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Efficient Synthesis of ESI-09, A Novel Non-cyclic Nucleotide EPAC Antagonist

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Abstract

A concise and efficient synthetic approach to producing a novel non-cyclic nucleotide EPAC antagonist **ESI-09** and its new analogs is reported. Key features of the synthesis include a mild and reliable one-pot procedure for an isoxazole synthon, as well as a modified one-pot protocol for the cyanomethyl ketone key intermediate. The synthesis requires inexpensive starting materials and only three linear steps for the completion in a total yield of 53%.

Keywords

EPAC antagonist; ESI-09; isoxazole; modified Kowalski's protocol; cyanomethyl ketone

Pancreatic ductal adenocarcinoma (PDA) is one of the leading causes of cancer death.[1] Despite extensive research over the years in terms of therapeutic strategies, no effective treatment has come forward for this universally lethal disease.[2] It is urgently needed to develop novel chemical probes and effective therapeutics that are based on new insights with better understanding of the molecular mechanism of pancreatic cancer. The exchange protein directly activated by cAMP (EPAC),[3,4] especially EPAC1, is over-expressed in human PDA specimens, but the functional roles of this over-expression have never been investigated.[5] Our team has previously reported the identification and characterization of a high throughput screening hit **ESI-09** (**1**, chemical structure shown in Figure 1), a non-cyclic nucleotide small molecule that specifically inhibits EPAC1 and EPAC2.[6,7] Using **ESI-09** as a small molecular chemical probe, we demonstrated that EPAC1 plays an important role in pancreatic cancer cell migration and invasion, suggesting EPAC1 may represent an attractive target for novel therapeutic strategies for treating PDA.[7]

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The authors declare no competing financial interest.

Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:xxx.

In our ongoing research to develop small molecules with high potency, specificity, and druglike properties based upon **ESI-09**, it is imperative to establish a practical and efficient method to readily access **1** suitable for a large scale as a reference compound as well as to further evaluate its pharmacological properties *in vivo*. Herein, we describe our optimized synthetic approaches to **1** starting from pinacolone in high overall yields.

Our retrosynthetic strategy is outlined in Scheme 1. Coupling of the key intermediate **8** with 3-chlorobenzenediazonium chloride **10** was expected to afford the desired product **1**.[8,9] Cyanomethyl derivative **8** could be formed from ethyl ester **6**.[10] Isoxazole **6** would be derived from the commercially available pinacolone **2** and oxalic acid diethyl ester **3**.[11]

We first prepared the 3,5-disubstituted isoxazole **6** in two steps (Scheme 2). Starting from the commercially available and inexpensive pinacolone **2**, oxalic acid diethyl ester **3** and NaH (60%), β-diketone **4** was readily prepared in 75% yield after purification. Further treatment of β-diketone **4** with hydroxylamine hydrochloride for about 2 h at room temperature afforded the intermediate oxime **5**. Upon stirring at 75 °C for another 4 h, followed by simple extraction procedures and short column silica gel chromatography, the desired product **6** was obtained in 90% yield. Despite the good yields for preparation of isoxazole **6**, this two-step procedure required two column purification steps. Based on the reagents which were used in this procedure, we reasoned that we could use hydroxylamine hydrochloride directly without further purification of β-diketone **4**. In this event, treatment of the reaction solution of β-diketone **4** with the solution of hydroxylamine hydrochloride in ethanol afforded the desired product **6** after refluxing at 75 °C for 24 h. To our delight, we found that only a single clear spot was detectable by TLC. After short column silica gel chromatography, isoxazole **6** was obtained in 87% yield.

Next, we followed Kowalski's protocol[12] to synthesize the key intermediate **7**, which was carried out at -78 °C in the presence of 2.0 equiv of LDA and 2.0 equiv of dibromomethane (Scheme 3). Based upon our findings, the reaction was incomplete after 1 h, and the product was a complex reaction mixture of several components. After purification with silica gel column, the desired bromomethyl ketone **7** was only obtained in 6% yield and we also isolated the trace amount of the dibromo by-product **11** (Table 1, entry 1). We then investigated the different bases for this reaction and the results showed that MeLi was the best base (entry 3), but the yield was still low (only 25% yield). It was noteworthy that this modified condition (entry 3) resulted in a clear reaction while approximately 50% of the starting material was recovered and the by-product **11** was also isolated in 25% yield. To improve the yield of this reaction, we increased the amount of MeLi and extended the reaction time from 1 h to 2 h (entry 4 to 6). To our delight, the reaction was also clear and the desired product **7** was obtained in 73% yield by using 4.0 equiv of MeLi (entry 6). Considering the mechanism of this reaction,[12] we reasoned that 1.2 equiv of dibromomethane would be enough for this reaction. As expected, the desired product **7** was obtained in 84% yield under an optimized condition by using 1.2 equiv of dibromomethane and 3.0 equiv of MeLi (entry 7).

With bromomethyl ketone **7** in hand, we used trimethylsilyl cyanide (TMSCN) as cyanide source to covert **7** into cyanomethyl derivative **8**.[13] In the presence of tetrabutylammonium fluoride (TBAF), cyanomethyl ketone **8** was smoothly generated in the solvent of CH_3CN at room temperature for about 3 h (Scheme 4). This reaction was clear with only a small polar spot observed via TLC. However, it was noteworthy that cyanomethyl ketone **8** could decompose upon purification by standard column chromatography (unstable in silica gel column or neutral Al_2O_3 column) and therefore, we used this intermediate directly for the next step without further purification.

Aryldiazonium salt **10** was prepared from 3-chloroaniline **9** by using sodium nitrite in the presence of hydrochloric acid at 0 °C for 2 min. Direct coupling of the crude cyanomethyl ketone **8** with aryldiazonium chloride **10** afforded the desired product **1** in 51% yield in two steps (Scheme 5). The chemical structure of **1** was further confirmed by single-crystal X-ray structural analysis.[14]

Despite the synthesis of **ESI-09** in moderate yields (an overall yield of 37% in four steps), we attempted to shorten the synthetic route by means of exploring one-pot procedure for converting isoxazole **6** to cyanomethyl ketone **8** according to our modified Kowalski's protocol for bromomethyl ketone (Table 1, entry 7). Replacing CH_3CN with dibromomethane, we found that **6** was converted to **8** in high yield by using 2.0 equiv MeLi as the base (Scheme 6).[15] Consistent with experimental observations from the synthesis of bromomethyl ketone **7**, it was noted that MeLi is the optimal base for preparation of ketonitrile **8** (less than 50% yields by using *n*-BuLi or LDA as the base under the same reaction condition). Using the same subsequent coupling reaction, desired product **1**[16] was obtained in 61% yield in two steps from isoxazole **6**. To further explore the synthetic potential for generating **ESI-09** analogs using this optimized approach, a series of new derivatives **12a-d** (Scheme 7) have been synthesized in good yields (62-76%, two steps).

In conclusion, we have developed a straightforward method to synthesize **1** starting from pinacolone in an excellent overall yield of 53% in three steps. The efficient approach included an one-pot procedure for the isoxazole synthon, followed by a modified protocol for the key cyanomethyl ketone and the subsequent coupling step. The described synthetic strategies and optimized reaction conditions may have general applications in the convenient preparation of various isoxazole and cyanomethyl ketone building blocks. Moreover, the established concise and efficient synthesis of the novel EPAC antagonist **ESI-09** will facilitate its further *in vitro* and *in vivo* pharmacological evaluations, as well as the ongoing synthesis of new analogs for the structure-activity relationship study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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14. The crystallographic data have been deposited in full at the Cambridge Crystallographic Data Centre, reference CCDC 913321, accessible at http://www.ccdc.cam.ac.uk.

15. ^{1}H and ^{13}C NMR spectra (see spectra of crude product in the Supporting Information) showed that the crude residue was only contaminated with some solvent impurities and suitable for direct use in the next step.

16. One-pot synthesis of 5-*tert*-butylisoxazole-3-carboxylic acid ethyl ester (**6**). To a solution of pinacolone (5.0 g, 50.0 mmol) in THF (100 mL) was added NaH (60%) (2.2 g, 55.0 mmol) at 0 °C. The reaction mixture was stirred at r.t. for about 30 min. Diethyl oxalate (7.3 g, 50.0 mmol) was added at 0 °C and the mixture was then stirred at r.t. overnight. To the resulting solution, hydroxylamine hydrochloride (3.8 g, 55.0 mmol) in ethanol (100 mL) was added dropwise. The mixture was heated at reflux for 16 h. After this time the sodium chloride was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was purified by short column chromatography on silica gel eluting with hexane/ethyl acetate (4/1) to provide the title compound as a colorless oil (8.57 g, 87%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.37 (s, 1H), 4.43 (q, 2H, J = 7.2 Hz), 1.41 (t, 3H, J = 7.2 Hz), 1.37 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 183.3, 160.5, 156.2, 99.3, 62.1, 33.1, 28.9, 14.3. HRMS (ESI) calcd for $C_{10}H_{16}NO_3$ 198.1125 $(M + H)^+$, found 198.1131.2-Bromo-1-(5-*tert*-butylisoxazol-3-yl)ethanone (**7**). To a solution of 5-*tert*-butylisoxazole-3-carboxylic acid ethyl ester (0.5 g, 2.5 mmol) in anhydrous tetrahydrofuran (10 mL) was added dibromomethane (0.52 g, 3.0 mmol) under nitrogen. The mixture was cooled to -78 °C and 1.6 M methyllithium in diethyl ether (4.7 mL, 7.5 mmol) was added dropwise. The solution was stirred at -78 °C for 40 min and then quenched with acetic acid (0.45 g, 7.5 mmol). The mixture was warmed to 0 °C and poured onto ice/water (20 mL) and extracted with ethyl acetate (50 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by short column chromatography on silica gel eluting with hexane/ethyl acetate (4/1) to provide the title compound as a colorless oil (524 mg, 84%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.40 (s, 1H), 4.57 (s, 2H), 1.38 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 185.7, 183.9, 159.7, 97.7, 33.2, 31.7, 28.9. HRMS (ESI) calcd for $C_9H_{13}BrNO_2$ 246.0124 $(M + H)^+$, found 246.0131.2,2-Dibromo-1-(5-*tert*-butylisoxazol-3-yl)ethanone (11). Colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.40 (s, 1H), 4.90 (s, 1H), 1.38 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 193.8, 183.6, 159.2, 97.2, 66.7, 33.1, 28.9. HRMS (ESI) calcd for $C_9H_{12}Br_2NO_2$ 323.9229 $(M + H)^+$, found 323.9320.3-(5-*tert*-Butylisoxazol-3-yl)-3-oxo-propionitrile (**8**). To a solution of 2-bromo-1-(5-*tert*-butylisoxazol-3-yl)ethanone (0.62 g, 2.5 mmol) in 10 mL of acetonitrile was added trimethylsilyl cyanide (0.25 g, 2.5 mmol), followed by 2.5 mL (2.5 mmol) of tetrabutylammonium fluoride solution (1 M in THF). The reaction mixture was stirred at r.t. for 0.5 h, and then diluted with brine (10 mL). The product was extracted with ethyl acetate (50 mL).

The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was obtained as a yellow oil and used directly for next step without further purification. It is noteworthy that cyanomethyl ketone 8 was unstable in silica gel column or neutral Al_2O_3 column. The pure compound for characterization was purified by preparative plate chromatography (silica gel, hexane/ethyl acetate 2:1) to obtain 8 as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.42 (s, 1H), 4.18 (s, 2H), 1.38 (s, 9H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 184.6, 182.0, 160.0, 112.9, 97.3, 33.2, 29.9, 28.8. HRMS (ESI) calcd for $C_{10}H_{13}N_2O_2$ 193.0972 $(M + H)^+$, found 193.1020.One-pot synthesis of 3-(5-*tert*-butylisoxazol-3-yl)-3-oxo-propionitrile (**8**) from 6. To a solution of CH_3CN (0.41 g, 10.0 mmol) in anhydrous THF (5 mL) was added 1.6 M methyllithium in diethyl ether (3.1 mL, 5.0 mmol) at -78 °C under nitrogen. The mixture was stirred at -78 °C for 0.5 h, and 5-tert-butylisoxazole-3-carboxylic acid ethyl ester (0.5 g, 2.5 mmol) in THF (5 mL) was then added dropwise. The solution was stirred at -78 °C for 1 h and then quenched with acetic acid (0.3 g, 5.0 mmol). The mixture was warmed to 0 °C and poured onto ice/water (10 mL) and extracted with ethyl acetate (50 mL). The organic lay was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue (490 mg) was obtained as a yellow oil and directly used for next step without further purification.3-(5-*tert*-Butylisoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-3-oxo-propionitrile (**1**). To a solution of 3-chloroaniline (30 mg, 0.24 mmol) in H_2O (1 mL cooled to -5°C) was added 0.24 mL of 1 N HCl (aq.). To the resulting acidic aniline solution, 1 mL solution of sodium nitrite (16 mg, 0.24 mmol) in H_2O was added dropwise to generate the aryldiazonium salt solution. To the aryldiazonium salt solution was added sodium acetate (33 mg, 0.4 mmol), followed by 1 mL solution of crude 3-(5-*tert*-butylisoxazol-3-yl)-3-oxo-propionitrile (38 mg, 0.2 mmol) in ethanol. The reaction mixture was stirred at 0 °C for 5 min, and then poured onto H_2O (2 mL) and extracted with ethyl acetate (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by short column chromatography on silica gel eluting with hexane/ethyl acetate (2/1) to provide the desired product **ESI-09** (40 mg, 61%, two steps from **6**) as a yellow solid (mp 146-147 °C). HPLC purity 99.6% (t_R = 21.72 min). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.70 (bs, 1H), 7.44-7.47 (m, 3H), 7.25-7.26 (m, 1H), 6.70 (s, 1H), 1.39 (s, 9H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 181.1, 179.4, 160.1, 143.6, 134.0, 131.2, 125.1, 116.2, 115.8, 113.4, 110.5, 100.4, 32.5, 28.5. HRMS (ESI) calcd for $C_{16}H_{16}ClN_4O_2$ 331.0956 $(M + H)^+$, found 331.0969.

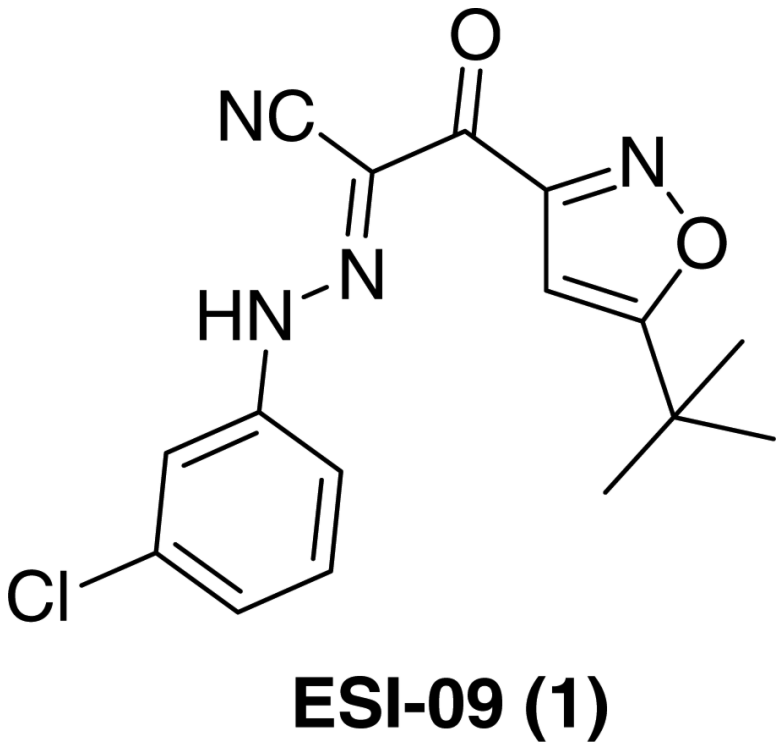
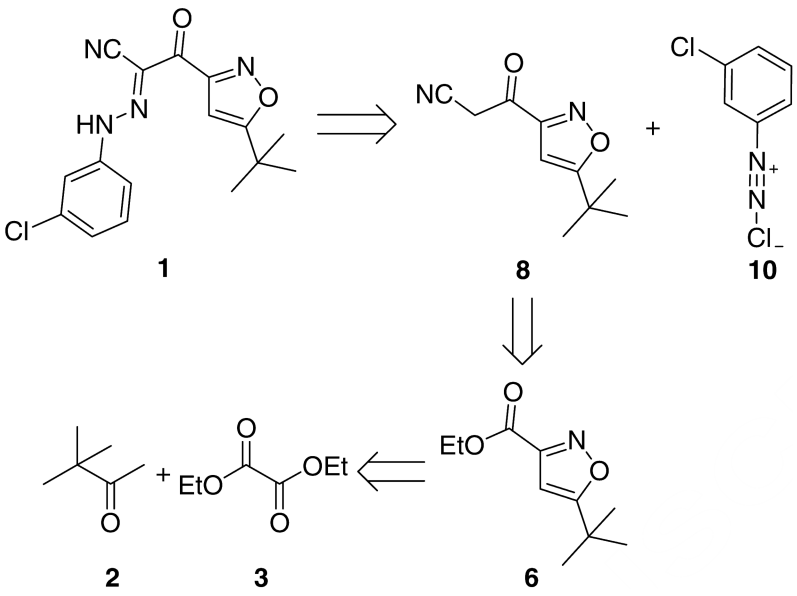
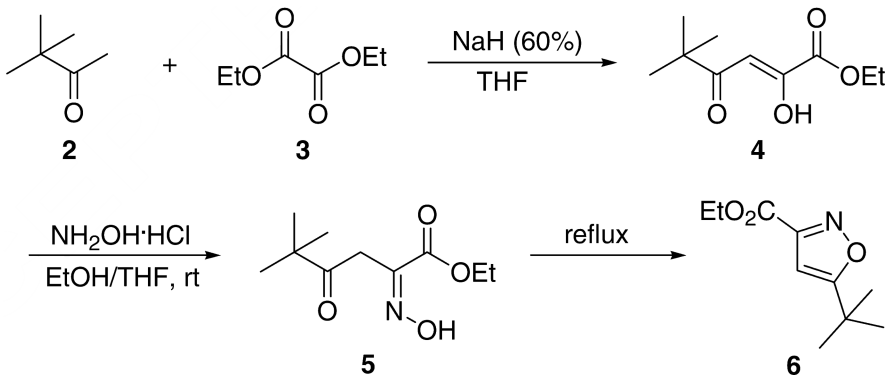


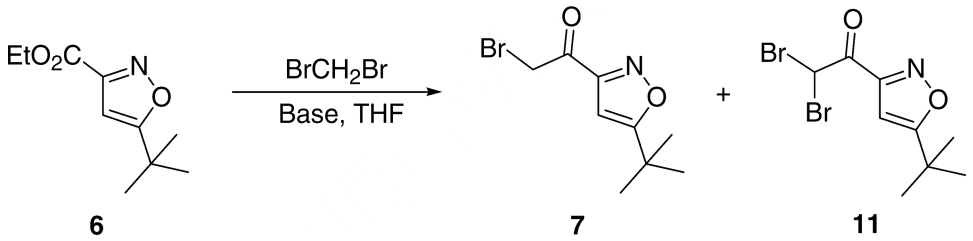
Figure 1.
Structure of **ESI-09** (**1**).



Scheme 1.
Retrosynthetic analysis of **ESI-09** (**1**).

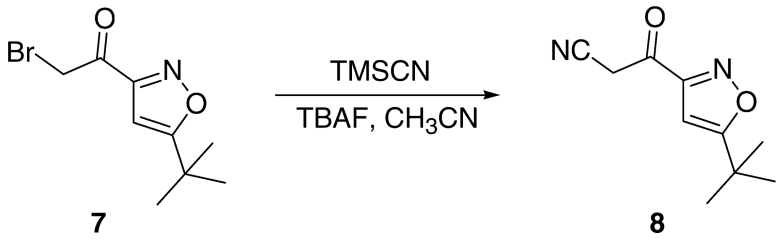


Scheme 2.
One-pot synthesis of 5-*tert*-butylisoxazole-3-carboxylic acid ethyl ester **6**.



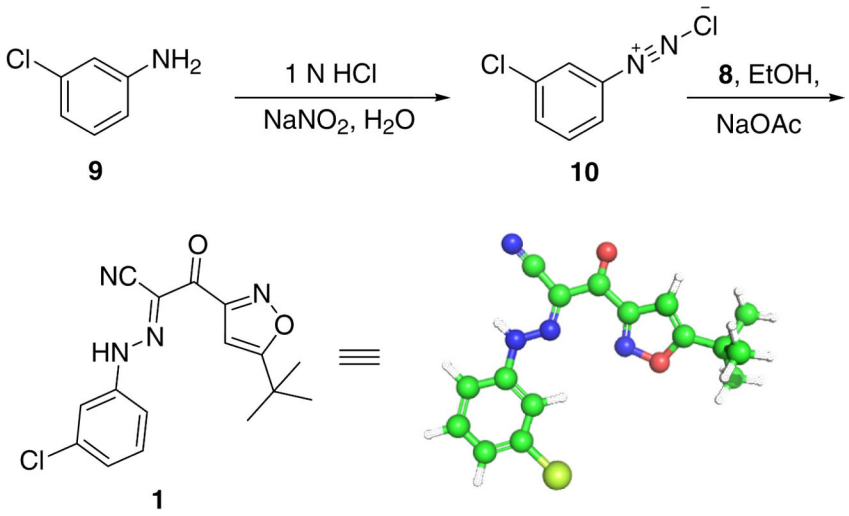
6 7 11

Scheme 3.
Synthesis of 2-bromo-1-(5-*tert*-butylisoxazol-3-yl)ethanone **7**.

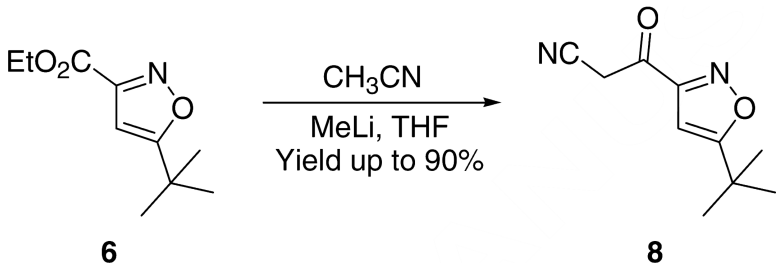


7 **8**

Scheme 4.
Synthesis of 3-(5-*tert*-butylisoxazol-3-yl)-3-oxo-propionitrile **8**.

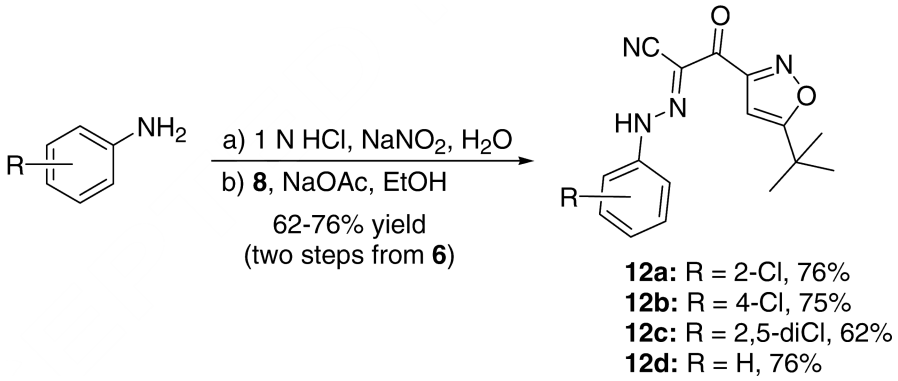


Scheme 5.
Synthesis of 3-(5-*tert*-butylisoxazol-3-yl)-2-[(3-chlorophenyl)-hydrazono]-3-oxo-propionitrile **1**.



6 **8**

Scheme 6.
Synthesis of 3-(5-*tert*-butylisoxazol-3-yl)-3-oxo-propionitrile **8** from **6**.



Scheme 7.
Synthesis of **ESI-09** analogs **12a-d**.

Table 1

Optimization of Reactions Conditions of Bromomethyl Ketone **7**[a]

entry	base (equiv)	$BrCH_2Br$ (equiv)	time	products (yield[b])
1	LDA (2.0)	2.0	1 h	**7** (6%), **11** (trace)
2	*n*-BuLi (2.0)	2.0	1 h	**7** (11%), **11** (trace)
3	MeLi (2.0)	2.0	1 h	**7** (25%), **11** (25%)
4	MeLi (2.5)	2.0	2 h	**7** (57%), **11** (5%)
5	MeLi (3.0)	2.0	2 h	**7** (65%), **11** (3%)
6	MeLi (4.0)	2.0	2 h	**7** (73%), **11** (trace)
7	MeLi (3.0)	1.2	2 h	**7** (84%), **11** (trace)

[a]The concentration was 0.25 M in THF.

[b]Isolated yield.