Bioorganic & Medicinal Chemistry Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Thioether-bridged arylalkyl-linked *N*-phenylpyrazole derivatives: Design, synthesis, insecticidal activities, structure-activity relationship and molecular-modeling studies

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A R T I C L E   I N F O

Article history:
Received 9 December 2017
Revised 4 April 2018
Accepted 11 April 2018
Available online xxxx

Keywords:
N-Phenylpyrazole derivatives
Thioether bridge
Insecticidal activities
Structure-activity relationship
Molecular-modeling studies

A B S T R A C T

Owing to thioether diverse physicochemical properties by non-covalent interactions with bio-macromolecules, thioether derivatives containing heterocyclic moiety are known for their interesting insecticidal bioactivities and attracting considerable attention as neuroactive insecticides. Here we synthesis a series of novel thioether bridged *N*-phenylpyrazole derivatives incorporating various (hetero)aromatic substituents into 4-position of the pyrazole ring. Structure-activity relationship (SAR) studies resulted in compounds **6d** and **7d** with the most potent insecticidal activity among the series containing various substituted benzene substituents (LC_{50} = 13.70–25.47 μg/g). Further optimization to increase the lipophilicity and charge density of aromatic substituents of compounds **6d** and **7d** resulted in compounds **12d**, **14d** and **16d** with sulfur-containing heterocycle substituents possessing good insecticidal activity against *Musca domestica* L. among the series (LC_{50} = 0.67–1.30 μg/g). The thioether bridge *N*-phenylpyrazole derivatives, which exhibit different length of the spacer arm introduced between *N*-phenylpyrazole moiety and the (hetero)aromatic substituents, were also prepared and evaluated. By contrast, the insecticidal activities of compounds containing the short thioether bridge, 1,2-bis((hetero)aromatic thio) ethane, are higher than that containing the long thioether bridge, 1,3-bis((hetero)aromatic thio) propane. The results of molecular docking and pharmacophore analyses indicated A299, T303, and L306 of a subunit were essential to form non-covalent interactions contacts with the ligands. Specially, the sulfur-containing heterocycle substituent derivatives **12d** and **14d** as the sterically favored areas could form the important hydrophobic interactions with the deeper residue P295.

N-Phenylpyrazole moiety has been shown to bestow biological activity, including insecticidal, miticidal, and herbicidal activity.[1] More specifically, *N*-phenylpyrazoles with a cyano or acetyl groups on 3-position, a sulfenyl, sulfinyl, thioalkyl, alkyl, acyl, alkynyl or cyano groups on 4-position, and an amino (or substituted amino) on 5-position of the pyrazole ring exhibit potent insecticidal activity.[2–6] Fipronil, an *N*-phenylpyrazole insecticide with a trifluoromethylsulfinyl substituent at 4-position, has attracted particular attention of researchers due to their more favorable selective toxicity in invertebrates relative to mammals.[7] The selective toxicity of fipronil is due in part to differ in binding between insect and mammalian GABA receptors[8], but is also dependent on the relative rates of conversion to the less selective sulfone fipronil metabolite at 4-positon of the pyrazole ring.[9] The change in single substituent, i.e., thioalkyl vs sulfinyl (Fig. 1), alters the lipophilicity and electronic properties and potentially also the photochemical and metabolic fate, effectiveness, and toxicology.[10] For example, high insecticidal activity and selective toxicity, low mammalian toxicity of *N*-phenylpyrazole insecticides was observed such as vaniliprole (Fig. 1A), pyrafluprole (Fig. 1B) and pyriprole (Fig. 1C) with 4-SCF_3, 4-SCH_2F and 4-SCF_2H instead of 4-$SOCF_3$ at 4-position of the pyrazole ring. However, fipronil with a trifluoromethylsulfinyl moiety probably exists in the cyclic hydrogen-bonded form allowing interaction of the sulfinyl and amino moieties, and may be the first step in extrusion of SO from fipronil to give higher toxic and persistent desulfinylfipronil, then lead to more toxic and persistent detrifluoromethylsulfinyl product than parent fipronil by photolysis reactions.[9] But the sulfide analogs of fipronil at 4-position of the pyrazole ring are much more stable and do not undergo an analogous photoextrusion reaction.[11] In addition, the sulfur-aromatic interaction is often described as improving the stability for modulation of protein interactions[12,13]

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https://doi.org/10.1016/j.bmcl.2018.04.022
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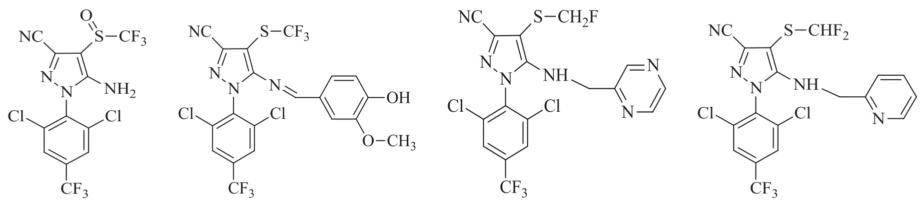


Fig. 1. Structure of thioalkyl fipronil derivatives.

and one-third of all known protein structures contain an energetically stabilizing methionine-aromatic motif.[14] Considering fipronil (trifluoromethylsulfinyl phenylpyrazole) have higher insecticidal activity than sulfone fipronil (trifluoromethylsulfonyl phenylpyrazole)[15] and intramolecular thioether-bridge formation is an effective way to protect compound against mixed function oxidases degradation *in vivo*, we speculated that thioalkyl fipronil derivatives may be increasing frequency near aromatic side-chains of γ-aminobutyric acid receptor proteins and altering bioavailability of drugs due to ligand(drug)-proteins interaction, and then improved insecticidal activity.

Meanwhile, after decades of intensive use, many target pests have developed higher resistance to fipronil, and owing in part to its high toxicity to beneficial organisms[16,17], fipronil was greatly limited to be used as a pesticide in China since 2009 and the European Union followed suit in 2013.[18] To reduce resistance and toxicological risk, we developed several fipronil derivatives by modifying the amino group at 3-position of the pyrazole ring with salicylide, substituted phenoxyacetyl, amino acid and inner salt groups.[19–24]

Inspired by these reports, herein we report a series of fipronil derivatives containing an arylalkyl thioether moiety at 4-position of the pyrazole ring (Scheme 1). In addition, the binding site of meta-diamides was demonstrated to be distinct from that of conventional noncompetitive antagonists such as fipronil. Thus, it is expected to become a prominent insecticide against pests with resistance to cyclodienes and fipronil.[25,26] So, different modes of actions of novel fipronil derivatives containing an arylalkyl thioether moiety at 4-position of the pyrazole ring may have appeared and it is expected to be effective against resistant pest insects.

The bioactivities of the target compounds were evaluated, the structure-activity relationships and molecular docking studies of these compounds are shown in this paper.

The target thioalkyl fipronil derivatives were prepared from 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazole (**b**) as shown in Scheme 1. The thiophenol or aromatic thiol was reacted with dibromoalkane in the presence of potassium carbonate and potassium iodide to afford the intermediates **1a**–**29a**. In addition, 1*H*-benzo[*d*]imidazole-2-thiol, 2-methoxybenzenethiol, 1*H*-1,2,4-triazole-3-thiol, thiazole-2-thiol and 1,3-dibromopropane; 4-methoxybenzenethiol and 1,2-dibromoethane were also tried but failed to give target products under similar conditions. We have tried to react with a more laborious reaction pathway, unfortunately the reaction was unsuccessful due to the complex formation between substrate and the catalyst. Compound **b** was converted to the key intermediate, bis(5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)pyrazol-4-*yl*) disulfide (**c**), according to the reported method.[3] The cleavage of disulfide **c** by intermediates **1a**–**29a** assisted with an alkali and a reducing reagent afforded the target thioalkyl fipronil derivatives **1d**–**29d**.[15]

According to experimental result, the aromatic halides such as phenyl bromide could not react with pyrazolyl disulfide.[15] We add a spacer linker between the *N*-phenylpyrazole moiety and thiophenol/aromatic thiol. The structure notably included a phenylpyrazole ring connected to the aromatic moiety via two thioether bond. Using the cleavage of S–S bond chemical reaction by intermediates **1a**–**29a**, the target fipronil derivatives were synthesized easily and quickly in good yields.

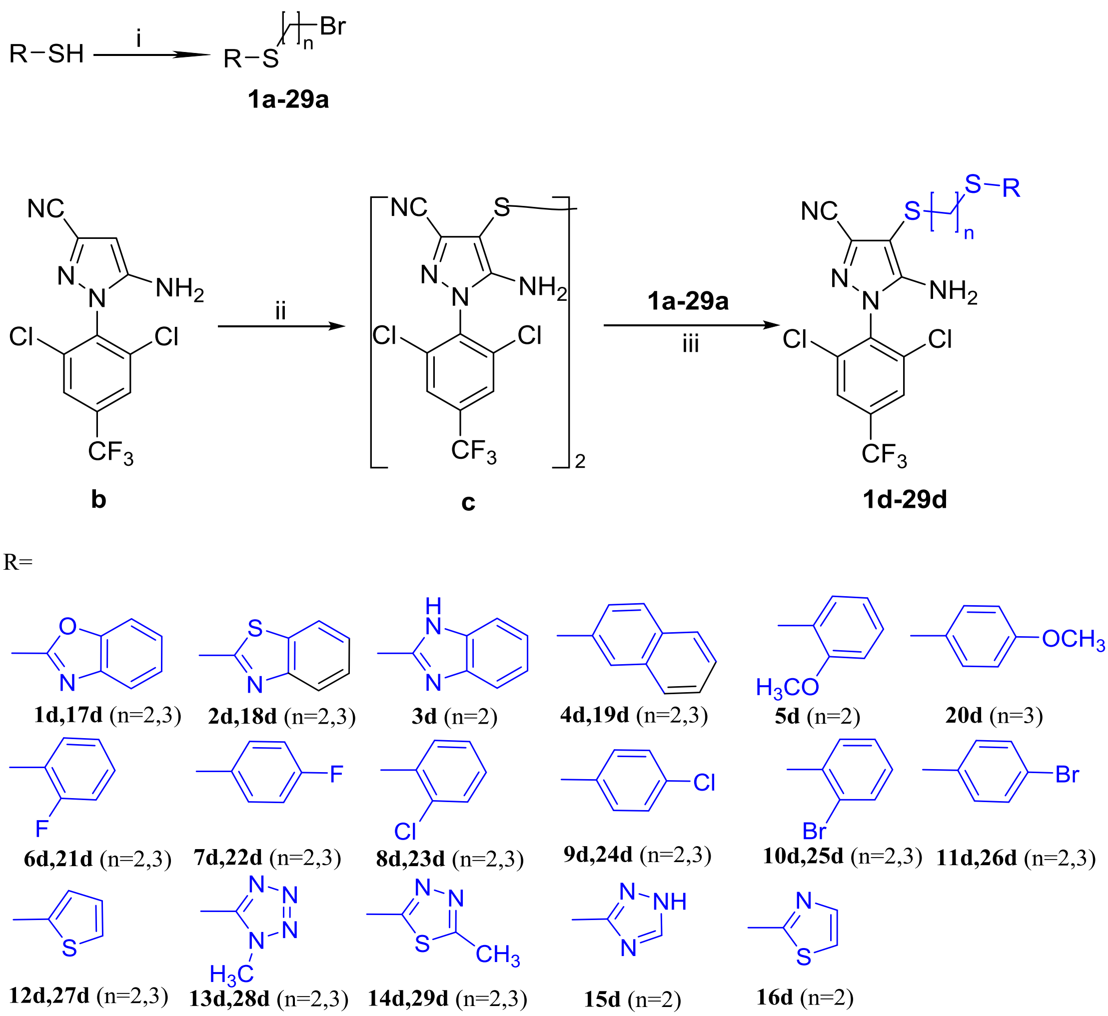
The structures of the synthetic compounds were confirmed by melting points, ^{1}H NMR, ^{13}C NMR and the structures of the title compounds **1d**–**29d** were confirmed by HRMS spectroscopic data additionally.

The preliminary insecticidal activities of compounds **1d**–**29d** were assessed against *Musca domestica* L. by the artificial diet dipping methods as the final mortality rates at 20 μg/g and fipronil as positive controls. The mortalities of *Musca domestica* L. were shown in Tables 1 and 2. Among all the tested compounds, compounds **12d**, **13d**, **14d**, **16d** and fipronil showed potent insecticidal activity (96.67%–100%); compounds **c**, **2d**, **3d**, **6d**, **7d** and **29d** showed moderate insecticidal activity (40.00%–53.33%). Insecticidal activities of other compounds were very low (<36.67%).

To gain further insight into potent toxicity of these compounds, fipronil and the ten compounds **2d**, **3d**, **6d**, **7d**, **12d**, **13d**, **14d**, **16d**, **29d** and **c** were investigated further at serial concentration gradient to determine their LC_{50}s (Table 3). The bioactivities of compounds **12d**, **14d** and **16d** being equipotent to fipronil against *Musca domestica* L. The LC_{50} values of fipronil and compounds **12d**, **14d** and **16d** were 0.68, 0.67, 0.90 and 1.30 μg/g, respectively. Comparable to fipronil, compound **13d** dramatically reduced the insecticidal activity (LC_{50} = 6.00 μg/g). Compounds **2d**, **3d**, **6d**, **7d**, **29d** and **c** displayed inferior activity as well, their LC_{50}s were 22.79, 22.47, 13.70, 25.47, 22.00 and 19.07 μg/g, respectively.

Initially, in order to find the optimal aromatic substitution, a series of thioether *N*-phenylpyrazole derivatives substituted with naphthalene, 2-methoxybenzene, 4-methoxybenzene, 2-fluorobenzene, 4-fluorobenzene, 2-chlorobenzene, 4-chlorobenzene, 2-bromobenzene, 4-bromobenzene were prepared and evaluated. Insecticidal activity data showed that derivatives containing the short thioether bridge, 1,2-bis((hetero)aromatic thio) ethane, far better than that of the long thioether bridge, 1,3-bis((hetero)aromatic thio) propane (**4d**–**11d** vs **19d**–**26d**). In particular, fluoro substituted benzene derivatives **6d** and **7d** possessed the most potent bioactivities, with the LC_{50}s of 13.70 μg/g and 25.47 μg/g, respectively. Inspired by the potent activity possessed by the fluoro substituted benzene derivatives, further research on heteroaromatic motif with strong lipophilicity and charge density were carried out.

Based on a rationally conceived pharmacophore model to increase the lipophilicity and charge density of aromatic substituents, nitrogen, oxygen, sulfur-containing heterocycle substituents were introduced to explore the SAR (Tables 2 and 3). The target compounds of nitrogen, oxygen-containing heterocycle substituents (except compound **13d**) showed much less potency compared to fipronil against *Musca domestica* L. The target compounds of sulfur-containing heterocycle substituents displayed



Scheme 1. Synthesis of derivatives **1d–29d**. Reagents and conditions: (i) $Br(CH_2)_nBr$, K_2CO_3, KI, acetone, rt, 5 h; (ii) S_2Cl_2, CH_2Cl_2, 0 °C-rt, 2 h; (iii) Na_2HPO_4, $Na_2S_2O_4$, DMF, H_2O, rt, 3 h.

enhanced insecticidal activity. Compounds **12d**, **14d** and **16d** were the most active compounds in this series, being equipotent to fipronil. However, compound **2d** containing sulfur-heterocycle substituent was much less active, but compound **13d** containing

Table 1
24-h effect of *N*-phenylpyrazole derivatives containing various substituted benzene substituents against *Musca domestica* L.

Compound	Mortality (%)*
4d	36.67 ± 1.20 d**
5d	33.33 ± 0.33 d
6d	43.33 ± 0.88 c
7d	43.33 ± 0.67 c
8d	6.67 ± 0.33 f
9d	33.33 ± 0.67 d
10d	26.67 ± 1.20 e
11d	36.67 ± 0.33 d
19d	3.33 ± 0.33 g
20d	0.00 ± 0.00 e
21d	3.33 ± 0.33
22d	3.33 ± 0.33 g
23d	6.67 ± 0.67 f
24d	6.67 ± 0.67 f
25d	0.00 ± 0.00 e
26d	0.00 ± 0.00 e
c	53.33 ± 0.88 b
Fipronil	100.00 ± 0.00 a
ck	0.00 ± 0.00 e

* Test concentration is 20 μg/g.
** Data followed by the same letter in a column are not significantly different at 5% level by DMRT.

Table 2
24-h effect of *N*-phenylpyrazole derivatives containing heteroaromatic substituents against *Musca domestica* L.

Compound	Mortality (%)*
1d	36.67 ± 0.33 d**
2d	40.00 ± 1.00 c
3d	46.67 ± 0.88 b
4d	36.67 ± 1.20 d
12d	100.00 ± 0.00 a
13d	100.00 ± 0.00 a
14d	96.67 ± 0.33 a
15d	33.33 ± 0.67 e
16d	100.00 ± 0.00 a
17d	3.33 ± 0.33 h
18d	10.00 ± 0.00 g
27d	30.00 ± 2.08 e
28d	20.00 ± 0.58 f
29d	46.67 ± 0.67 b
Fipronil	100.00 ± 0.00 a
ck	0.00 ± 0.00 i

* Test concentration is 20 μg/g.
** Data followed by the same letter in a column are not significantly different at 5% level by DMRT.

Table 3
The LC_{50} of thioether bridged N-phenylpyrazole derivatives against *Musca domestica* L. after 24-h.

Compound	Linear regression equation (y = a + bx)	Correlation coefficient (r)	LC_{50}* (μg/g)	95% CI** (μg/g)
2d	y = 1.2431 + 2.7672x	0.9997	22.79	18.32–25.17
3d	y = 2.3534 + 1.9581x	0.9913	22.47	15.48–32.63
6d	y = 3.5069 + 1.3135x	0.9930	13.70	8.03–23.37
7d	y = 1.3087 + 2.6253 x	0.9988	25.47	14.62–44.39
12d	y = 5.3067 + 1.7778 x	0.9880	0.67	0.24–1.88
13d	y = 2.3026 + 3.4669 x	0.9994	6.00	4.51–7.98
14d	y = 5.0747 + 1.7078 x	0.9990	0.90	0.40–2.06
16d	y = 4.7396 + 2.2676 x	0.9941	1.30	0.83–2.05
29d	y = 1.2850 + 2.7672 x	0.9940	22.00	13.89–34.97
c	y = 1.1000 + 3.0461 x	0.9930	19.07	13.23–27.48
Fipronil	y = 5.2932 + 1.7501 x	0.9945	0.68	0.48–0.97

* LC_{50}: concentration required to kill 50%.
** CI: confidence interval.

nitrogen-heterocycle substituent showed good insecticidal activity.

Similar trends were observed with the thioether N-phenylpyrazole derivatives of aromatic substituents (Tables 2 and 3). In general, the N-phenylpyrazole derivatives of sulfur-containing heterocycle substituents containing the short thioether bridge displayed excellently insecticidal activity (Tables 2 and 3). In particular, compounds **12d**, **14d** and **16d** bearing a thiophene, 5-methyl-1,3,4-thiadiazole, thiazole substituent respectively, were equivalent to or better than that of fipronil against *Musca domestica* L. Compound **12d** and **14d** containing the short thioether bridge, bearing a thiophene and 5-methyl-1,3,4-thiadiazole substituent respectively, showed a 2- to 3-fold higher activity compared to its long thioether bridge derivatives (**12d** vs **27d**; **14d** vs **29d**). As a similar result, compound **13d** containing the short thioether bridge showed a 5-fold higher activity compared to compound **28d** containing long thioether bridge derivative.

To understand the interaction between compound **12d**, **14d** and the amino acid residues in the acting sites of *M. domestica* GABA receptor, ligand-docking studies were performed using an *M. domestica* GABA receptor homology model. The model was built using the X-ray crystal structure of pentameric ligand-gated ion channel of *Gloeobacter violaceus* as a template. The *M. domestica* sequence similarity in M1-4 regions aligned with *Gloeobacter violaceus* was 44.1%. Compound **12d**, **14d** and fipronil were docked into the pore formed by M2s with the predicted closed conformation model (Fig. 2). Compounds docked with the inter-subunit cavity of *M. domestica* GABA receptor homology model in the closed conformation with the best scores (Fig. 3), indicating compounds potentially fit to the inter-subunit cavity.[27] Thus, these are possible to hypothesize that it would bind and stabilize a closed state of the receptor.

The results showed that compound **12d**, **14d** and fipronil are well embedded in the binding site. Compound **12d**, **14d** and fipronil are capable of non-covalent interactions with residues A299, T303, and L306, which are equivalent to A302, T306 and L309 in *Drosophila* GABA receptor subunit repectively[25], to stabilize the complexes (Fig. 4A–C). The newly introduced thioether moiety and sulfur-containing heterocycles of compound **12d** and **14d** would be extend and form hydrophobic interactions with the deeper residue P295 (Fig. 4B and C).

When the length of the spacer arm introduced between N-phenylpyrazole moiety and the (hetero)aromatic substituents equal to two carbons could form a new hydrophobic interaction between sulfur-containing heterocycles and the residue P295. However, the length of the spacer arm more than two carbons, the hydrophobic interaction would disappear. At this point, it could be convinced by the docking results of fipronil, compounds **12d** and **14d** (Fig. 4A–C). The docking mode of compound **12d**

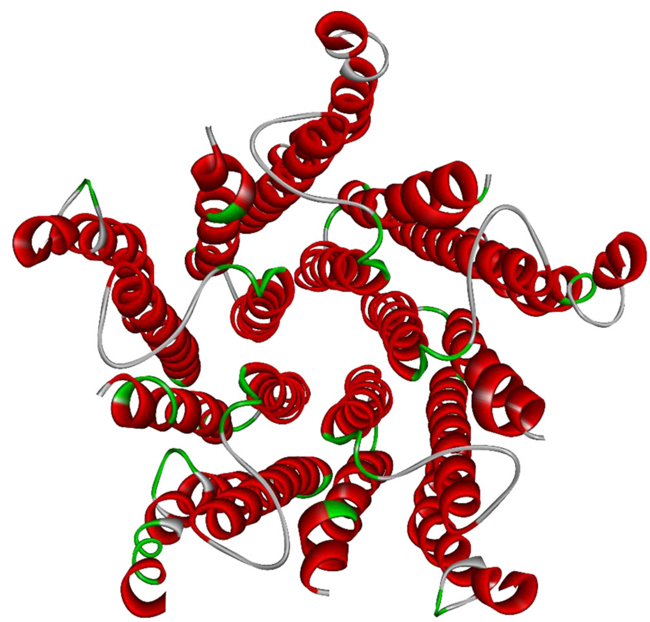


Fig. 2. Top view of the homology model of *Musca domestica* GABA receptor subunit homomer in closed state without the N-terminal and C-terminal extracellular domains.

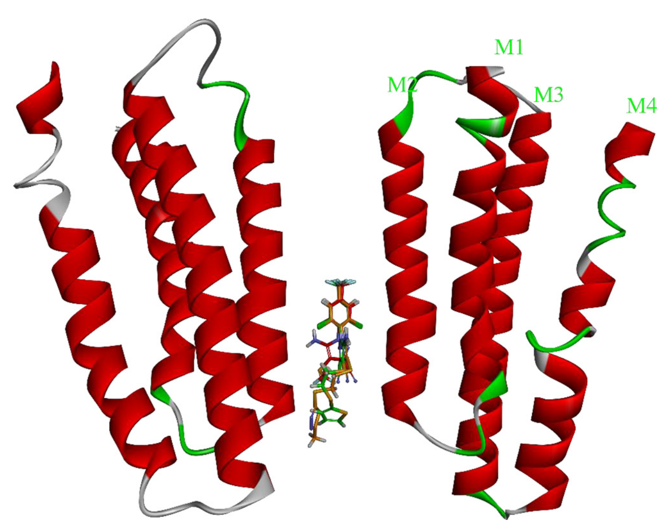


Fig. 3. Three superimposed best scoring docking poses for compound **12d**, **14d** and the control fipronil in the internal pocket as a green, yellow and red carbon stick model, respectively.

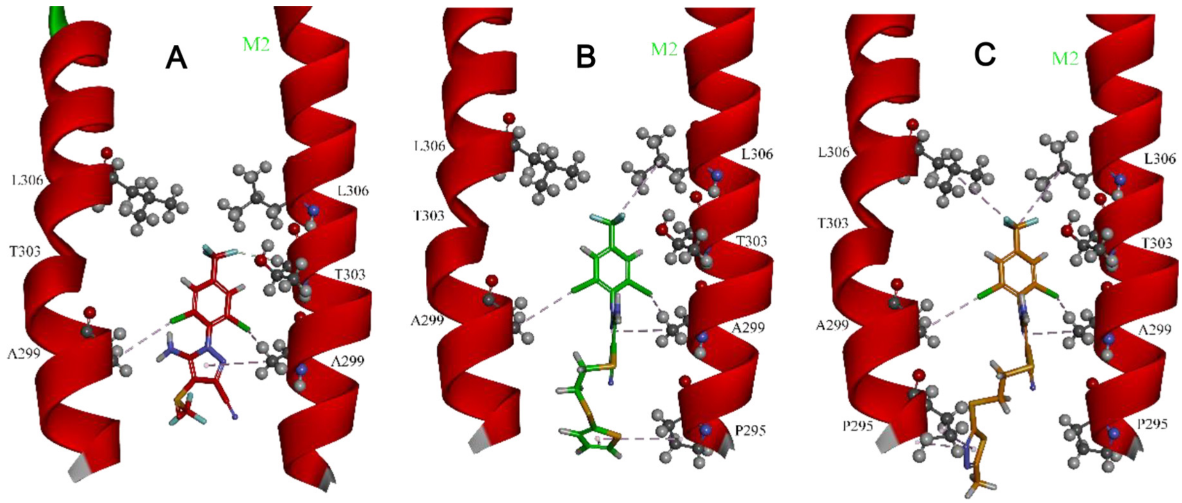


Fig. 4. Binding modes of *Musca domestica* M2 regions in complex with fipronil (A), **12d** (B) and **14d** (C) as a red, green, and yellow carbon stick model respectively. The closed conformation are rendered as red ribbon models. The amino acid residues interacted with compound are shown as ball and stick models, and the non-covalent interactions are presented as the dashed line.

exhibited the lowest CDOCKER energy of 1.95435 kcal mol^{-1}. Meanwhile, compare with fipronil (3.10111 kcal mol^{-1}), compound 14d displayed the CDOCKER energy of 10.7804 kcal mol^{-1}. According to Fig. 3, compound **12d** and **14d** were still located in the vicinity of the residue P295. The hydrophobic interaction with P295 might be the main reason for the high activity of compound **12d** and **14d** (Fig. 4B and C).

In summary, a series of novel thioether *N*-phenylpyrazole derivatives incorporating various (hetero)aromatic substituents into 4-position of the pyrazole ring have been reported. SAR studies have shown that the insecticidal activity of thioether *N*-phenylpyrazole derivatives substituted with heteroaromatic substituents into 4-position of the pyrazole ring was higher than that of various substituted benzene substituents, and containing the short thioether bridge, 1,2-bis((hetero)aromatic thio) ethane, far better than that of the long thioether bridge, 1,3-bis((hetero)aromatic thio) propane against *Musca domestica* L. Molecular docking analyses allowed us to speculate the binding mechanism of thioether-bridged arylalkyl-linked *N*-phenylpyrazole derivatives and the sulfur-containing heterocycle substituents of the ligands were the sterically favored areas, which mainly formed hydrophobic interactions with the residue P295. Among the derivatives prepared, thioether *N*-phenylpyrazole derivatives substituted with sulfur-containing heterocycle substituents into 4-position of the pyrazole ring leads to excellent insecticidal activity. This indicates that further investigation is need to improve the lipophilicity and charge density of the heteroaromatic substituents into 4-position of the pyrazole ring. The discovery of compound **12d** and the SAR and the insecticidal activity studies reported here will provide insight into further optimization of thioether *N*-phenylpyrazole derivatives for the prevention and treatment of hygienic and agricultural pest insect.

Acknowledgments

The authors are grateful for financial support from the National Natural Science Foundation of China (No. 31171871), the Science and Technology Planning Project of Guangdong Province (2016A020210082), the Science and Technology Planning Project of Guangzhou (201607010181), and the Science and Technology Program of Zhongshan, China (2016F2FC0016).

A. Supplementary data

Supplementary data (synthetic procedure, ^{1}H and ^{13}C NMR, HRMS spectra and biological assay method and molecular docking scheme) associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bmcl.2018.04.022.

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