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NOVEL PROTEIN KINASE C INHIBITORS: α-TERTHIOPHENE DERIVATIVES

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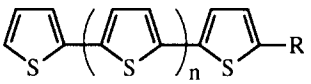
Abstract: A series of α-terthiophene derivatives were prepared and their protein kinase C inhibitory activity were evaluated. The aldehyde derivatives were most potent inhibitors (IC_{50} < 1 μM). α-Terthiophene monoaldehyde was inactive in the inhibitions of protein kinase A, mitogen activated protein kinase and protein tyrosine kinase.

Protein kinase C (PKC) is a calcium-activated and phospholipid-dependent protein kinase.[1] It is known to represent a family of proteins encoded by multiple genes and is endogenously activated by diacylglycerol, which is produced from mitogen induced hydrolysis of inositol phospholipids by phospholipase C. Inositol triphosphate is also formed in the phospholipase C reaction, which releases calcium. Activated protein kinase C phosphorylates proteins and induces many intracellular reponses, including proliferation, differentiation, gene expression and tumor promotion. Therefore, protein kinase C plays a key role in signal transduction. In our search for novel PKC inhibitors from medicinal plants, we observed good inhibitory activity in the extract of *Eclipta prostrata*, an ancient Chinese medicinal plant used in the treatment of inflamatory diseases and kidney ailments.[2] On the basis of this inhibitory activity, we have discovered a series of polythiophenes (n = 0-1) as novel PKC inhibitors.



Plants containing polythiophenes have been used in traditional medicine.[2-4] For example, the juice of *Eclipta alba* leaves has reportedly been used in India for the treatment of vitiligo, atheletes foot, ringworm, and some chronic skin diseases.[4] Whether any pharmacological activity can be attributed to α-terthiophene or congeners present in this plant has not been established with certainty. Many of the isolated and synthesized α-terthiophene derivatives exhibit phototoxic activity against nematodes, larvae and eggs of insects, bacteria, algae, fungi, and viruses, respectively.[5] No naturally occurring terthiophenes are currently used as drugs. The unique biological

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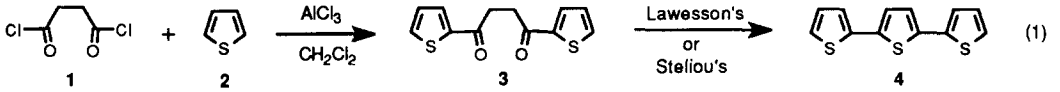
activity of α-terthiophenes, that frequently occur in plants belonging to the family of Asteraceae,[6] has stimulated numerous synthetic efforts by us and others.[7]

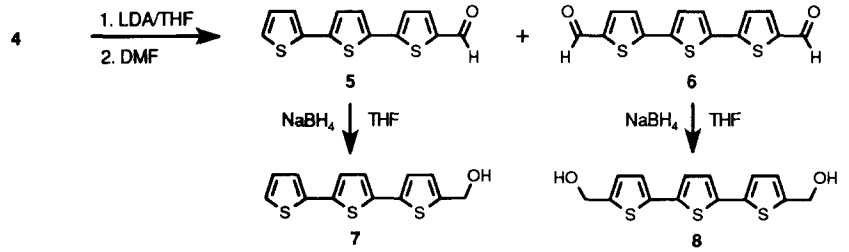
Preparation of α-Terthiophene Derivatives

A series of α-terthiophene derivatives were prepared in order to establish a structure-PKC inhibitory activity relationship. Succinyl chloride **1** was reacted with thiophene **2** in the presence of aluminum chloride ($AlCl_3$) in CH_2Cl_2 at 0 °C to afford the dithiophene-1,4-diketone **3**[7e,7f] in 80% yield. Thionation using Lawesson's reagent[8] afforded α-terthiophene **4** in 90% yield while Steliou's thionation method [$((C_6H_{11})_3Sn)_2S/BCl_3$/toluene][9] afforded α-terthiophene **4** in 95% yield. The work up procedure using Steliou's reagent was much easier than that of Lawesson's reagent and the byproduct [$(C_6H_{11})_3SnCl$] was reuseable while the Lawesson's was not.



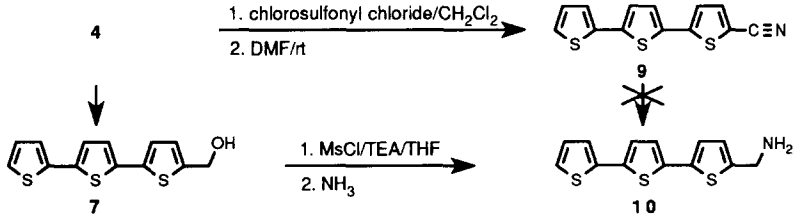
(1)

α-Terthiophene **4** was formylated [(i), LDA (1.2 equiv)/THF/-78 °C/2 h; (ii), DMF][10] to give α-terthiophene-monoaldehyde **5** and α-terthiophenedialdehyde **6** which were reduced ($NaBH_4$/THF) to their corresponding alcohols **7** and **8**.



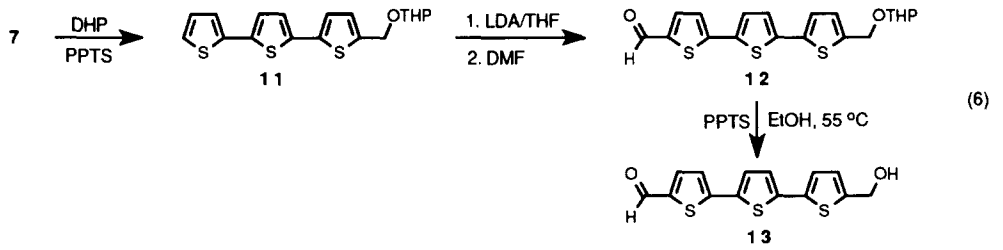
(2)

Reacting α-terthiophene **4** with chlorosulfonyl chloride in CH_2Cl_2, followed by DMF treatment[11] afforded 2-cyano-α-terthiophene **9** (57%). Reduction of 2-cyano-α-terthiophene **9** under various reducing condition ($NaBH_4$, $NaBH_4/TiCl_4$, LAH etc.) to 2-aminomethyl-α-terthiophene **10** was unsuccessful. To obtain 2-aminomethyl-α-terthiophene **10**, 2-hydroxymethyl-α-terthiophene **7** was mesylated (methanesulfonyl chloride/TEA/THF/0 °C)[12] and NH_3 was bubbled through for 15 minutes at 0 °C to afford the desired product **10**[13] as a photosensitive light yellow powder (75%, eq 3).



(3)

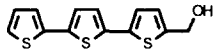
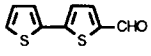
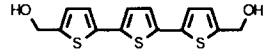
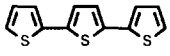
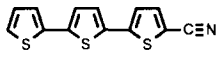
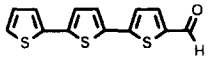
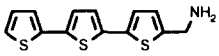
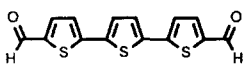
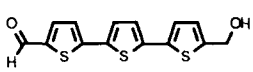
2-Hydroxymethyl-α-terthiophene **7** was protected as a THP ether **11** by using DHP in the presence of pyridinium *p*-toluenesulfonate (PPTS)[15] and was formylated [(i), LDA (1.1 equiv)/THF/-78 °C/2 h; (ii), DMF][10] to afford the THP ether α-terthiophene aldehyde **12** in 90% yield. The removal of THP protection (PPTS/EtOH/55 °C)[16] afforded a mixed functional group attached 2-formyl-5''-hydroxymethyl-α-terthiophene **13** (eq 6).



PKC Inhibitory Activity of α-Terthiophene Derivatives

PKC inhibitory activity of α-terthiophene derivatives is presented in Table 1. 2-Formyl-α-terthiophene **5** inhibited PKC with IC_{50} = 7 x 10^{-7} M while monohydroxymethyl-α-terthiophene **7** inhibited PKC with approx. 10 times less potency (IC_{50} = 4 x 10^{-6} M). α-Terthiophene dialdehyde **6** (IC_{50} = 3 x 10^{-7} M) and hydroxymethyl-α-terthiophene aldehyde **7** (IC_{50} = 7 x 10^{-7} M) were as active as compound **5** while dihydroxymethyl-α-terthiophene **8** (IC_{50} = 2 x 10^{-4} M) showed a drastic loss of PKC inhibitory activity. An interesting observation was that as the number of thiophene ring system increased in the aldehyde series (thiophene-2-carboxaldehyde **14**, 2-formyl-α-dithiophene **15**, and 2-formyl-α-terthiophene **5**) the PKC inhibitory activity also increased (IC_{50} = 4 x 10^{-3} M, 7 x 10^{-4} M, and 7 x 10^{-7} M), respectively. Converting hydroxymethyl compound **7** (IC_{50} = 4 x 10^{-6} M) to aminomethyl compound **10** (IC_{50} = 7 x 10^{-6} M) did not show any change in PKC inhibitory activity. In addition, the polythiophene inhibitors have also been demonstrated to be noncompetitive inhibitor with respect to ATP. Furthermore, the observed protein kinase inhibitory activity of α-terthiophene monoaldehyde was specific to PKC, with no cross inhibition of protein kinase A, mitogen-activated protein kinase or protein-tyrosine kinase.[18]

Table 1. Inhibition of Protein Kinase C[16]

Compounds		IC_{50} (M)	Compounds		IC_{50} (M)
	**14**	> 4 x 10^{-3}		**7**	4 x 10^{-6}
	**15**	7 x 10^{-4}		**8**	2 x 10^{-4}
	**4**	1 x 10^{-5}		**9**	2 x 10^{-6}
	**5**	7 x 10^{-7}		**10**	7 x 10^{-6}
	**6**	3 x 10^{-7}		**13**	7 x 10^{-7}

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16. PKC inhibition was determined as previously described[17], except the reaction was incubated at room temperature for 30 min with the additional or changed reaction components: 0.1 mg/mL phosphatidylserine, 10 nM 12-O-tetradecanoyl phorbol 3-acetate, 0.1 mM $CaCl_2$, 0.2 mg/mL bovine serum albumin, 10 μg/mL leupeptin, and a 1:1 mixture of recombinant PKC_{α} and $PKC_{\beta 2}$, partially purified after expression in Sf9 insect cells.Trifluoperazine (IC_{50}: 5 x 10^{-4} M) and staurosporine (IC_{50}: 2 x 10^{-8} M) were used as standard inhibitors.
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