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NOVEL PROTEIN KINASE C INHIBITORS: SYNTHESIS AND PKC INHIBITION OF β-SUBSTITUTED POLYTHIOPHENE DERIVATIVES[1]

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Abstract: A series of β-substituted polythiophene derivatives was synthesized through palladium-catalyzed coupling reaction. Their structure-protein kinase C (PKC) inhibitory activity relationship was studied. The carboxaldehyde and hydroxymethyl derivatives of α-terthiophene were potent PKC inhibitors (IC_{50} = 10^{-7} M).

Protein kinase C (PKC) is a key enzyme in signal transduction in animal cells.[2] It is a family of phospholipids-dependent serine/threonine kinases that are activated by second messengers, such as diacylglycerol (DAG) and Ca^{2+}. Once activated, PKC phosphorylates proteins and triggers many cellular responses, including cell proliferation, differentiation, and gene expression.[3] It is therefore believed that PKC plays an important role in tumorgenesis and tumor metastasis, and may serve as an attractive target for antitumor agents. We recently discovered that α-substituted polythiophene compounds were potent protein kinase C (PKC) inhibitors.[1] This discovery led us to study the PKC inhibition by β-substituted polythiophenes.

Synthesis of β-substituted polythiophenes

Stille coupling reaction can tolerate many functional groups, and thus allows a one-pot preparation of analogs without using protecting groups.[4] They have been used in the synthesis of various dithienopyridines[5] and thiophene containing terheterocyclic compounds.[6] In the synthesis of α-substituted polythiophene compounds, the functional group can be introduced by direct functionalization of bithiophene or terthiophene since the α-position is more reactive than the β-position. This electrophilic substitution often can not be used in the introduction of β-functional group. An alternative approach must thus be taken for the preparations of β-substituted polythiophene derivatives.[7] In this paper, we report the synthesis of β-substituted polythiophene derivatives from the halogenated β-substituted thiophene or bithiophene derivatives by Stille coupling reaction.

Mono-β-substituted polythiophenes: Thiophene or bithiophene was treated with *n*-BuLi and tributylstannylchloride afforded the stanneous reagent **1** or **2**, which was then coupled with 2-bromo-4-thiophenecarboxyaldehyde[8] in the presence of $Pd(PPh_3)_4$. When **1** was used in the coupling reaction, **3** was obtained in only 40% yield. With the addition of Ph_3P (15% mol equiv.), the reaction yield was significantly improved (67% from thiophene). β-Formyl-α-terthiophene **4** was similarly prepared in good yield (two steps, 85%). Reduction of **3** or **4** provided **5**[9] or **6**, respectively. (Scheme 1)

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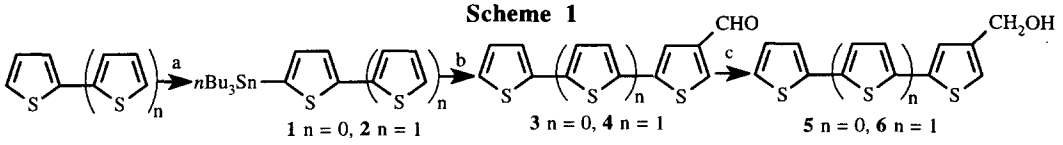
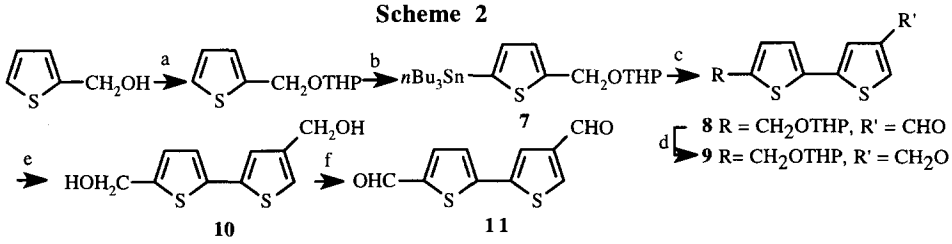
Scheme 1



Conditions: (a) i. *n*-BuLi, THF, -78 °C, 0.5 h, 0 °C, 15 min; ii. *n*-Bu_3SnCl, -78 °C, rt, 2 h; (b) 2-bromo-4-thiophenecarboxyaldehyde, $Pd(Ph_3P)_4$ (5% mol equiv.), Ph_3P(15% mol equiv.), DMF, 80 °C, 16 h, **3** (67%, two steps from thiophene), **4** (85%, two steps from bithiophene); (c) $NaBH_4$, EtOH, rt, 0.5 h, **5** (98%), **6** (96%).

Di-α,β-substituted polythiophenes: The coupling of stanneous reagent **7** with 2-bromo-4-thiophenecarboxyaldehyde afforded **8**. Reduction of **8** gave compound **9** quantitatively, and subsequent deprotection yielded **10**. Further oxidation of **10** with DDQ afforded **11**.[10] (Scheme 2)

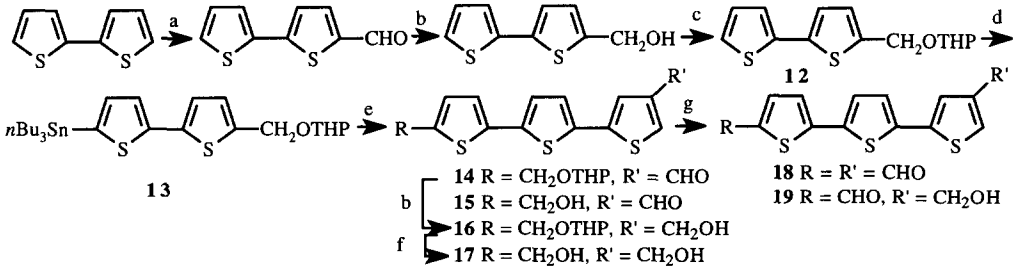
Scheme 2



Conditions: (a) Dihydropyran, PPTS, CH_2Cl_2, rt, 2 h, 92%; (b) i. *n*-BuLi, THF, -78 °C, 0.5 h, -20 °C, 1 h; ii. *n*-Bu_3SnCl, -78 °C, rt, 2 h; (c) 2-bromo-4-thiophenecarboxyaldehyde, $Pd(Ph_3P)_4$ (5% mol equiv.), Ph_3P(15% mol equiv.), DMF, 80 °C, 16 h, 60% (two steps); (d) $NaBH_4$, EtOH, rt, 0.5 h, 99%; (e) PPTS, EtOH:H_2O = 10:1, 40 °C, 16 h, 90%; (f) DDQ, CH_2Cl_2:Acetone:H_2O = 4:1:0.5, rt, 5 h, 90%.

The stanneous reagent **13** was prepared from bithiophene in 4 steps. The coupling of **13** with 2-bromo-4-thiophene carboxaldehyde gave the major product **15**. The THP protecting group was simultaneously removed by *n*Bu_3SnBr formed in the reaction[11] (53% from **12**). With the addition of Ph_3P, **14** was obtained as a major product in 60% yield from **12**. **14** was then subjected to $NaBH_4$ reduction and deprotection to yield **17**, which could also be obtained by direct reduction of **15**. **17** was then oxidized with DDQ to afford **18** and **19** (3:1). (Scheme 3)

Scheme 3

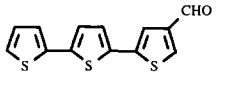
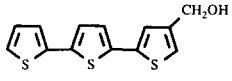
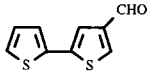
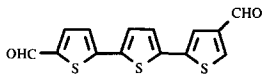
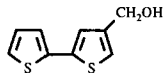
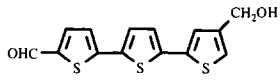
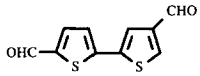
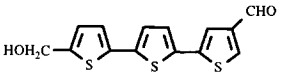
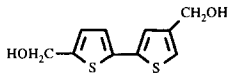
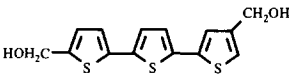


Conditions: (a) i. LDA, THF, -78 °C, 0.5 h, then 0 °C 1 h; ii. DMF, -78 °C, 0.5 h, then rt, 3 h, 80%; (b) $NaBH_4$, EtOH, rt, 0.5 h, 98%; (c) Dihydropyran, PPTS, CH_2Cl_2, rt, 2 h, quant; (d) i. *n*-BuLi, THF, -78 °C, 0.5 h, -20 °C, 1 h; ii. *n*-Bu_3SnCl, -78 °C, rt, 2 h; (e) 2-bromo-4-thiophenecarboxyaldehyde, $Pd(Ph_3P)_4$ (5% mol equiv.), DMF, 80 °C, 16 h, **15** (60% from **12**); with the addition of Ph_3P (15% mol equiv.), **14** (60% from **12**); (f) PPTS, EtOH:H_2O = 8:1, 40 °C, 5 h, 90%; (g) DDQ, CH_2Cl_2:Acetone:H_2O = 4:2:0.5, rt, 5 h, **18** (48%), **19** (16%).

PKC Inhibitory Activity of α-Terthiophene Derivatives

The PKC inhibitory activity of β-substituted α-terthiophene derivatives are summarized in Table 1. It is evident that the number of thiophene rings increased in the aldehyde series (3-formylthiophene **20**, 4-formyl-α–bithiophene **3**, and 4-formyl-α-terthiophene **4**), the PKC inhibitory activity steadily increased. The conversion of the formyl group to a hydroxymethyl group in the α-substituted α-terthiophene series resulted in the reduction of activity.[1] However, in the β-substituted α-terthiophene series, the hydroxymethyl derivatives were slightly more active than the corresponding formyl derivatives (**6** vs **4**, **19** vs **18**) except 4,5″-dihydroxymethyl-α-terthiophene **17**, which was ten-fold less potent than the formyl analog **15**. The inhibitory potency for the β-hydroxymethyl derivatives was proportional to the number of thiophene rings (**21**, **5**, and **6**). Among all β-substituted α-terthiophenes, the most potent PKC inhibitors were 4-hydroxymethyl-5″-formyl-α-terthiophene **19** (IC_{50} = 3 × 10^{-7} M), and 4,5″-diformyl-α–terthiophene **18** (IC_{50} = 7 × 10^{-7} M). It is also interesting that the retention of an α-carboxaldehyde functional group is critical for the inhibitory activity for disubstituted α-terthiophene derivatives [**18** (**19**) vs **15** (**17**)].

Table 1. Inhibition of Protein Kinase C*

Compounds		IC_{50} (M)	Compounds		IC_{50} (M)
	**20**	>4 x 10^{-3}		**4**	5 x 10^{-6}
	**21**	>4 x 10^{-3}		**6**	3 x 10^{-6}
	**3**	5 x 10^{-4}		**18**	7 x 10^{-7}
	**5**	>1 x 10^{-3}		**19**	3 x 10^{-7}
	**11**	1 x 10^{-3}		**15**	3 x 10^{-5}
	**10**	>1 x 10^{-3}		**17**	3 x 10^{-4}

*PKC inhibition was determined as previously described,[1] except the compounds were mixed with phosphatidylserine at room temperature for 30 min prior to addition of PKC enzymes, and then incubated with the enzyme for 60 min before the addition of histone and ATP. Trifluoperazine (IC_{50} = 5 × 10^{-4} M) and staurosporine (IC_{50} = 1 × 10^{-8} M) were used as standard inhibitors.

In conclusion, we have synthesized a series of new β-substituted polythiophene derivatives using the Stille coupling reaction with the addition of Ph_3P, by which the yield of some coupling reactions could be improved significantly. We have also established that polythiophenes are a novel class of PKC inhibitors. The carboxaldehyde and hydroxymethyl derivatives are important lead compounds for further elaboration of the inhibitory specificity and potency.

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