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2-Trifluoroacetylthiophene oxadiazoles as potent and selective class II human histone deacetylase inhibitors

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ABSTRACT

Trifluoroacetylthiophene carboxamides have recently been reported to be class II HDAC inhibitors, with moderate selectivity. Exploration of replacements for the carboxamide with bioisosteric pentatomic heteroaromatic like 1,3,4-oxadiazoles, 1,2,4-oxadiazoles and 1,3-thiazoles, led to the discovery that 2-trifluoroacetylthiophene 1,3,4-oxadiazole derivatives are very potent low nanomolar HDAC4 inhibitors, highly selective over class I HDACs (HDAC 1 and 3), and moderately stable in HCT116 cell culture.

Histone deacetylase (HDAC) and histone acetyltransferase (HAT) enzymes regulate the acetyl removal/addition to the N-terminal Lys residues on histones. The acetylation status of histones is directly related to gene expression, with hyperacetylation being associated with a less condensed chromatin state, a conformation that induces activation of gene transcription.

HDACs are also involved in the deacetylation of other non-histone proteins (HSP90, α-tubuline), and in interactions with transcription factors and nuclear receptors (p53, Erα).[1]

The involvement of HDACs in regulating cell proliferation, together with the experimental evidence that inhibition of HDACs induces growth arrest or apoptosis in various tumor cell lines,[2] has highlighted HDACs as an appealing target for cancer therapy.

The 18 known human HDACs can be divided into four classes: class I (HDAC1, 2, 3 and 8), class II, which can be further divided into the two subclasses HDAC IIa (HDAC 4, 5, 7, 9) and IIb (HDAC 6 and 10) and class IV (HDAC11) are Zn-dependent HDACs, while class III, or Sirtuins (SIRT1-7), are structurally unrelated NAD-dependent deacetylases.

Class I, II and IV HDACs are sensitive to the classical HDAC inhibitor trichostatin A (TSA), whereas those of class III are insensitive to this inhibitor.[1b]

The cellular function of each HDAC class is not fully understood and also the specific role in the antitumor activity of the single isoforms is still not completely deciphered. Notwithstanding, the urgency for alternative therapies for cancer has allowed a rapid development of broad spectrum HDAC inhibitors. Indeed, most of the HDAC inhibitors that are known or in clinical trials are unselective, or are just partially selective for one class of HDACs[2,3] (e.g. Vorinostat, formerly SAHA, approved by the FDA in 2006, is an inhibitor of HDAC1, 2, 3 and 6).[4] Elucidation of the function of each HDAC subtype would potentially address toxicity issues and clinical adverse effects of HDAC inhibitors. Consequently, there remains a widely recognized need to identify selective HDAC inhibitors.[5] Selective HDAC inhibitors would also provide more focused cancer therapies and new clinical opportunities. For instance, it has been demonstrated for HDAC inhibitors to be additive or synergistic with conventional anticancer chemotherapeutics and to produce cellular sensitization to ionizing radiation.[6] The former seems to be correlated to the inhibition of class I[7] HDACs, while studies on modulation of cellular response to radiation provide evidence of class II HDAC involvement, implying a role for the HDAC4 isoform.[8]

In our company a research program was initiated aiming at developing a portfolio of different chemotypes, each with a different HDAC selectivity profile. This effort led to the discovery of potent and selective class I HDAC inhibitors, belonging to various chemical classes, including methyl ketone **A** (Fig. 1)[9] and aminobenzamides **B**[10] and **C**,[11] which have been demonstrated to cause tumor growth inhibition in a HCT-116 xenograft model.

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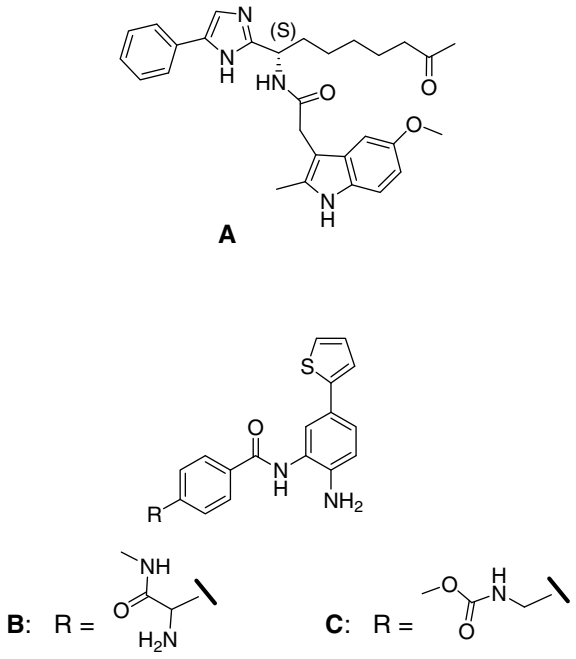


Figure 1. Class I selective HDAC inhibitors.

Research directed at the identification of selective class II HDAC inhibitors, targeting in particular HDAC4, led to the discovery that 5-(trifluoroacetyl)thiophene-2-carboxamides of general structure **1** (Fig. 2) [12] are active site class II HDAC inhibitors,[13] with moderate selectivity over class I HDACs. SAR studies on the amide moiety of **1** showed that various substitutions are tolerated.

The lack of cellular activity displayed by these inhibitors can be rationalized in terms of the metabolic instability of the trifluoroacetyl ketone moiety of **1**. This functionality is readily reduced in the presence of HCT116 cells to the corresponding alcohol, which is inactive against HDAC4.[12,14] The half-life of the amide inhibitors **1** was shown to vary depending on the nature of the amide substituents, and to be in the range of 20 min to 2 h.

The modest selectivity and the instability in cells prompted us to seek alternative structures, aiming to obtain more potent and selective HDAC4 inhibitors. Structural modification could also possibly address the metabolic instability issue by reducing the molecule recognition by the intracellular carbonyl reductases, thus decreasing the susceptibility of the trifluoroacetyl group towards reduction.

We knew that, among amide substitutions, the benzyl and heterobenzyl substitutions produced the most active compounds when screened on class II HDACs (**2**, Fig. 2).[12] The simplest benzyl derivative **3** (Table 1) showed basically no selectivity over class I HDACs (HDAC1 and 3). The presence of a methyl in the benzylic position was tolerated (**4** and **5**), showing a marked difference between the two enantiomers, the (S)-enantiomer displaying a higher selectivity for HDAC4 over HDAC1 and 3.

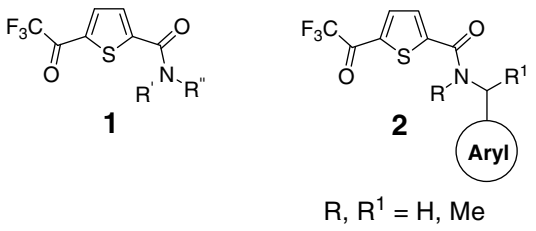
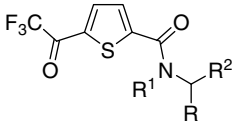


Figure 2. Trifluoroacetylthiophene 2-carboxamides class II HDAC inhibitors.

Table 1
Inhibitory activity of trifluoroacetylthiophene carboxamides against HDAC 4, 1, 3 and 6



Compound	R	R^1	R^2	HDAC IC_{50}[a] (nM)			
				4WT[b]	1	3	6
3	Ph	H	H	243	467	520	370
4	Ph(R)	H	Me	76	320	230	360
5	Ph(S)	H	Me	170	5300	9400	210
6	Ph	Me	H	510	1800	1200	230
7	Me	H	H	1700	7100	>10000	310

[a] Values are means of >2 experiments.
[b] His-tagged HDAC4 catalytic domain (653–1084) from *Escherichia coli* (Ref. 15).

Methylation of the amide nitrogen as in **6** was also tolerated, suggesting that the NH was not essential for HDAC4 activity. The simple ethylamide **7** proved to be only a weak inhibitor of HDAC4 and a submicromolar HDAC6 inhibitor.

Based on the above observations, we reasoned that it should be possible to substitute the amide bond in **8** (Fig. 3) with pentatomic heteroaromatic bioisosteres such as 1,2,4-oxadiazoles **9a**, 1,3,4-oxadiazoles **9b** and 1,3-thiazole **9c**.

We were pleased to see that 1,2,4-oxadiazole **10a** retained HDAC4 potency, and displayed appreciable selectivity over HDAC1 and 3. Submicromolar activity on HDAC6 was also detected. The isomeric 1,3,4-oxadiazole **10b** proved to be 4-fold less active than **10a** on HDAC4 WT and less selective, and thiazole **10c** was only a weak class II HDAC inhibitor.

The trend of 1,2,4-oxadiazole being a more potent HDAC4 inhibitor than 1,3,4-oxadiazole was confirmed for other pairs of isomers, such as **11a**–**b** and **12a**–**b**. This evidence prompted us to concentrate our efforts on a wider investigation of the 3-position of the oxadiazole nucleus in the 1,2,4-oxadiazole series (Table 3), with the aim to improve activity and selectivity.

Aromatic substitutions proved to be tolerated both in terms of the position and the nature of the substituents, as demonstrated by compounds **13-18** (Table 3). 4-*t*-Bu derivative (**19**) showed micromolar activity on all tested HDAC isoforms.

The introduction of heteroaryl moieties as R, (such as in **20** and **21,** R = 2-pyridyl and 3-pyridyl, respectively), essentially retained the HDAC4 activity of phenyl analog **10a**, with somewhat reduced selectivity over HDAC1. This is in line with what was observed previously for thiophene derivative **12a**. Isomeric 4-pyridyl derivative **22** displayed a marked drop in HDAC4 potency, though maintaining the same level of activity on HDAC6.

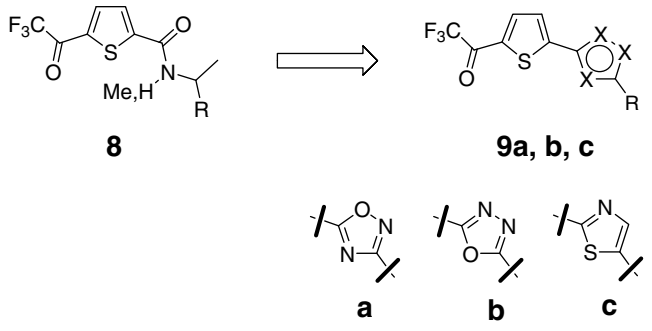
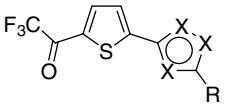


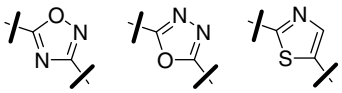
Figure 3. Isosteres of the alkylamide moiety of trifluoroacetylthiophene carboxamides: 1,2,4-oxadiazole, 1,3,4-oxadiazole, 1,3-thiazole derivatives.

Interestingly, a methylene spacer between the oxadiazole core and thiophene (**23**) proved very favorable (compare **23** with **12a**). **23** displayed 15 nM activity on HDAC4, and a 70-fold selectivity with respect to HDAC1, maintaining high HDAC6 activity. This result prompted us to further explore alkyl substitutions, and some of the most relevant results are reported in Table 3. A variety of different functionalities provided HDAC4 active compounds, such as alkylamide **24** and benzoxazolone **25**, which showed excellent activity on HDAC4 (IC_{50} = 15 nM), high selectivity over HDAC1 (>75-fold), and a >20-fold class IIa selectivity. Phenoxy derivative **26** proved to be the most potent HDAC4 inhibitor and displayed the highest selectivity in the series (>600-fold and >100-fold, respectively, HDAC1/HDAC4 and HDAC6/HDAC4 selectivity). Alkyl and aryl sulfones were also favorable substitutions, providing potent and selective HDAC4 inhibitors, such as **27** and **28** (HDAC4 IC_{50} = 30 nM, >70-fold HDAC4 *vs* HDAC1 selectivity), sulfonylthiophene derivative **29** (>100-fold HDAC4/HDAC1 selectivity) and benzyl sulfone **30**. Surprisingly, we discovered that the simple methyl oxadiazole **31** was a very potent HDAC4 inhibitor and highly selective over HDAC1. This result, in a head-to-head comparison with the corresponding carboxamide **7** (Table 1), confirmed the high potential of the oxadiazole series as potent and selective HDAC4 inhibitors.

The most active/selective compounds were then analyzed for metabolic stability in cellular S9 fraction,[14] which has been demonstrated to be a fast and reliable test to rank stability towards reduction of the trifluoroacetyl moiety to the alcohol. Pleasingly, some of the oxadiazoles tested (Table 4) showed a half-life significantly higher than that observed for amide derivatives (amide **6** as reference),[15,16] ranging from 18–20 min (**25**, **26**) to more than 2 h (**17**, **27**, **31**). Compounds **27–30**, which showed half-lives ranging from 35 to >120 min in the S9 preliminary test, and high potency on HDAC4 (IC_{50}: 30–60 nM), were then tested in the standard HCT116 cell based assay conditions (in the presence of 10% FBS, Table 4). The data obtained confirmed the stability trend observed in

Table 2
Comparison of 1,3,4-oxadiazoles, 1,2,4-oxadiazoles and 1,3-thiazole activities on HDACs



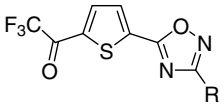


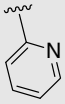
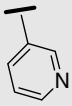
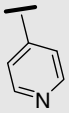
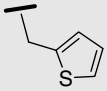
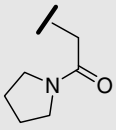
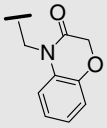
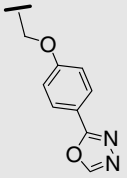
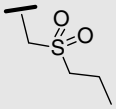
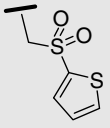
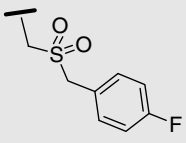
a b c

Compound	het	R	HDAC IC_{50} (nM)[a]			
			4WT	1	3	6
10	a	Ph	110	7400	>1000	580
10	b	Ph	420	2970	500	330
10	c	Ph	1500	>10000	nd	1350
11	a	4-Me-Ph	110	6800	nd	860
11	b	4-Me-Ph	270	1800	>1000	600
12	a		95	2900	nd	670
12	b		450	2600	500	160

[a] See notes a–b in Table 1.

Table 3
HDAC activity of 1,3,4-oxadiazole derivatives



Compound	R	HDAC IC_{50}[a] (nM)			
		4WT	1	3	6
13	3-Me-Ph	280	6540	>1000	680
14	3-CF_3-Ph	320	17500	>1000	2450
15	4-CF_3-Ph	200	>10000	nd	11000
16	4-F-Ph	220	5900	>1000	270
17	2,4-diF-Ph	120	4200	>1000	580
18	2,4-diCl-Ph	240	>5000	nd	3000
19	4-*t*Bu-Ph	>2000	16600	>1000	3540
20		90	2800	nd	500
21		70	2200	nd	840
22		590	>10000	nd	570
23		15	990	630	170
24		60	5900	nd	710
25		15	1170	>1000	410
26		7	4800	>1000	760
27		30	2470	>1000	320
28		30	2220	>1000	240
29		35	3890	>1000	520
30		60	6550	>1000	700
31	Me	75	7600	7100	650

[a] See notes a–b in Table 1.

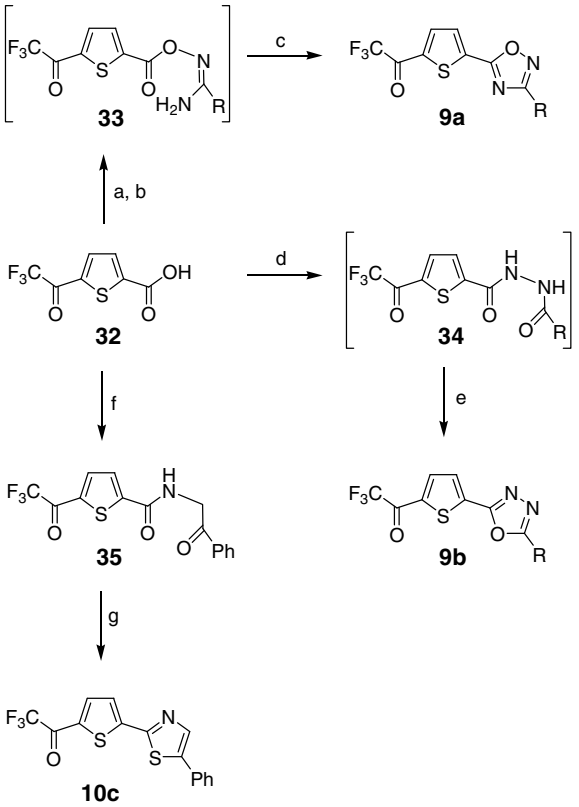
the S9 preliminary test, leading to the identification of compounds with half-life longer than 10 h, such as **27** (half-life 13 h).

As the cellular substrate for HDAC4 is unknown, no cellular target engagement assay was available. HDAC6 has been demonstrated to be a specific α-tubulin deacetylase,[17] so that α-tubulin hyperacetylation in HCT116 cells was used as surrogate activity marker.[12] Two of the most stable compounds from Table 4, **27** and **28**, were tested for the inhibition of α-tubulin deacetylation and showed values (**27**: IC_{50} = 640 nM, **28**: IC_{50} = 500 nM) that were compatible with the corresponding values for HDAC6 inhibition reported in Table 3, thus confirming the ability of these inhibitors to inhibit class II HDACs in cells.

Table 4
Stability of 1,3,4-oxadiazole derivatives in the presence of S9 fraction and HCT116 cells

Compound	Half life	
	S9[a] (min)	HCT116[b] (h)
6	15[c]	
17	>120	
23	35	
25	25	
26	18	
27	>120	13
28	45	12
29	44	6
30	35	5.5
31	>120	

[a] S9 fraction of HCT116 cells, in the presence of NADPH and regenerating system (Ref. 14).
[b] Compounds (5 μM) were incubated with HCT116 cells in cell culture medium containing 10% FBS (Fetal Bovine Serum) (Ref. 14).
[c] Ref. 14.



Scheme 1. Synthesis of compounds in Tables 2 and 3. Reagents and conditions: (a) CDI, DMF, rt, 30 min; (b) R-C(NOH)NH_2, rt, 12 h; (c) CDI, DMF, μw, 140 °C, 2 min; (d) PS-carbodiimide, DCM/DMF, R-$CONHNH_2$, rt, 12 h; (e) $SOCl_2$, μw, 100 °C, 5 min; (f) PS-carbodiimide, DCM, 2-aminoacetophenone hydrochloride, TEA, rt, 12 h; (g) Lawesson's reagent, toluene, μw, 100 °C, 10 s.

All the derivatives described in Tables 2 and 3 were prepared as shown in Scheme 1 from the common intermediate 5-(trifluoroacetyl)thiophene-2-carboxylic acid **32**.[18] To obtain the 1,2,4-oxadiazoles **9a**[19] a modification of a literature procedure was used.[20] Acid **32**, upon activation with 1,1'-carbonyldiimidazole, followed by treatment with the appropriate amidoxime, provided *O*-acyl amidoxime **33**, which was dehydrated in one pot by treatment with a further equivalent of CDI and microwave irradiation for 2 min to give **9a**.

When **32** was treated with the appropriate acyl hydrazide and polymer-supported carbodiimide in amide coupling conditions, intermediate **34** was obtained, which was cyclized to the corresponding 1,3,4-oxadiazoles **9b**[21] by treatment with thionyl chloride under microwave irradiation conditions. Thiazole **10c**[22] was prepared reacting amide **35** with Lawesson's reagent in toluene under microwave irradiation.

In conclusion, substitution of the carboxamide moiety of trifluoroacetylthiophene carboxamides HDAC4 inhibitors with bioisosteric pentatomic heteroaromatic 1,2,4-oxadiazole, 1,3,4-oxadiazole and 1,3-thiazole led to the discovery of 1,2,4-oxadiazole derivatives as potent low nanomolar inhibitors of HDAC4, highly selective with respect to class I HDAC1 and 3 isoforms and with appreciable subclass (class IIa/IIb) selectivity. Some of the oxadiazole inhibitors displayed significant stability in the presence of HCT116 cells and oxadiazole **27** demonstrated good inhibition of class II HDAC, HDAC6, in cells.

These compounds represent an important tool to elucidate the role and possible therapeutic implications of HDAC4 as a target in cancer therapy.

Acknowledgments

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18. Carboxylic acid **32** was obtained by $LiOH/H_2O$/MeOH hydrolysis of the corresponding ethyl ester (purchased from Rieke).
19. As an example, synthesis of **27**: To 5-(trifluoroacetyl)thiophene-2-carboxylic acid **32** in DMF (2 mL/mmol) 1,1′-carbonyldiimidazole (1.1 equiv) in DMF (1 mL/mmol), was added. The mixture was stirred at room temperature for 30 min, then *N*-hydroxy-2-(methylsulfonyl)ethanimidamide (1.1 equiv) in anhydrous DMF (2 mL/mmol) was added and the mixture stirred at room temperature overnight. The intermediate formed was not isolated, and reacted by addition of CDI (1.1 equiv) in DMF (1 mL/mmol) and heating (Biotage Initiator60 microwave apparatus, sealed tube, 140 °C, 2 min). From the reaction mixture the product was isolated (yield: 34%) by preparative RP-HPLC, using water (0.1% TFA) and acetonitrile (0.1% TFA) as eluents (column: C18). ^{1}H NMR (300 MHz, DMSO-d_6, 300 K): δ 8.22 (s, 2 H), 4.98 (s, 1 H), 3.21 (s, 3 H), hydrate form 8.34 and 8.33 (2s, 0.18H), 7.98 (d, J = 4 Hz, 0.15 H), 7.38 (d, J = 4 Hz, 0.16 H). ^{19}F NMR proton decoupled (282 MHz, DMSO-d_6, 300 K): δ −75.04 (s, 2.3F, keto form), −86.42 (s, 0.7F, hydrate form). MS m/z (EI+): 341 $(M + H)^+$, 359 $(M + 18 + H)^+$. MS m/z (ES-): 339 $(M + e)^-$, 357 $(M + 18 + e)^-$.
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21. As an example, synthesis of **10b**: A solution of 5-(trifluoroacetyl)thiophene-2-carboxylic acid **32** in DCM (18 mL/mmol) was added to PS-carbodiimide (1.7 equiv), and the suspension stirred at room temperature for 30 min. Benzoic hydrazide (1.3 equiv) dissolved in DCM/DMF 1:1 (3 ml/mmol) was added, and the resulting suspension was stirred at room temperature overnight. The suspension was filtered and the filtrate evaporated. Crude carboxyhydrazide **34** was used as such. It was dissolved in thionyl chloride (9 mL/mmol) and the solution was heated (Biotage Initiator60 microwave apparatus, sealed tube, 100 °C, 5 min), then the thionyl chloride was evaporated. From the reaction mixture **10b** was isolated (yield: 15%) by preparative RP-HPLC, as reported in Ref. 19 ^{1}H NMR (300 MHz, DMSO-d_6, 300 K): δ 8.25 (s, ^{1}H), 8.21–8.05 (m, 3 H), 7.75–7.60 (m, 3 H). ^{19}F NMR proton decoupled (282 MHz, DMSO-d_6, 300 K): δ −74.09 (s, 2.8F, keto form), −85.56 (s, 0.2F, hydrate form). MS m/z (EI+): 325 $(M + H)^+$, 343 $(M + 18 + H)^+$. MS m/z (ES-): 323 $(M + e)^-$.
22. Synthesis of **10c**: To a solution of 5-(trifluoroacetyl)thiophene-2-carboxylic acid **32** in DCM (22 mL/mmol) was added to PS-carbodiimide (2 equiv), and the suspension stirred at room temperature for 30 min. A mixture of 2-aminoacetophenone hydrochloride (1.1 equiv) in DCM (1.5 mL/mmol) and TEA (1.1 equiv) was added and the mixture stirred at room temperature overnight. The suspension was filtered, the filtrate evaporated and the residue purified by flash chromatography (silica gel/dichloromethane) to isolate **35** (yield: 68%). ^{1}H NMR (300 MHz, CD_3CN, 300 K): δ 8.1–8.0 (m, 3 H), 7.8–7.50 (m, 5 H), 4.85 (d, J = 6 Hz, 2 H). A mixture of the above amide in toluene (24 mL/mmol) and Lawesson's reagent (4 equiv) was heated (Biotage Initiator60 microwave apparatus, sealed tube, 100 °C, 10 s), then the reaction mixture was purified by flash chromatography (silica gel, gradient of petroleum ether/AcOEt) to provide **10c** (yield: 50%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K): δ 8.03 (s, 1 H), 7.93–7.85 (m, 1 H), 7.61–7.55 (m, 3 H); 7.48–7.34 (m, 3 H). MS m/z (EI+): 340 (M + H)+, 358 $(M + 18 + H)^+$.