Brief Report

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Design, synthesis and biological evaluation of 4-chromanone derivatives as I_{Kr} inhibitors

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Summary **Cardiac arrhythmia is a major cause of death in the world. Among many delayed rectifier potassium currents, the rapid delayed rectifier K current (I_{Kr}) plays an important role in the repolarization of cardiac tissue. The inhibition of I_{Kr} can delay repolarization and lead to an increase in the QT interval of the electrocardiogram, which is the treatment mechanism of Class III antiarrhythmic agents. Therefore, I_{Kr} can be considered as the drug target for the treatment of cardiac arrhythmia. In the current study, a series of 4-chromanone compounds (WR1-WR12) were well designed and synthesized as I_{Kr} inhibitors. The results disclosed that two compounds displayed potent inhibitory activities against I_{Kr}. Moreover, our structure-activity relationship results might provide necessary information for the rational design of inhibitors for I_{Kr}.**

Keywords: I_{Kr}, inhibitors, 4-chromanone, arrhythmia

1. Introduction

Potassium ion (K^+) channels consist of a ubiquitous family of membrane proteins that play critical roles in a wide variety of physiological processes, such as the regulation of neuronal excitability, cell proliferation, muscle contraction, and insulin secretion (*1*). K^+ channels have long been attractive targets for the rational drug design, due to their pivotal functions in biological systems (*2*). So far, various small-molecule compounds and toxins have been discovered as K^+ channel modulators (*3*).

Multiple delayed rectifier potassium currents play an important role in the repolarization and termination of the cardiac action potential. Inhibition of these potassium currents prolongs action potential duration (*4*), delays repolarization, and produces an antiarrhythmic effect (*5*). As the rapid component of cardiac delayed rectifier potassium current, the I_{Kr} potassium channel is mainly encoded by hERG (human ether-a-go-go-related gene) (*6,7*), and its electrophysiological properties can be regulated by its auxiliary subunit KCNE1 and KCNE2 (*8*). Besides in heart, it was reported that the expressing level of hERG in cancer cells was greatly increased (*9*). As a result, I_{Kr} encoded by hERG channel may be a potential cancer therapeutic target (*10*).

I_{Kr} is highly sensitive to blockade by many structurally diverse compounds (*11*), such as astemizole, imipramine, and dofetilide (*12*). However, there is still an urgent demand on I_{Kr} inhibitors for examining the mechanism of inhibition of I_{Kr}. In the current research, we designed and synthesized a series of 4-chromanone compounds, and evaluated their inhibitory activity against I_{Kr} using a radio-ligand based assay. The experimental results revealed that several compounds exhibited respectable activity against I_{Kr}. Moreover, analysis of the structure–activity relationship on these compounds could contribute to designing new I_{Kr} blocker for preventing arrhythmia and/or cancer therapy.

2. Materials and Methods

2.1. *Chemicals*

In summary, a series of 4-chromanone derivatives were well designed and synthesized. The synthetic route is outlined in Scheme 1. In this case, 4-substituted phenol

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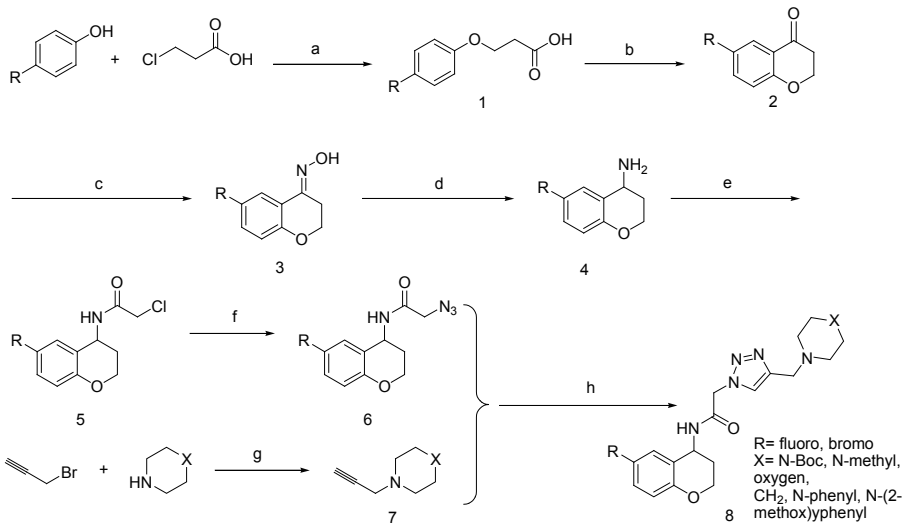


Scheme 1. Synthetic route of WR1-WR12. Reagents and conditions: **(a)** KOH, H_2O, reflux 4 h; **(b)** PPA, 60°C, 4 h; **(c)** Hydroxylamine hydrochloride, Na_2CO_3, EtOH, H_2O, 0°C→ r.t., 12 h; **(d)** CH_3COOH, Zn, 0°C→ r.t., 24 h; **(e)** Chloroacetyl chloride, K_2CO_3, CH_2Cl_2, 0°C→ r.t., 4 h; **(f)** NaN_3, KI, CH_3CN, reflux 6 h; **(g)** K_2CO_3, CH_2Cl_2, 0°C→ r.t., 4 h; **(h)** Sodium ascorbate, $CuSO_4$, MeOH, 0 °C→ r.t., 24 h.

and 3-chloropropionic acid gave compound 1, which was cyclized to form compound 2 by polyphosphoric acid (PPA). Compound 2 is then conveniently converted to compound 3 under oximation reaction. Hydrogenation of compound 3 with CH_3COOH/Zn gave compound 4, subsequently provided the key intermediate compound 6 *via* acylation with chloroacetyl chloride and substitution with sodium azide. Finally, reaction of compound 6 with the corresponding alkyne presented compound WR1-WR12 *via* click reaction, which was catalyzed by $CuSO_4$ and sodium ascorbate.

2.2. I_{Kr} *inhibition assay*

The inhibitory activities of these 12 compounds against I_{Kr} were evaluated by testing their affinities with hERG in the presence of 9 nM [^{3}H] dofetilide. Astermizole (Cat. No. #A2861-10MG; Sigma-Aldrich, St. Louis, MO, USA) and atropin was selected as positive and negative controls, respectively. The affinity with *h*ERG potassium channel was accessed in the presence of 9 nM [^{3}H]-dofetilide. Their binding abilities with the *h*ERG were exhibited and compared with the positive and negative controls.

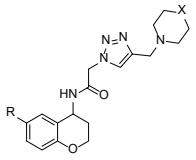
In brief, each compound was dissolved in DMSO as a stock solution (1 mM), which was diluted with binding buffers (10 folds, 6 points) when applied to the binding assays. Cell membranes were prepared as the instruction (GenScript USA Inc.). First, each well of Uni-filter 96 GF/C microplate was incubated with 100 µL 0.5% PEI (Polyethyleneimine，Sigma-Aldrich, dissolved in milli-Q water) at 4°C for 30-60 min. PEI was then discarded, and plates were washed with 2 mL/well wash buffer (50 mM Tris-HCl, pH 7.4; filtered and stored at 4°C). The reaction mixtures, including membrane (10 µg/well), each compound and [^{3}H]-dofetilide ligand (9 nM), were prepared in 24-well plates in a final volume of 100 µL (binding buffer: 10 mM Hepes, 130 mM NaCl, 60 mM KCl, 0.8 mM $MgCl_2$, 1 mM NaEGTA, 10 mM glucose, 0.1% BSA, pH7.4; filtered and stored at 4°C) and incubated at 25°C for 2 h with a shaking speed of 530 RPM. The reaction system was transferred into the filter plates and filtered with a Millipore vacuum manifold. The wells were washed with 2 mL/well cold wash buffer and dried at room temperature for 120 min. The bottom of the plates was sealed with Bottom seal™ (opaque) (Perkin Elmer) and 50 µL MicroScint 20™ (Perkin Elmer) was added to each well. Finally, the plates were sealed with Topseal A (Perkin Elmer) and counted on TopCount NXT for 1 min/well. IC_{50} values were calculated by GraphPad Prism 4 using the Cheng-Prusoff equation.

The binding data were converted to % displacement according to the below equation: % displacement = 100 × (1 – (sample CPM/Total binding CPM)) (in which total binding CPM values were obtained by testing binding of [^{3}H]-dofetilide to the target channel without competitors).

3. Results and Discussion

In order to study the influence of different substituents in the phenyl ring and piperazine ring in 4-chromanone-based compounds on the inhibitory activity, we design and synthesize twelve compounds (WR1-WR12) with different R and X group to examine the importance of phenyl ring and piperazine ring, respectively. All inhibition results are presented in Table 1. No compound exhibits a comparable replacement percentage (56% at 10 µM) with astemizole; however, several compounds could still prevent the binding

Table 1. Structures and inhibitory activities against I_{Kr} of compounds.



Numbers	R	X	IC_{50}/(μM)	% Displacement at 10 μM
Astemizole			0.0084	56.61 ± 4.29
Atropine				7.41 ± 3.53
WR1	F	N-phenyl	NA	29.08 ± 0.27
WR2	F	N-(2-methoxyl)phenyl	NA	24.69 ± 0.27
WR3	F	O	NA	2.26 ± 0.89
WR4	F	C	NA	31.66 ± 1.29
WR5	F	N-methyl	NA	3.22 ± 1.27
WR6	F	N-Boc	NA	6.20 ± 2.36
WR7	Br	N-phenyl	NA	34.98 ± 2.33
WR8	Br	N-(2-methoxyl)phenyl	9.16	25.79 ± 2.81
WR9	Br	O	NA	23.49 ± 0.06
WR10	Br	C	NA	17.95 ± 5.86
WR11	Br	N-methyl	NA	11.08 ± 9.16
WR12	Br	N-Boc	1.07	25.85 ± 2.88

NA: No significant dose response curve fitted.

of the ligand to the channel with > 20% replacement percentage when their concentrations are 10 μM (Table 1). We then calculated the inhibitory IC_{50} values for providing the exact value of inhibitory activity against I_{Kr}. As a result, two compounds with bromine rather than fluorine displayed potent inhibitory activities against I_{Kr}, which proposes that R group should be low electronegativity. While compare the X group we can find that phenyl and 2-methoxyphenyl are superior to other substituents to a piperazine ring, such as O, N-methyl, *etc*., which suggests that phenylpiperazine is highly fitted with the binding pocket of hERG.

On the basis of the result of I_{Kr} inhibition assay, we harvested several compounds with I_{Kr} inhibitory activities. In our opinions, these compounds are a new series of I_{Kr} inhibitors with novel chemical structures, so that can serve as lead compounds for the development of new I_{Kr} inhibitors.

4. Conclusion

In conclusion, a series of 4-chromanone derivatives were well designed and synthesized in the current study. After biological evaluation, two compounds exhibited moderate inhibition against I_{Kr}. It should be noted that these compounds are new I_{Kr} inhibitors with novel structure, so that can serve as lead compounds for further development. SAR analysis revealed that both the R group and the X group might play an important role in anti-I_{Kr} activity, and substituents of a phenyl ring should have a high influence on the activity. The proof-of-concept in this study may provide essential information for the future design of inhibitors for I_{Kr}. In our follow-up study, we will continue to manipulate the chemical structures of these compounds for improving the activity against I_{Kr}, as well as to take an attempt to employ other methods to test the inhibition of compound against I_{Kr} and to explore their possibility of preventing cardiac arrhythmia.

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Appendix

Chemistry: general procedures

All materials were purchased from commercial suppliers and used without further purification. Twice-distilled water was used throughout all experiments. Solvents were distilled prior to use, and all the reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60GF-254) and visualized with UV light or ninhydrin. Mass spectra were performed by the analytical and the mass spectrometry facilities in Drug Analysis Center at Shandong University on Agilent Technologies 1100 infinity HPLC, Applied Biosystems API4000. ^{1}H-NMR spectra were recorded on the Bruker 300 MHz NMR and 600 MHz NMR spectrometers. Chemical shifts are reported in delta (δ) units, parts per million (ppm) downfield from trimethylsilane. Melting points were determined on an electrothermal melting point apparatus (uncorrected).

1.1. *Phenyl-4-propynylpiperazine (7a)*

Propargyl bromide (1.19 g, 10 mmol) was added slowly to a mixture of 1-phenylpiperazine (1.95 g, 12 mmol) and K_2CO_3 (2.76 g, 20 mmol) in acetone. The mixture was stirred for 16 h at room temperature, filtrated, and the precipitation was washed with acetone. The combined filtrate was evaporated under vacuum. Water was added to the residue, and the mixture was extracted with CH_2Cl_2 (40 mL). The combined organic layer was washed with 5% $NaHCO_3$ (3 × 50 mL), saturated brine (2 × 50 mL), and then dried over $MgSO_4$. The solution was filtrated and evaporated. The crude product was then purified by column chromatography to obtain a white solid, yield 80%, m.p: 46-48°C. ESI-MS calcd for $C_{13}H_{16}N_2$ ($M + H^+$): 201.1; found: 201.4. ^{1}H-NMR (600 MHz, DMSO-d6): δ = 7.19 (dd, J_1 = 9.0, 7.8 Hz, 2H), 6.92 (d, J = 7.8 Hz, 2H), 6.76 (t, J = 7.2 Hz, 1H), 3.36 (s, 2H), 3.22 (s, 1H), 3.15 (s, 4H), 2.62 (s, 4H).

1.2. *1-(2-Methoxyphenyl)-4-propynylpiperazine (7b)*

Compound 7b was synthesized following the procedure described in 1.1. White solid, yield 76%, m.p: 75-77°C. ESI-MS calcd for $C_{14}H_{18}N_2O$ ($M + H^+$): 231.1; found: 231.2. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 7.01 (m, 1H), 6.96 (d, J = 1.2 Hz, 1H), 6.92 (t, J = 7.2 Hz, 1H), 6.86 (d, J = 7.8 Hz, 1H), 3.87 (s, 3H), 3.36 (d, J = 1.8 Hz, 4H), 2.79 (s, 4H), 2.27 (s, 1H).

1.3. *N-propargylmorpholine (7c)*

Compound 7c was synthesized following the procedure described in 1.1. White solid, yield 51%, m.p: 161-163°C. ESI-MS calcd for $C_7H_{11}NO$ ($M + H^+$): 126.1; found: 126.0. ^{1}H-NMR (600 MHz, D_2O) δ: 3.95-3.90 (m,

4H), 3.77 (s, 2H), 3.43 (s, 2H), 3.13 (s, 2H), 2.95 (t, J = 2.4 Hz, 1H).

1.4. *1-(Prop-2-ynyl)piperidine (7d)*

Compound 7d was synthesized following the procedure described in 1.1. White solid, yield 46%, m.p: 178-180°C. ESI-MS calcd for $C_8H_{13}N$ (M + H^+): 124.1; found: 124.3. ^{1}H-NMR (600 MHz, D_2O) δ: 3.79 (s, 2H), 3.45-3.43 (m, 2H), 2.90-2.83 (m, 3H), 1.80-1.78 (m, 2H), 1.65-1.63 (m, 1H), 1.57-1.50 (m, 2H), 1.29-1.26 (m, 1H).

1.5. *1-Methyl-4-propargylpiperazine (7e)*

Compound 7e was synthesized following the procedure described in 1.1. White solid, yield 72%, m.p: 210°C. ESI-MS calcd for $C_8H_{14}N_2$ (M + H^+): 139.1; found: 139.1. ^{1}H-NMR (600 MHz, D_2O) δ: 3.96 (s, 2H), 3.69 (s, 4H), 3.35 (s, 4H), 2.98 (d, J = 2.4 Hz, 1H), 2.85 (s, 3H).

1.6. *Tert-butyl 4-propargylpiperazine-1-carb-oxylate (7f)* (*13*)

A solution of di-*tert*-butyl dicarbonate (4.34 g, 20 mmol) in CH_2Cl_2 (25 mL) was slowly added to a stirring solution of piperazine (3.46 g, 40 mmol) in CH_2Cl_2 (50 mL) at 0°C. The mixture was then stirred for 24 h at room temperature, and the solvent removed in vacuum. The crude solid was redissolved in diethyl ether (100 mL) with warming, and the white precipitate was filtered to. The product was extracted from the mother liquor with 1M citric acid solution (3 × 50 mL), and the aqueous layer was washed with CH_2Cl_2 (3 × 50 mL), basified with Na_2CO_3 (pH 11), and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layer was dried over $MgSO_4$ and evaporated in vacuum to give *tert*-butyl 1-piperazinecarboxylate as a waxy white solid (2.82 g, yield 75.6%). Propargyl bromide (2.38 g, 20 mmol) was added slowly to a mixture of *tert*-butyl 1-piperazinecarboxylate (4.4 mg, 24 mmol) and K_2CO_3 (3.32 g, 24 mmol) in acetone. The mixture was stirred for 12 h at room temperature. CH_2Cl_2 (40 mL) was then added, and the solution obtained was washed with 5% $NaHCO_3$ (3 × 50 mL), saturated brine (2 × 50 mL), and then dried over $MgSO_4$. The solution was filtrated and evaporated to dryness. The residue was crystallized from ethanol and gave *tert*-butyl 4-propargylpiperazine-1-carboxylate *7f* (4.2 g, yield 78%), m.p: 98-100°C. ESI-MS calcd for $C_{12}H_{20}N_2O_2$ (M + H^+): 224.1; found: 225.1. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 3.41(s, 4H), 3.26 (s, 2H), 2.46 (s, 4H), 2.22 (s, 1H), 1.42 (s, 9H).

2.1. *3-(4-Fluorophenoxy)propanoic acid (1a)* (*14*)

A mixture of potassium hydroxide (12.34 g, 220 mmol), 4-fluorophenol (11.21 g, 100 mmol), 3-chloropropionic acid (10.85 g, 100 mmol) and ethanol (2 mL) in water (40 mL) were refluxed 6 h. After cooling, the solution was acidified with concentrated hydrochloric acid to pH = 2 and extracted with ethyl acetate. The organic layer was washed with saturated aqueous solution of sodium bicarbonate. The aqueous phase was then acidified with concentrated hydrochloric acid and extracted with ethyl acetate. The final organic layer was dried over magnesium sulfate, filtrated, and evaporated under vacuum. The title product was recrystallized in ethanol. The resulting precipitate was collected by filtration, washed with hexane, and dried (3.87g, yield 21%), m.p: 84-86°C (Lit (*15*) m.p: 84-86°C). ESI-MS calcd for $C_9H_9FO_3$ (M + H^+): 185.1; found: 185.3.

2.2. *3-(4-Bromophenoxy)propanoic acid (1b)*

Compound 1b was synthesized following the procedure described in 2.1. White solid, yield 24%, and m.p: 148-150°C (Lit (*16*) m.p: 145-147°C). ESI-MS calcd for $C_9H_9BrO_3$ (M + H^+): 243.9; found: 243.8.

3.1. *6-Fluorochroman-4-one (2a)*

A mixture of 3-(4-fluorophenoxy)propanoic acid (3.68 g, 20 mmol) and polyphosphoric acid (PPA) (100 g) was placed into a three-neck round-bottom flask. The mixture was heated and stirred with a blade stirrer at 60°C for 4 h. One hundred mL ice water was added to the mixture after the temperature of the mixture was equal to room temperature, and then the aqueous layer was extracted with ethyl acetate for three times. The combined organic layer was dried overnight with anhydrous magnesium sulphate, filtrated, and evaporated under vacuum. The title product was recrystallized in ethanol. The resulting precipitate was collected by filtration, washed with hexane, and dried (2.76 g, yield 83%), and m.p: 118-120°C (Lit (*15*) m.p: 113-116°C). ESI-MS calcd for $C_9H_7FO_2$ (M + H^+): 167.0; found: 167.0.

3.2. *6-Bromochroman-4-one (2b)*

Compound 2b was synthesized following the procedure described in 3.1. White solid, yield 78%, and m.p: 77-78°C (Lit (*17*) m.p: 77°C). ESI-MS calcd for $C_9H_7BrO_2$ (M + H^+): 227.0; found: 227.1.

4.1. *(E)-6-fluorochroman-4-one oxime (3a)* (*18*)

To a suspension of hydroxylamine hydrochloride (1.39g, 20 mmol) in ethanol (50 mL), 6-fluorochroman-4-one (1.66g, 10 mmol) was added. Then, a solution of Na_2CO_3 (1.05 g, 20 mmol) in water (10 mL) was added dropwise. The resulting solution was stirred overnight. Most of the ethanol was evaporated, and then 60 mL water was added. The aqueous phase was extracted

with ethyl acetate (3 × 50 mL), and the combined organic layer was dried over Na_2SO_4. The solvents were removed under vacuum, and the residue was recrystallized. The resulting precipitate was collected by filtration, washed with hexane, and dried (1.54 g, yield 85%), m.p: 108-110°C. ESI-MS calcd for $C_9H_8FNO_2$ (M + H^+): 182.1; found: 182.0. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.25 (s, 1H), 7.51 (dd, *J* = 9.3 Hz, *J* = 3 Hz, 1H), 7.01 (ddd, *J* = 10.8 Hz, *J* = 7.8 Hz, *J* = 3.0 Hz, 1H), 6.89 (dd, *J* = 9 Hz, *J* = 4.5 Hz, 1H), 4.25 (t, *J* = 6.3 Hz, 2H), 3.00 (t, *J* = 6.0 Hz, 2H).

4.2. *(E)-6-bromochroman-4-one oxime (3b)*

Compound 3b was synthesized following the procedure described in 4.1. White solid, yield 91%, m.p: 139-141°C. ESI-MS calcd for $C_9H_8BrNO_2$ (M + H^+): 241.9; found: 241.7. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.46 (s, 1H), 7.95 (d, *J* = 2.4 Hz, 1H), 7.36 (dd, *J* = 9.0 Hz, *J* = 2.4 Hz, 1H), 6.81 (d, *J* = 8.7 Hz, 1H), 4.26 (t, *J* = 6.3 Hz, 2H), 3.00 (t, *J* = 6.3 Hz, 2H).

5.1. *2-Chloro-N-(6-fluorochroman-4-yl)acetamide (5a)* (*19,20*)

To a suspension of (E)-6-fluorochroman-4-one oxime (1.81 g, 10 mmol) in CH_3COOH (20 mL) was added the Zn (dust, 3.27 g, 50 mmol). The resulting solution was stirred 24 h. The Zn dust was filter off and washed by ethanol (20 mL) three times. The filtrate was collected and evaporated under vacuum. Then the product without purification was transferred to a mixture of K_2CO_3 (2.76 g，20 mmol) and CH_2Cl_2 (30 mL), and chloroacetyl chloride (2.26 g，20 mmol) was added in the mixture at room temperature. The reaction mixture was stirred for 4 h, subsequently slowly poured into 100 mL of ice water. The aqueous solution was extracted with CH_2Cl_2 (40 mL × 3), the organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated to furnish a solid residue which was purified by crystallization from ethanol. The resulting precipitate was collected by filtration, washed with hexane, and dried (1.27 g, yield 52%), m.p: 129-131°C. ESI-MS calcd for $C_{11}H_{11}ClFNO_2$ (M + H^+): 244.1; found: 244.2. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.95-6.89 (m, 2H), 6.83-6.80 (m, 2H), 5.19 (dt, *J* = 13.8 Hz, *J* = 6.3 Hz, 1H), 4.29-4.22 (m, 1H), 4.19-4.15 (m, 1H), 4.12 (d, *J* = 2.4 Hz, 2H), 2.29-2.19 (m, 1H), 2. 90-2.00 (m, 1H).

5.2. *2-Chloro-N-(6-bromochroman-4-yl)acetamide (5b)*

Compound 5b was synthesized following the procedure described in 5.1. White solid, yield 50%, m.p: 176-178°C. ESI-MS calcd for $C_{11}H_{11}BrClNO_2$ (M + H^+): 304.0; found: 304.2. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.31-7.26 (m, 2H), 6.76 (d, *J* = 6.0 Hz, 2H), 5.18 (dt, *J* = 13.5 Hz, *J* = 6.3 Hz, 1H), 4.31-4.24 (m, 1H), 4.20-4.15(m, 1H), 4.13 (d, *J* = 4.2 Hz, 2H), 2.29-2.18 (m, 1H), 2.10-2.00 (m, 1H).

6.1. *N-(7-fluorochroman-4-yl)-2-(4-((4-phenylpiperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (WR1)*

Compound 5a (0.24 g, 1.0 mmol), KI (0.17 g, 1 mmol), sodium azide (2.2 mmol), 15 mL acetonitrile and 10 mL H_2O were added to a round bottom flask. After stirring overnight at 80°C, the reaction mixture was cooled to room temperature and most of the acetonitrile was evaporated and then 60 mL water was added. The product (6a) was extracted by CH_2Cl_2 and removed the solvent under reduced pressure. Without purification, 6a was dissolved into 20 mL methanol and 2 mL water, and compound 7a (0.2 g, 1.0 mmol), an aqueous sodium ascorbate solution (0.1 g, 0.5 mmol, dissolved in 2 mL water) and an aqueous solution of copper(II) sulfate pentahydrate (0.1 mL, 0.05 mmol) was added to this stirred solution. After another 24 h of stirring, the solvent was removed under vacuum, and the crude product was purified by flash chromatography (CH_2Cl_2/MeOH, 10:1) to afford the product WR1 (0.2 g, yield 52%), and m.p: 97-99°C. ESI-HRMS calcd for $C_{24}H_{27}FN_6O_2$ (M + H^+): 451.2258; found: 451.2277. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.75 (s, 1H), 7.03-6.98 (m, 2H), 6.93 (d, *J* = 4.2 Hz, 2H), 6.89-6.83 (m, 2H), 6.80-6.72 (m, 2H), 6.70 (d, *J* = 7.8 Hz, 1H), 5.17-5.11(m, 3H), 4.23-4.16 (m, 1H), 4.11-4.04 (m, 1H), 3.78 (s, 2H), 3.09 (br.s, 4H), 2.75 (br.s, 4H), 2.24-2.14 (m, 1H), 2.03-1.93 (m, 1H).

6.2. *N-(6-fluorochroman-4-yl)-2-(4-((4-(2-methoxyphenyl) piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (WR2)*

Compound WR2 was synthesized as white solid following the procedure described in 6.1, yield 41%, m.p: 152-154°C. ESI-HRMS calcd for $C_{25}H_{29}FN_6O_3$ (M + H^+): 481.2363; found: 481.2389. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.80 (s, 1H), 7.04-6.67 (m, 1H), 6.93 (d, *J* = 4.2 Hz, 2H), 6.90-6.82 (m, 2H), 6.80 (dt, *J* = 8.1 Hz, *J* = 3 Hz, 2H), 6.59 (d, *J* = 8.1 Hz, 1H) 5.18-5.12 (m, 3H), 4.23-4.16 (m, 1H), 4.11-4.04 (m, 1H), 3.86 (s, 3H), 3.82 (s, 2H), 3.12 (br.s, 4H), 2.78 (br.s, 4H), 2.25-2.14 (m, 1H), 2.04-1.93 (m, 1H).

6.3. *N-(6-fluorochroman-4-yl)-2-(4-(morpholinomethyl)-1H-1,2,3-triazol-1-yl)acetamide (WR3)*

Compound WR3 was synthesized following the procedure described in 6.1. White solid, yield 38%, m.p: 152-154°C. ESI-HRMS calcd for $C_{18}H_{22}FN_5O_3$ (M + H^+): 376.1785; found: 376.1787. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.02 (d, *J* = 8.1 Hz, 1H), 8.37 (s, 1H), 7.07-7.00 (m, 2H), 6.85 (dd, *J* = 8.7 Hz, *J* = 4.8 Hz, 1H), 5.36 (dt, *J* = 25.2 Hz, *J* = 6.3 Hz, 2H), 5.04 (dt, *J* = 13.2 Hz,

J = 6.3 Hz, 1H), 4.49 (s, 2H), 4.26-4.15 (m, 2H), 3.98-3.95 (m, 2H), 3.77-3.70 (m, 2H), 3.38-3.30 (m, 2H), 3.11-3.02 (m, 2H), 2.11-2.03 (m, 1H), 1.96-1.86 (m, 1H).

6.4. *N-(6-fluorochroman-4-yl)-2-(4-(piperidin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)acetamide (WR4)*

Compound WR4 was synthesized following the procedure described in 6.1. White solid, yield 46%, m.p: 138-140°C. ESI-HRMS calcd for $C_{19}H_{24}FN_5O_2$ (M + H^+): 374.1992; found: 374.1994. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.90 (d, *J* = 7.8 Hz, 1H), 7.96 (s, 1H), 7.07-7.00 (m, 2H), 6.85 (dd, *J* = 8.7 Hz, *J* = 5.1 Hz, 1H), 5.20 (dt, *J* = 26.7 Hz, *J* = 16.2 Hz, 2H), 5.04 (dt, *J* = 13.2 Hz, *J* = 6.3 Hz, 1H), 4.27-4.14 (m, 2H), 3.55 (s, 2H), 2.50 (br.s, 4H), 2.12-2.02 (m, 1H), 1.95-1.85 (m, 1H), 1.52-1.45 (m, 4H), 1.38-1.36 (m, 2H).

6.5. *N-(6-fluorochroman-4-yl)-2-(4-((4-methylpiperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (WR5)*

Compound WR5 was synthesized following the procedure described in 6.1. White solid, yield 39%, and m.p: 223-225°C. ESI-HRMS calcd for $C_{19}H_{25}FN_6O_2$ (M + H^+): 389.2101; found: 389.2113. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.06 (d, *J* = 8.1 Hz, 1H), 8.36 (s, 1H), 7.07-7.00 (m, 2H), 6.85(dd, *J* = 8.7 Hz, *J* = 4.8 Hz, 1H), 5.33 (dt, *J* = 24.6 Hz, *J* = 15.0 Hz, 2H), 5.04 (dt, *J* = 13.5 Hz, *J* = 6.3 Hz, 1H), 4.48 (s, 2H), 4.28-4.17 (m, 2H), 3.80-3.77 (m, 4H), 3.62-3.58 (m, 4H), 2.81 (s, 3H), 2.13-2.03 (m, 1H), 1.97-1.87 (m, 1H).

6.6. *Tert-butyl4-((1-(2-((6-fluorochroman-4-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)piperazine-1-carboxylate (WR6)*

Compound WR6 was synthesized following the procedure described in 6.1. White solid, yield 44%, m.p: 224-226°C. ESI-HRMS calcd for $C_{23}H_{31}FN_6O_4$ (M + H^+): 475.2469; found: 475.2473. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.06 (d, *J* = 7.8 Hz, 1H), 8.38 (d, *J* = 4.2 Hz, 1H), 7.07-7.00 (m, 2H), 6.85(dd, *J* = 8.7 Hz, *J* = 4.8Hz, 1H), 5.34 (dt, *J* = 26.4 Hz, *J* = 15.9 Hz, 2H), 5.04 (dt, *J* = 13.2 Hz, *J* = 6.3 Hz, 1H), 4.49 (s, 2H), 4.26-4.17 (m, 2H), 3.51-3.01 (m, 8H), 2.13-2.03 (m, 1H), 1.95-1.88 (m, 1H), 1.47(s, 9H).

6.7. *N-(6-bromochroman-4-yl)-2-(4-((4-phenylpiperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (WR7)*

Compound WR7 was synthesized following the procedure described in 6.1. White solid, yield 40%, and m.p: 148-150°C. ESI-HRMS calcd for $C_{24}H_{27}BrN_6O_2$ (M + H^+): 511.1457, 513.1437; found, 511.1448, 513.1430. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.89 (d, *J* = 8.1 Hz, 1H), 8.01 (s, 1H), 7.36-7.32 (m, 2H), 7.27-7.17 (m, 2H), 6.94 (d, *J* = 8.1 Hz, 2H), 6.80-6.74 (m, 2H), 5.23 (dt, *J* = 31.2 Hz, *J* = 15.9 Hz, 2H), 5.04 (dt, *J* = 12.9 Hz, *J* = 5.7 Hz, 1H), 4.30-4.15 (m, 2H), 3.64 (s, 2H),3.14-3.11 (m, 4H), 2.58-2.55 (m, 4H), 2.13-2.03 (m, 1H), 1.98-1.88 (m, 1H).

6.8. *N-(6-bromochroman-4-yl)-2-(4-((4-(2-methoxyphenyl) piperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (WR8)*

Compound WR8 was synthesized following the procedure described in 6.1. White solid, yield 42%, m.p: 106-108°C. ESI-HRMS calcd for $C_{25}H_{29}BrN_6O_3$ (M + H^+): 541.1485, 543.1464; found, 541.1540, 543.1527. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.89 (d, *J* = 8.1 Hz, 1H), 8.03 (s, 1H), 7.35-7.32 (m, 2H), 6.94-6.85 (m, 4H), 6.82-6.77 (m, 1H), 5.23 (dt, *J* = 31.2 Hz, *J* = 16.2 Hz, 2H), 5.04 (dt, *J* = 13.5 Hz, *J* = 6.3 Hz, 1H), 4.26-4.16 (m, 2H), 3.76 (s, 3H), 3.64 (s, 2H), 2.95 (br.s, 4H), 2.56 (br.s, 4H), 2.10-2.03 (m, 1H), 1.95-1.88 (m, 1H).

6.9. *N-(6-bromochroman-4-yl)-2-(4-(morpholinomethyl)-1H-1,2,3-triazol-1-yl)acetamide (WR9)*

Compound WR9 was synthesized following the procedure described in 6.1. White solid, yield 39%, m.p: 166-168°C. ESI-HRMS calcd for $C_{18}H_{22}BrN_5O_3$ (M + H^+): 436.0984, 438.0964; found, 436.0999, 438.0980. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.01 (d, *J* = 7.8 Hz, 1H), 8.36 (s, 1H), 7.36-7.32 (m, 2H), 6.81-6.77 (m, 1H), 5.34 (dt, *J* = 31.5 Hz, *J* = 16.2 Hz, 2H), 5.04 (dt, *J* = 13.5 Hz, *J* = 6.3 Hz, 1H), 4.49 (s, 2H), 4.30-4.17 (m, 2H), 4.07-3.95 (m, 2H), 3.76-3.69 (m, 2H), 3.37-3.30 (m, 2H), 3.12 (s, 2H), 2.13-2.03 (m, 1H), 1.97-1.87 (m, 1H).

6.10. *N-(6-bromochroman-4-yl)-2-(4-(piperidin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)acetamide (WR10)*

Compound WR10 was synthesized following the procedure described in 6.1. White solid, yield 43%, m.p: 215-216°C. ESI-HRMS calcd for $C_{19}H_{24}BrN_5O_2$ (M + H^+): 434.1192, 436.1171; found, 434.1203, 436.1185. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.71 (s, 1H), 7.26-7.22 (m, 1H), 7.18 (d, *J* = 2.1 Hz, 1H), 6.72-6.67 (m, 2H), 5.15-5.10 (m, 3H), 4.24-4.17 (m, 1H), 4.11-4.03 (m, 1H), 3.64 (s, 2H), 2.44 (br.s, 4H), 2.22-2.12 (m, 1H), 2.06-1.93 (m, 1H), 1.61-1.54 (m, 4H), 1. 46-1.42 (m, 2H).

6.11. *N-(6-bromochroman-4-yl)-2-(4-((4-methylpiperazin-1-yl)methyl)-1H-1,2,3-triazol-1-yl)acetamide (WR11)*

Compound WR11 was synthesized following the procedure described in 6.1. White solid, yield 34%, m.p: 202-204°C. ESI-HRMS calcd for $C_{19}H_{25}BrN_6O_2$ (M + H^+): 449.1301, 451.1280; found, 449.1293, 451.1270.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.03 (d, *J* = 7.8 Hz, 1H), 8.32 (s, 1H), 7.36-7.32 (m, 2H), 6.81-6.78 (m, 1H), 5.34 (dt, *J* = 30.9 Hz, *J* = 16.2Hz, 2H), 5.04 (dt, *J* = 13.5 Hz, J = 6.3 Hz, 1H), 4.65 (s, 2H), 4.24-4.17 (m, 2H), 3.60-3.40 (m, 4H), 3.30-3. 04 (m, 4H), 2.81 (s, 3H), 2.16-1.99 (m, 1H), 1.97-1.87 (m, 1H).

6.12. *Tert-butyl4-((1-(2-((6-bromochroman-4-yl)amino)-2-oxoethyl)-1H-1,2,3-triazol-4-yl)methyl)piperazine-1-carboxylate (WR12)*

Compound WR12 was synthesized following the procedure described in 6.1. White solid, yield 42%, m.p: 206-207°C. ESI-HRMS calcd for $C_{23}H_{31}BrN_6O_4$ (M + H^+): 535.1668, 537.1648; found, 535.1654, 537.1635. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 9.58 (s, 1H), 9.03 (d, *J* = 8.4 Hz, 1H), 7.36-7.32 (m, 2H), 6.81-6.77 (m, 1H), 5.34 (dt, *J* = 31.2 Hz, *J* = 16.2Hz, 2H), 5.04 (dt, *J* = 13.2 Hz, *J* = 6.0 Hz, 1H), 4.49 (s, 2H), 4.30-4.13 (m, 2H), 4.06-4.02 (m, 2H), 3.47-3. 00 (m, 6H), 2.13-2.00 (m, 1H), 1.97-1.87 (m, 1H), 1.41(s, 9H).