Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron 62 (2006) 9554–9570
Tetrahedron

Tandem versus single C–C bond forming reaction under palladium–copper catalysis: regioselective synthesis of α-pyrones fused with thiophene☆

Sirisilla Raju,[a,c] Venkateswara Rao Batchu,[b] Nalivela Kumara Swamy,[b] R. Vasu Dev,[b] Bukkapattanam R. Sreekanth,[b] J. Moses Babu,[b] K. Vyas,[b] P. Rajender Kumar,[a] K. Mukkanti,[c] Pazhanimuthu Annamalai[b] and Manojit Pal[b,*]

[a]*Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd, Bollaram Road, Miyapur, Hyderabad 500049, India*
[b]*Discovery Research, Dr. Reddy's Laboratories Ltd, Bollaram Road, Miyapur, Hyderabad 500049, India*
[c]*Chemistry Division, Institute of Science and Technology, JNT University, Kukatpally, Hyderabad 500072, India*

Received 11 May 2006; revised 7 July 2006; accepted 27 July 2006
Available online 22 August 2006

Abstract—We herein report a highly convenient protocol for rapid construction of α-pyrone fused with thiophene. This includes one-pot and regioselective synthesis of 4,5-disubstituted and 5-substituted thieno[2,3-*c*]pyran-7-ones, 6,7-disubstituted and 6-substituted thieno[3,2-*c*]pyran-4-ones. The synthesis of thieno[2,3-*c*]pyran-7-ones involves palladium mediated cross coupling of 3-iodothiophene-2-carboxylic acid with terminal alkynes in a simple synthetic operation. The coupling–cyclization reaction was initially studied in the presence of $Pd(PPh_3)_2Cl_2$ and CuI in a variety of solvents. 5-Substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones were isolated in good yields when the reaction was performed in DMF. Similarly, 6-substituted 7-alkynylthieno[3,2-*c*]pyran-4-ones were synthesized via palladium-catalyzed cross coupling of 2-bromothiophene-3-carboxylic acid with terminal alkynes. A tandem C–C bond forming reaction in the presence of palladium catalyst rationalizes the formation of coupled product in this apparently three-component reaction. The cyclization step of this coupling–cyclization–coupling process occurs in a regioselective fashion to furnish products containing six-membered ring only. This sequential C–C bond forming reaction however, can be restricted to the formation of single C–C bond by using 10% Pd/C–Et_3N–CuI–PPh_3 as catalyst system in the cross coupling reaction. 5-Substituted thieno[2,3-*c*]pyran-7-ones were obtained in good yields when the coupling reaction was performed under this condition. Some of the compounds synthesized were tested in vitro for their anticancer activities.

1. Introduction

α-Pyrones[1a] and their benzo derivatives, e.g., isocoumarins[2a] are of considerable synthetic and pharmacological interest because of their wide range of activities[2b–d] such as antifungal, antimicrobial, phytotoxic and other effects. 3-Substituted isocoumarins in particular have shown promising pharmacological activities.[2e] The angiogenesis inhibitor NM-3,[2f] which belongs to this class is presently undergoing Phase-I clinical trials. More recently, promising cytotoxic activities of substituted pyrones especially 4-alkynyl substituted 2-pyrones (A, Fig. 1) have been reported.[2g] In continuation of our research under the new drug discovery program, we were in need of a combinatorial library based on the scaffold of α-pyrone fused with five-membered heterocycles (B, Fig. 1). The library model, as shown in Figure 1, has three centers for the introduction of diversity into α-pyrone molecule. While much effort has been devoted toward the introduction/modification of C-3 and C-4 substituents on the benzo derivative, i.e., isocoumarin ring,[2h–j] replacing the benzene ring by a suitable heterocyclic moiety is not common in the literature. We envisioned that α-pyrone fused with a five- or six-membered heterocycle might lead to a novel class of compounds useful for the Structure–Activity Relationship (SAR) studies. The thiophene moiety is common in many bioactive agents and drugs[3a] and is considered as a bioisostere of the benzene ring.[3a] (The distance between two neighboring carbon atoms in benzene is roughly equivalent to the diameter of the sulfur atom in thiophene and the latter often displays pharmacological properties similar to those of benzene.[3b]) On the other hand pyrazole fused with pyran and benzopyran rings has shown affinity and selectivity toward A_1 adenosine receptor.[3c,d] Thus, one can anticipate that

☆ DRL publication no. 522.

Keywords: α-Pyrone; 4,5-Disubstituted thieno[2,3-*c*]pyran-7-one; 6,7-Disubstituted thieno[3,2-*c*]pyran-4-ones; Palladium catalyst; Terminal alkynes; 3-Iodothiophene-2-carboxylic acid; 2-Bromothiophene-3-carboxylic acid.

* Corresponding author. Tel.: +91 40 23045439; fax: +91 40 23045438/23045007; e-mail: manojitpal@drreddys.com

0040–4020/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2006.07.095

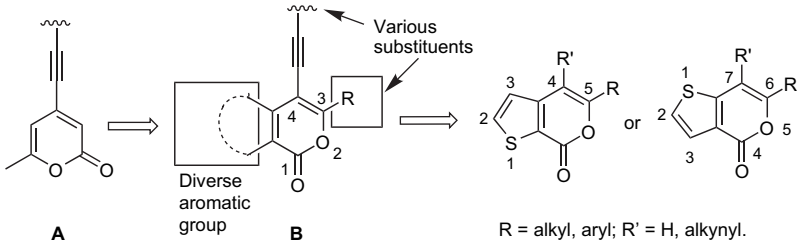


Figure 1. Design of α-pyrones fused with 5-membered heterocycles derived from diversity based isocoumarin scaffold.

replacing the benzene ring of isocoumarin by a five-membered ring such as thiophene would afford compounds (i.e., thienopyranones, Fig. 1) of potential pharmacological interest.[3e,f] Additionally, thiophene derivatives are excellent synthetic intermediates because of the unique electronic properties of sulfur as well as the steric constraints of a five-membered ring.[3g] However, as a class of compounds the thienopyranones are rather unusual. Only a few number of thieno[2,3-*c*]pyran-4-ones were synthesized and evaluated for their antileishmanial and antifungal activities.[3h]

Based on above considerations and our continuing interest in the synthesis of oxygen containing heterocycles we decided to synthesize pyranones fused with thiophene. A thorough literature search revealed that methodologies devised for the elaboration of this framework are generally limited and not investigated extensively.[4a–e] Moreover, synthesis of thieno[2,3-*c*]pyran-7-ones/thieno[3,2-*c*]pyran-4-ones (and their 4-/7-alkynyl analogues) has not been reported thus far. Therefore, to synthesize a library of isocoumarins[5a] for biological screening we became interested in the synthesis of pyranones fused with five-membered heterocycles.[5b]

The construction of functionalized α-pyrone ring incorporated into the isocoumarin system is the most commonly used strategy for a rapid assemblage of isocoumarin derivatives. On the other hand palladium-catalyzed reactions have become most attractive and powerful tool for C–C bond forming reaction due to their generality and superiority over many tedious classical methods. Thus, among the many methods reported for the synthesis of isocoumarins one widely used process is Sonogashira-type coupling followed by electrophilic or transition metal mediated cyclization of the resulting alkynes possessing a carboxylate or an equivalent group in proximity to the triple bond.[6] Attractive features of this process include its versatility and ability to tolerate a wide range of important organic functional groups. Thus, isocoumarins have been prepared in a efficient manner by reacting *o*-iodobenzoic acid with terminal alkynes in the presence of $Pd(PPh_3)_4$, Et_3N, and a stoichiometric amount of $ZnCl_2$.[7a] The use of $ZnCl_2$ in place of CuI[7b,c] was found to be responsible for the predominant formation of isocoumarins over phthalides. However, very recently we have shown that isocoumarins can be obtained as major products even in the presence of CuI when the coupling reaction was performed in the presence of 10% Pd/C–Et_3N–CuI–PPh_3 as catalyst system in ethanol (Fig. 2).[5a] More recently, we have noted that 3-iodothiophene-2-carboxylic acid (**1a**) reacts smoothly with terminal alkynes in the presence of $PdCl_2(PPh_3)_2$–Et_3N–CuI as a catalyst system affording 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones (**2a–j**) in good yields (Scheme 1). This palladium-catalyzed

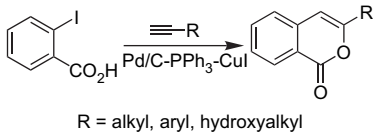
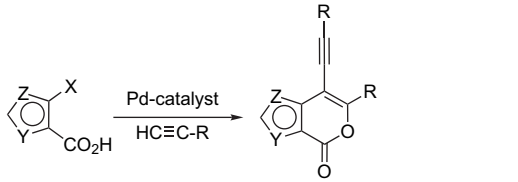


Figure 2. Pd/C-mediated synthesis of 3-substituted isocoumarins.

transformation is particularly interesting, because apart from providing an easy access to thieno[2,3-*c*]pyran-7-one ring it affords novel conjugated enynes that could be utilized further as precursors to construct even more complex molecules. Only four examples of analogue 3-substituted 4-alkynyl isocoumarins have been reported during Pd–Zn mediated synthesis of 3-substituted isocoumarins where these derivatives were isolated as minor products in 3–5% yield.[7a] Alternatively, 4-alkynyl isocoumarins/coumarins have been prepared via multistep synthesis that usually involves ring construction followed by Sonogashira coupling.[8] Being arguably the most versatile transition metal for catalysis palladium features prominently in a number of tandem transformations[9] and we have recently shown that terminal alkynes participate in tandem coupling reactions depending on the reaction condition employed.[9g] Herein, we report further study on our previously communicated one-step synthesis of 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones[5b] along with the synthesis of isomeric 6-substituted-7-alkynylthieno[3,2-*c*]pyran-4-ones under palladium catalysis. Additionally, we report detailed study on Pd/C-mediated synthesis of 6-substituted thieno[3,2-*c*]pyran-4-ones and 5-substituted thieno[2,3-*c*]pyran-7-ones where no significant formation of corresponding alkynyl analogues was observed.



Scheme 1. Synthesis of thienopyranones having disubstitutions on the pyranone ring.

2. Results and discussion

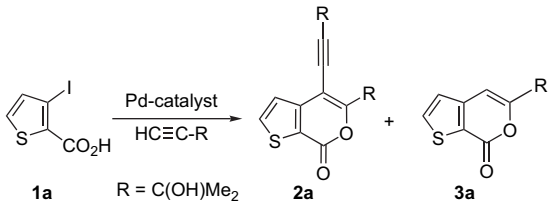
2.1. Optimization of reaction conditions and product characterization

To evaluate the potential of the use of palladium mediated coupling cyclization methodology[10] for the synthesis of 5-substituted thieno[2,3-*c*]pyran-7-ones (**3**), we first selected

1a as a model substrate with 2-methyl-3-butyn-2-ol as the terminal alkyne. We observed that Pd-catalyzed coupling of **1a**[11] with 2-methyl-3-butyn-2-ol in ethanol afforded 5-alkylthieno[2,3-*c*]pyran-7-ones (**3a**, R=–C(CH$_3$)$_2$OH, Table 1) in 24% yield and the unexpected 4-(3-hydroxy-3-methylbut-1-ynyl)-5-(1-hydroxy-1-methylethyl)thieno[2,3-*c*]pyran-7-one (**2a**) in 50% purified yield, with a 2/1 ratio of **2a** and **3a** (Entry 1, Table 1). Compound **2a** was characterized by ^{1}H and ^{13}C NMR and other spectroscopic methods and identified as an alkyne possessing the thieno[2,3-*c*]-pyran-7-one ring at one end. This was supported by the molecular structure of **2b** (R=–CH$_2$CH$_2$OH), which was confirmed by X-ray analysis.[5b] The ORTEP diagram of **2b** (Fig. 3) shows a planar thieno[2,3-*c*]pyran-7-one core with a disordered hydroxyl group due to the alkynyl side chain along with the other hydroxy group oriented in the opposite direction. The molecular structure of **3a** was also confirmed by X-ray analysis (Fig. 3).[12] The noteworthy features of this structure include (a) two independent molecules of **3a** in the unit cell are differentiated by the presence of one molecule of water, (b) the water molecule present in the lattice bridges the C=O of one molecule and O–H of other one, by inter molecular hydrogen bonding. Nevertheless, the unexpected formation of **2a** prompted us to investigate this tandem C–C bond forming reaction in a more systematic manner.

The initial reaction was carried out in ethanol in the presence of PdCl$_2$(PPh$_3$)$_2$ (0.048 equiv), CuI (0.06 equiv), Et$_3$N (5.0 equiv), and 2.0 equiv of the terminal alkyne at 75 °C. A series of optimization experiments on the reaction of **1a** with 2-methyl-3-butyn-2-ol were carried out by keeping the molar ratio of Pd/Cu at 1/1.3 and changing a number of parameters, e.g., effect of catalysts, solvent, base, and temperature (Table 1). Change of solvent from ethanol to a non-protic solvent such as 1,4-dioxane or dimethylacetamide (DMA) suppressed the formation of **3a** dramatically (Entries 2 and 3, Table 1) and **2a** was isolated in 30 and 55% yield, respectively. While the reason for these observations was not clear the non-protic solvents perhaps allowed the participation of second equivalent of alkyne used in the crucial cyclization process. The yield of **2a** was increased significantly when DMF was used (Entry 4, Table 1). To assess the role of solvent we carried out this reaction using a lesser amount of terminal alkyne (1.0 equiv) in DMF where **2a** was isolated in 28% yield and the reaction did not reach completion. This observation clearly suggests that formation of **2a** was not dependent on the concentration of alkyne used but was favored by the non-polar solvent employed. Both Pd and Cu catalysts played crucial roles as no reaction was observed when either was omitted. The use of other Pd catalysts, e.g., Pd(PPh$_3$)$_4$, Pd(OAc)$_2$ or PdCl$_2$(dppf)$_2$ was investigated (Entries 5–7, Table 1) where **2a** was isolated as major product albeit in low yield (35–40%). Significant amount of **3a** was isolated when PdCl$_2$(dppf)$_2$ was used as catalyst (Entry 7, Table 1). Thus, PdCl$_2$(PPh$_3$)$_2$ was identified as the best catalyst for the synthesis of **2a** and was used for further studies. The use of commercially available 3-bromothiophene-2-carboxylic acid however, did not afford **2a** in good yield even after 48 h when reacted with 2-methyl-3-butyn-2-ol in the presence of PdCl$_2$(PPh$_3$)$_2$ and CuI (Entry 8, Table 1). Remarkably, the use of 0.035 equiv of 10% Pd/C

Table 1. Effect of reaction conditions on the palladium-catalyzed coupling reaction of 3-iodothiophene-2-carboxylic acid with 2-methyl-3-butyn-2-ol[a]



Entry	Pd catalyst	Solvent; time	Yield (%)[b]	
			2a	**3a**
1	PdCl$_2$(PPh$_3$)$_2$	EtOH; 12 h	50	24
2	PdCl$_2$(PPh$_3$)$_2$	1,4-Dioxane; 12 h	30	0
3	PdCl$_2$(PPh$_3$)$_2$	DMA; 12 h	55	5
4	PdCl$_2$(PPh$_3$)$_2$	DMF; 8 h	80	0
5	Pd(PPh$_3$)$_4$	DMF; 12 h	40	0
6[c]	Pd(OAc)$_2$–PPh$_3$	DMF; 12 h	35	0
7	PdCl$_2$(dppf)$_2$	DMF; 12 h	35	28
8[d]	PdCl$_2$(PPh$_3$)$_2$	DMF; 48 h	12	0
9[c]	10% Pd/C–PPh$_3$	EtOH; 12 h	8	68
10[c,e]	10% Pd/C–PPh$_3$	1,4-Dioxane; 12 h	6	80

[a] Reaction conditions: **Ia** (1.0 equiv), terminal alkyne (2.0 equiv), Pd(II)-catalyst (0.048 equiv) or Pd/C (0.035 equiv), CuI (0.06 equiv), Et$_3$N (5 equiv) in a solvent at 70–80 °C under N$_2$.
[b] Isolated yield of **2a** and **3a**.
[c] PPh$_3$ used: 0.3 equiv.
[d] 3-Bromothiophene-2-carboxylic acid was used in place of **1a**.
[e] Terminal alkyne used: 1.5 equiv.

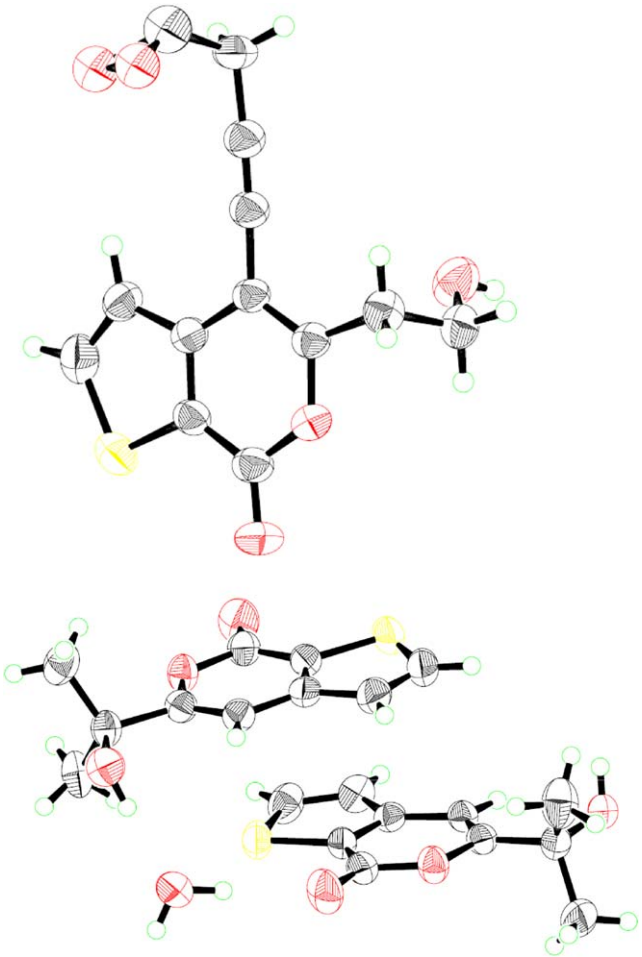


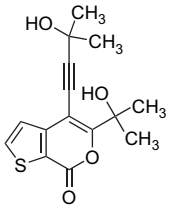
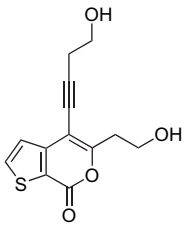
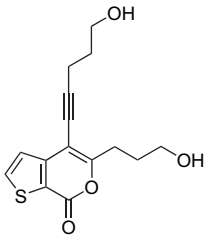
Figure 3. X-ray crystal structure of **2b** and **3a** (ORTEP diagram).

in place of $PdCl_2(PPh_3)_2$ in ethanol during coupling reaction of **1a** with 2-methyl-3-butyn-2-ol afforded **3a** as major product in good yield with trace amount of **2a** (Entry 9, Table 1). Indeed, a better yield of **3a** was achieved in 1,4-dioxane using lesser quantity, i.e., 1.5 equiv of terminal alkyne (Entry 10, Table 1). Less than 0.035 equiv of 10% Pd/C can be used to afford **3a**, but the yield of product was found to be often irreproducible. The use of 5% Pd/C also afforded lower yield of **3a**. All the reactions were usually carried out at 70–80 °C under nitrogen. The use of higher or lower reaction temperature led to the inferior results. Originally, we speculated that the formation of **2a** might first involve the formation of **3a**, which subsequently reacted with another mole of the terminal alkyne under Pd–Cu catalysis. However, formation of **2a** was not observed when **3a** was subjected to the same Pd-catalyzed reaction conditions.

2.2. Scope of the reaction

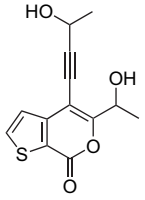
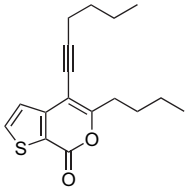
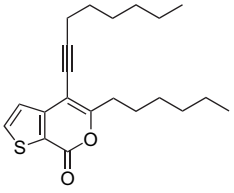
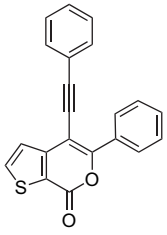
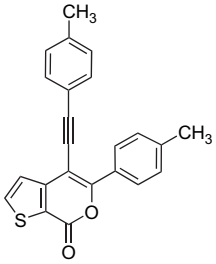
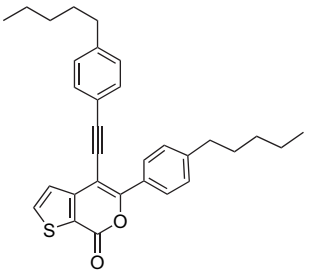
2.2.1. Synthesis of 5-substituted 4-alkynyl thieno[2,3-*c*]pyran-7-ones and 6-substituted 7-alkynyl thieno[3,2-*c*]pyran-4-ones. To examine the effect on the yield of the substituents on the terminal alkynes we next tested the optimized reaction conditions from Entry 4 of Table 1 (Method A: $PdCl_2(PPh_3)_2$, CuI, Et_3N in DMF) with other terminal alkynes (Entries 1–10, Table 2). Like *o*-iodobenzoic acid, 3-iodothiophene-2-carboxylic acid showed good reactivity toward the present coupling reaction and various functional groups including aryl, alkyl, hydroxyl, ether etc. present in terminal alkynes were well tolerated. This allowed the preparation of a variety of 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones (**2**) under mild condition. However, yields of compound **2** varied depending on the nature of alkynes used. While alkynes bearing a tertiary and secondary hydroxyl group on the carbon next to the triple bond provided best yields of **2** (Entries 1 and 4, Table 2), the presence of long alkyl chains on the triple bond did not affect the yields drastically (Entries 5 and 6, Table 2). A hydroxyl group at the end of the long alkyl chain of the alkyne however, lowered the yields of **2** slightly (Entries 2 and 3, Table 2). Among the aryl acetylenes used, phenyl acetylene afforded only a modest 57% yield of **2** under the reaction condition studied. A mild electron-donating group at the *para* position of the benzene ring however, improved the yield of **2** compared to phenyl acetylene (Entries 8 and 9 vs Entry 7, Table 2). The use of alkyne where $–CH_2O–$ moiety linked the triple bond with the phenyl group provided better yield of **2** than phenyl acetylene (Entry 10 vs Entry 7, Table 2). All these facts clearly indicated that yields of **2** were partially dependent on the nature of the substituent present on the terminal alkynes. Generally, **2** was isolated as the sole product in all cases except when 1-hexyne and 1-octyne were used. Compound **3** was also isolated as minor product in these cases (Entries 5 and 6, Table 2). In contrary to the 3-bromothiophene-2-carboxylic acid, isomeric and commercially available 2-bromothiophene-3-carboxylic acid **1b** provided

Table 2. Pd-mediated synthesis[a] of 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones and 6-substituted 7-alkynylthieno[3,2-*c*]pyran-4-ones

Entry	Alkyne (R)	Time (h)	Products (**2**)	Yield (%)	
				2	**3**
1	**a**; $–C(CH_3)_2OH$	8	 **2a**	80	0
2	**b**; $–(CH_2)_2OH$	12	 **2b**	53	0
3	**c**; $–(CH_2)_3OH$	12	 **2c**	61	0

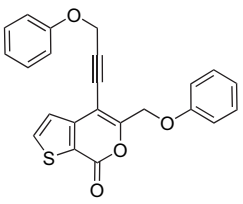
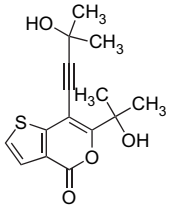
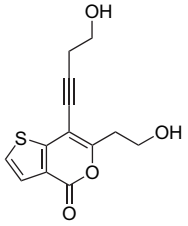
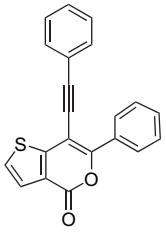
(*continued*)

Table 2. (*continued*)

Entry	Alkyne (R)	Time (h)	Products (**2**)	Yield (%)	
				2	**3**
4	**d**; –CH(OH)CH_3	10	 **2d**	82	0
5	**e**; –$(CH_2)_3CH_3$	12	 **2e**	65	15
6	**f**; –$(CH_2)_5CH_3$	12	 **2f**	62	35
7	**g**; –C_6H_5	12	 **2g**	57	0
8	**h**; –$C_6H_4CH_3$-*p*	12	 **2h**	62	0
9	**i**; –$C_6H_4C_5H_{11}$-*p*	8	 **2i**	73	0

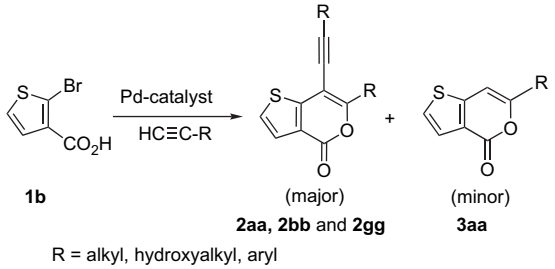
(*continued*)

Table 2. (*continued*)

Entry	Alkyne (R)	Time (h)	Products (**2**)	Yield (%)	
				2	**3**
10	**j**; $-CH_2OC_6H_5$	8	 **2j**	75	0
11[b]	**a**	10	 **2aa**	55	40
12[b]	**b**	10	 **2bb**	88	0
13[b]	**g**	10	 **2gg**	60[c]	0

[a] All reactions were carried out using **1** (1.0 equiv), terminal alkyne (2.0 equiv), $PdCl_2(PPh_3)_2$ (0.048 equiv), CuI (0.06 equiv) and Et_3N (5 equiv) in DMF at 70–80 °C under nitrogen.
[b] 2-Bromothiophene-3-carboxylic acid was used in place of **1**.
[c] 1,4-Diphenyl-1,3-butadiyne was isolated in 35% yield in addition to **2gg**.

better yield of corresponding 6,7-disubstituted thieno[3,2-*c*]pyran-4-ones (Scheme 2), e.g., **2aa**, **2bb**, and **2gg** (Entry 8 of Table 1 vs Entries 11–13 of Table 2) under the condition studied. Higher reactivity shown by 2-bromo derivative over 3-bromo toward the present palladium-catalyzed reaction was perhaps aided by the electron withdrawing inductive effect of sulfur at the nearby position. Nevertheless, the success of this tandem reaction is presumably due to the in situ generation of the Pd(II)-complex after normal Sonogashira coupling, which facilitates the insertion of another alkyne moiety on the intermediate generated upon cyclization (see later for mechanistic discussion).

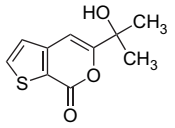
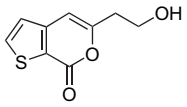
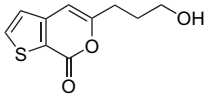
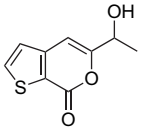
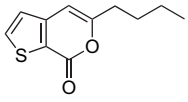
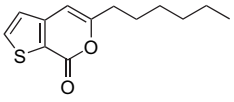
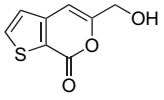
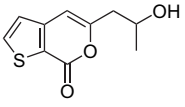
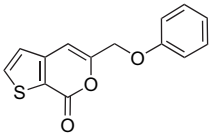
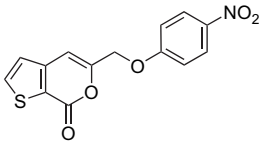


Scheme 2. Synthesis of 6-substituted 7-alkynylthieno[3,2-*c*]pyran-4-ones.

2.2.2. Synthesis of 5-substituted thieno[2,3-*c*]pyran-7-ones. We have found that 5-substituted thieno[2,3-*c*]pyran-7-one (**3**) can also be prepared as a major product by coupling **1a** with terminal alkynes using 10% Pd/C–Et_3N–CuI–PPh_3 (Method B) as a catalyst system (Entries 9 and 10, Table 1). Pd/C catalyzed reactions are particularly attractive because the catalyst can be removed easily by filtration at the end of the reaction allowing product isolation without transition metal impurities, the removal of which could be tedious and cumbersome. Thus, to assess the generality of this approach, the scope of the Pd/C-mediated coupling of **1a**

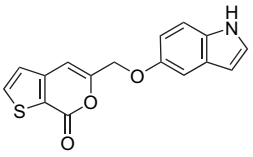
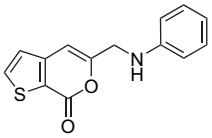
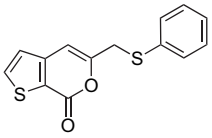
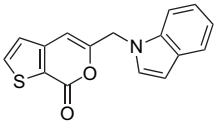
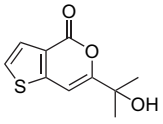
has been studied. Treatment of **1a** with a variety of terminal alkynes under the condition described in Table 1 [1.0 equiv of 3-iodothiophene-2-carboxylic acid, 1.5 equiv of terminal alkyne, 10% Pd/C (0.035 equiv), PPh_3 (0.3 equiv), CuI (0.06 equiv), and Et_3N (5 equiv) in 1,4-dioxane at 70–80 °C under nitrogen] afforded moderate to good yields of 5-substituted thieno[2,3-*c*]pyran-7-ones (Table 3). In addition to alkynes used earlier a number of other alkynes

Table 3. Pd/C-mediated synthesis of 5-substituted thieno[2,3-*c*]pyran-7-ones[a]

Entry	Alkyne (R)	Time (h)	Products (**3**)	Yield (%)	
				2	**3**
1	**a**; $-C(CH_3)_2OH$	12	 **3a**	6	80
2	**b**; $-(CH_2)_2OH$	12	 **3b**	30	60
3	**c**; $-(CH_2)_3OH$	12	 **3c**	10	60
4	**d**; $-CH(OH)CH_3$	12	 **3d**	0	55
5	**e**; $-(CH_2)_3CH_3$	12	 **3e**	17	50
6	**f**; $-(CH_2)_5CH_3$	12	 **3f**	0	55
7	**k**; $-CH_2OH$	12	 **3g**	0	35
8	**l**; $-CH_2CH(OH)CH_3$	12	 **3h**	0	55
9	**j**; $-CH_2OC_6H_5$	10	 **3i**	0	72
10	**m**; $-CH_2OC_6H_4NO_2$-*p*	10	 **3j**	0	60

(*continued*)

Table 3. (*continued*)

Entry	Alkyne (R)	Time (h)	Products (**3**)	Yield (%)	
				2	**3**
11	**n**; 5-Indolyloxy	10	 **3k**	0	70
12	**o**; $-CH_2NC_6H_5$	10	 **3l**	0	65
13	**p**; $-CH_2SC_6H_5$	10	 **3m**	0	65
14	**q**; 1-Indolyl	10	 **3n**	0	68
15	**a**; $-C(CH_3)_2OH$	12	 **3aa**	0	72

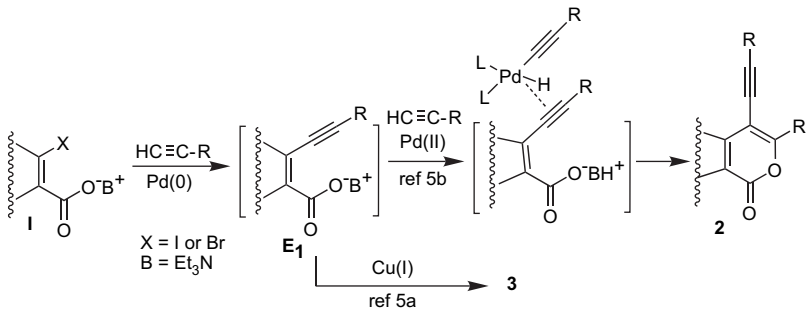
[a] All reactions were carried out by using **I** (1.0 equiv), terminal alkyne (1.5 equiv), 10% Pd/C (0.035 equiv), PPh_3 (0.3 equiv), CuI (0.06 equiv), Et_3N (5 equiv) in 1,4-dioxane at 70–80 °C under nitrogen.

containing ether, amine, thioether, and indole functionality were tested and the reaction proceeded well affording good yields of product **3** (Entries 10–14, Table 3). The use of 2-bromothiophene-3-carboxylic acid afforded 6-substituted thieno[3,2-*c*]pyran-4-one in good yield (Entry 15, Table 3). Except in four cases (Entries 1–3 and 5, Table 3), no significant amount of **2** was isolated from the reaction mixture indicating the high product selectivity of this coupling–cyclization process under Pd/C–Cu catalysis. Presumably, participation of the copper salt in the cyclization step was responsible for the predominant formation of **3** over **2** (see later for mechanistic discussion). Notably, the use of Pd/C as a source of 'ligandless palladium' in arylation of thiophene was found to be unsuccessful, which was thought to be due to the poisoning of the heterogenous catalyst.[13]

We have shown that 5-substituted thieno[2,3-*c*]pyran-7-ones (**3**) and their 4-alkynyl analogues (**2**) can be prepared via coupling reaction of 3-iodothiophene-2-carboxylic acid with terminal alkynes depending on the reaction condition employed (Method A or B). The nature of the palladium catalysts played an important role in these coupling–cyclization reactions. The use of Pd(II) or Pd(0) complexes generally led to the formation of 4-alkynyl analogues (**2**) via tandem coupling–cyclization–coupling reaction whereas the use of Pd/C facilitated only coupling–cyclization to afford **3** in good yields. Nevertheless, the reaction showed very high regioselectivity in both cases as no isomeric thieno[2,3-*c*]-furan-6-ones resulting from '5-*exo-dig*' cyclization were detected under the reaction conditions studied. This is in sharp contrast to the earlier observations[7] where the coupling–cyclization followed 5-*exo-dig* ring closure predominantly under Pd–Cu catalysis in DMF.

2.3. Proposed reaction mechanism

A reasonable pathway for $PdCl_2(PPh_3)_2$ mediated tandem reaction leading to compound **2** and Pd/C-mediated coupling–cyclization to compound **3** is shown in Scheme 3. The possibility of generating **2** via **3** can be ruled out, because it requires palladium mediated C–H activation at C-4 of thieno[2,3-*c*]pyran-7-one ring (**3**) followed by interaction of the resulting Pd(II) intermediate with the copper acetylide. This is not only an energetically unfavorable process, but also would afford copper hydride as a side product, which is unlikely. Moreover, formation of **2a** was not observed when **3a** was reacted with another mole of terminal alkyne under Pd–Cu catalysis. Thus, the reaction seems to



Scheme 3. A plausible mechanism for Pd-catalyzed formation of **2** and **3**.

proceed via in situ generation of intermediate $\mathbf{E_1}$ according to a typical Sonogashira pathway.[7b,14] Once formed this acid then undergoes intramolecular cyclization aided by the Pd(II)-complex[6a] or copper salt[5a] to give **2** or **3**. However, formation of **2** clearly suggests that the corresponding pathway is a Pd(II)-mediated process. Presumably, this proceeds via insertion of the Pd(0) complex into the acetylenic C–H bond of the terminal alkyne leading to a Pd(II) intermediate[15] ($\mathbf{E_2}$) that catalyzes the '6-*endo-dig*' ring closure. Although we have no actual proof for the generation of $\mathbf{E_2}$, insertion of Pd(0) complex into the acetylenic C–H bond however, has been suggested by Trost and co-workers earlier.[16] Additionally, a closely related intramolecular oxypalladation of the complex formed by the coordination of organo Pd(II)-complex [generated by oxidative addition of the aryl halide to Pd(0) in situ] with the C–C triple bond of the 2-(1-alkynyl)benzoate anion has also been proposed by Rossi and co-workers.[2i] Thus, reductive elimination of Pd(0) followed the '6-*endo-dig*' ring closure to afford thieno[2,3-*c*]pyran-7-one derivative. A '5-*exo-dig*' ring closure, although allowed by Baldwin's rule, was not observed in the present case because of the favorable geometry associated with the 5-6 ring formation rather than the 5-5 ring. This also accounts for observation of no solvent effect on using 1,4-dioxane and ethanol in the present synthesis of **3**, which is in contrast with the earlier synthesis of isocoumarin.[5a] It is noteworthy that due to the electron-donating resonance effect of thiophene moiety the carbon–carbon triple bond of $\mathbf{E_1}$ is more nucleophilic than that of analogue 2-(1-alkynyl)benzoic acid and therefore interacts better with the Pd(II)-complex ($\mathbf{E_2}$) generated in situ under the condition studied. This perhaps accounts for the isolation of 4-alkynyl isocoumarins as side products in poor yield during the preparation of isocoumarins under palladium–zinc catalysis. Although the reason for preferential interaction of $\mathbf{E_1}$ with Pd(II)-complex ($\mathbf{E_2}$) over CuI is not clear at this stage a possible explanation for the change of product selectivity on going from Method A to Method B is a change in the reaction mechanism in the cyclization step (Scheme 3). While generation of **3** is arguably feasible via Pd(II)-mediated cyclization of corresponding 1-alkynyl substituted thiophene carboxylic acid, isolation of **3** as only product under Pd/C–Cu catalysis in most of the cases suggests that perhaps copper salt plays a major role in Pd/C-mediated coupling–cyclization process.[5a] This was further supported by the isolation of **3e** as a sole product when 3-hex-1-ynyl thiophene-2-carboxylic acid[17] was treated with CuI in DMF at 70–80 °C for 2 h.

2.4. In vitro anticancer activity

Based on the promising cytotoxic activities reported for substituted pyrones especially 4-alkynyl substituted 2-pyrones earlier[2g] we evaluated some of the thienopyranones synthesized for in vitro anticancer activity. Selected compounds were tested on a panel of cancer cell lines, e.g., HT-29 (colon), NCI-H460 (lung), and LoVo (colon) using the NCI standard protocol for screening anticancer molecules.[18] After treating the cells with compounds at 100 μM concentration initially the percentage growth of cells was measured, which is shown in Table 4. Based on the result obtained for compound **2g** against LoVo cell line we tested this compound further at lower concentrations such as 10, 1.0, 0.1, and 0.01 μM against the same cancer cell line and the percentage growth was noted as 77, 86, 97, and 102, respectively. The GI_{50} value (the concentration that causes 50% inhibition of cancer cell growth against a cell line is expressed as GI_{50}) for compound **2g** was found to be 83.4 μM compared to 23 μM of Glevec™, an well known anticancer drug developed by Novartis. Additionally, the LC50 (Lethal Concentration 50 is the concentration of a compound that kills 50% of cells treated) of **2g** was noted as 100 μM. The present study thus indicates that alkynyl substituted thienopyranone moiety could be a new and potential scaffold that needs further exploration for design and SAR studies for the development of novel anticancer agents.

Table 4. In vitro anticancer activities of thienopyranone derivatives

Compound no	Cell line	Percentage growth @ 100 μM
2g	LoVo	48
	H460	71
	HT-29	71
2h	LoVo	61
	H460	87
	HT-29	61
3a	LoVo	68
	H460	84
	HT-29	77
3i	LoVo	72
	H460	73
	HT-29	72

3. Conclusions

In summary, a catalytic approach to thienopyranones of potential pharmacological interest has been developed through the coupling of bromo or iodo substituted thiophenecarboxylic acid with terminal alkynes under palladium–copper catalysis. A detailed study related to the effect of reaction conditions on product distribution was carried out. Conditions were developed that allow for the selective synthesis of either 4,5-disubstituted or only 5-substituted thieno-[2,3-*c*]pyran-7-ones. The best process for the preparation of 4,5-disubstituted derivatives involved the use of $PdCl_2(PPh_3)_2$ as a catalyst source and was found to be quite general and highly regioselective, placing the alkynyl moiety at the C-4 position of the thieno[2,3-*c*]pyran-7-one ring. This process was extended to the regioselective synthesis of 7-alkynylthieno[3,2-*c*]pyran-4-ones successfully. 5-Substituted thieno[2,3-*c*]pyran-7-ones on the other hand was obtained easily by using Pd/C-mediated coupling–cyclization of 3-iodothiophene-2-carboxylic acid with terminal alkynes. All these processes worked well with a broad range of terminal alkynes to afford the corresponding products in good isolated yields. The scope and limitations of both the process along with the mechanism of the reaction have been discussed. The sequencing of two or more reactions in a one-pot process, as illustrated in this report, not only makes better use of precious reagents as well as solvents but also has the benefit of eliminating cumbersome separation and purification after each step. Since the use of single catalyst source for tandem reaction is a powerful strategy for generating complex structures, we believe that the novel palladium-catalyzed transformation described here would find wide usage for the synthesis of similar class of compounds.

4. Experimental

4.1. General methods

Unless stated otherwise, reactions were performed under nitrogen atmosphere. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (60–120 mesh) using distilled petroleum ether and ethyl acetate. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$, DMSO-d_6 or MeOH-d_4 solution on 200 and 400, and 50 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ=0.00) as internal standard and expressed in parts per million. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as br (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FTIR spectrometer. Melting points were determined by using melting point apparatus and are uncorrected. Thermal analysis data [Differential Scanning Calorimetry (DSC)] were generated with the help of DSC-50 detector. MS spectra were obtained on a mass spectrometer. Chromatographic purity by HPLC was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, retention times. All terminal alkynes, 3-bromothiophene-2-carboxylic acid and 2-bromothiophene-3-carboxylic acid used are commercially available.

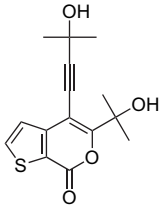
4.2. Preparation of 3-iodo thiophene-2-carboxylic acid[19] (1a)

To a solution of thiophene-2-carboxylic acid (5.0 g, 39 mmol) in dry THF (50 mL) *n*-butyl lithium (6.25 g, 41.6 mL, 97 mmol) was added slowly and dropwise at −78 °C under nitrogen atmosphere over a period of 1 h. The mixture was stirred for 1 h at the same temperature (−78 °C) and a solution of iodine (11.8 g, 46 mmol) in THF (25 mL) was added slowly by maintaining the temperature at −78 °C. The mixture was stirred initially for 8 h at −78 °C and then at 25–35 °C for 30 h. After completion of the reaction the mixture was diluted with 5% aqueous HCl (10 mL) followed by water (100 mL) and extracted with ethyl acetate (3×100 mL). The organic layers were collected, combined, washed with 10% aq sodium thiosulfate (3×50 mL) followed by water (3×50 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The crude compound was triturated with light petroleum ether (distillation range 60–80 °C) to get the title compound as light brown solid (6 g, 60% yield); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.26 (d, J=5.6 Hz, 1H), 7.52 (d, J=5.1 Hz, 1H); IR (cm^{-1}, $CHCl_3$) 3092, 2852, 1670; *m*/*z* (ES Mass) 255 (M^+, 100%).

4.3. Preparation of 5-substituted 4-alkynyl thieno[2,3-*c*]pyran-7-ones (2)

4.3.1. General procedure. A mixture of 3-iodo thiophene-2-carboxylic acid (0.787 mmol), $PdCl_2(PPh_3)_2$ (0.038 mmol), CuI (0.047 mmol), and Et_3N (4 mmol) in DMF (10 mL) was stirred for 1 h under nitrogen. The acetylenic compound (1.57 mmol) was added and the mixture was stirred at room temperature for 1 h and then at 70–80 °C for 8–12 h. After completion of the reaction, DMF was removed under reduced pressure and the residue was extracted with ethyl acetate (3×50 mL). The organic layers were collected, combined, washed with saturated aq $NaHCO_3$ (2×25 mL) followed by water (2×25 mL), dried over anhydrous Na_2SO_4, and concentrated under vacuum. The crude compound was purified by column chromatography on silica gel using light petroleum ether (60–80 °C)–ethyl acetate.

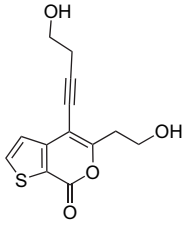
4.3.2. 4-(3-Hydroxy-3-methyl-but-1-ynyl)-5-(1-hydroxy-1-methyl-ethyl)thieno[2,3-*c*]pyran-7-one (2a).



Low melting light brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.85 (d, J=5.0 Hz, 1H), 7.34 (d, J=5.0 Hz, 1H), 2.24 (br s, –OH), 1.61 (s, 12H, CH_3); IR (cm^{-1}, $CHCl_3$) 3391, 2981, 1715 (C=O), 1626; *m*/*z* (ES Mass) 293 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 167.4 (C=O), 148.3, 144.0,

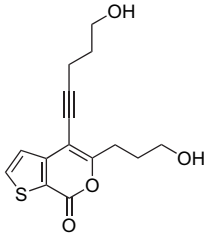
138.8 (2C), 124.7, 105.6, 104.0, 74.1, 73.6, 65.6, 31.1 (2C, CH_3), 28.5 (2C, CH_3); UV (nm, MeOH) 312.0, 252.8, 238.4, 214.2; HPLC 96.3%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 11.3 min; Elemental analysis found C, 61.77; H, 5.45; $C_{15}H_{16}O_4S$ requires C, 61.62; H, 5.52.

4.3.3. 4-(4-Hydroxy-but-1-ynyl)-5-(2-hydroxy-ethyl)-thieno[2,3-*c*]pyran-7-one (2b).



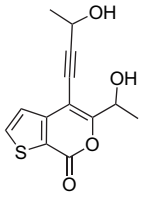
White solid; mp 116–116.5 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, J=5.0 Hz, 1H), 7.32 (d, J=5.0 Hz, 1H), 4.03 (t, J=5.7 Hz, 2H, CH_2), 3.86 (t, J=5.7 Hz, 2H, CH_2), 3.08 (t, J=5.7 Hz, 2H, CH_2), 2.74 (t, J=6.0 Hz, 2H, CH_2), 1.66 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3390, 3015, 2927, 1715 (C═O), 1602; *m*/*z* (ES Mass) 265 (M^+, 100); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 160.4 (C═O), 157.4, 157.2, 136.8, 124.6, 123.9, 104.2, 94.0, 72.3, 61.1 (CH_2OH), 60.4 (CH_2OH), 29.7 (CH_2), 23.9 (CH_2); UV (nm, MeOH) 315.2, 313.4, 252.8, 238.4, 201.6; HPLC 98.8%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 8.4 min; Elemental analysis found C, 59.15; H, 4.51; $C_{13}H_{12}O_4S$ requires C, 59.08; H, 4.58.

4.3.4. 4-(5-Hydroxy-pent-1-ynyl)-5-(3-hydroxy-propyl)-thieno[2,3-*c*]pyran-7-one (2c).



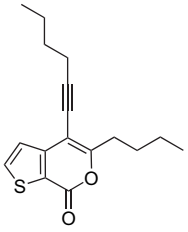
Low melting light brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, J=4.7 Hz, 1H), 7.23 (d, J=4.7 Hz, 1H), 3.85 (t, J=4.7 Hz, 2H, CH_2), 3.75 (t, J=4.6 Hz, 2H, CH_2), 2.9 (t, J=4.6 Hz, 2H, CH_2), 2.6 (t, J=4.68 Hz, 2H, CH_2), 2.10–1.90 (m, 2H, CH_2), 1.85–1.80 (m, 2H, CH_2), 1.69 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3429, 3019, 2400, 1719 (C═O), 1601; *m*/*z* (ES Mass) 293 (M^+, 100); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 163.7 (C═O), 157.2, 153.8, 136.7, 132.1, 124.5, 106.6, 94.6, 72.4, 61.4 (CH_2OH), 58.3 (CH_2OH), 30.2 (CH_2), 29.6 (CH_2), 28.6 (CH_2), 16.1 (CH_2); UV (nm, MeOH) 317.2, 253.0, 235.4, 210.8; HPLC 98.2%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 11.0 min; Elemental analysis found C, 61.32; H, 5.57; $C_{15}H_{16}O_4S$ requires C, 61.62; H, 5.52.

4.3.5. 4-(3-Hydroxy-but-1-ynyl)-5-(1-hydroxy ethyl)-thieno[2,3-*c*]pyran-7-one (2d).



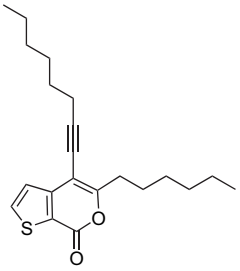
Low melting light brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.87 (d, J=5.0 Hz, 1H), 7.33 (d, J=5.0 Hz, 1H), 5.14 (q, J=6.3 Hz, 1H, CH), 4.82 (q, J=6.7 Hz, 1H, CH), 1.61 (d, J=3.4 Hz, 3H, CH_3), 1.57 (d, J=3.7 Hz, 3H, CH_3), 1.75 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3307, 2919, 1713 (C═O), 1600; *m*/*z* (CI Mass) 265 (M^+, 100); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 160.7 (C═O), 153.7, 144.2, 137.1, 127.4, 124.6, 104.2, 99.4, 75.4, 66.0 (CH), 58.8 (CH), 24.3 (CH_3), 21.2 (CH_3); UV (nm, MeOH) 313.4, 253.2, 235.8, 201.8; HPLC 96.4%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 9.7 min; HRMS Calcd for $C_{13}H_{12}O_4S$ ($M+H^+$): 265.0534. Found: 265.0540.

4.3.6. 5-Butyl-4-hex-1-ynyl-thieno[2,3-*c*]pyran-7-one (2e).



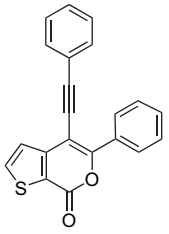
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, J=5.1 Hz, 1H), 7.28 (d, J=5.1 Hz, 1H), 2.79 (t, J=7.5 Hz, 2H, CH_2), 2.48 (t, J=7.2 Hz, 2H, CH_2), 1.74–1.66 (m, 2H, CH_2), 1.61–1.59 (m, 2H, CH_2), 1.45–1.50 (m, 2H, CH_2), 1.41–1.36 (m, 2H, CH_2), 0.93–0.99 (m, 6H, CH_3); IR (cm^{-1}, $CHCl_3$) 2958, 1729 (C═O); *m*/*z* (ES Mass) 289 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 155.2 (C═O), 154.6, 152.3, 136.4, 124.6, 123.4, 105.6, 96.6, 71.5, 31.8, 30.8, 29.7, 29.5, 22.2, 22.0, 19.2 (CH_3), 13.7 (CH_3); UV (nm, MeOH) 325.2, 252.6, 238.4, 209.6; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 18.3 min; Elemental analysis found C, 70.87; H, 6.95; $C_{17}H_{20}O_2S$ requires C, 70.80; H, 6.99.

4.3.7. 5-Hexyl-4-oct-1-ynyl-thieno[2,3-*c*]pyran-7-one (2f).



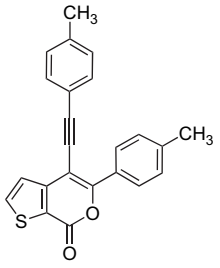
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.79 (d, J=5.1 Hz, 1H), 7.29 (d, J=5.1 Hz, 1H), 2.78 (t, J=7.8 Hz, 2H, CH_2), 2.47 (t, J=6.9 Hz, 2H, CH_2), 1.79–1.65 (m, 2H, CH_2), 1.68–1.58 (m, 2H, CH_2), 1.61–1.4 (m, 6H, CH_2), 1.39–1.26 (m, 6H, CH_2), 0.91–0.87 (m, 6H, CH_3); IR (cm^{-1}, $CHCl_3$) 2929, 1727 (C═O), 1600; *m/z* (ES Mass) 345 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) 136.3 (C═O), 124.6, 32.1 (2C), 31.5 (2C), 31.3 (2C), 29.7 (2C), 28.7 (2C), 28.6 (2C), 27.4 (2C), 22.50 (2C), 19.5 (2C), 14.0; UV (nm, MeOH) 322.0, 252.8, 239.4, 214.8; HPLC 98.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 19.9 min; Elemental analysis found C, 73.37; H, 8.10; $C_{21}H_{28}O_2S$ requires C, 73.21; H, 8.19.

4.3.8. 5-Phenyl-4-phenyl ethynyl-thieno-[2,3-*c*]pyran-7-one (2g).



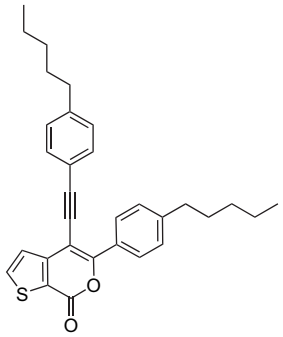
Pale yellow solid; mp 122–123 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.25–8.22 (m, 2H), 7.90 (d, J=5.1 Hz, 1H), 7.90 (d, J=5.1 Hz, 1H), 7.50–7.30 (m, 8H); IR (cm^{-1}, KBr) 3107, 1723 (C═O); *m/z* (ES Mass) 329 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.6 (C═O), 148.4, 136.7, 131.9, 313.6 (2C), 131.4, 131.2, 130.5, 130.2, 129.5, 128.8, 128.5, 128.4, 128.3 (2C), 125.3, 122.6, 105.1, 96.6, 88.3; UV (nm, MeOH) 313.4, 217.4, 247.8; HPLC 99.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 18.0 min; Elemental analysis found C, 76.87; H, 3.66; $C_{21}H_{12}O_2S$ requires C, 76.81; H, 3.68.

4.3.9. 5-*p*-Tolyl-4-*p*-tolylethynyl-thieno[2,3-*c*]pyran-7-one (2h).



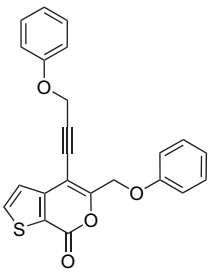
Pale yellow solid; mp 171.5–171.8 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.15 (d, J=8.3 Hz, 2H), 7.87 (d, J=5.1 Hz, 1H), 7.53 (d, J=5.1 Hz, 1H), 7.40 (d, J=8.1 Hz, 2H), 7.29 (d, J=8.1 Hz, 2H), 7.19 (d, J=8.1 Hz, 2H), 2.35–2.43 (m, 6H, CH_3); IR (cm^{-1}, KBr) 3072, 1727 (C═O), 1584; *m/z* (CI Mass) 358 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 158.9 (C═O), 148.6, 140.9, 139.1, 136.5, 133.3 (2C), 131.2, 129.3 (2C), 128.9, 128.7 (2C), 126.2, 125.2 (2C), 123.3, 119.6, 103.6, 96.8, 82.3, 22.6 (CH_3), 21.5 (CH_3); UV (nm, MeOH) 317.4, 252.8; HPLC 96.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 18.7 min; Elemental analysis found C, 77.37; H, 4.54; $C_{23}H_{16}O_2S$ requires C, 77.50; H, 4.52.

4.3.10. 5-(4-Pentyl-phenyl)-4-(4-pentyl-phenylethynyl)-thieno[2,3-*c*]pyran-7-one (2i).



Low melting brown solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.18 (d, J=8.3 Hz, 2H), 7.87 (d, J=5.1 Hz, 1H), 7.53 (d, J=5.1 Hz, 1H), 7.43 (d, J=8.3 Hz, 2H), 7.30 (d, J=8.3 Hz, 2H), 7.19 (d, J=8.1 Hz, 2H), 2.69–2.61 (m, 4H, CH_2), 1.67–1.61 (m, 4H, CH_2), 1.37–1.25 (m, 8H, CH_2), 0.92–0.87 (m, 6H, CH_3); IR (cm^{-1}, $CHCl_3$) 2928, 1736 (C═O), 1598; *m/z* (CI Mass) 469 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) 158.6 (C═O), 148.7, 147.5, 145.9, 144.1, 136.5, 132.4, 131.3 (2C), 129.3 (2C), 128.6 (2C), 128.3 (2C), 127.3, 123.2, 120.8, 103.8, 96.9, 82.4, 35.9 (2C), 31.4 (2C), 30.8 (2C), 22.5 (2C), 14.0 (2C); UV (nm, MeOH) 317.8, 254.0, 202.0; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 23.4 min; HRMS Calcd for $C_{31}H_{32}O_2S$ (M+H^+): 469.2201. Found: 469.2216.

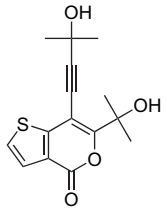
4.3.11. 5-Phenoxymethyl-4-(3-phenoxy-prop-1-ynyl)-thieno[2,3-*c*]pyran-7-one (2j).



White solid; mp 115.2 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (d, J=7.5 Hz, 1H), 7.34–7.23 (m, 6H), 7.22–7.0 (m,

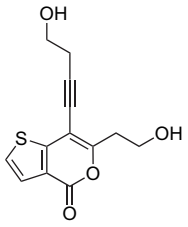
5H), 4.93 (s, 2H, CH_2), 5.03 (s, 2H, CH_2); IR (cm^{-1}, KBr) 2925, 1733 (C=O), 1598, 1240; *m/z* (CI Mass) 389 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) 157.9 (C=O), 156.9, 154.5, 153.8, 152.7, 137.0, 129.6 (2C), 129.5 (2C), 126.6, 124.7, 121.8, 121.7, 115.0 (2C), 114.9 (2C), 101.2, 92.7, 78.1, 65.0, 56.2; UV (nm, MeOH) 313.0, 255.8, 202.8; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.0 mL/min, UV 254 nm, retention time 17.4 min; Elemental analysis found C, 71.22; H, 4.10; $C_{23}H_{16}O_4S$ requires C, 71.12; H, 4.15.

4.3.12. 6-(1-Hydroxy-1-methyl-ethyl)-7-(3-hydroxy-3-methyl-but-1-ynyl)-thieno[3,2-*c*]pyran-4-one (2aa).



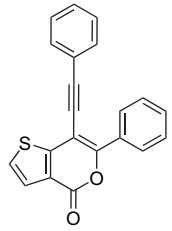
Low melting light brown solid; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.54 (d, *J*=5.2 Hz, 1H), 7.37 (d, *J*=5.3 Hz, 1H), 2.24 (br s, –OH), 1.68 (s, 12H); IR (cm^{-1}, $CHCl_3$) 3272, 1722 (C=O); *m/z* (CI Mass) 293 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.9 (C=O), 156.7, 153.9, 132.1, 128.3, 126.3, 125.7, 104.4, 95.8, 73.6, 65.4, 30.8 (2C, CH_3), 28.5 (2C, CH_3); UV (nm, MeOH) 314.8, 264.4, 231.8, 203.4; HPLC 96.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 12.5 min; Elemental analysis found C, 61.32; H, 5.58; $C_{15}H_{16}O_4S$ requires C, 61.62; H, 5.52.

4.3.13. 6-(2-Hydroxy-ethyl)-7-(4-hydroxy-but-1-ynyl)-thieno [3,2-*c*]pyran-4-one (2bb).



Brown color solid; mp 101–102 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.54 (d, *J*=5.6 Hz, 1H), 7.34 (d, *J*=5.6 Hz, 1H), 4.02 (t, *J*=5.9 Hz, 2H), 3.88 (t, *J*=5.9 Hz, 2H), 3.08 (t, *J*=5.9 Hz, 2H), 2.75 (t, *J*=5.9 Hz, 2H), 1.66 (br s, –OH); IR (cm^{-1}, KBr) 3381, 2919, 1712 (C=O); *m/z* (CI Mass) 265 (M^+, 100%); ^{13}C ($CDCl_3$, 50 MHz) δ 160.3 (C=O), 158.1, 152.4, 132.1, 128.4, 125.8, 102.1, 95.3, 73.6, 60.8 (CH_2OH), 59.9 (CH_2OH), 35.6 (CH_2), 23.9 (CH_2); UV (nm, MeOH) 316.4, 276.0, 264.8, 232.8; HPLC 98.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 8.9 min; Elemental analysis found C, 59.17; H, 4.47; $C_{13}H_{12}O_4S$ requires C, 59.08; H, 4.58.

4.3.14. 6-Phenyl-7-phenyl ethyl-thieno-[3,2-*c*]pyran-4-one (2gg).

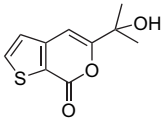


Pale yellow solid; mp 183–184 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.25 (m, 2H), 7.63 (d, *J*=5.4 Hz, 1H), 7.43 (d, *J*=5.4 Hz, 1H), 7.55–7.50 (m, 5H), 7.40–7.38 (m, 3H); IR (cm^{-1}, KBr) 3421, 2962, 1738 (C=O); *m/z* (CI Mass) 329 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 157.0 (C=O), 131.4 (2C), 130.6 (2C), 129.0 (2C), 128.5 (2C), 128.3, 128.2, 126.6 (2C), 126.0 (2C), 125.4, 122.6, 122.2, 101.6, 97.6 (2C); UV (nm, MeOH) 340.6, 300.0, 239.0, 203.2; HPLC 98.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 18.0 min; Elemental analysis found C, 76.63; H, 6.77; $C_{21}H_{12}O_2S$ requires C, 76.81; H, 3.68.

4.4. Preparation of 5-substituted thieno[2,3-*c*]pyran-7-ones (3)

4.4.1. General procedure. A mixture of 3-iodo thiophene-2-carboxylic acid (1.18 mmol), 10% Pd/C (0.035 mmol), PPh_3 (0.14 mmol), CuI (0.07 mmol), and triethylamine (6.0 mmol) in 1,4-dioxane (10 mL) was stirred at 25–30 °C for 30 min under nitrogen and acetylinic compound (1.8 mmol) was added. The mixture was then stirred at room temperature for 1 h and then at 75–80 °C for 10–12 h. After completion of the reaction the mixture was cooled to room temperature, diluted with EtOAc (50 mL), and filterd through Celite. The filtrate was washed with saturated aq sodium hydrogen carbonate (2×25 mL) followed by water (2×25 mL), dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by column chromatography on silicagel, using light petroleum (distillation range 60–80 °C)–ethylacetate as eluent.

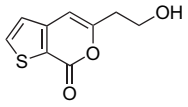
4.4.2. 5-(1-Hydroxy-1-methyl-ethyl)-thieno-[2,3-*c*]-pyran-7-one (3a).



White solid, mp 87–88 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.82 (d, *J*=5.3 Hz, 1H), 7.17 (d, *J*=5.0 Hz, 1H), 6.8 (s, 1H, CH=C), 2.24 (br s, –OH), 1.61 (s, 6H, CH_3); IR (cm^{-1}, KBr) 3499, 1736 (C=O); *m/z* (ES Mass) 211 (M^+, 100%); ^{13}C ($CDCl_3$, 50 MHz) δ 164.7 (C=O), 155.7, 147.3, 136.8, 124.7 (2C), 97.3, 71.2, 28.4 (2C, CH_3); UV (nm, MeOH) 360.0, 353.8, 310.4, 282.0, 230.8; HPLC 99.2%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70,

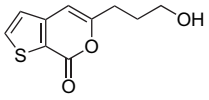
15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 7.2 min; Elemental analysis found C, 57.24; H, 4.75; $C_{10}H_{10}O_3S$ requires C, 57.13; H, 4.79.

4.4.3. 5-(2-Hydroxy-ethyl)-thieno[2,3-*c*]pyran-7-one (3b).



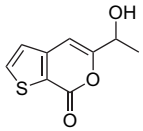
Low melting brown solid; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.82 (d, J=5.0 Hz, 1H), 7.14 (d, J=5.3 Hz, 1H), 6.58 (s, 1H), 4.02 (t, J=6.0 Hz, 2H), 2.84 (t, J=6.0 Hz, 2H), 1.65 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3430, 1717 (C=O); m/z (ES Mass) 197 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 157.6 (C=O), 152.2, 147.4, 136.7, 124.2 (2C), 102.2, 59.6 (CH_2), 29.6 (CH_2); UV (nm, MeOH) 312.4, 231.4, 210.0; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 5.2 min; Elemental analysis found C, 55.15; H, 4.10; $C_9H_8O_3S$ requires C, 55.09; H, 4.11.

4.4.4. 5-(3-Hydroxy-propyl)-thieno[2,3-*c*]pyran-7-one (3c).



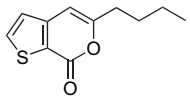
Low melting brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.80 (d, J=5.1 Hz, 1H), 7.12 (d, J=5.1 Hz, 1H), 6.49 (s, 1H), 2.70 (t, J=7.5 Hz, 2H), 2.39 (t, J=6.9 Hz, 2H), 2.05–1.94 (m, 2H), 1.56 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3361, 1713 (C=O); m/z (CI Mass) 211 (M^+, 100%); UV (nm, MeOH) 308.8, 231.2, 209.8; HPLC 96.5%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 8.1 min; Elemental analysis found C, 57.24; H, 4.77; $C_{10}H_{10}O_3S$ requires C, 57.13; H, 4.79.

4.4.5. 5-(1-Hydroxy-ethyl)-thieno[2,3-*c*]pyran-7-one (3d).



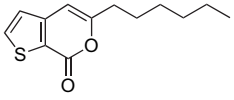
Low melting brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, J=5.0 Hz, 1H), 7.16 (d, J=5.0 Hz, 1H), 6.75 (s, 1H), 4.75–4.66 (m, 1H), 2.58 (br s, –OH), 1.56 (d, J=6.7 Hz, 3H); IR (cm^{-1}, $CHCl_3$) 3331, 1712 (C=O); m/z (CI Mass) 197 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.0 (C=O), 151.0, 147.0, 136.8, 124.6 (2C), 98.6, 66.8 (CHOH), 21.5 (CH_3); UV (nm, MeOH) 309.4, 231.4, 209.8; HPLC 98.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 6.4 min; HRMS Calcd for $C_9H_8O_3S$ ($M+H^+$): 197.0272. Found: 197.0271.

4.4.6. 5-Butyl-thieno[2,3-*c*]pyran-7-one (3e).



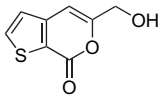
^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.78 (d, J=5.1 Hz, 1H), 7.10 (d, J=5.1 Hz, 1H), 6.43 (s, 1H), 2.56 (t, J=7.3 Hz, 2H), 1.74–1.66 (m, 2H), 1.46–1.36 (m, 2H), 0.94 (t, J=7.3 Hz, 3H); IR (cm^{-1} $CHCl_3$) 2957, 1720 (C=O); m/z (ES Mass) 209 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 161.0 (C=O), 156.7, 147.5, 136.4, 124.0 (2C), 100.3, 33.1, 29.6, 22.0, 13.7 (CH_3); UV (nm, MeOH) 315.0, 281.6, 231.2, 210.4; HPLC 98.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 16.9 min; Elemental analysis found C, 63.39; H, 5.83; $C_{11}H_{12}O_2S$ requires C, 63.43; H, 5.81.

4.4.7. 5-Hexyl-thieno [2,3-*c*] pyran-7-one (3f).



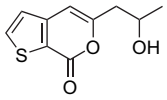
^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.79 (d, J=5.0 Hz, 1H), 7.11 (d, J=5.0 Hz, 1H), 6.43 (s, 1H), 2.59–2.52 (m, 2H), 1.78–1.70 (m, 2H), 1.40–1.25 (m, 6H), 0.92–0.85 (m, 3H); IR (cm^{-1}, $CHCl_3$) 3390, 1718 (C=O); m/z (CI Mass) 237 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 161.0 (C=O), 156.3, 147.5, 136.4, 124.0 (2C), 100.3, 33.4, 31.4, 28.5, 27.0, 22.4, 13.9; UV (nm, MeOH) 314.8, 231.4, 210.0; HPLC 96.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 17.1 min; HRMS Calcd for $C_{13}H_{16}O_2S$ ($M+H^+$): 237.0949. Found: 237.0943.

4.4.8. 5-Hydroxymethyl-thieno[2,3-*c*]pyran-7-one (3g).



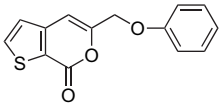
Low melting brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.84 (d, J=5.1 Hz, 1H), 7.18 (d, J=5.1 Hz, 1H), 6.74 (s, 1H), 4.54 (s, 2H), 1.65 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3388, 2926, 1696 (C=O); m/z (CI Mass) 183 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 156.3 (C=O), 144.7, 142.5, 136.9, 124.5 (2C), 100.3, 61.4 (CH_2OH); UV (nm, MeOH) 306.6, 231.0, 209.2; HPLC 95.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 4.9 min; Elemental analysis found C, 52.69; H, 3.34; $C_8H_6O_3S$ requires C, 52.74; H, 3.32.

4.4.9. 5-(2-Hydroxy-propyl)-thieno[2,3-*c*]pyran-7-one (3h).



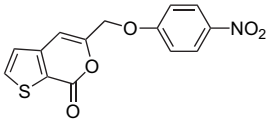
Low melting brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.80 (d, J=5.1 Hz, 1H), 7.12 (d, J=5.1 Hz, 1H), 6.54 (s, 1H), 4.31–4.25 (m, 1H), 2.77–2.66 (m, 5H), 1.83 (br s, –OH); IR (cm^{-1}, $CHCl_3$) 3366, 1713 (C=O); *m/z* (CI Mass) 211 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 159.1 (C=O), 157.7, 147.3, 136.7, 124.2 (2C), 102.4, 66.2, 43.0 (CHOH), 23.1 (CH_3); UV (nm, MeOH) 237.6, 231.4, 210.6; HPLC 96.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention of time 7.3 min; Elemental analysis found C, 57.24; H, 4.78; $C_{10}H_{10}O_3S$ requires C, 57.13; H, 4.79.

4.4.10. 5-Phenoxymethyl-thieno[2,3-*c*]pyran-7-one (3i).



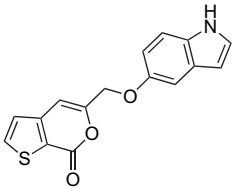
Pale yellow solid; mp 105.2–105.5 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, J=2.4 Hz, 1H), 7.34–7.30 (m, 2H), 7.19 (d, J=2.9 Hz, 1H), 7.17–6.99 (m, 3H), 6.86 (s, 1H), 4.93 (s, 2H); IR (cm^{-1}, $CHCl_3$) 3411, 1722 (C=O); *m/z* (CI Mass) 259 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 157.7 (C=O), 155.1, 146.7, 136.9, 130.7, 129.9 (2C), 129.5, 124.6, 121.8, 114.6 (2C), 101.2, 65.7; UV (nm, MeOH) 304.2, 231.6, 209.8; HPLC 95.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention of time 15.3 min; Elemental analysis found C, 65.21; H, 3.88; $C_{14}H_{10}O_3S$ requires C, 65.10; H, 3.90.

4.4.11. 5-(4-Nitro-phenoxymethyl)-thieno-[2,3-*c*]pyran-7-one (3j).



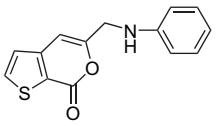
Pale yellow solid; mp 212–213 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.24 (d, J=9.4 Hz, 2H), 7.87 (d, J=5.1 Hz, 1H), 7.23 (d, J=5.1 Hz, 1H), 7.06 (d, J=9.4 Hz, 2H), 6.81 (s, 1H), 5.01 (s, 2H); IR (cm^{-1}, KBr) 3106, 1713 (C=O), 1591, 1338, 1265; *m/z* (CI Mass) 304 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 156.5 (C=O), 154.8, 144.9, 142.5 (2C), 130.6 (2C), 124.9, 121.3 (2C), 114.2 (2C), 104.6, 64.3 (CH_2); UV (nm, MeOH) 308.4, 228.8, 219.4; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention of time 14.5 min; Elemental analysis found C, 55.49; H, 3.05; N, 4.59; $C_{14}H_9NO_5S$ requires C, 55.44; H, 2.99; N, 4.62.

4.4.12. 5-(1*H*-Indol-5-yloxymethyl)-thieno-[2,3-*c*]pyran-7-one (3k).



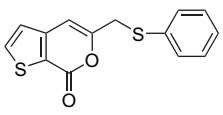
White crystalline solid; mp 130–131 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.82 (d, J=5.1 Hz, 1H), 7.34–6.91 (m, 5H), 6.46 (d, J=11.8 Hz, 1H), 6.85 (s, 1H), 4.98 (s, 2H); IR (cm^{-1}, KBr) 2924, 2854, 1710 (C=O); *m/z* (CI Mass) 298 (M^+, 100%); UV (nm, MeOH) 360.4, 298.6, 211.6; Elemental analysis found C, 64.69; H, 3.72; N, 4.68; $C_{16}H_{11}NO_3S$ requires C, 64.63; H, 3.73; N, 4.71.

4.4.13. 5-Phenyl aminomethyl-thieno-[2,3-*c*]pyran-7-one (3l).



Pale yellow solid; mp 156–157 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.79 (d, J=5.1 Hz, 1H), 7.08–7.23 (m, 4H), 6.80–6.63 (m, 3H), 4.27 (s, 2H); IR (cm^{-1}, KBr) 3389, 1709 (C=O), 1603; *m/z* (CI Mass) 258 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 157.5 (C=O), 146.7 (2C), 136.7 (2C), 129.3 (2C), 124.4 (2C), 118.4, 112.9 (2C), 100.3, 45.2 (CH_2); UV (nm, MeOH) 310.6, 283.8, 240.0, 204.2; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention of time 12.4 min; Elemental analysis found C, 65.53; H, 4.26; N, 5.33; $C_{14}H_{11}NO_2S$ requires C, 65.35; H, 4.31; N, 5.44.

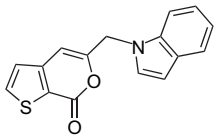
4.4.14. 5-Phenylsulfanylmethyl-thieno[2,3-*c*]pyran-7-one (3m).



Low melting brown solid; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.78 (d, J=5.1 Hz, 1H), 7.39–7.29 (m, 5H), 7.07 (d, J=5.1 Hz, 1H), 6.53 (s, 1H), 3.94 (s, 2H); IR (cm^{-1}, $CHCl_3$) 3347, 2924, 1719 (C=O); *m/z* (CI Mass) 275 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 155.9 (C=O), 146.7, 136.7, 134.5, 131.1, 130.8, 129.4, 129.1, 128.2, 127.3, 124.3 (2C), 101.8, 36.6; UV (nm, MeOH) 315.4, 238.2, 206.4; HPLC 96.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 15.6 min; Elemental analysis

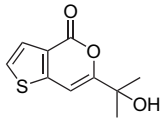
found C, 61.37; H, 3.65; $C_{14}H_{10}O_2S_2$ requires C, 61.29; H, 3.67.

4.4.15. 5-Indol-1-ylmethyl-thieno[2,3-*c*]pyran-7-one (3n).



Pale yellow solid; mp 179–180 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.75 (d, J=5.1 Hz, 1H), 7.67 (d, J=6.9 Hz, 1H), 7.32 (d, J=7.5 Hz, 1H), 7.25–7.14 (m, 3H), 6.98 (d, J=5.3 Hz, 1H), 6.62 (s, 1H), 6.05 (d, J=1.3 Hz, 1H), 5.20 (s, 2H); IR (cm^{-1}, KBr) 3364, 2931, 1709 (C=O); *m/z* (CI Mass) 282 (M^+, 100%); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 162.8 (C=O), 155.3, 146.5 (2C), 136.9 (2C), 128.2, 124.5, 122.3, 121.2 (2C), 120.1, 109.2, 102.9, 100.3, 47.1 (CH_2); UV (nm, MeOH) 360.0, 291.0, 281.0, 219.6; HPLC 98.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention of time 15.7 min; Elemental analysis found C, 68.52; H, 3.90; N, 4.85; $C_{16}H_{11}NO_2S$ requires C, 68.31; H, 3.94; N, 4.98.

4.4.16. 6-(1-Hydroxy-1-methyl-ethyl)-thieno[3,2-*c*]-pyran-4-one (3aa).



Off-white solid; mp 115–116 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.56 (d, J=5.3 Hz, 1H), 7.30 (d, J=5.3 Hz, 1H), 6.9 (s, 1H), 2.24 (br s, –OH), 1.60 (s, 6H); IR (cm^{-1}, KBr) 3231, 2981, 1723 (C=O); *m/z* (CI Mass) 211 (M^+, 100%); ^{13}C ($CDCl_3$, 50 MHz) δ 163.6 (C=O), 158.2, 147.3, 125.5, 125.2, 96.4, 83.9, 65.5, 31.0 (2C, CH_3); UV (nm, MeOH) 310.6, 283.4, 230.6, 210.5; HPLC 97.0%, column: Zorbax Eclipse XDB C-18 (150×4.6 mm), mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in methanol, gradient (T/%B): 0/30, 13/70, 15/100, 25/100, flow rate: 1.5 mL/min, UV 254 nm, retention time 8.1 min; Elemental analysis found C, 57.40; H, 4.75; $C_{10}H_{10}O_3S$ requires C, 57.13; H, 4.79.

4.5. Protocol for in vitro cell growth assay

Anticancer activity of selected compounds has been tested in HT-29 (ATCC NO# HTB-38 Colon adenocarcinoma), NCI-H460 (ATCC NO# HTB-177 Large cell lung cancer), and LoVo (ATCC NO# CCL-229 Colon adenocarcinoma) cell lines by using Sulforhodamine B (SRB) assay.[18] Cells were maintained in RPMI 1640 with 10% FBS (Fatal Bovine Serum), Penicillin (50 μg/mL), and Streptomycin (100 μg/mL). Cells were seeded in a 96-well cell culture plates at a concentration of 10,000 cells per well and incubated at 37 °C in CO_2 incubator. Twenty-four hours later cells were treated with different concentrations (100, 10, 1, 0.1, and 0.01 μM) of compound dissolved in DMSO and incubated for 48 h. Cells were fixed by adding ice-cold 50% trichloroacetic acid (TCA) and incubating for 1 h at 4 °C. The plates were washed with distilled water, air-dried, and stained with SRB solution (0.4% wt/vol in 1% acetic acid) for 30 min at room temperature. Unbound SRB was removed by washing thoroughly with 1% acetic acid and the plates were air-dried. The bound SRB stain was solubilized with 10 mM Tris buffer, and the optical densities were read on a spectrophotometric plate reader at 515 nm. At the time of drug addition separate reference plate for cell growth at time 0 h (the time at which drugs were added) was also terminated as described above. From the optical densities the percentage growths were calculated using the following formulae: if T is greater than or equal to T_0, percentage growth= $100\times[(T-T_0)/(C-T_0)]$ and if T is less than T_0, percentage growth=$100\times[(T-T_0)/T_0)]$, where T is optical density of test, C is the optical density of control, and T_0 is the optical density at time zero. From the percentage growths a dose response curve was generated and GI_{50} values were interpolated from the growth curves.

Acknowledgements

The authors thank Dr. A. Venkateswarlu, Dr. R. Rajagopalan, Professor J. Iqbal and Dr. Vilas Dahanukar for their encouragement and the analytical group for spectral data. S. Raju thanks DRL for allowing him to pursue this work as a part of his Ph.D. dissertation. M.P. thanks DRL for allowing him to attend the Symposium on Chemistry, Biology and Medicine organized on the occasion of the 60th Birthday of Professor K. C. Nicolaou, from May 28 till June 1, 2006, in Paphos, Cyprus.

References and notes

1. (a) Posner, G. H.; Nelson, T. D.; Kinter, C. M.; Johnson, N. *J. Org. Chem.* **1992**, *57*, 4083; The chemistry of α-pyrones has been investigated intensively, see for example: (b) Kvita, V.; Fischer, W. *Chimia* **1992**, *46*, 457; (c) Kvita, V.; Fischer, W. *Chimia* **1993**, *47*, 3; (d) Katritzky, A. R.; Wang, Z.; Wang, M.; Hall, C. D.; Suzuki, K. *J. Org. Chem.* **2005**, *70*, 4854; For potent and non peptidic HIV protease inhibitory effects of α-pyrones, see: (e) Vara Prasad, J. V. N.; Para, K. S.; Lunney, E. A.; Ortwine, D. F.; Dunbar, J. B., Jr.; Ferguson, D.; Tummino, P. J.; Hupe, D.; Tait, B. D.; Domagala, J. M.; Humblet, C.; Bhat, T. N.; Liu, B.; Guerin, D. M. A.; Baldwin, E. T.; Erickson, J. W.; Sawyer, T. K. *J. Am. Chem. Soc.* **1994**, *116*, 6989.
2. (a) Barry, R. D. *Chem. Rev.* **1964**, *64*, 229; (b) Nozawa, K.; Yamada, M.; Tsuda, Y.; Kawai, K.; Nakajima, S. *Chem. Pharm. Bull.* **1981**, *29*, 2491; (c) Hussain, M. T.; Rama, N. H.; Malik, A. *Indian J. Chem., Sect. B* **2001**, *40*, 372; (d) Sato, H.; Konoma, K.; Sakamura, S. *Agric. Biol. Chem.* **1981**, *45*, 1675; (e) See for example: *Drug Data Rep.* **1999**, *21*, 176; (f) Oikawa, T.; Sasaki, M.; Inose, M.; Shimamura, M.; Kuboki, H.; Hirano, S.-I.; Kumagai, H.; Ishizuka, M.; Takeuchi, T. *Anticancer Res.* **1997**, *17*, 1881; (g) Fairlamb, I. J. S.; Marrison, L. R.; Dickinson, J. M.; Lu, F.-J.; Schmidt, J. P. *Bioorg. Med. Chem.* **2004**, 4285; (h) Larock, R. C.; Yum, E. K.; Doty, M. J.; Sham, K. K. C. *J. Org. Chem.* **1995**,

60, 3270; (i) Larock, R. C.; Doty, M. J.; Han, X. *J. Org. Chem.* **1999**, *64*, 8770; (j) Rossi, R.; Bellina, F.; Biagetti, M.; Catanese, A.; Mannina, L. *Tetrahedron Lett.* **2000**, *41*, 5281.

3. (a) Campaigne, E. *Comprehensive Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Pergamon: New York, NY, 1984; Vol. 4, pp 911–913; (b) Burger, A. *Prog. Drug Res.* **1991**, *37*, 287; (c) Colotta, V.; Catarzi, D.; Varano, F.; Melani, F.; Filacchioni, G.; Cecchi, L.; Trincavelli, L.; Martini, C.; Lucacchini, A. *Il Farmaco* **1998**, *53*, 189; (d) Catarzi, D.; Cecchi, L.; Colotta, V.; Filacchioni, G.; Martini, C.; Tacchi, P.; Lucacchini, A. *J. Med. Chem.* **1995**, *38*, 1330; (e) For example, thiophene isosteres of the potassium channel opener cromakalim were found to be 10-fold more potent than cromakalim when tested for oral antihypertensive activity in spontaneously hypertensive rats, see: Sanfilippo, P. J.; McNally, J. J.; Press, J. B.; Fitzpatrick, L. J.; Urbanski, M. J.; Katz, L. B.; Giardino, E.; Falotico, R.; Salata, J.; Moore, J. B., Jr.; Miller, W. *J. Med. Chem.* **1992**, *35*, 4425; (f) For synthesis and analgesic activity of pyrano[2,3-*c*]pyrazoles, see: Ueda, T.; Mase, H.; Oda, N.; Ito, I. *Chem. Pharm. Bull.* **1981**, *12*, 3522; (g) For example, thiophenes can undergo Diels–Alder reactions through their 1,1-dioxide derivatives, see: Press, J. B.; Russell, R. K. *Progress in Heterocyclic Chemistry*; Suschitzky, H., Scriven, E. F. V., Eds.; Pergamon: Oxford, 1992; Vol. 4, pp 62–80; (h) Ram, V. J.; Goel, A.; Shukla, P. K.; Kapil, A. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3101.
4. For synthesis of related derivatives, see: (a) Mladenovic, S. A.; Castro, C. E. *J. Heterocycl. Chem.* **1968**, *5*, 227; (b) Mentzer, B. *Bull. Soc. Chim. Fr.* **1945**, *12*, 292; (c) Zhang, Y.; Herndon, J. W. *J. Org. Chem.* **2002**, *67*, 4177; For other examples of the thienopyrone ring system, see: (d) Jackson, P. M.; Moody, C. J.; Shah, P. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2909; (e) Jackson, P. M.; Moody, C. J. *J. Chem. Soc., Perkin Trans. 1* **1990**, 681.
5. (a) Subramanian, V.; Batchu, V. R.; Barange, D.; Pal, M. *J. Org. Chem.* **2005**, *70*, 4778; (b) A preliminary report on part of this work has appeared, see: Raju, S.; Batchu, V. R.; Swamy, N. K.; Dev, R. V.; Babu, J. M.; Kumar, P. R.; Mukkanti, K.; Pal, M. *Tetrahedron Lett.* **2006**, *47*, 83.
6. For leading references, see: (a) Sashida, H.; Kawamukai. *Synthesis* **1999**, 1145; (b) Yao, T.; Larock, R. C. *J. Org. Chem.* **2003**, *68*, 5936; (c) Biagetti, M.; Bellina, F.; Carpita, A.; Stabile, P.; Rossi, R. *Tetrahedron* **2002**, *58*, 5023; (d) Rossi, R.; Carpita, A.; Bellina, F.; Stabile, P.; Mannina, L. *Tetrahedron* **2003**, *59*, 2067; (e) Peng, A.-Y.; Ding, Y.-X. *Org. Lett.* **2004**, *6*, 1119.
7. (a) Liao, H.-Y.; Cheng, C.-H. *J. Org. Chem.* **1995**, *60*, 3711; (b) Kundu, N. G.; Pal, M.; Nandi, B. *J. Chem. Soc., Perkin Trans. 1* **1998**, 561; (c) Kundu, N. G.; Pal, M. *J. Chem. Soc., Chem. Commun.* **1993**, 86.
8. See for example: (a) Wu, J.; Liao, Y.; Yang, Z. *J. Org. Chem.* **2001**, *66*, 3642; (b) Fu, X.; Zhang, S.; Yin, J.; Schumacher, D. P. *Tetrahedron Lett.* **2002**, *43*, 6673; (c) Kabalka, G. W.; Dong, G.; Venkataiah, B. *Tetrahedron Lett.* **2004**, *45*, 5139; (d) Catellani, M.; Chiusoli, G. P.; Fagnola, M. C.; Solari, G. *Tetrahedron Lett.* **1994**, *35*, 5923. See also Ref. 6b; For the synthesis of 4-alkynyl-2-pyrones, see: (e) Marrison, L. R.; Dickinson, J. M.; Ahmed, R.; Fairlamb, I. J. S. *Tetrahedron Lett.* **2002**, *43*, 8853; (f) Fairlamb, I. J. S.; O'Brien, C. T.; Lin, Z.; Lam, K. C. *Org. Biomol. Chem.* **2006**, *4*, 1213; (g) Fairlamb, I. J. S.; Lee, A. F.; Loe-Mie, F.; Niemelä, E. H.; O'Brien, C. T.; Whitwood, A. C. *Tetrahedron* **2005**, *61*, 9827; (h) Collings, J. C.; Parsons, A. C.; Porrès, L.; Beeby, A.; Batsanov, A. S.; Howard, J. A. K.; Lydon, D. P.; Low, P. J.; Fairlamb, I. J. S.; Marder, T. B. *Chem. Commun.* **2005**, 2666; (i) Niemelä, E. H.; Lee, A. F.; Fairlamb, I. J. S. *Tetrahedron Lett.* **2004**, *45*, 3593; (j) Fairlamb, I. J. S.; Lu, F.-J.; Schmidt, J.-P. *Synthesis* **2003**, 2564; (k) Marrison, L. R.; Dickinson, J. M.; Fairlamb, I. J. S. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3509.
9. (a) Tietze, L. F. *Chem. Rev.* **1996**, *96*, 115; (b) Lira, R.; Wolfe, J. P. *J. Am. Chem. Soc.* **2004**, *126*, 13906; (c) Loones, K. T. J.; Maes, B. U. W.; Dommisse, R. A.; Lemiere, G. L. F. *Chem. Commun.* **2004**, 2466; (d) Zhu, G.; Zhang, Z. *Org. Lett.* **2004**, *6*, 4041; (e) Tietze, L. F.; Sommer, K. M.; Zinngrebe, J.; Stecker, F. *Angew. Chem., Int. Ed.* **2004**, *44*, 257; (f) Wasilke, J.-C.; Obrey, S. J.; Baker, R. T.; Bazan, G. C. *Chem. Rev.* **2005**, *105*, 1001; (g) Pal, M.; Dakarapu, R.; Parasuraman, K.; Subramanian, V.; Yeleswarapu, K. R. *J. Org. Chem.* **2005**, *70*, 7179.
10. For our earlier studies on Sonogashira-type reactions, see: (a) Pal, M.; Parasuraman, K.; Gupta, S.; Yeleswarapu, K. R. *Synlett* **2002**, 1976; (b) Pal, M.; Subramanian, V.; Parasuraman, K.; Yeleswarapu, K. R. *Tetrahedron* **2003**, *59*, 9563; (c) Pal, M.; Subramanian, V.; Yeleswarapu, K. R. *Tetrahedron Lett.* **2003**, *44*, 8221; (d) Pal, M.; Subramanian, V.; Batchu, V. R.; Dager, I. *Synlett* **2004**, 1965; (e) Batchu, V. R.; Subramanian, V.; Parasuraman, K.; Swamy, N. K.; Kumar, S.; Pal, M. *Tetrahedron* **2005**, *41*, 9869.
11. For the synthesis of 3-iodo-thiophene-2-carboxylic acid (**1**), see: Duffault, J.-M.; Tellier, F. *Synth. Commun.* **1998**, *28*, 2467.
12. Crystallographic data (excluding structure factors) for **3a** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 607010.
13. Glover, B.; Harvey, K. A.; Liu, B.; Sharp, M. J. *Org. Lett.* **2003**, *5*, 301.
14. (a) Coupling of 3-iodothiophenes with terminal alkynes under Sonogashira conditions are common in the literature, see for example: Ye, X.-S.; Wong, H. N. C. *J. Org. Chem.* **1997**, *62*, 1940; (b) Padwa, A.; Chiacchio, U.; Fairfax, D. J.; Kassir, J. J.; Litrico, A.; Semones, M. A.; Xu, S. L. *J. Org. Chem.* **1993**, *58*, 6429.
15. Conversion of Pd(0) to Pd(II) species under Sonogashira condition has been proposed by Lu et al., see: Lu, X.; Huang, X.; Ma, S. *Tetrahedron Lett.* **1993**, *34*, 5963; This Pd(II) species was thought to be responsible for the cyclization of ynenoic acid via the σ-vinyl-palladium intermediate, see also: Yanagihara, N.; Lambert, C.; Iritani, K.; Utimoto, K.; Nozaki, H. *J. Am. Chem. Soc.* **1986**, *108*, 2753.
16. Trost, B. M.; Chan, C.; Ruhter, G. *J. Am. Chem. Soc.* **1987**, *109*, 3486.
17. 3-Hex-1-ynyl thiophene-2-carboxylic acid was prepared by Sonogashira coupling[14b] of 3-iodo thiophene-2-carboxylic acid methyl ester with 1-hexyne in the presence of Et_3N in DMF at 60 °C for 6 h followed by hydrolysis of the resulting ester in aqueous methanol.
18. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
19. Duffault, J.-M.; Tellier, F. *Synth. Commun.* **1998**, *28*, 2467.