Journal Pre-proofs

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PII: S0960-894X(19)30931-X
DOI: https://doi.org/10.1016/j.bmcl.2019.126945
Reference: BMCL 126945

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 16 November 2019
Revised Date: 21 December 2019
Accepted Date: 28 December 2019

Please cite this article as: Li, M., Zan, N., Huang, M., Jiang, D., Hu, D., Song, B., Design, Synthesis and Anti-TMV Activities of Novel Chromone Derivatives Containing Dithioacetal Moiety, *Bioorganic & Medicinal Chemistry Letters* (2019), doi: https://doi.org/10.1016/j.bmcl.2019.126945

Design, Synthesis and Anti-TMV Activities of Novel Chromone Derivatives Containing Dithioacetal Moiety

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ABSTRACT: Thirty-five novel chromone derivatives containing dithioacetal moiety were designed, synthesized, and their anti-TMV activities were evaluated through half-leaf method. The results showed compound **c23** illustrates highly curative, protective and inactivating activities against TMV at 500 mg/L, with the values of 68.8%, 58.8%, 86.0% respectively, which were superior to that of Ribavirin (42.3%, 49.8%, 68.4%, respectively) and similar to that of Ningnanmycin (59.4%, 52.4%, 88.4%, respectively). The EC_{50} value of inactivating activities of compound **c23** is 9.3 mg/L, which was better than that of Ribavirin (135.2 mg/L), and equivalent to that of Ningnanmycin (8.8 mg/L). Furthermore, compound **c23** can destroy the integrity of TMV-CP, resulting in reduced infectivity of TMV. Meanwhile, compound **c23** can combine with TMV protein coat and hydrolyze TMV protein coat to impact the process of self-assembling of TMV, with the association constant (K_d) 4.5 mg/L. This finding suggests that chromone derivatives containing dithioacetal moiety can be used as new antiviral agent.

KEYSWORDS: chromone derivatives, dithioacetal moiety, anti-TMV activities, mechanism

Plant virus disease can cause seriously damage to global agricultural industry. Tobacco mosaic virus (TMV) as a paradigm in virology can infect more than 400 species of plants belonging to 36 families[1-3], which causes huge economic losses and food security pressures[4]. Ningnanmycin and Ribavirin are the most widely used antiviral agents, while there are disadvantages of them. For example, Ningnanmycin shows 52.4% curative effect at 500 mg/L in vivo but lose activity in the field. Ribavirin as a plant viral inhibitor, its antiviral activities are poor (inhibitory activity is lower than 50.0% at 500 mg/L in vivo). In fact, there is no super chemical pesticide that can completely inhibit TMV including Ningnanmycin and Ribavirin[5]. Hence the controlling of TMV may open the way to effective treatment of phytoviruses.

Natural products are extremely important resources of drug development. Chromones are benzoannelated γ-pyrone heterocyclic compounds that widely exists in nature, especially in plants. Chromone has attracted a lot of interest from researcher in recent years because of its extensive biological activity, such as anti-inflammatory, antimicrobial, antitumor, antidiabetic and antioxidant[6-9]. Many chromone derivatives were reported to possess superior anti-TMV activity. However, they are generally natural source products that are difficult to be commercialized[10-14](Fig. 1). Until now, simple chromones have been an up-and-coming area of exploration because of their interesting biological activities or from a synthetic point of view. In addition, many antivirus mechanism studies of chromone derivatives are about the interaction between chromone derivatives and human viral protein but seldomly involve in plant

virus[15]. Therefore, the mechanism of anti-TMV action of chromone derivatives needs further investigation.

Our group reported that dithioacetal has anti-plant virus activities for the first time, finding xiangcaoliusuobingmi had brilliant anti-plant virus activities [16]. And we found that the dithioacetal derivatives **C14** and **C24** (Fig. 2) with antiviral activities against TMV were synthesized[17,18], however, the inhibitory activities of the compounds were limited for development of new antiviral agent. Considering that chromone and dithioacetal group both have potential anti-TMV activity, we intend to combine chromone with dithioacetal (Fig. 2) by using active compound derivatization method to obtain a series of target compounds **c1-c35** (Fig. 3). We systematically evaluated their anti-TMV activities and studied its anti-TMV mechanism.

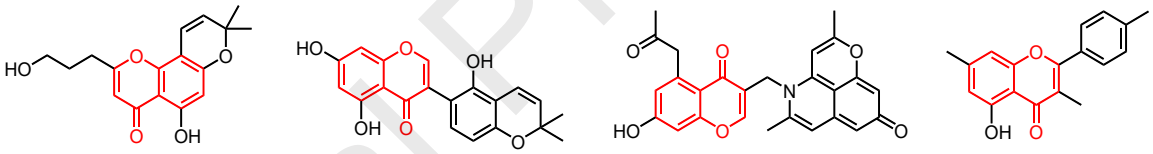


Fig. 1. Antitobacco mosaic virus agents from natural sources.

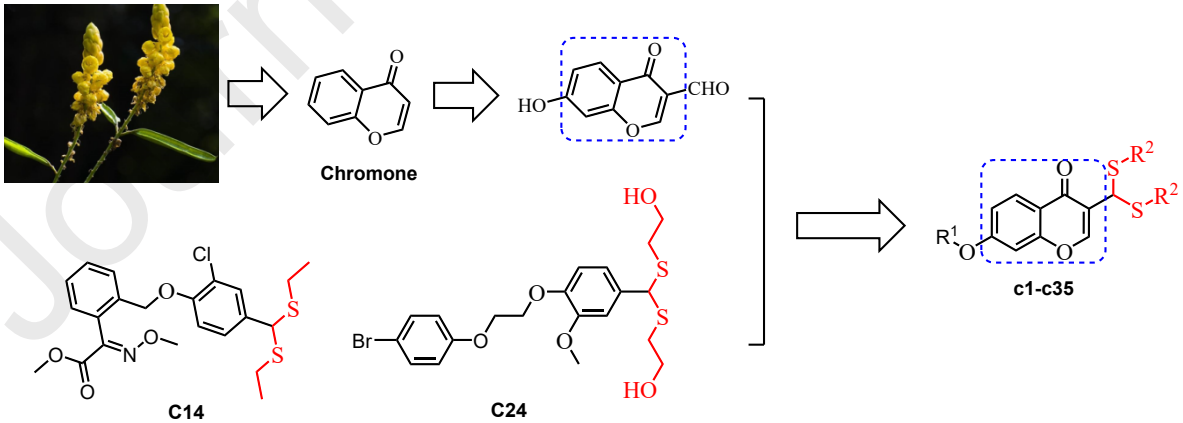
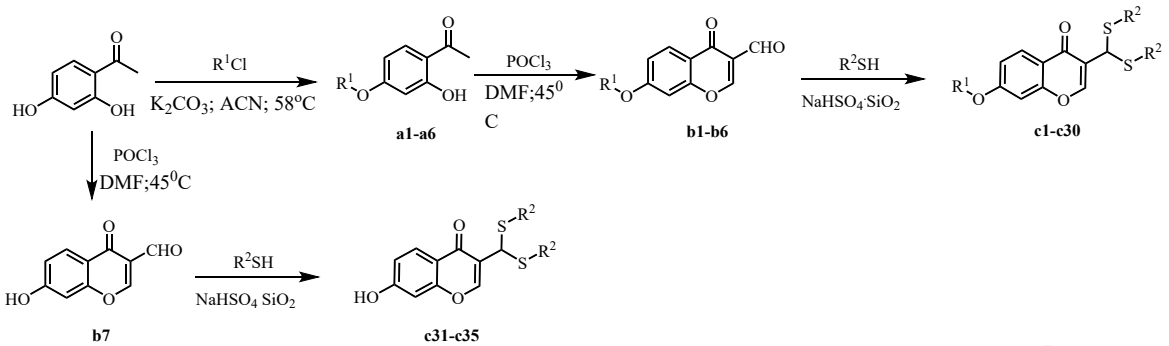


Fig. 2. Design of the target compounds



c1: R^1= 4-Chlrobenzyl; R^2= CH_2CH_3;
c2: R^1= 4-Chlrobenzyl; R^2= $CH(CH_3)_2$;
c3: R^1= 4-Chlrobenzyl; R^2= $CH_2CH_2CH_3$;
c4: R^1= 4-Chlrobenzyl; R^2= $C(CH_3)_3$;
c5: R^1= 4-Chlrobenzyl; R^2= $CH_2CH_2CH_2CH_2CH_3$;
c6: R^1= Benzyl; R^2= CH_2CH_3;
c7: R^1= Benzyl; R^2= $CH(CH_3)_2$;
c8: R^1= Benzyl; R^2= $CH_2CH_2CH_3$;
c9: R^1= Benzyl; R^2= $C(CH_3)_3$;
c10: R^1= Benzyl; R^2= $CH_2CH_2CH_2CH_2CH_3$
c11: R^1= 4-Methylbenzyl; R^2= CH_2CH_3;
c12: R^1= 4-Methylbenzyl; R^2= $CH(CH_3)_2$;
c13: R^1= 4-Methylbenzyl; R^2= $CH_2CH_2CH_3$;
c14: R^1= 4-Methylbenzyl; R^2= $C(CH_3)_3$;
c15: R^1= 4-Methylbenzyl; R^2= $CH_2CH_2CH_2CH_2CH_3$;
c16: R^1= 2-Chlrobenzyl; R^2= CH_2CH_3;
c17: R^1= 2-Chlrobenzyl; R^2= $CH(CH_3)_2$;
c18: R^1= 2-Chlrobenzyl; R^2= $CH_2CH_2CH_3$;
c19: R^1= 2-Chlrobenzyl; R^2= $C(CH_3)_3$;
c20: R^1= 2-Chlrobenzyl; R^2= $CH_2CH_2CH_2CH_2CH_3$;
c21: R^1= 4-Fluorobenzyl; R^2= CH_2CH_3;
c22: R^1= 4-Fluorobenzyl; R^2= $CH(CH_3)_2$;
c23: R^1= 4-Fluorobenzyl; R^2= $CH_2CH_2CH_3$;
c24: R^1= 4-Fluorobenzyl; R^2= $C(CH_3)_3$;
c25: R^1= 4-Fluorobenzyl; R^2= $CH_2CH_2CH_2CH_2CH_3$;
c26: R^1= 4-Bromobenzyl; R^2= CH_2CH_3;
c27: R^1= 4-Bromobenzyl; R^2= $CH(CH_3)_2$;
c28: R^1= 4-Bromobenzyl; R^2= $CH_2CH_2CH_3$;
c29: R^1= 4-Bromobenzyl; R^2= $C(CH_3)_3$;
c30: R^1= 4-Bromobenzyl; R^2= $CH_2CH_2CH_2CH_2CH_3$;
c31: R^2= CH_2CH_3;
c32: R^2= $CH(CH_3)_2$;
c33: R^2= $CH_2CH_2CH_3$;
c34: R^2= $C(CH_3)_3$;
c35: R^2= $CH_2CH_2CH_2CH_2CH_3$;

Fig. 3. The synthesis route of target compound **c1-c35**.

The activity of the target compounds towards TMV is listed in Table 1. As we see, most of target compounds show better inhibitory activity (activities mentioned in table.1) than Ribavirin (49.8%, 42.3%, 68.4%). Besides, the EC_{50} values of inactivating activities of some compounds were superior to that of Ribavirin (135.2 mg/L). Furthermore, the protective effect of **c1**, **c6**, **c14**, **c23** and **c30** (60.2%, 63.3%, 61.7%, 68.8% and 61.5%, respectively) were better than that of Ningnanmycin (59.4%). The curative effect of **c7**, **c8**, **c10**, **c17**, **c19**, **c23**, **c27**, **c33**, **c35** (55.2%, 58.3%, 56.4%, 63.4%, 56.2%, 57.9%, 58.8%, 60.7% and 53.2% respectively) were better than that of Ningnanmycin (52.4%). The inactivating effect of **c6**, **c23**, **c24** and **c34** (83.8%, 86.0%, 80.0% and 86.9%, respectively) were equivalent to that of Ningnanmycin (88.4%). The EC_{50} of inactivating activities of **c23** (9.3 mg/L) was equivalent to that of Ningnanmycin (8.8 mg/L).

79 **Table 1.** Antiviral activities of target compounds **c1**-**c35** against TMV *in vivo*.

Compd.	protective activity[a]（%）	curative activity[a]（%）	inactivating activity[a]（%）	EC_{50} for TMV inactivating activity[a]（mg/L）
c1	60.2±6.6	51.5±3.7	64.6±6.1	161.4±2.1
c2	23.1±3.4	50.6±2.8	53.4±4.2	317.8±1.5
c3	45.8±3.3	43.5±4.7	76.2±3.9	79.5±2.9
c4	42.7±5.5	44.0±6.6	57.1±4.8	243.8±2.1
c5	38.6±1.4	43.5±4.3	70.1±4.9	115.0±2.5
c6	63.3±4.9	49.3±4.8	83.8±3.5	26.1±4.1
c7	43.4±1.4	55.2±3.1	68.3±6.1	137.3±3.5
c8	49.2±4.3	58.3±5.6	55.7±3.7	198.6±2.8
c9	37.9±1.1	37.4±6.7	55.5±4.3	290.7±1.8
c10	58.3±0.5	56.4±2.4	62.0±2.6	180.2±2.1
c11	47.5±0.2	52.0±2.0	58.4±2.8	226.3±1.5
c12	40.3±6.3	63.4±2.6	61.9±0.9	183.1±2.3
c13	33.8±3.9	38.0±4.7	60.9±3.5	200.4±1.4
c14	61.7±2.9	34.3±6.1	63.9±3.1	168.2±2.5
c15	39.4±5.3	47.6±5.9	58.6±5.5	223.3±2.3
c16	43.4±5.3	50.1±5.1	61.9±4.1	188.8±2.0
c17	42.4±0.9	56.2±3.7	78.1±4.3	66.2±2.9
c18	38.9±2.2	31.5±6.0	50.4±1.1	373.1±2.5
c19	39.9±3.5	57.9±2.6	64.9±4.3	166.6±2.4
c20	39.4±5.3	45.5±4.0	62.0±4.2	183.2±2.7
c21	58.1±4.9	42.0±4.7	53.2±3.8	315.7±2.2
c22	39.4±5.3	46.2±0.9	59.0±2.4	218.4±2.2
c23	68.8±3.7	58.8±2.1	86.0±0.8	9.3±3.9
c24	46.1±3.4	27.5±4.4	80.0±1.4	54.3±5.5
c25	47.1±6.5	54.7±3.9	78.4±3.3	65.6±2.7
c26	47.3±5.4	50.8±2.0	57.4±5.7	238.3±3.0
c27	57.1±5.2	52.8±4.8	52.2±1.5	338.4±1.4
c28	55.0±4.1	35.9±2.6	67.1±5.4	145.7±2.8
c29	47.1±4.8	52.8±1.1	69.6±5.4	128.4±2.3
c30	61.5±1.5	38.1±4.8	58.4±4.0	225.8±3.2
c31	43.6±4.1	39.8±5.2	64.6±4.0	170.6±2.5
c32	45.7±4.3	47.8±5.0	73.0±3.3	102.7±1.8
c33	30.6±6.9	60.7±3.0	75.1±3.9	88.4±2.6
c34	20.3±3.5	41.7±5.5	86.9±2.0	11.0±3.0
c35	43.3±4.1	53.2±4.7	70.0±1.0	120.4±3.2
Ribavirin[b]	42.3±2.3	49.8±3.2	68.4±1.4	135.2±1.3
Ningnanmycin[b]	59.4±5.8	52.4±3.9	88.4±2.5	8.8±2.9

80 Footnotes: [a] average of three replicates; [b]Ningnanmycin and Ribavirin was used as the control.

The TEM image was gained by using FEI Talors F200C made by America. We can see the granular morphology of TMV (Fig. 4A) is straight, well-proportioned, rod-shaped and some of them are array regularly. The treated TMV particles interact with compound **c23** for 30 min (Fig. 4D). The morphology of TMV granular became smaller, shorter and arrayed in messy. TMV particles interact with negative control Ningnanmycin for 30 min (Fig. 4B). The change of morphology of TMV granular was just as same as the change mentioned above. TMV particles were broken after interacting with Ribavirin (Fig. 4C), but the fracture of TMV particles is not serious to **c23** and Ningnanmycin. The change of morphology of TMV was possibly due to the ability of small molecule which can crush the interaction between coat protein subunits. Resulting in breaking viral particle, loosing structure and disordered arrangement.

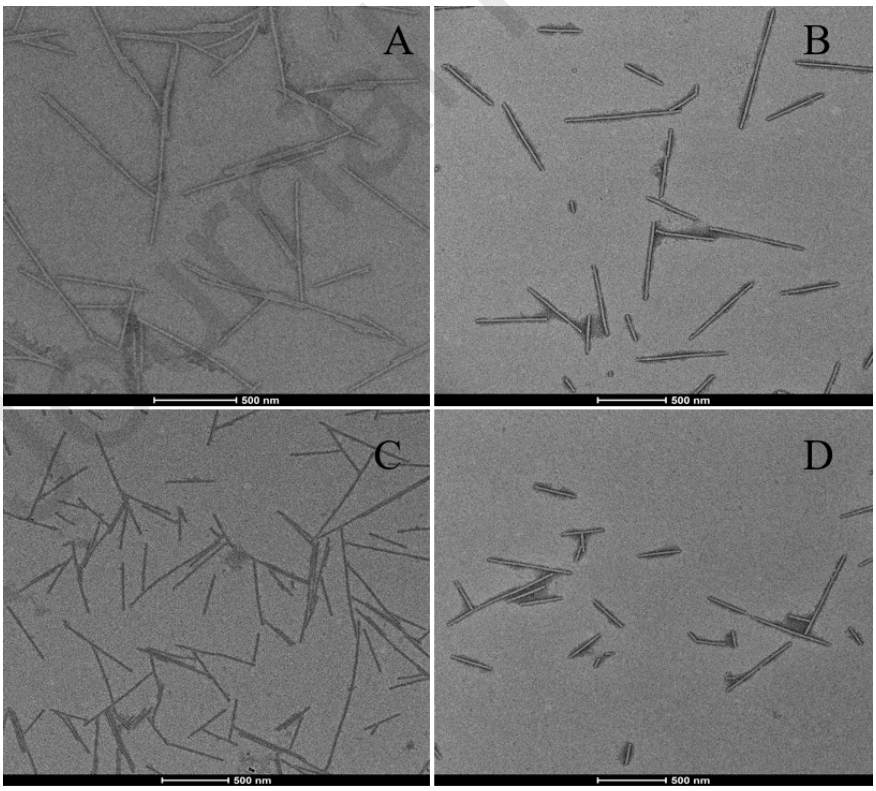


Fig. 4. The effect on the morphology of TMV particles of compounds **CK** (A), **Ningnanmycin** +

TMV(B), **Ribavirin**+ TMV (C), **c23**+ TMV (D).

Molecular docking studies (Fig. 5) for compound **c23** with TMV-CP (PDB code:1EI7) have shown that the compounds had the ability of embedding between the two subunits of TMV-CP[19] (ASP219, LYS253, GLU222, VAL251, LYS268, PRO254, VAL75, SER138, TYR139). As we know, there are 2130 coat protein subunits in TMV structure. These subunits contact each other by the forming non-covalent. For this kind of special bonding model multiple replication finally resulted in TMV's symmetrical structure[20]. Therefore, coat protein subunits played a critical role in self-assembly of TMV. As we see, the result shown in molecular docking on the software of Discovery Studio 4.5. ASP219 showed strong hydrogen bond interaction with compound **c23**. Following is LYS253, also showed hydrogen bond with **c23**. Others coat protein subunits can bind with **c23** through a variety of ways as well. The interaction of these two subunits of TMV-CP was probably weaken by the interaction between small molecular and TMV-CP leading to the TMV particle would be unable to self-assembly. The result of Molecular docking studies of compound **c23** with TMV-CP suggests that compound **c23** can influence the process of replication of TMV. This influence may be the reason that compound **c23** possess excellent antiviral activity.

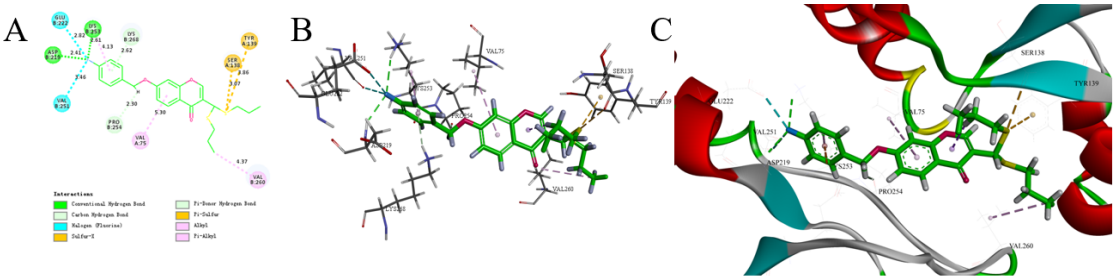


Fig. 5. Molecule docking results of compounds **c23** (A-C).

To study the combine capacity of the target compound to TMV-CP, the binding constant (K_d) was performed via MST and the results were listed in Table. 2 and Fig. 6. The results evidence that **c23** which showed excellent activity towards TMV in vivo also showed excellent activity towards TMV in vitro. The K_d = 4.5 ± 1.3 μM. The K_d of compound **c23** is similar to Ningnanmycin (K_d= 4.9± 2.2 μM). Ribavirin showed moderate bonding ability with TMV-CP. The K_d = 134.9±54.5 μM. The activity in vitro results as determined by MST are consistent with the activity in vivo.

Table 2 Interaction results of the commercial antiviral agents and **c23** with TMV CP

Compd.	K_d [a] (μM)	EC_{50} for TMV inactivating activity [a] (mg/L)
c23	4.5±1.3	9.3±3.9
Ribavirin[b]	134.9±54.5	135.2±1.3
Ningnanmycin[b]	4.9±2.2	8.8±2.9

Footnotes: [a]average of three replicates; [b]Ningnanmycin and Ribavirin was used as the control.

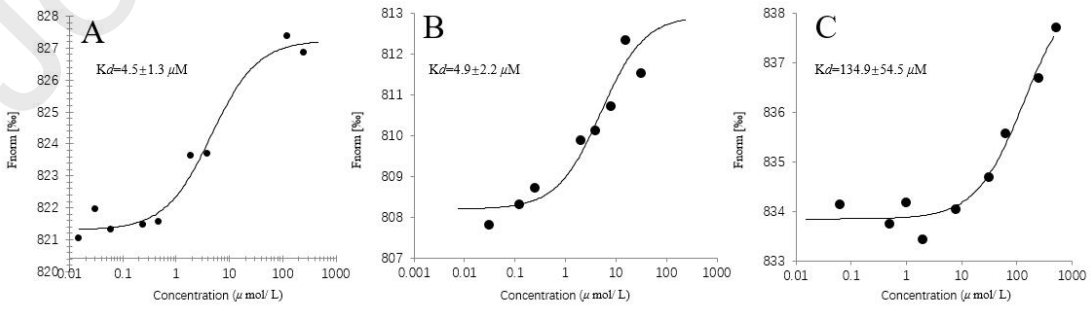


Fig. 6 Microscale thermophoresis (MST) test results of compound **c23** (A), **Ribavirin** (B),

Ningnanmycin (C).

In this study, a series of chromone derivatives containing dithioacetal moiety were designed and synthesized. The bioassay indicated that the great majority of target compounds show good anti-TMV activities at 500 mg/L in vivo. Among them, compound **c23** shows excellent inactivating activities to TMV, with EC_{50} value of 9.3 mg/L. therefore, **c23** was selected as a new lead for further mechanism studies. Compound **c23** can bind with coat protein subunits (ASP219 and LYS253) in a strong hydrogen bonding mode by molecular docking software simulating. This bonding destroys the integrity of TMV and leads it lose its infectivity. The binding constant K_d was obtained in MST, with the value of 4.5μM. Besides, the granular morphology of TMV was suffered substantial fracture after reacting with compound c23 under TEM observation. The results suggest that Chromone derivatives containing dithioacetal were important substructure for the novel pesticide development. This finding could be important foundation and basis for the research of antiviral agents.

Acknowledgement

This work has received the financial support of the National Natural Science Foundation of China (No. 21732002).

Supporting Information

The synthesis, physical analysis, ^{1}H NMR spectra, ^{13}C NMR spectra and high resolution mass spectrum (HRMS) of compounds **c1-c35** can be found in the supplementary data.

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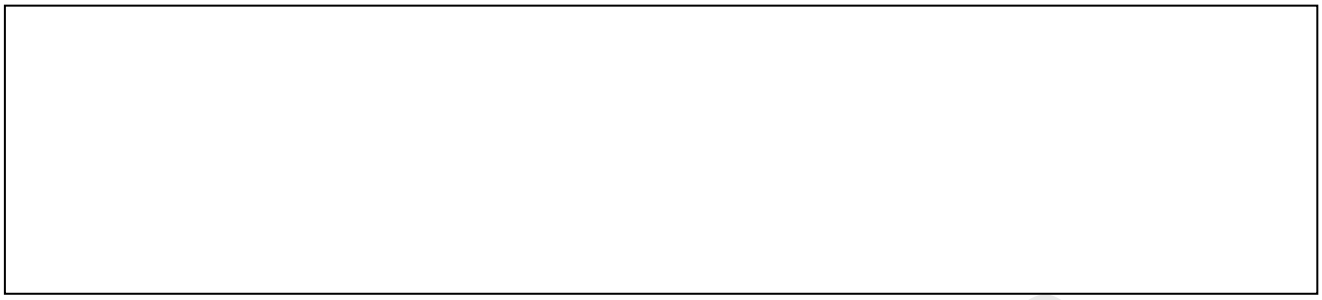
1. Thirty-five novel chromone derivatives containing dithioacetal moiety were designed and synthesized.
2. Novel chromone derivative **c23** with excellent anti-TMV was synthesized.
3. The mechanism to TMV of chromone derivatives were reported for the first time.

[21]

Declaration of interests

☑ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:



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