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Design, synthesis and biological evaluation of novel substituted benzoylguanidine derivatives as potent Na^+/H^+ exchanger inhibitors

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ABSTRACT

A novel series of substituted benzoylguanidine derivatives were designed and synthesized as potent NHE1 inhibitors. Most compounds can significantly inhibit NHE1-mediated platelet swelling in a concentration-dependent manner, among which compound **5f** (IC_{50} = 3.60 nM) and **5l** (IC_{50} = 4.48 nM) are 18 and 14 times respectively more potent than cariporide (IC_{50} = 65.0 nM). Furthermore, when tested in vivo and in vitro, compound **5f** showed superior cardioprotective effects against SD rat myocardial ischemic-reperfusion injury over cariporide, representing a promising lead compound for further exploration.

The Na^+/H^+ exchanger (NHE) is an integral membrane protein ubiquitously expressed in mammalian cells. It regulates intracellular pH by removing a proton in exchange for an extracellular sodium ion. To date, nine isoforms of the exchanger have been identified and are designated NHE1–NHE9. NHE1 was the first isoform discovered and is ubiquitously expressed in the plasma membrane of mammalian cells. Other isoforms have more restricted tissue distributions and some have predominantly intracellular localization.[1,2] In mammals, NHE1 is the predominant isoform in heart and is involved in numerous physiological processes, including regulation of intracellular pH, cell-volume control, cytoskeletal organization, heart disease and cancer.[3,4] The activity of NHE1 is elevated in animal models of myocardial infarcts and in left ventricular hypertrophy.[5] During ischemia and reperfusion of the myocardium, NHE activity catalyzes increased uptake of intracellular Na^+. This in turn is exchanged for extracellular calcium by the Na^+/Ca^{2+} exchanger resulting in calcium overload and damage to the myocardium, such as myocardial infarction activation, stunning and tissue necrosis.[6,7]

NHE1 inhibitors, in their cation form, combine with NHE1 at the extracellular Na^+ binding site to competitively inhibit NHE1 function and reduce Na^+ and Ca^{2+} influx, and hence abolish the post-ischemia Ca^{2+} overload in myocardial cells and lower the risk of cell dysfunction and injury.

Aroylguanidine, a subunit typically presented in most of the known NHE1 inhibitors, such as cariporide and sabiporide (Fig. 1),[8] is well-considered as the possible pharmacophore among the reported NHE1 inhibitors.[9] Upon analyzing the structures of cariporide and sabiporide, we chose benzoylguanidine as core structure, and introduced 4-(2,3,4-trimethoxy-benzyl)-piperazin-1-ylmethyl group to the ortho, meta or para position of benzoylguanidine, and then synthesized our target compounds **5a**–**l** (Table 1).

The synthetic route for target compounds is depicted in Scheme 1. Substituted bromo-methyl benzoic acid ethyl esters **2a**–**l**, obtained by bromination of methyl (or ethyl)-substituted benzoic acid ethyl esters **1a**–**l**, were reacted with 1-(2,3,4-trimethoxy-benzyl)-piperazine (**3**, trimetazidine) to give intermediate **4a**–**l**. Treatment of **4a**–**l** with excessive guanidine in anhydrous THF or isopropyl alcohol afforded the free base of the target compounds **5a**–**l**. The crude bases were purified by silica gel column chromatography (petroleum ether/acetone, 2–5:1), followed by salt formation with saturated HCl (g) in anhydrous acetone.

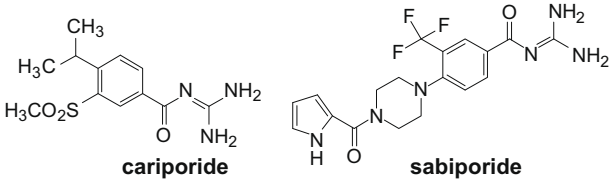

Figure 1. Structures of cariporide and sabiporide.

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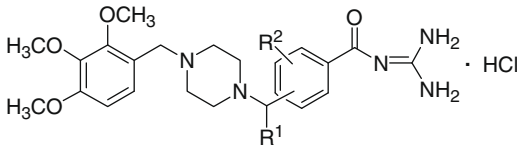
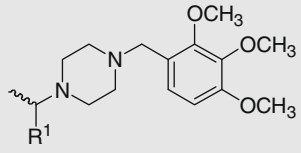
Table 1
NHE1 inhibitory activity of compounds **5a–l**



Compound	R^1	R^2	Position of 	IC_{50}[a] (nM)
Cariporide				65.0
5a	H	H	4	36.4
5b	H	H	3	36.5
5c	H	H	2	11.1
5d	CH_3	H	4	40.5
5e	H	3-Br	4	248
5f	H	3-NO_2	4	3.60
5g	H	4-NO_2	3	14.2
5h	CH_3	3-NO_2	4	18.7
5i	H	3-SO_2NH_2	4	11.5
5j	H	3-$SO_2NHC_3H_7$	4	1150
5k	H	3-SO_2CH_3	4	15.0
5l	CH_3	3-SO_2CH_3	4	4.48

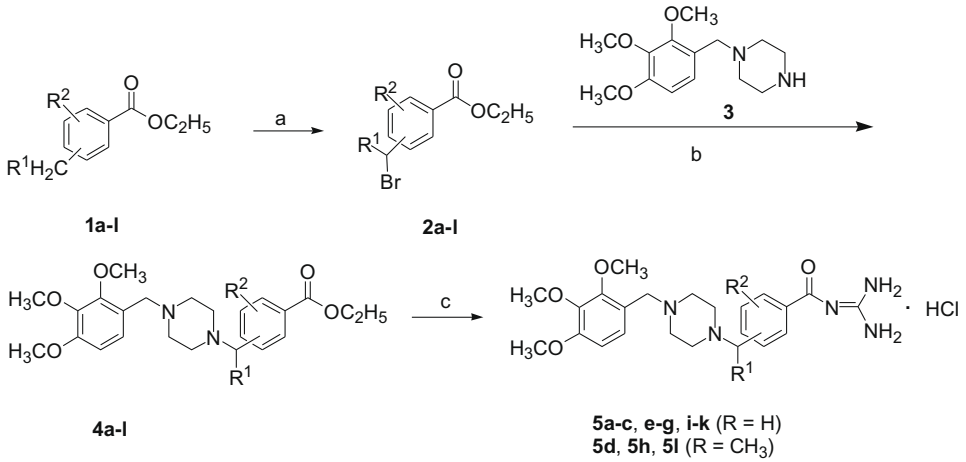
[a] Drug concentration to achieve half-maximal inhibition of acid-induced swelling in rat platelets.

According to the synthetic method of **5f** described in Scheme 1, 1 equiv of **4f** was needed to react with 20 equiv of guanidine in order to obtain moderate yield of crude **5f**.[10] The crude product could only be purified by silica gel column chromatography (petroleum ether/acetone, 2–5:1) two times to be free from guanidine. The improved synthetic route for compound **5f**[11] is depicted in Scheme 2. The crude ester **4f** was hydrolyzed with K_2CO_3 in MeOH/H_2O (V/V, 1:1) to give the corresponding acid **4f–1**, which is a zwitterionic compound and can be separated from the reaction mixture simply by adjusting its isoelectric point using 0.1 mol/L HCl. Compound **5f** can be obtained by reaction of the acid **4f–1** with 3 equiv of guanidine in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotrizole (HOBt),[12] and purified by silica gel column chromatography (petroleum ether/acetone, 3:1) one time to be free from by-products and guanidine.

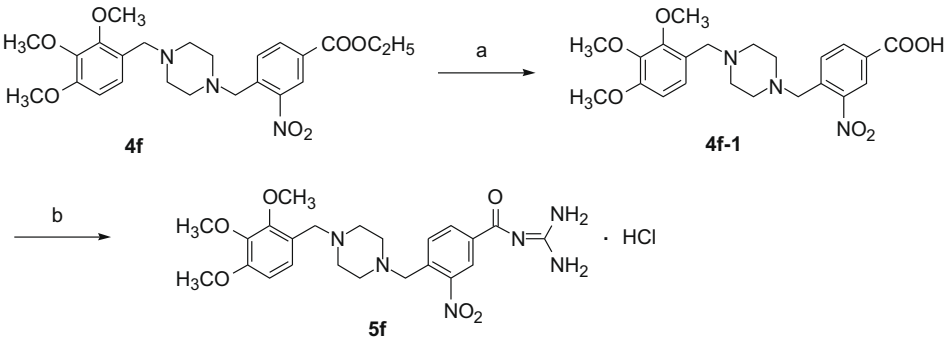
All synthesized compounds, along with reference compound cariporide, were evaluated in rat platelet swelling assay (PSA) for NHE1 inhibitory activity screening. The experiment procedure was similar as in the literature,[13] with only minor modifications. The IC_{50} of the tested compounds was obtained from the linear part of the relationship between the log concentration and NHE activity using linear regression analysis. The PSA results showed that most of the tested compounds did inhibit rat platelet NHE1 in a concentration-dependent manner. The potential of **5f** and **5l** is superior to that of cariporide. Preliminary structure–activity relationship analysis indicated that, compounds with electron-withdrawing group possess better NHE1 inhibitory activity. The introduction of nitro or methylsulfonyl group led to a significant increase in NHE1 inhibitory potency.

Next, compound **5f**, which showed a good NHE1 inhibitory activity, was evaluated for its cardioprotective efficacy against ischemia-reperfusion injury in vivo (Figs. 2 and 3). The protective effect was determined in rat heart coronary artery occlusion and reperfusion model.[14] After 45 min of occlusion, the coronary artery was reperfused by loosing the ligature for 90 min. **5f** (1 mg/kg, 0.5 mg/kg, 0.25 mg/kg), cariporide were intravenously given 5 min before reperfusion. The evaluation of cardioprotective effect was measured as an index of the ratio of myocardial infarction size to left ventricle area (IS/LV). The activity of creatine kinase (CK), lactate dehydrogenase (LDH), superoxide dismutase (SOD) and the content of malondialdehyde (MDA) in prepared serum were also measured as the indicators of myocardial injury. Compared with the model group, **5f** could significantly diminish the myocardial infarct size, decrease the release of CK and LDH, the content of MDA and increase the activity of SOD in serum. These protective effects were in a dose-dependent manner. There was no significant difference between **5f** (1 mg/kg) and cariporide (1 mg/kg) in the myocardial protective effect.

The protective effect of compound **5f** in vitro was also determined in improved Langendorff system (Figs. 4 and 5).[15] The isolated rat hearts were experienced a 20 min equilibration period, perfused at a low rate (0.5 ml/min) for 30 min, and then reperfused for 30 min. **5f** (50 μM, 10 μM, 2 μM), cariporide(10 μM) contained in perfusate were administered during the low rate perfusion period. For the evaluation of their cardioprotective effect, the ratios of the coronary flow at the beginning and the end of reperfusion to the end of equilibration value were measured. Additionally, the content of ATP, lactic acid (LD) and the



Scheme 1. Reagents and conditions: (a) NBS, benzoyl peroxide, CCl_4, reflux; (b) trimetazidine, $(C_2H_5)_3N$, CH_3CN, 20–80 °C; (c) (i) Guanidine, THF or isopropyl alcohol, reflux; (ii) satd HCl (g) in anhydrous acetone.



Scheme 2. Reagents and conditions: (a) K_2CO_3, MeOH/H_2O, reflux, 92%; (b) (i) EDCI, HOBt, guanidine, DMF, rt; (ii) satd HCl (g) in anhydrous acetone, 60%.

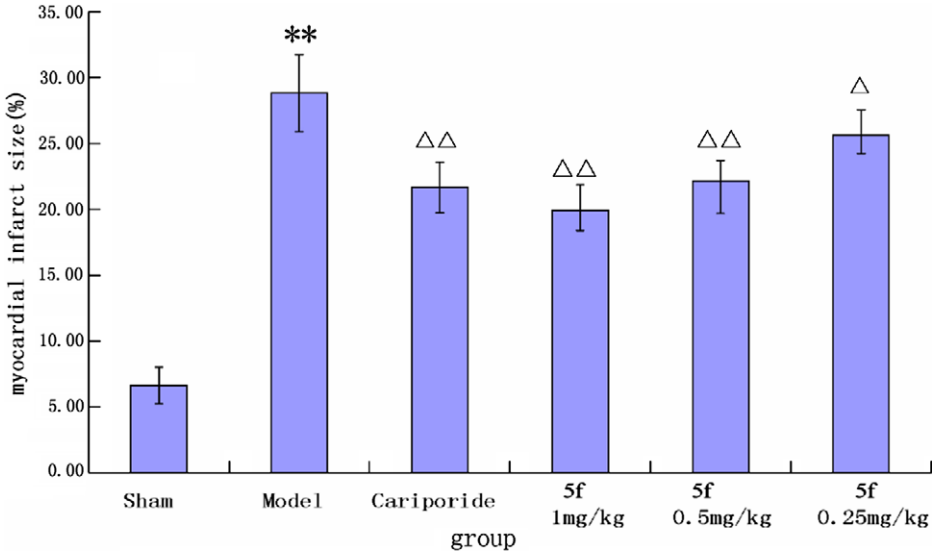


Figure 2. Effect of compound **5f** on myocardial infarct size in rats subjected to 45 min occlusion followed by 90 min reperfusion. Drugs were given by iv bolus at 5 min before reperfusion. The myocardial infarct size was expressed as a percentage of the left ventricle area (IS/LV%) ($n = 8$). $^{*}P$ <0.05, $^{**}P$ <0.01 versus sham group; $^{\triangle}P$ <0.05, $^{\triangle\triangle}P$ <0.01 versus model group.

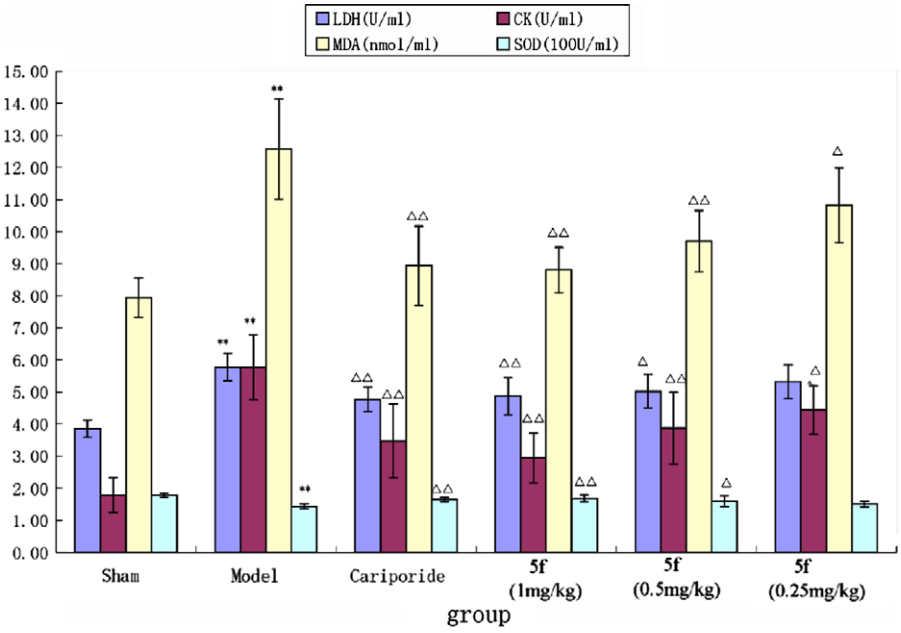


Figure 3. Effect of compound **5f** on LDH, CK, MDA and SOD included in the prepared serum of rats subjected to 45 min occlusion followed by 90 min reperfusion. Drugs were given by iv bolus at 5 min before reperfusion ($n = 8$). $^{*}P$ <0.05, $^{**}P$ <0.01 versus sham group; $^{\triangle}P$ <0.05, $^{\triangle\triangle}P$ <0.01 versus model group.

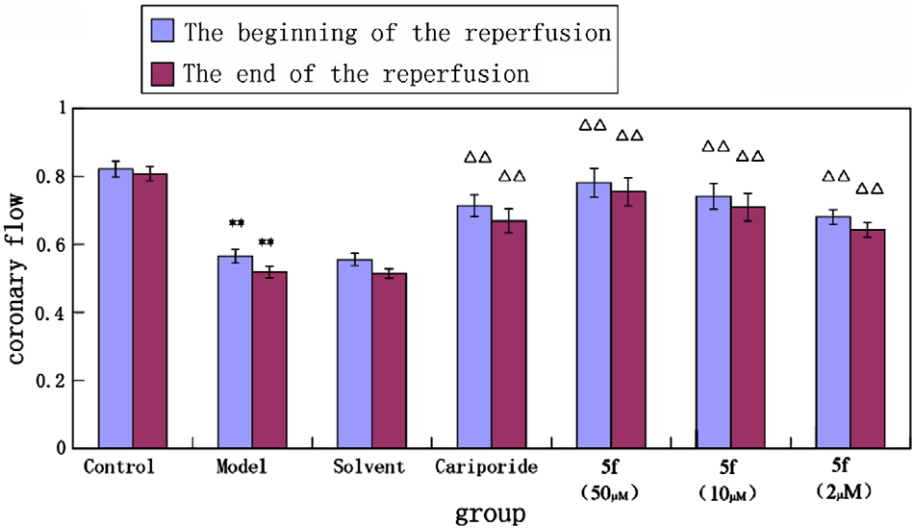


Figure 4. Effect of NHE1 inhibitor **5f** on coronary flow of isolated rat hearts subjected to 30 min low rate perfusion followed by 30 min reperfusion. Drugs were contained in the perfusate. The coronary flow at the beginning and the end of reperfusion was expressed as the ratio to the end of equilibration value ($n = 10$). $^{*}P$ <0.05, $^{**}P$ <0.01 versus sham group; $^{\triangle}P$ <0.05, $^{\triangle\triangle}P$ <0.01 versus model group.

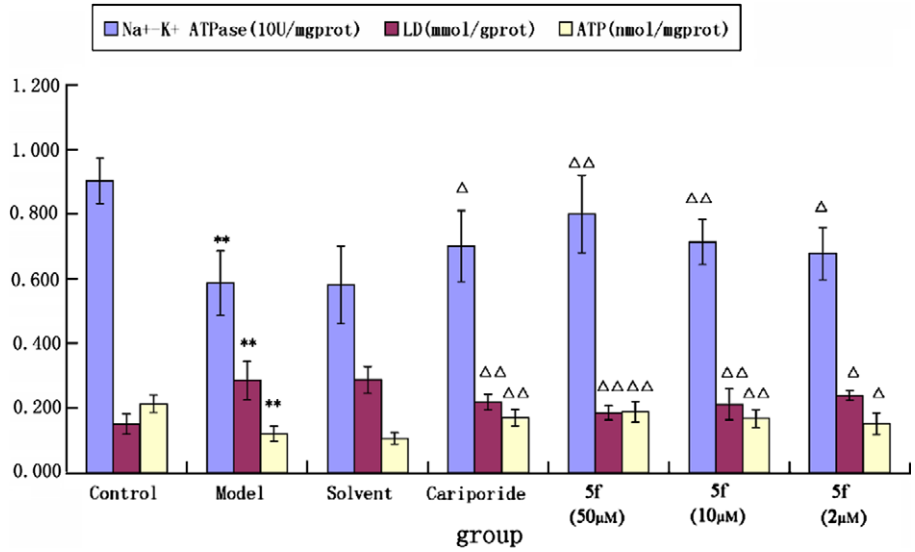


Figure 5. Effect of NHE1 inhibitor **5f** on the activity of Na^{+}–K^{+}ATPase, the content of LD and ATP included in the tissue homogenate of isolated rat hearts subjected to 30 min low rate perfusion followed by 30 min reperfusion ($n = 10$). $^{*}P$ <0.05, $^{**}P$ <0.01 versus sham group; $^{\triangle}P$ <0.05, $^{\triangle\triangle}P$ <0.01 versus model group.

activity of Na^{+}–K^{+} ATPase in prepared heart homogenate were measured to determine the cardioprotective effect. The testing results showed that, **5f** could significantly increase the coronary flow, the content of ATP, the activity of Na^{+}–K^{+} ATPase and reduce the content of LD in tissue homogenates. These effects of relaxing coronary artery and improving myocardial energy metabolism were in a dose-dependent manner. In improving myocardial energy metabolism, **5f** (10 μM) was better than cariporide(10 μM). In relaxing coronary artery, **5f** (50 μM) and **5f** (10 μM) showed better effect. But there was no significant difference between **5f** (10 μM) and cariporide (10 μM).

All values are expressed as mean ± S.E.M. Data were analyzed by one-way analysis of variance (ANOVA) and unpaired Student's *t*-test. In all comparison, the difference was considered to be statistically significant at P <0.05 and extremely significant at P <0.01.

In summary, a series of novel substituted benzoylguanidine derivatives were designed, synthesized and evaluated for their NHE1 inhibitions. Most compounds show more potent NHE1 inhibitory activity than cariporide, and the most potent one was **5f**. Further testing showed that, **5f** could excellently improve the cardiac function and reduce infarct size against ischemia-reperfusion injury. Preliminary structure–activity relationship showed that, the introduction of electron-withdrawing group led to an increase in NHE1 inhibitory potency. Further biological evaluation of these derivatives and extensive lead optimization are ongoing in our laboratory and will be reported in due course.

Acknowledgments

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10. To a solution of ethyl 4-methyl-3-nitrobenzoate (2.0 g, 9.5 mmol) in anhydrous CCl_4 (40 ml) was added NBS (2.15 g, 9.5 mmol) and benzoyl peroxide in catalystic amount. The mixture was heated to reflux for 8 h under light, then filtered and concentrated to give the bromide **2f**. To a mixture of compound **3** (1 g, 3 mmol) and Et_3N (4 ml) in anhydrous CH_3CN (15 ml) was added dropwise a solution of **2f** (0.8 g, 3 mmol) in anhydrous CH_3CN (10 ml) at room temperature. The reaction mixture was stirred at 50 °C for 3 h and filtered. The filtrate was evaporated in vacuo, and the residue was purified by column chromatography (petroleum ether/acetone, 7:1) to afford **4f**. Then, the mixture of **4f** (0.47 g, 1.0 mmol) and guanidine (1.1 g, 20 mmol) in THF (20 ml) was stirred under reflux for 5 h. The solvent was removed in vacuum, and the residue was purified by column chromatography (petroleum ether/acetone, 3:1). The product was dissolved in acetone, and followed by hydrochlorination with gaseous HCl to give **5f** as light yellow solid. Yield: 35%; mp: 230–233 °C; IR (KBr, cm^{-1}): 3401, 2936, 1703(C=O), 1612, 1582, 1543(NO_2), 1345(NO_2), 1106(OCH_3); ^1H NMR (500 MHz, $CDCl_3$): 2.53–3.29 (8H, m, –N⌒N–); 3.77 (3H, s, OCH_3); 3.82 (3H, s, OCH_3); 3.87 (3H, s, OCH_3); 3.94 (2H, s, CH_2); 4.20 (2H, s, CH_2); 6.88 (1H, d, ArH, *J* = 8.7 Hz); 7.26 (1H, d, ArH, *J* = 8.6 Hz); 7.93 (1H, d, ArH, *J* = 8.1 Hz); 8.47 (1H, dd, ArH, *J* = 8.0 Hz, *J* = 1.7 Hz); 8.64 (1H, d, ArH, *J* = 1.7 Hz); 8.50 (3H, br s, NH); 8.70 (3H, br s, NH); 9.94 (1H, br s, NH); 12.36 (1H, br s, NH). MS (ESI(+)70 V, *m/z*): 487$(M+H)^+$.
11. To a solution of **4f** (1.0 g, 2.1 mmol) in 30 ml MeOH/H_2O (1:1) was added K_2CO_3 (0.87 g, 6.3 mmol), and the mixture was heated under reflux for 1.5 h. The methanol was removed in vacuo, and the residue was neutralized with 0.1 mol/L hydrochloric acid until pH value reached 5–6, then filtrated to give carboxylic acid **4f**–**1** (0.89 g, 92%). Without further purification, **4f**–**1**(0.3 g, 0.67 mmol), EDCI (0.13 g, 0.67 mmol) and HOBt (0.09 g, 0.67 mmol) were added to anhydrous DMF (4 ml), then guanidine (0.12 g, 2.0 mmol) were added at room temperature. The reaction was allowed to stir for 5 h, then diluted with 20 ml H_2O, extracted with EtOAc (50 ml). The organic layers was washed with brine, dried (Na_2SO_4), evaporated under reduced pressure, and purified by column chromatography (petroleum ether/acetone, 3:1). The resulting oil was dissolved in acetone and treated with gaseous HCl to afford **5f**. Yield: 60%; mp: 231–233 °C.
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