± 0.20 (2)	6.89	< 4.48 (0%)
6	*n*-Bu	5.83	–0.04	4.17	6.37 ± 0.08 (2)	7.04	< 4.00 (0%)
7	*i*-Bu	5.70	–0.03	2.98	7.35 ± 0.13 (2)	7.25	< 4.00 (0%)
8	*t*-Bu	5.57	–0.07	2.03	7.07 ± 0.24 (2)	7.14	< 6.00 (0%)

9	$N(CH_3)_2$	4.28	0.06	2.05	6.10 ± 0.01 (2)	5.68	< 4.30 (0%)
10	SCH_3	4.44	0.25	2.17	7.39[g]	6.83	< 4.30 (0%)[g]
11	$S(O)CH_3$	2.44	0.49	2.03	4.79[g]	4.81	< 4.00 (0%)[g]
12	SO_2CH_3	2.26	0.59	2.26	4.76[g]	4.93	< 4.30 (0%)[g]
13	CF_3	4.63	0.40	1.24	8.03[g]	8.17	5.15 ± 0.01 (2)[h]
14	CF_2CF_3	5.00	0.41	2.67	8.35[g]	8.37	4.94 ± 0.04 (2)[h]
15	$CF_2CF_2CF_3$	5.23	0.39	3.48	8.36 ± 0.13 (3)[h]	8.39	4.45 ± 0.01 (2)
16	RH-5849	2.48	-	-	6.44[i]	-	4.41[g]
17	Tebufenozide	4.51	-	-	8.81[i]	-	6.47[g]
18	20E	−1.21	-	-	6.78[i]	-	inactive
19	PonA	1.00	-	-	8.05[i]	-	inactive

[a] Calculated by CLOGP program (BioByte Corp., Claremont, CA, USA). [b] Taken from ref.[14] [c] Calculated for the energy-minimized structures (see Supplementary Materials). [d] Mean ± standard deviation. Values in parentheses are the number of replications. [e] Calculated by Eq. 3. [f] Percentages in parentheses are the proportion killed at the corresponding dose. [g] Taken from ref.[9] [h] Reevaluated in this study. [i] Taken from ref.[12]

We also evaluated larvicidal activity [pLD_{50} (mmol/larva)] of the synthesized compounds against the Oriental leafworm, *Spodoptera litura* (Table 1). Unfortunately, none of the newly synthesized compounds (**3**–**9**) gave pLD_{50} values, although the highest dose of compound **8** was only 1/100 those of other compounds due to its limited solubility in dimethyl sulfoxide. The larvicidal activity of compounds with fluorinated alkyl groups (**13**–**15**), which were evaluated in our earlier publication,[9] were measured again to confirm the reproducibility. They showed the moderate larvicidal activity with small standard deviation. Compound **13**, which showed the highest larvicidal activity among the ITD congeners, is approximately 20 times less potent than the commercialized ecdysone agonist, tebufenozide (**17**).

To identify the physicochemical factors governing the ligand–receptor interaction, we performed the Hansch–Fujita QSAR analysis. We previously obtained Eq. 1 for the binding affinity of a small set of compounds (**1**, **2**, **10**–**15**)[9]:

$$pIC_{50} = 1.519\ (\pm 0.315)\ \Delta\text{Clog}\ P + 3.923\ (\pm 1.643)\ F + 4.872\ (\pm 0.361) \qquad (1)$$

$$n = 8,\ s = 0.349,\ r = 0.985,\ F_{2,5} = 81.754$$

where ΔClog P is the hydrophobic parameter which represents the Clog P value of a compound relative to that of the unsubstituted compound [ΔClog P = Clog P (X) − Clog P (H)], and F is the electronic parameter defined by Swain and Lupton.[15] In this and the following equations, values in parentheses are the 95% confidence intervals of the regression coefficients, n is the number of compounds, s is the standard deviation, and r is the correlation

coefficient. $F_{m,n-m-1}$ is the value of ratio between regression and residual variances, where m is the number of independent variables. Equation 1 gave the satisfactory correlation; however, the number of parameters used to derive the equation was two (ΔClog P and F), which is not statistically favored. In general, at least 5 compounds are required per parameter.

We reanalyzed the binding affinity of the expanded set of compounds (**1**–**15**) to obtain Eq. 2:

$$\mathrm{pIC_{50}} = 1.386\ (\pm 0.296)\ \mathrm{Clog}\ P + 4.157\ (\pm 1.365)\ \sigma_\mathrm{I} - 0.405\ (\pm 1.515) \qquad (2)$$

$$n = 15,\ s = 0.431,\ r = 0.947,\ F_{2,12} = 52.280$$

where σ_I is the inductive component of Hammett substituent constant σ, which by definition is equivalent to Swain–Lupton F. Indeed, the replacement of σ_I with F also yielded the similar correlation (s = 0.447, r = 0.943). The intercept was much smaller than that in Eq. 1, because here we utilized intact Clog P instead of ΔClog P. The significance of Eq. 2 was justified above 99.9% by F-test; however, the standard deviation was still large compared to the maximal standard deviation in the data set (0.24). In our previous QSAR study, the binding affinity of DAHs with various *para*-substituents Y (Figure 1; X = 2-Cl) was reasonably explained by hydrophobic, electronic, and steric parameters.[16] This experience prompted us to add a steric parameter, formulating Eq. 3:

$$\mathrm{pIC_{50}} = 1.626\ (\pm 0.316)\ \mathrm{Clog}\ P + 4.914\ (\pm 1.292)\ \sigma_\mathrm{I} - 0.315\ (\pm 0.263)\ \Delta L - 0.930\ (\pm 1.326) \qquad (3)$$

$$n = 15,\ s = 0.352,\ r = 0.968,\ F_{3,11} = 54.497$$

where ΔL represents the STERIMOL length parameter for a substituent relative to hydrogen.[17] Equation 3 indicates that hydrophobic and inductively electron-withdrawing substituents enhance the binding affinity, while long substituents not. The physicochemical parameters and the calculated pIC_{50} values by Eq. 3 are listed in Table 1. Below we discuss the implications of the above QSAR model in detail.

The large, positive coefficient of the Clog *P* term in Eq. 3 indicates that the hydrophobic substituents drastically enhance the binding affinity. It is generally accepted that the slope of log *P* term implies the location of the ligand binding site, since log *P* corresponds to the desolvation free energy of a compound upon its partitioning from the aqueous phase into the 1-octanol phase.[18] The slope of 1.0 means the complete desolvation of water molecules from the ligand, suggesting the ligand is completely buried in the receptor, while the slope of 0.5 means the half-desolvation, suggesting that the ligand binds to the surface of the receptor. In Eq. 3, the coefficient (1.626) of the Clog *P* term is much greater than 1.0. This might suggest that the binding cavity of ITDs is located inside the receptor and composed of hydrophobic amino acid side chains, wherein hydrophobic interaction is predominant.

The positive coefficient of the σ_I term in Eq. 3 means that inductively electron-withdrawing substituents are favorable for the ligand–receptor interaction. Two assumptions are possible for this positive contribution of the σ_I term: (1) the electron-withdrawing effect toward the imidazothiadiazole ring system enhances the binding, (2) the electronegative substituent participates in the interaction with electropositive residues

of the EcR. If the assumption (1) is true, the resonance electronic effect should be significant; however, addition of the resonance component, σ_R, did not improved the correlation. We therefore concluded that the assumption (2) is reasonable, which means that the electronegative atoms (e.g. fluorine) participate in the electrostatic interaction with the receptor.

The negative coefficient of the ΔL term in Eq. 3 indicates that long substituents are sterically unfavorable for the binding. Replacement of the ΔL term with other steric parameters, such as substituent width (ΔB_5) and volume (ΔV_w), did not improve the correlation. This negative steric effect is prominent in the case of compounds with linear alkyl chains. With elongation of the carbon chain, the binding affinity showed a gradual increase for **1**–**4**, but suddenly dropped for **6**. These results suggest the presence of the receptor wall that limits the binding of long substituents.

Equation 3 rationally explains the change in binding affinity of ITDs. A notable example is the activity difference between compounds **5** and **9**: compound **5** with *i*-Pr group is 5 times more active than compound **9** with $N(CH_3)_2$ group. As shown in Table 1, these two substituents are almost equivalent in terms of σ_I and ΔL. By contrast, the Clog *P* value of compound **5** is about 8 times higher than that of compound **9**. This difference in hydrophobicity leads to the higher binding affinity of compound **5** than that of compound **9**.

Figure 2 shows the possible binding model of ITDs based on the above QSAR model and our previous study.[9] Holmwood and Schindler reported the possible binding mode of ITDs, although they did not provide any experimental data supporting it.[8] According to their structures, ITDs and DAHs roughly share the same binding position, and substituent X

of ITDs and *tert*-butyl group of DAHs are closely located. In the crystal structure of *Heliothis virescens* EcR/USP complex bound to a DAH analog (PDB: 1R20), the *tert*-butyl group is surrounded by hydrophobic amino acid residues, such as F336, M413, L511, and L518.[7] In particular, F336 seems to function as a cap that separates the ligand binding cavity from the bulk water, limiting the size of the ligand molecule. Furthermore, located at the edge of the binding pocket is T340 that is able to form hydrogen bond with electronegative atoms like fluorine. These observations are in good accordance with the present QSAR results.

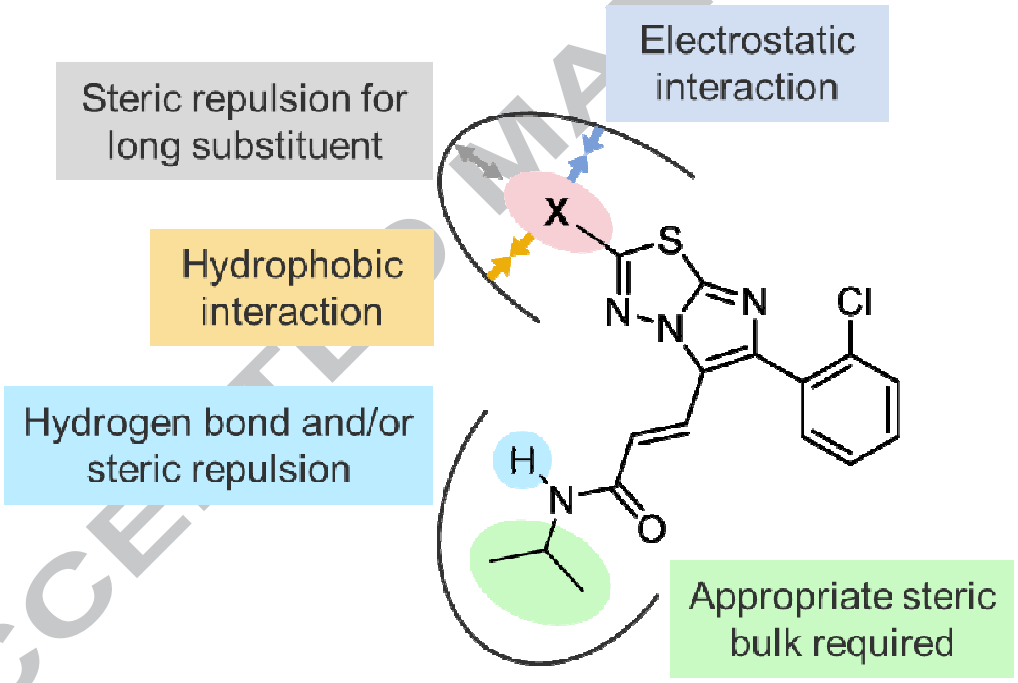


Figure 2. The possible binding model of imidazothiadiazoles against the receptor.

As shown in Table 1, compounds with fluoroalkyl groups (**13**–**15**) were moderately toxic to *S. litura*, while the other compounds (**1**–**12**) showed no substantial

activity. This is probably due to the metabolic detoxification in insect body after application. Compounds with alkyl and sulfide moieties (**2–8**, **10**) can undergo the oxidative metabolism to lower the insecticidal activity. It is likely that the presence of fluorine atom is effective not only for improving the intrinsic activity but also for suppressing the metabolic detoxification. Meanwhile, there is no clear correlation between the larvicidal and binding activity among compounds with fluoroalkyl groups (**13–15**). Instead, the larvicidal activity is negatively correlated to the molecular hydrophobicity. Increased hydrophobicity seems to act as a limiting factor of their concentration at the target site. Further synthetic efforts, especially to improve their pharmacokinetic properties, are required to bring their larvicidal potency into the practical level.

In summary, the present QSAR study identified the key physicochemical factors of ITDs that are important for the ligand–receptor interaction: molecular hydrophobicity, inductive electron-withdrawing effects, and substituent length. Comparison of the binding and larvicidal activities demonstrated the importance of the fluoroalkyl substituent on the imidazothiadiazole ring, in terms of improving their intrinsic activity and metabolic stability. These findings provide a valuable information for the rational design of novel ecdysone agonists.

Acknowledgements

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Supplementary Materials

Detailed experimental procedures and chemical characterization of new compounds are provided in the Supplementary Materials.

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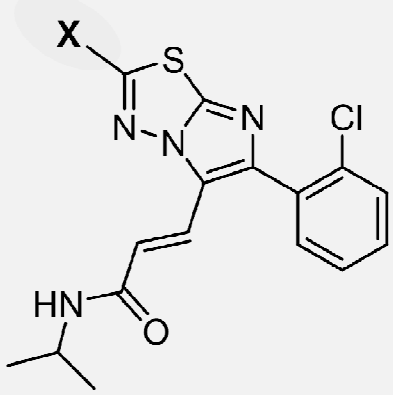

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