Synthesis and biological activity of the novel indanedione anticoagulant rodenticides containing fluorine

Feng Chen, Liping Liu, Zengguo Bai, Tianhua Zhang, and Keke Zhao

Department of Chemistry, Northeastern University, Shenyang City, People’s Republic of China

ABSTRACT
Here, 3 fluorinated intermediates of drug were synthesized: (M1), (M2), (M3). Three new anticoagulant rodenticides were designed which were based on 4-hydroxycoumarin or 1,3-indandione, added acute toxicity groups containing fluorine. The structures of synthesized compounds were analyzed and proved by FT-IR spectroscopy and $^1$H nuclear magnetic resonance ($^1$H-NMR). The compounds were also evaluated for their anticoagulant and acute biologic activity. In addition, both the acute orally toxicity and the feeding indexes of R1 and R2 were tested. The result of the experiment proved that the new synthesis of 1, 3 - indan diketone for maternal new anticoagulant rodenticide can replace the current 4 - hydroxyl coumarin as the mother of the second generation anticoagulant rodenticide and 1, 3 - indan diketone for maternal new anticoagulant rodenticides will have a good development prospect.

KEYWORDS
One-bromo-2-fluoroethane; 1,3-indandione; 4-hydroxycoumarin; anticoagulant rodenticid; chalcone; fluoroacetylchloride

Introduction
Nowadays, anticoagulant rodenticides (ARs) are commonly used in many parts of the world for the control of pest rodents for plant protection and environmental governance in both rural and urban environments.1 Second-generation anticoagulant rodenticides are more acutely toxic than first-generation compounds and capable of killing the target rodent after a single feeding.2-6 However, the second-generation anticoagulant rodenticides are relatively expensive.7 At present, the 1, 3 - indan anti-coagulant rodenticide is reported little, and because the price of 4 - hydroxyl coumarin is obviously higher than that of phthalate esters, the synthesis of a new type of rodenticide replacing the current of the second generation anticoagulant rodenticide will greatly save the cost.8 And the cycle of killing rats for the second-generation anticoagulant rodenticides last for a long time and the average death time of rats was about after 7 d.9-11 So shortening the time of killing rats is the focus of our research. The toxicity groups of ethanol fluoroacetic acid sodium, fluorine acetamide, fluorine were introduced into the molecular structure of the chronic anticoagulant rodenticides to make new material structure had the effect of the acute and chronic.12-14 In our lab, we tried to synthesize 3 novel rodenticides which were based on 1,3-indandione by introducing acute toxicity groups containing fluorine.15-17 The results showed that the synthesized compound had good palatability, the acute and chronic toxicity and the cost was low. The fluoride groups played an important role in killing the rat quickly and shortening the rat’s death time so as the novel rodenticides will be widely used for killing rat.

Materials and methods
Synthesis of anticoagulant rodenticides R1, R2, R3
Acetamide acetophenone (1)
Four-aminoacetophenone (10 g), glacial acetic acid (9 g) and acetic anhydride (1.6 g) were added to the 100 mL of 3 flasks. And then, the solution was stirred and refluxed for 8 h. After reaction, the reaction liquid was stirred in the ice water and then crystallized, filtered, washed and dried to get 1 (12.6 g): yield, 96.4%.

Amino chalcone ketones (2)
Acetyl amino chalcone ketones (6 g) and 5% of HCl aqueous solution (150 mL) were added to the 3 flasks
and reluxed for 3 h and then cooled and filtered to get the red solid. The solid was wated repeatedly by K$_2$CO$_3$ solution until liquid was litmusless and dried to get 2 (3.4 g): mp, 101.0°C; yield, 68.0%; IR (KBr, cm$^{-1}$) 3487, 3328, 1661, 1610, 1598-1450 cm$^{-1}$.

**Fluoracyl chloride (3)**

PCl$_5$ (125 g) and natrium fluoroaceticum (50 g) were added to the 3 flasks and stirred under the protection of ice until the mixture was liquid. After removing the ice bath for 3 h, the reaction devive was insteded by distillation divice and was heated under oil (temperature doesn’t exceed 120 °C) to get fraction (78°C) and then was distillated to get fraction (72°C-73°C). Fluorine acetyl chloride was getted (30.1 g): mp, 101.0°C; yield, 62.4%; IR (KBr, cm$^{-1}$) 1780, 1450, 1010 cm$^{-1}$.

**Fluoroacetamide chalcone ketones (M$_2$)**

Fluorine acetyl chloride (0.13 mol) was added to Amino chalcone ketones (0.11 mol) with a constant pressure drop funnel slowly and the mixture was stirred under the protection of ice for 3 h under the protection of ice. Then the mixture was distillated under reduced pressure (about 7.24 × 104 Pa) to remove the unreacted fluorine acetyl chloride and other low boiling. Crude products were watered in 30 mL 2% hydrochloric acid solution and was filtered to get fluoroacetamide chalcone ketones (23.8 g): mp, 126.3°C; yield, 69.4%; IR (KBr, cm$^{-1}$) 3297, 1671, 1646, 1605-1453 cm$^{-1}$.

**Three - [3 - (4 - fluorine acetamide) - phenyl ethyl benzene formyl - 1] - 4 - hydroxyl coumarin(4)**

Dioxane (100 mL), piperidine (5 mL) and 4-Hydroxy-coumarin (12.5 g) and 4 - benzene propylene acyl - N - fluorine acetanilide were added to the 250 mL of 3 flasks and reluxed for 7 h. Then the mixture was distillated under reduced pressure (about 5.33 × 103 Pa) and watered with 50 mL 95% ethanol and filtrated, dried to get light yellow product 3 - [3 - (4 - fluorine acetamide) - phenyl ethyl benzene formyl - 1] - 4 - hydroxyl coumarin: mp, 131.6°C; yield, 52.6%; IR (KBr, cm$^{-1}$) 3362, 1706, 1682, 1621, 1605-1442, 1039, 840, 758, 699 cm$^{-1}$.

**Novel Indanedione Anticoagulant Rodenticides R$_1$**

Dehydrated alcohol (100 mL), 3 - [3 - (4 - fluorine acetamide) - phenyl ethyl benzene formyl - 1] - 4 - hydroxyl coumarin (23.5 g) and pyridine (10 mL) were added into 3 flasks and stirred under 30 ~35 °C. Then sodium borohydride was added to the mixture once every 5 min and reacted for 3 ~4 h. Take the sample to verify end point (ketone group and 2, 4 - dinitrobenzene hydrazine generated red brown which wasn’t dissolved in ethanol precipitation). After the reaction, the mixture was vacuum distillated to get solid. The solid was slowly added into water (200 mL). Dilute hydrochloric acid wasn’t added until be acid and filtered, recrystallized with ethanol to get product R1: yield, 76.0%; IR (KBr, cm$^{-1}$) 3411, 1700, 1681, 1602-1444, 1037, 840, 758, 699 cm$^{-1}$; 1 HNMR (CDCl$_3$, 300 Hz, δppm) 7.20-7.80 (m, 13H, ArH), 11.54 (s, 1H, H-5), 4.05-4.20 (m, 1H, H-6), 2.10-2.25 (m, 2H, H-10), 4.35-4.50 (m, 1H, H-11), 4.60-4.72 (s, 1H, H-12), 8.03 (m, 1H, H-15), 4.87, 5.03 (m, 2H, H-16). Rf=0.6 (cyclohexane:ethyl acetate=1:7).

**Ethyl Fluoride (5)**

Two-fluoroethanol (19.2 g) and 1, 4 - dioxane (50 mL) were added to the 250 mL of 3 flasks and stirred. Phosphorus tribromide was added dropwise to the mixture under ice for 1 h. Then the solution was heated and reluxed for 1 ~2 h to get distillate (71~90°C). The mixture was watered with sodium carbonate and water, then dried with anhydrous calcium chloride and distillated to get distillate (71~74°C, 18.34 g): yield, 57.0%; IR (KBr, cm$^{-1}$) 3616, 2980-2842, 1458, 712 cm$^{-1}$.

**The hydroxyl benzylidene acetone (6)**

Sodium hydroxide (20 mL, 5%), acetone (15 mL), p-hydroxy benzaldehyde (12.2 g), sodium hydroxide (4 g), H$_2$O (60 mL) and acetone (15 mL) were added to the 250 mL of 3 flasks. Reaction temperature was controlled not to exceed 30 °C and was dropped out for 2 h, and reacted for 4 h. The mixture was neutralized to pH = 6 ~8 with hydrochloric acid. Acetone was distillated under atmospheric and water phase was static cooled. The mixture was filtered and recrystallized with ethanol recrystallization to get yellow solid: mp,162.2°C; yield, 52%; IR (KBr, cm$^{-1}$) 3137, 1670, 1627, 1597-1441, 834 cm$^{-1}$.

**Intermediate 4 - (2 - fluorine ethoxy) acetophenone (M$_3$)**

P-Hydroxyacetophenone (13.6 g), alcohol (80 mL), potassium hydroxide (5.6 g), potassium iodide were
added to the 250 mL of 3 flasks and stirred fully for 30 min. Then ethyl fluoride was added to the mixture and refluxed for 5 h. The reaction liquid was added to the water in the beaker. The mixture was filtered and was washed to be neutral. The solid was recrystallized with ethanol to get white crystal: yield, 83.2%; IR (KBr, cm$^{-1}$) 3079, 2996–2886, 1681, 1602–1453, 1254, 1051, 836 cm$^{-1}$.

**Four - (2 - fluorine ethoxy) benzylidene acetone (M$_4$)**

P-hydroxychalcones (12.9 g, 0.08 mol) and alcohol (60 mL) were added to 3 flasks and stirred. Then potassium hydroxide (4.5 g) and potassium iodide (0.5 g) of aqueous solution (10 mL) were stirred slowly for 30 min and then 1, 2 - fluorine bromine ethane (10 mL) were added to the solution and stirred under normal temperature for 1 ~ 2 h and refluxed for 16 h. The reaction mixture was added to water in the beaker to get solid. The solid was filtered and washed to be neutral. The solid recrystallized with ethanol to get white crystal: yield, 83.2%; IR (KBr, cm$^{-1}$) 3137, 1670, 1627, 1597–1441, 834 cm$^{-1}$.

**Novel Indanedione Anticoagulant Rodenticides R$_2$**

Sodium methoxide (5.4 g, 0.1 mol) and dimethyl phthalate (19.4 g, 0.1 mol) and methylbenzene (60 mL) were added to 3 flasks and dissolved under stirration. Methylbenzene (40 mL) containing fluoroethoxybenzylideneacetone (18.2 g, 0.1 mol) was added to the solution for 1 ~ 2 h and refluxed for 6 ~ 8 h. After cooling, the solution was hydrolysised with sodium hydroxide solution under stirring, and then was separated. Organic phase was washed with 2% sodium hydroxide solution until the water phase was colorless. Water phase was merged and hydrochloricd with acid neutralization and extracted twice with ether. Ether was steamed out from organic phase to get the coarse product B. The crude product was recrystallized with ether to get pale yellow product R$_2$: mp, 162.4°C; yield, 42.3%; IR (KBr, cm$^{-1}$) 3083, 2973–2858, 1696, 1648, 1594–1446, 1269, 1042, 843 cm$^{-1}$. 1HNMR (CDCl$_3$ 300 Hz, δppm) 7.05–8.31 (m, 8H, ArH), 6.05 (s, 1H, H-3), 4.29–4.40 (m, 2H, H-6), 4.73–4.91 (m, 2H, H-7). Rf=0.4(cyclohexane:ethyl acetate=1:3).

**Novel Indanedione Anticoagulant Rodenticides R$_3$**

Sodium methoxide (1.62 g) and dimethyl phthalate (6 g) and methylbenzene (60 mL) were added to 3 flasks and dissolved under stirration. Methylbenzene (30 mL) containing fluoroethoxybenzylideneacetone (6.24 g, 0.03 mol) was added to the solution for 1~2 h and refluxed for 6~8 h. After cooling, the solution was hydrolysised with sodium hydroxide solution under stirring, and then was separated. Toluene layer was separated and distilled. The remnant was recrystallized with ethanol to get croci product R$_3$:mp, 165.6°C; yield, 45.1%; IR (KBr, cm$^{-1}$) 3040, 2954–2888, 1713, 1657, 1616, 1607–1454, 1251, 1051, 834 cm$^{-1}$. Rf=0.5 (cyclohexane:ethyl acetate=1:3).

### Results

**Bioactivity assays**

In the no-choice and choice experiments, compounds R$_1$, R$_2$ were applied as rodenticide bait of laboratory-prepared. Mice were divided into 5 groups, and each group had 5 males and 5 females according to gender and weight. Mice were KunMing closed group and they were 8~10 d old and 15 g-20 g weight. The following was concrete operations.

1. **No-choice experiment**: Baits comprised about 0.005% wheat baits and water. These mice were fed baits for 2 d. Record rat dead condition and bait consumption.

2. **Choice experiment**: Baits comprised about 0.005% wheat baits, wheat and water. Mice chose food voluntarily. These mice were fed baits for 2 d. Record rat dead condition and bait consumption and calculated mortality and feeding coefficient.

**Feeding coefficient of novel indanedione anticoagulant rodenticides R$_1$, R$_2$**

Feeding coefficient that represent rat palatability is known as the selective feeding rate. Namely in the test environment, the poison bait and blank bait were provided simultaneously, and exchanged place regularly. $^{18-20}$ Rat accessed to food selectively. The ratio of free poison bait consumption gap and consumption to bait is feeding coefficient. It is generally believed that under the test of mice death. Feeding coefficient that is greater than 0.30 is A level. The greater, the better palatability.

The feeding experiment of 0.005% R$_1$, the result was in Table 1.
The Table 1 shows that killing rat rate of 0.005\% new anticoagulant rodenticide R1 was 100\%, and the average feeding coefficient was 0.87 (> 0.30) and poison bait palatability was good.

The killing rat rate of 0.005\% new anticoagulant rodenticide R1, the result was in Table 2.

The Table 2 shows that killing rat rate of 0.005\% new anticoagulant rodenticide R1 was 100\%, and the average feeding coefficient was 0.7 (> 0.30) and poison bait palatability was good.

The test of acute oral LD50 R1, R2, the result was in Table 3 and Table 5.

LD50 of R1 = 0.718(mg/kg)

When acute oral LD50 was determined, we found in different dosage of new anticoagulant rodenticide, rat death time and death number of relationships with other anticoagulant rodenticide was different, and when novel anticoagulant rodenticide was compared with the former 2 kinds of synthesis of new anticoagulant rodenticide, acute toxicity was strengthened. The result was shown in Table 4.

Table 4. The relation of obituary time and quantity of big rat under different dosage of rodenticide R1.

| Death Number | rodenticide concentration (mg/Kg) | 0.4 | 0.56 | 0.79 | 1.12 | 1.58 |
|--------------|----------------------------------|-----|------|------|------|------|
| Date (D)     | 1                               | 0   | 0    | 0    | 1    | 1    |
|              | 2                               | 0   | 0    | 1    | 1    | 3    |
|              | 3                               | 0   | 1    | 2    | 3    | 2    |
|              | 4                               | 0   | 1    | 1    | 2    | 0    |
|              | 5                               | 1   | 1    | 0    | 1    | 2    |
|              | 6                               | 1   | 1    | 0    | 1    |      |
|              | 7                               | 0   | 0    | 0    | 0    | 0    |
|              | 8                               | 0   | 0    | 0    | 0    | 0    |
|              | 9                               | 0   | 0    | 0    | 1    | 0    |
|              | 10                              | 0   | 0    | 0    | 0    | 0    |
|              | 11                              | 0   | 0    | 0    | 0    | 0    |
|              | 12                              | 0   | 0    | 0    | 0    | 0    |
|              | 13                              | 0   | 0    | 0    | 0    | 0    |
|              | 14                              | 0   | 0    | 0    | 0    | 0    |
|              | 15                              | 0   | 0    | 0    | 0    | 0    |

LD50 of R2 = 0.693(mg/kg)

LD50 of R1 was less than the LD50 of R2 showed R1 was more toxic than R2.

When acute oral LD50 was determined, we found in different dosage of new anticoagulant rodenticide, rat death time and death number of relationships with other anticoagulant rodenticide was different, and when novel anticoagulant rodenticide was compared with the former 2 kinds of synthesis of new anticoagulant rodenticide, acute toxicity was strengthened. The result was shown in Table 6.

The relation of obituary time and quantity of big rat under different dosage of rodenticide R1 and R2, the result was in Table 4 and Table 6.

Table 5. The oral acute virulence of R2 for big white rat.

| group | dosage (mg/kg) | number | fatality number | mortality rate (%) | survival rate (%) |
|-------|----------------|--------|-----------------|--------------------|-------------------|
| 1     | 0.40           | 10     | 2               | 20                 | 80                |
| 2     | 0.56           | 10     | 4               | 40                 | 60                |
| 3     | 0.79           | 10     | 5               | 50                 | 50                |
| 4     | 1.12           | 10     | 8               | 80                 | 20                |
| 5     | 1.58           | 10     | 9               | 90                 | 10                |

Pesticide effect comparison of novel anticoagulant rodenticide R1, R2
Rodenticide R1: acute oral LD50 = 0.718 mg/kg; Average feeding coefficient was 0.87. Rodenticide R2: acute oral LD50 = 0.693 mg/kg; Average feeding coefficient was 0.70.

Table 3. The oral acute virulence of R1 for big white rat.

| group | dosage (mg/kg) | number | fatality number | mortality rate (%) | survival rate (%) |
|-------|----------------|--------|-----------------|--------------------|-------------------|
| 1     | 0.40           | 10     | 2               | 20                 | 80                |
| 2     | 0.56           | 10     | 4               | 40                 | 60                |
| 3     | 0.79           | 10     | 5               | 50                 | 50                |
| 4     | 1.12           | 10     | 8               | 80                 | 20                |
| 5     | 1.58           | 10     | 9               | 90                 | 10                |
Three new fluoride anticoagulant rodenticide LD$_{50}$ values showed that they made a strong poison effect to the rat, and they had good palatability (achieves A level). The experiment showed that mice’s death peak was at about 72 h and showed drug had good characteristics of acute medicine. Acute oral LD$_{50}$ values also showed that the synthesis of new type of fluoride anticoagulant rodenticide had the characteristics of both acute and chronic rodenticide and achieved the expected effect. Due to the average feeding coefficient of R$_1$ was greater than R$_2$, which explained palatability of R$_1$ was best.

**Reagent and company**

Ethylene glycol: Tianjin Bo Di Chemical Company  
Phosphorus pentachloride: Beijing Shunyiwei New Chemical Factory

| Date (D) | Death Number | 0.4 | 0.56 | 0.79 | 1.12 | 1.58 |
|----------|--------------|-----|------|------|------|------|
| 1        | 0            | 0   | 0    | 0    | 0    | 0    |
| 2        | 0            | 0   | 0    | 1    | 2    | 4    |
| 3        | 0            | 0   | 0    | 1    | 2    | 4    |
| 4        | 0            | 0   | 2    | 3    | 4    |      |
| 5        | 0            | 1   | 1    | 1    | 0    |      |
| 6        | 1            | 1   | 0    | 2    | 1    |      |
| 7        | 1            | 1   | 1    | 0    | 1    |      |
| 8        | 1            | 1   | 1    | 1    | 0    |      |
| 9        | 0            | 0   | 0    | 0    | 0    |      |
| 10       | 0            | 0   | 0    | 0    | 0    |      |
| 11       | 0            | 0   | 0    | 0    | 0    |      |
| 12       | 0            | 0   | 0    | 0    | 0    |      |
| 13       | 0            | 0   | 0    | 0    | 0    |      |
| 14       | 0            | 0   | 0    | 0    | 0    |      |
| 15       | 0            | 0   | 0    | 0    | 0    |      |

**Figure 1.** Synthesis of anticoagulant rodenticide R$_1$, R$_2$, R$_3$. Reagents and conditions: (A) Acetic anhydride; (B) NaOH, HCl, H$_2$O, K$_2$CO$_3$; (C) NaOH; (D) PCl$_5$; (E) 4-hydroxyl coumarin; (F) NaBH$_4$; (G) KF, PBr, tetrahydrofuran; (H) KOH, KI; (I) Dimethyl phthalate; (J) acetone.
Conclusion

We designed 3 new anticoagulant rodenticides, which were based on 4-hydroxycoumarin or 1,3-indandione, added introducing containing fluorine acute toxicity groups. By the test of FT-IR, 1H-NMR, and other physical and chemical methods, we concluded that the target product-novel anticoagulant rodenticides R1,R2,R3 and 3 intermediates were synthesized.

LD50 value of R2 was minimum, virulence of R2 was greater than 1,3-indandione, which showed the fluoride acetyl played an important role in killing rat and most importantly, the novel indanedione anticoagulant rodenticides was more toxic than 1,3 - indan diketone. Feeding coefficient (>0.3) of novel indanedione anticoagulant rodenticides showed palatability was good. Average feeding coefficient of R1 was biggest which showed R1 palatability was best. The result of the experiment proved that the new synthesis of 1,3 - indan diketone for maternal new anticoagulant rodenticides can replace the current 4 - hydroxyl coumarin as the mother of the second generation anticoagulant rodenticide.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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