Thienylimidazo[2,1-*b*]thiazoles as Inhibitors of Mitochondrial NADH Dehydrogenase

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The synthesis of 6-substituted 5-(thienylvinyl)imidazo[2,1-*b*]thiazoles and 6-thienylimidazo-[2,1-*b*]thiazoles is reported. These compounds were tested as specific inhibitors of the NADH: ubiquinone (UBQ) reductase activity of NADH dehydrogenase in mitochondrial membranes. The 6-thienylimidazo[2,1-*b*]thiazoles were more potent in mammalian than in nematode mitochondria and had an average titer of 0.11 mM for 2-methyl-6-(2-thienyl)imidazo[2,1-*b*]-thiazole (**10**). This compound is noncompetitive with the ubiquinone substrate and interacts with a site which is mutually exclusive with that of rotenone but nonexclusive with that of piericidin and several other inhibitors of NADH dehydrogenase. In the series of 5-(thienylvinyl)-imidazothiazoles, the hydrobromide of (*E*)-6-chloro-5-(2-thienylvinyl)imidazo[2,1-*b*]thiazole (*E*-**5**·HBr) was found to be more potent as an inhibitor of the NADH:UBQ activity (IC_{50} = 15–17 μM) than the 6-thienylimidazoles such as **10**. The inhibitory action of *E*-**5**·HBr and its analogs is different from that of compound **10** as indicated by the mutual exclusivity with other inhibitors and the relative inhibition of the activity with various electron acceptors.

Introduction

In the scrutiny of the biological properties of synthetic (thienylvinyl)indoles, i.e., indoles bearing the same group present in the well-known anthelmintic agent pyrantel (**1**),[1] some derivatives were found to inhibit mitochondrial NADH dehydrogenase.[1,2] This enzyme complex (see refs 3 and 4 for recent reviews) can be inhibited by a large variety of chemically different compounds, many of which have important biomedical or agrochemical applications (Chart 1). The inhibitors of NADH dehydrogenase with greatest biomedical interest are drugs such as the analgesic agent meperidine (demerol)[5] and in particular its related methylphenylpyridine derivatives which induce Parkinson-like symptoms in humans.[4,6] Although these compounds are relatively weak inhibitors of mitochondrial NADH dehydrogenase *in vitro*, they are actively concentrated *in vivo* within certain brain structures and lead to cell death by progressively inhibiting the bioenergy production associated with mitochondrial NADH oxidation.[4,6] Neurodegenerative conditions similar to Parkinson's disease are also induced in man by a variety of chemicals inhibiting mitochondrial NADH dehydrogenase, e.g., rotenone (see refs 3 and 4 and references therein). The mechanism by which NADH dehydrogenase inhibitors have neurotoxic or narcotic action remains to be established, but it is presumably related to the energetic function of this enzyme and the prevalence of NAD-linked metabolism in nervous cells.[3,4,6] Research on inhibitors of mitochondrial NADH dehydrogenase is now assuming a broader significance because several natural and synthetic compounds have been recently developed as insecticides and acaricides (some are already in commerce), and they all function as inhibitors of mitochondrial NADH dehydrogenase.[7,8] The potential impact on human health of these new pesticides demands a better knowledge on their mode of action.

It is now emerging that many inhibitors of NADH dehydrogenase interact at different sites even if they all seem to behave as the classical inhibitor rotenone.[7–10] The chemical determinants which discriminate the action of inhibitors toward one or more of these sites are however unclear.[4,8] In order to better understand this issue, we planned the synthesis of compounds with an imidazo[2,1-*b*]thiazole moiety, which is the same ring system present in levamisole (**2**) (another well-known anthelmintic agent) and is also similar to the ring of potent inhibitors of NADH dehydrogenase such as benzimidazole.[8,11]

Herein, we describe the synthesis and properties of a series of 5-(thienylvinyl)imidazo[2,1-*b*]thiazoles and a series of 6-thienylimidazo[2,1-*b*]thiazoles. Compounds of the latter series inhibit the activity of NADH: ubiquinone reductase, with potencies depending upon small changes in structure. The most effective new compounds have been carefully characterized with respect to the action of several established inhibitors of mitochondrial NADH dehydrogenase. Compounds of the 5-thienylvinyl series were found to be more potent than those of the 6-thienyl series but to interact in a different way with mitochondrial NADH dehydrogenase.

Chemistry

The 5-(2-thienylvinyl)imidazo[2,1-*b*]thiazoles **3**–**5** (Chart 2) were prepared from the 6-substituted imidazo-[2,1-*b*]thiazole-5-carboxaldehydes **16** [12] (Scheme 1) by means of the Wittig reaction with (2-thienylmethyl)-triphenylphosphonium chloride (**17**).[1] The reaction gave a mixture of *E* and *Z* isomers: in the case of compounds **3** and **4**, the *E* isomer was predominant whereas the

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Chart 1. Known Inhibitors of NADH Dehydrogenase Mentioned in the Text

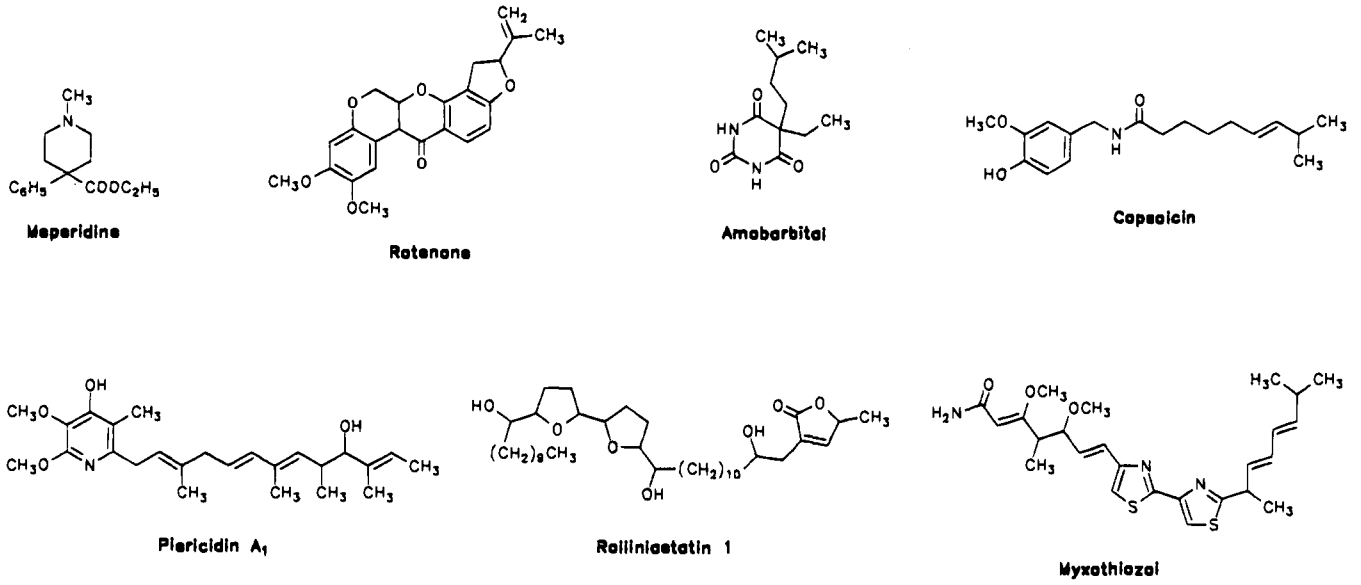
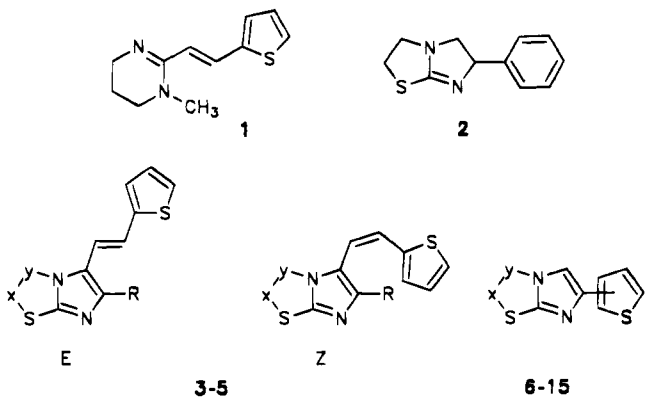


Chart 2[a]



[a] For x, y, and R, see Table 1.

mixture of **5** was about equimolar and the two isomers were isolated and characterized. For the purposes reported under Biochemical Results, the corresponding methyl iodides (**3**–**5**·CH_3I) and hydrobromides (**3**–**5**·HBr) were also prepared; under the experimental conditions employed, compound *Z*-**5** gave mainly the hydrobromide of *E*-**5** (Table 1).

The 6-thienylimidazo[2,1-*b*]thiazoles were synthesized by treating the appropriate 2-aminothiazole **18** with the appropriate bromo ketone **19**: 2-(bromoacetyl)thiophene[13] for compounds **6**, **8**–**10**, **12**, and **14** and 3-(bromoacetyl)-thiophene[14] for compounds **7**, **11**, **13**, and **15**. The intermediate salt **20** was identified by the typical IR carbonyl absorption band and, without further purification, was refluxed with dilute hydrobromic acid to yield the expected derivatives **6**–**15**. For the purposes of comparison, compound **10** was isolated as free base, as methyl iodide and as hydrobromide. Compounds **6**,[15,16] **10**,[17] **12**,[16] and **14**[16] were already reported, without spectroscopic data.

Compounds **3**–**15** gave ^{1}H-NMR spectra (Table 2) in agreement with the assigned structures. In the thienylvinyl derivatives **3**–**5**, the CH=CH group appears as two doublets in the range 6.1–7.5 ppm with the expected coupling constant of ~16 Hz for the *E* and ~11 Hz for the *Z* isomers; the thienyl group gives three sets of bands in the range 7.0–7.6 ppm. In the 6-thienyl derivatives **6**–**15**, the position 5 is free and a sharp singlet is always evident in the range 7.6–8.1 ppm; the thienyl group falls in the same region as above, but it is possible to distinguish 2-thienyl (**6**, **8**–**10**, **12**, **14**: 7.0–7–4 ppm) from 3-thienyl derivatives (**7**, **11**, **13**, **15**: 7.4–7.7 ppm). As far as the salts are concerned, only the spectra of the methyl iodides are reported in

Scheme 1

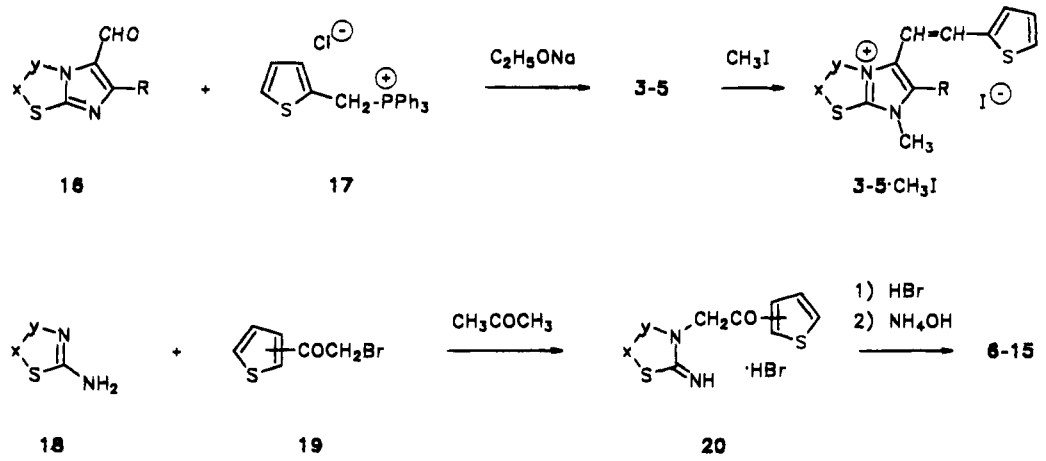


Table 1. Imidazo[2,1-*b*]thiazoles **3**–**15**

compd	x–y	R	thienyl	formula (MW)	mp, °C	ν_{max}, cm^{-1}
***E*-3**	HC=CH	C_6H_5		$C_{17}H_{12}N_2S_2$ (308.4)	105–108	1325, 1245, 1190, 935, 770, 700
***E*-3·CH_3I**	HC=CH	C_6H_5		$C_{18}H_{15}IN_2S_2$ (450.4)	245–250 dec	940, 810, 770, 720, 690, 655
***E*-3·HBr**	HC=CH	C_6H_5		$C_{17}H_{13}BrN_2S_2$ (389.3)	240–245	1500, 1300, 1200, 1115, 765, 690
***E*-4**	H_2C-CH_2	C_6H_5		$C_{17}H_{14}N_2S_2$ (310.4)	141–143	1330, 1045, 940, 915, 770, 690
***E*-4·CH_3I**	H_2C-CH_2	C_6H_5		$C_{18}H_{17}IN_2S_2$ (452.4)	201–204 dec	1535, 1210, 940, 775, 730, 700
***E*-4·HBr**	H_2C-CH_2	C_6H_5		$C_{17}H_{15}BrN_2S_2$ (391.3)	232–235	1625, 1500, 955, 910, 760, 690
***E*-5**	H_2C-CH_2	Cl		$C_{11}H_9ClN_2S_2$ (268.8)	116–118	1235, 1190, 1160, 935, 700, 690
***E*-5·CH_3I**	H_2C-CH_2	Cl		$C_{12}H_{12}ClIN_2S_2$ (410.7)	213–215 dec	1565, 1530, 1195, 1040, 945, 730
***E*-5·HBr**	H_2C-CH_2	Cl		$C_{11}H_{10}BrClN_2S_2$ (349.7)	247–250 dec	1620, 1500, 1315, 950, 705, 685
***Z*-5**	H_2C-CH_2	Cl		$C_{11}H_9ClN_2S_2$ (268.8)	105–108	1260, 1215, 1035, 855, 820, 705
***Z*-5·CH_3I**	H_2C-CH_2	Cl		$C_{12}H_{12}ClIN_2S_2$ (410.7)	208–212 dec	1625, 1580, 1530, 1195, 855, 730
6	HC=CH		2	$C_9H_6N_2S_2$ (206.3)	138–141[a]	1550, 1270, 1245, 1180, 845, 720
7	HC=CH		3	$C_9H_6N_2S_2$ (206.3)	145–148	1550, 1280, 960, 855, 785, 730
8	H_2C-CH_2		2	$C_9H_8N_2S_2$ (208.3)	125–128	1295, 1255, 845, 740, 720, 700
9	ClC=CH		2	$C_9H_5ClN_2S_2$ (240.7)	146–149	1265, 1170, 990, 845, 720, 700
10	H_3CC=CH		2	$C_{10}H_8N_2S_2$ (220.3)	122–125[b]	1600, 1270, 1180, 910, 840, 720
10·CH_3I	H_3CC=CH		2	$C_{11}H_{11}IN_2S_2$ (362.2)	216–222 dec	1540, 1240, 1215, 1175, 1095, 745
10·HBr	H_3CC=CH		2	$C_{10}H_9BrN_2S_2$ (301.2)	225–228 dec	1610, 1500, 1260, 1100, 935, 725
11	H_3CC=CH		3	$C_{10}H_8N_2S_2$ (220.3)	143–146	1260, 1170, 955, 850, 780, 715
12	HC=CCH_3		2	$C_{10}H_8N_2S_2$ (220.3)	136–138[c]	1270, 1200, 1160, 840, 810, 700
13	HC=CCH_3		3	$C_{10}H_8N_2S_2$ (220.3)	123–127	1275, 1180, 1160, 855, 785, 710
14	H_3CC=CCH_3		2	$C_{11}H_{10}N_2S_2$ (234.3)	167–170[b,d]	1270, 1205, 1170, 1040, 845, 720
15	H_3CC=CCH_3		3	$C_{11}H_{10}N_2S_2$ (234.3)	143–145	1255, 1160, 960, 850, 790, 715

[a] Lit.[15] mp 143–144 °C. Lit.[16] mp 141–153 °C. [b] Lit.[17] mp not reported in the abstract. [c] Lit.[16] mp 138–139 °C. [d] Lit.[16] mp 170 °C.

Table 2. ^{1}H-NMR of Compounds **3**–**15**

compd	δ, ppm; *J*, Hz in DMSO-d_6 (T = thiophene, ar = aromatic)
***E*-3**	7.03 (1H, d, CH, *J* = 16.6), 7.07 (1H, dd, T), 7.24 (1H, d, T), 7.40 (1H, d, CH, *J* = 16.6), 7.42 (1H, d, T), 7.47 (1H, d, H2, *J* = 4.5), 7.5 (3H, m, ar), 7.67 (2H, m, ar), 8.42 (1H, d, H3, *J* = 4.5)
***E*-3·CH_3I**	3.77 (3H, s, CH_3), 6.81 (1H, d, CH, *J* = 16.6), 7.08 (1H, dd, T), 7.25 (1H, d, T), 7.32 (1H, d, CH, *J* = 16.6), 7.55 (1H, d, T), 7.65 (2H, m, ar), 7.70 (3H, m, ar), 7.95 (1H, d, H2, *J* = 4.3), 8.86 (1H, d, H3, *J* = 4.3)
***E*-4**	4.01 (2H, t, H2, *J* = 7.5), 4.50 (2H, t, H3, *J* = 7.5), 6.85 (1H, d, CH, *J* = 16.5), 7.07 (1H, dd, T), 7.15 (1H, d, CH, *J* = 16.5), 7.20 (1H, d, T), 7.33 (1H, d, T), 7.45 (3H, m, ar), 7.56 (2H, m, ar)
***E*-4·CH_3I**	3.55 (3H, s, CH_3), 4.29 (2H, t, H2, *J* = 7.5), 4.86 (2H, t, H3, *J* = 7.5), 6.49 (1H, d, CH, *J* = 16.5), 7.07 (1H, dd, T), 7.21 (1H, d, CH, *J* = 16.5), 7.25 (1H, d, T), 7.55 (3H: 2H, m, ar + 2H, d, T), 7.65 (3H, m, ar)
***E*-5**	3.95 (2H, t, H2, *J* = 7.5), 4.45 (2H, t, H3, *J* = 7.5), 6.64 (1H, d, CH, *J* = 16.5), 7.07 (1H, dd, T), 7.19 (1H, d, CH, *J* = 16.5), 7.25 (1H, d, T), 7.49 (1H, d, T)
***E*-5·CH_3I**	3.68 (3H, s, CH_3), 4.21 (2H, t, H2, *J* = 7.5), 4.75 (2H, t, H3, *J* = 7.5), 6.70 (1H, d, CH, *J* = 16.5), 7.13 (1H, dd, T), 7.45 (1H, d, T), 7.50 (1H, d, CH, *J* = 16.5), 7.65 (1H, d, T)
***Z*-5**	3.80 (2H, t, H2, *J* = 6.5), 3.90 (2H, t, H3, *J* = 6.5), 6.15 (1H, d, CH, *J* = 11.3), 7.04 (2H: 1H, d, CH, *J* = 11.3 + 1H, dd, T), 7.18 (1H, d, T), 7.52 (1H, d, T)
***Z*-5·CH_3I**	3.72 (3H, s, CH_3), 4.15 (2H, t, H2, *J* = 7.5), 4.35 (2H, t, H3, *J* = 7.5), 6.18 (1H, d, CH, *J* = 11.7), 7.10 (1H, dd, T), 7.29 (1H, d, CH, *J* = 11.7), 7.35 (1H, d, T), 7.67 (1H, d, T)
6	7.07 (1H, m, T), 7.26 (1H, d, H2, *J* = 4.4), 7.38 (1H, m, T), 7.42 (1H, m, T), 7.91 (1H, d, H3, *J* = 4.4), 8.10 (1H, s, H5)
7	7.24 (1H, d, H2, *J* = 4.4), 7.48 (1H, m, T), 7.57 (1H, m, T), 7.72 (1H, m, T), 7.92 (1H, d, H3, *J* = 4.4), 8.07 (1H, s, H5)
8	3.89 (2H, t, H2, *J* = 7), 4.18 (2H, t, H3, *J* = 7), 7.01 (1H, m, T), 7.20 (1H, m, T), 7.31 (1H, m, T), 7.58 (1H, s, H5)
9	7.08 (1H, m, T), 7.41 (1H, m, T), 7.45 (1H, m, T), 8.11 (1H, s, H5), 8.27 (1H, s, H3)
10	2.39 (3H, s, CH_3), 7.06 (1H, m, T), 7.34 (1H, m, T), 7.40 (1H, m, T), 7.67 (1H, s, H3), 8.01 (1H, s, H5)
10·CH_3I	2.57 (3H, s, CH_3-2), 3.91 (3H, s, CH_3-7), 7.32 (1H, m, T), 7.59 (1H, m, T), 7.91 (1H, m, T), 8.08 (1H, s, H3), 8.45 (1H, s, H5)
11	2.39 (3H, s, CH_3), 7.46 (1H, m, T), 7.56 (1H, m, T), 7.69 (2H: 1H, s, H3 + 1H, m, T), 7.99 (1H, s, H5)
12	2.41 (3H, s, CH_3), 6.89 (1H, s, H2), 7.08 (1H, m, T), 7.38 (1H, m, T), 7.42 (1H, m, T), 8.12 (1H, s, H5)
13	2.41 (3H, s, CH_3), 6.87 (1H, s, H2), 7.50 (1H, m, T), 7.58 (1H, m, T), 7.72 (1H, m, T), 8.10 (1H, s, H5)
14	2.32 (6H, s, CH_3), 7.07 (1H, m, T), 7.35 (1H, m, T), 7.40 (1H, m, T), 8.04 (1H, s, H5)
15	2.32 (6H, s, CH_3), 7.48 (1H, m, T), 7.57 (1H, m, T), 7.68 (1H, m, T), 8.02 (1H, s, H5)

Table 2, since the spectra of the hydrobromides are quite similar to those of the parent compounds.

Biochemical Results

Screening of the Imidazothiazoles 3–5 (Methyl iodides) and 6–15 as Inhibitors of NADH Dehydrogenase. The biological activity of compounds **3**–**13** on mitochondrial NADH dehydrogenase has been initially tested at the relatively high concentrations (0.5 mM) used previously for thienylvinyl derivatives.[1,2] Since at these concentrations many compounds were poorly soluble in water, they were first tested on the activity of NADH:dichlorophenolindophenol (DCIP) reductase in the presence of exogenous ubiquinone substrate (UBQ) to accelerate the enzymatic rates.[2,18] Although DCIP is a relatively unspecified acceptor for assaying the redox function of NADH dehydrogenase,[4] its high extinction coefficient at 600 nm allows the measurement of activity even in the presence of the turbidity generated by addition of the hydrophobic compounds under test.[2] Addition of UBQ, moreover, rendered the reduction of DCIP more sensitive to rotenone, the classical inhibitor of the quinone function of NADH dehydrogenase.[3,4]

As shown in Table 3, the methyl iodides of compounds **3**–**5** were apparently ineffective as inhibitors of NADH:UBQ:DCIP reductase activity in both beef and *Parascaris* mitochondrial particles. All the 6-thienyl derivatives had some inhibitory action, however, in beef mitochondria, especially compounds **9**–**11**. The 6-thienyl derivatives were also good inhibitors of the NADH:UBQ reductase activity as followed by the oxidation of

Table 3. Screening of Thienylimidazo[2,1-*b*]thiazoles as Inhibitors of Mitochondrial NADH Dehydrogenase

compd[a]	residual activity of NADH:UBQ:DCIP reductase,[b] %	
	Parascaris mitochondrial particles	beef mitochondrial particles
E-**3**·CH_3I	94	81
E-**4**·CH_3I	92	77
E-**5**·CH_3I	88	91
Z-**5**·CH_3I	92	85
6	79	57
7	83	68
8	88	68
9	75	40
10	59	28
11	73	40
12	70	38
13	75	43
rotenone (1 μM)	65	34

[a] At 0.5 mM final concentration. [b] The numbers are the average of at least two separate experiments. DCIP was 50 μM and UBQ 30 μM. Protein concentration was 0.216 mg/mL for *Parascaris* and 0.07 mg/mL for beef heart mitochondrial particles. Assay conditions were as described previously.[1,2]

Table 4. Titer of the 6-Thienylimidazo[2,1-*b*]thiazoles **10**–**13** on the Specific Activity of NADH:UBQ Reductase[a]

compd	IC_{50} (mM)	
	Parascaris mitochondrial particles	beef mitochondrial particles
10	0.22	0.07
11	*b*	0.14
12	0.38	0.09
13	*b*	0.21

[a] Experimental conditions were the same as in Table 3 and ref 9. [b] Concentrations yielding significant inhibition also produced high turbidity that hampered accurate measurements of the rates, so no reasonable IC_{50} value could be determined.

NADH promoted by UBQ: Table 4 shows the titer for compounds **10**–**13**. The NADH:UBQ reductase assay could be performed accurately only in beef mitochondria in which these compounds were effective at concentrations well below their solubility limit, and thus turbidity interferences with the near UV absorbance of NADH were minimal. Indeed, all the compounds were clearly more powerful in beef than in *Parascaris* mitochondria (Tables 3 and 4). Information on the chemical specificity for the substituents in the imidazothiazole ring could be obtained from the data in Table 4. First, 2-methyl derivatives were more effective than their 3-methyl analogs. Secondly, the 3-thienyl derivatives were always less inhibitory than the 2-thienyl derivatives (Table 4 and also Table 3). Preliminary results with the 2,3-dimethyl derivatives **14** and **15** indicated that they are about as potent as the corresponding 2-methyl derivatives. However, such compounds were too much hydrophobic to allow accurate titrations of the NADH:UBQ reductase activity, and their inhibition was always incomplete (results not shown).

Effect of Methylation and Protonation on the Inhibitory Properties of Thienylimidazo[2,1-*b*]-thiazole Derivatives. From compounds *E*-**3**–**5** and *Z*-**5** were prepared the corresponding methyl iodides in order to render them more water soluble and thus suitable for the biochemical assays of NADH dehydrogenase. In view of their poor inhibition of the NADH:UBQ:DCIP reductase activity with respect to the 6-thienyl-substituted compounds **6**–**13** (Table 3), we investigated whether this modification was responsible

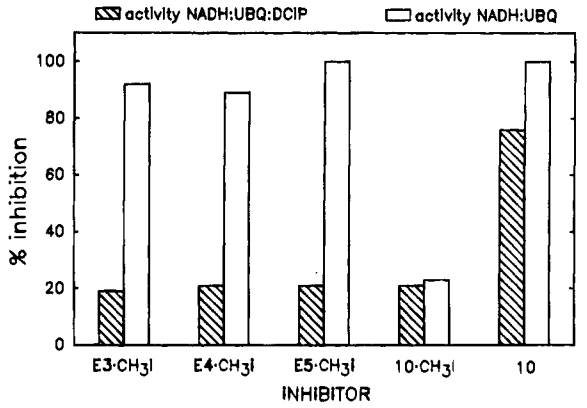


Figure 1. Inhibition of the NADH:UBQ:DCIP reductase activity (hatched histograms) and of the NADH:UBQ reductase activity (white histograms) by *E*-**3**–**5**·CH_3I, **10**, and **10**·CH_3I (cf. Table 1). Final concentrations were 1 mM for *E*-**3**–**5**·CH_3I and 0.5 mM for **10** and **10**·CH_3I. Beef heart submitochondrial particle protein (0.04 mg/mL) was assayed with 50 μM DCIP, 30 μM UBQ, and 150 μM NADH, for the NADH:UBQ:DCIP reductase activity, and with 150 μM NADH and 30 μM UBQ, for the NADH:UBQ reductase activity. The assay buffer was 50 mM K_2HPO_4, 1 mM EDTA, and 10 mM KCN (pH = 7.6).[9]

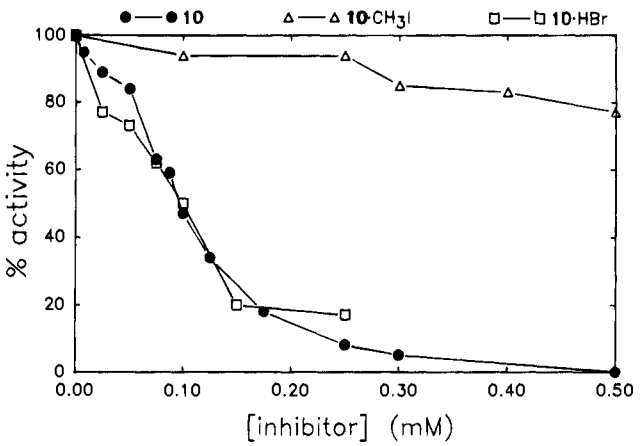


Figure 2. Effect of methylation and protonation of compound **10** on its inhibition of the NADH:UBQ reductase activity of beef heart submitochondrial particles. Conditions were the same as those described in Figure 1.

per se for the different inhibition. The methylation of compound **10** with CH_3I, indeed, greatly reduced the inhibitory action both of the NADH:UBQ reductase activity and of the NADH:UBQ:DCIP reductase activity (Figures 1 and 2). On the contrary, compounds *E*-**3**–**5**·CH_3I were much more potent in inhibiting the NADH:UBQ reductase activity than the NADH:UBQ:DCIP reductase activity (Figure 1). Interestingly, *Z*-**5**·CH_3I was 13-fold less potent in terms of IC_{50} than its *E*-**3**·CH_3I isomer in inhibiting the NADH:UBQ reductase activity (data not shown). The inhibitory action of compounds *E*-**3**–**5** (free bases) on this reductase activity, however, could not be tested because of their extremely low solubility in water.

Given that methylation with CH_3I affects the biological properties of the compounds reported in Figures 1 and 2, we decided to test the same compounds as hydrobromides. The hydrobromide of **10** was as efficient as the unmodified parent compound in inhibiting both the NADH:UBQ reductase activity (Figure 2) and the NADH:UBQ:DCIP reductase activity (data not shown). Contrary to the 6-thienyl derivatives such as **10**, the

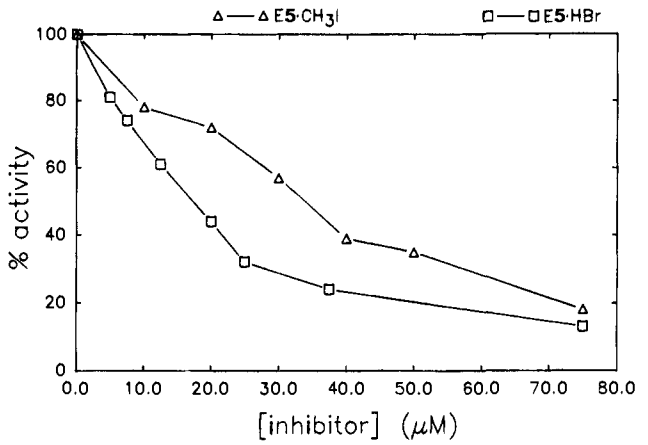


Figure 3. Titrations of compounds *E*-**5**·HBr and *E*-**5**·CH_3I on the NADH:UBQ reductase activity of beef submitochondrial particles. Conditions were the same as those in Figures 1 and 2.

hydrobromides of compounds *E*-**3**–**5** were about 2-fold more powerful than the methyl iodides in inhibiting the NADH:UBQ reductase activity (Figure 3) and much more effective inhibitors of the NADH:UBQ:DCIP reductase activity than the methyl iodides (data not shown). In particular, compound *E*-**5**·HBr had a behavior most similar to that of **10**·HBr in inhibiting the two reductase activities (data not shown; cf. Figure 2). For this reason, *E*-**5**·HBr and **10** were chosen as representative compounds of their respective series for characterizing their mode of action on the NADH dehydrogenase of mammalian mitochondria.

Inhibitory Properties of Compounds *E*-5·HBr and 10. The synthesis of 2-methyl-6-(2-thienyl)imidazo[2,1-*b*]thiazole (**10**) has been already reported,[17] but its biological activity at the mitochondrial level has not been considered before. Compound **10** was slightly superior to its analogs in inhibitory potency (Table 4), with an average IC_{50} of 0.11 ± 0.05 mM for the specific NADH:UBQ reductase activity. This titer was apparently independent upon the protein concentration in the assay and generally lower than the average in fresher or more active preparations. In our experience with several inhibitors of mitochondrial NADH:quinone reductase, these properties of compound **10** were similar to those of amobarbital (average IC_{50} = 0.5 mM[2]), meperidine (with an average IC_{50} of 0.2 mM[2,10]), and also capsaicin (average IC_{50} ca. 0.05 mM[2,10,19]). The hydrobromide of (*E*)-6-chloro-5-(2-thienylvinyl)imidazo-[2,1-*b*]thiazole (*E*-**5**·HBr) had typical IC_{50} values of 15–17 μM for the NADH:UBQ reductase activity in beef mitochondrial particles. Its analogs, *E*-**3**·HBr and *E*-**4**·HBr, exhibited similar IC_{50} values. Thus compounds *E*-**3**–*E*-**5** are the most powerful inhibitors of imidazothiazole structure that have been reported so far.[1,2,17,23]

UBQ as a substrate is not commercially available (see ref 9 and references therein), and more commonly the activity of NADH dehydrogenase has been measured as NADH oxidase[4,19,21–23] or NADH:ubiquinone-1 (Q-1) reductase.[9,19] Therefore, the inhibitory action of compounds *E*-**5**·HBr and **10** was studied with these and other assays to better define the site of inhibition in the NADH dehydrogenase complex as well as to compare our data more directly with those reported in the literature employing beef submitochondrial particles.

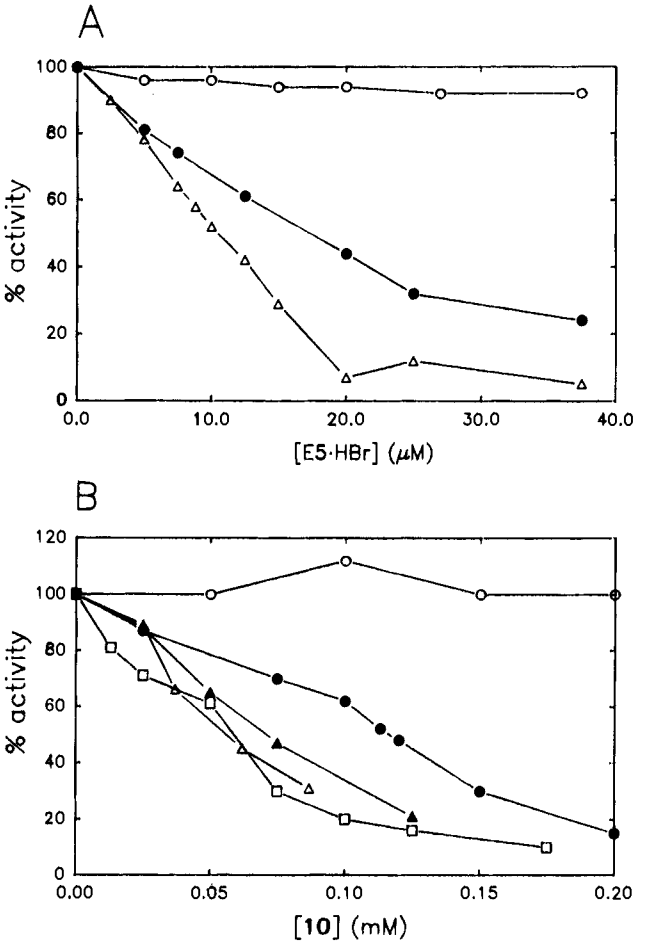


Figure 4. Titrations of compounds *E*-**5**·HBr (A) and **10** (B) on various activities of mitochondrial NADH dehydrogenase of beef heart submitochondrial particles. Conditions were similar to those in Figure 1 but with 0.03 mg/mL beef heart submitochondrial particle protein in B. (○–○) NADH:ferricyanide reductase activity. Control activities were 3.65 μmol/min mg for A and 10.5 μmol/min mg for B. (●–●) NADH:UBQ reductase activity. Control activities were 0.547 μmol/min mg for A and 0.325 μmol/min mg for B. (▲–▲) NADH:cytochrome *c* reductase activity. Control activity was 0.159 μmol/min mg. (△–△) NADH:Q-1 reductase activity (rotenone sensitive). Control activities were 0.197 μmol/min mg for A and 0.554 μmol/min mg for B. (□–□) NADH oxidase activity. Control activity was 0.252 μmol/min mg.

Compound *E*-**5**·HBr had the lowest titer (10 μM) for NADH:Q-1 reductase activity and was basically ineffective as inhibitor of the NADH:ferricyanide activity (Figure 4A). Similarly, compound **10** had the lowest titer (0.05 mM) for the NADH:Q-1 reductase activity and, also, for the NADH oxidase activity. Alike *E*-**5**·HBr, compound **10** basically did not inhibit the NADH:ferricyanide reductase activity (Figure 4). This indicates that the site of action of the 6-thienyl and 5-thienylvinyl derivatives presented here is at the ubiquinone junction in NADH dehydrogenase as in the case of most other inhibitors of this enzyme.[2,4,21,23] Moreover, the 6-thienyl derivatives did not significantly interfere with the function of complex III (ubiquinol:cytochrome *c* reductase), since the titer of NADH:cytochrome *c* reductase was superimposable with that of NADH:Q-1 reductase (Figure 4B). Hence, the compounds studied here can be considered specific inhibitors of the first phosphorylating site of mitochondria. Previously, benzothiadiazole derivatives, which are chemi-

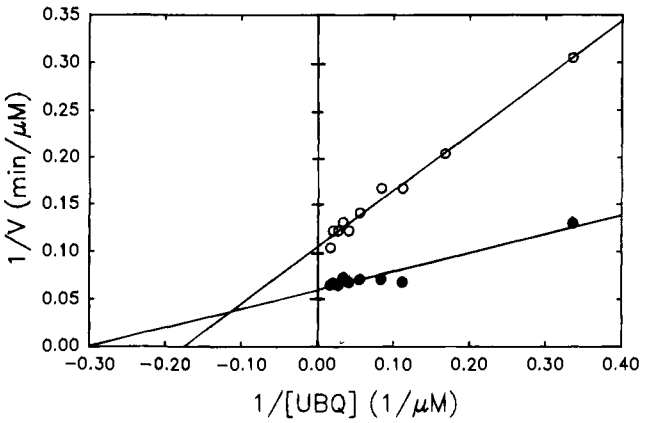


Figure 5. Effect of compound **10** on the UBQ titration of the NADH:Q reductase activity. The assay conditions were the same as those Figure 1 with 0.064 mg/mL beef heart particle protein. (●−●) Control. (○−○) In the presence of compound **10** (0.1 mM)

cally related to compounds **3**−**13**, were also found to specifically inhibit mitochondrial NADH dehydrogenase, even if they were about 10-fold less potent than compound **10** in inhibiting the NADH oxidase activity of beef submitochondrial particles.[23]

The inhibitory power of both *E*-**5**·HBr and **10** was influenced by the nature of the quinone substrate, since these compounds were more powerful with the hydrophilic Q-1 than with the much more hydrophobic UBQ (Figure 4 and Table 4). Similar differences were found for inhibitors such as rotenone, piericidin, and amobarbital (results not shown). Other inhibitors of mitochondrial NADH:Q reductase, however, were influenced in a different way by the quinone substrates. For instance, the action of the acetogenins extracted from *Annonaceae* plants such as rolliniastatin-2 was relatively indifferent to the nature of the quinone,[9] whereas other compounds were reported to be competitive with ubiquinone-2.[8] Compound **10** (Figure 5) and compound *E*-**5**·HBr (data not shown) essentially behaved as noncompetitive inhibitors versus UBQ, since they modified both the slope and the intercepts in the reciprocal plots of the quinone titrations. Similar behavior was shown by several other inhibitors including capsaicin and rotenoids (results not shown; see refs 8 and 9).

The theory of Chou and Talalay[20] has been recently applied to identify the mutual overlapping in the interaction site of a number of inhibitors of the NADH dehydrogenase complex.[9,10] This approach, which was based on the additivity of inhibition of NADH:UBQ reductase activity as described in refs 9 and 10, has been applied here to locate the action site of *E*-**5**·HBr and **10** as representatives of either class of compounds described here (Table 5). Compound **10** was found to compete for the same site to which rotenone binds. However, other inhibitors known to interact with this rotenone site, notably piericidin,[4] were mutually nonexclusive with **10** (Table 5). Moreover, all the annonaceous acetogenins tested (rolliniastatin-2, rolliniastatin-1, squamocin, and otivarin; see ref 9 and references therein) were also mutually nonexclusive with **10** (Table 5). These results could be rationalized by assuming that 6-thienylimidazothiazoles such as compound **10** bind to the portion of the rotenone binding pocket which does not overlap the interaction site of piericidin and annonaceous acetogenins. Such a rationalization would

Table 5. Mutual Exclusivity of Compound **10** (Inhibitor 1) with Other Inhibitors of NADH:UBQ Reductase (I = % inhibition; A = % residual activity of NADH:UBQ reductase)

I/A (1)	inhibitor 2	I/A (2)	I/A (1 + 2)	exclusive[a]	nonexclusive[b]
2.01	rotenone	2.38	2.87	4.40	9.20
1.86	rotenone	2.03	4.99	3.89	7.65
2.01	piericidin	2.87	12.50	4.89	10.70
2.01	rolliniastatin-2	2.87	12.50	4.89	10.70
1.62	rolliniastatin-1	2.34	10.76	3.95	7.74
1.00	squamocin	1.35	3.52	2.36	3.71
2.01	otivarin	2.01	8.00	4.02	8.07
1.00	amobarbital	1.23	15.10	2.24	3.47
1.24	meperidine	1.80	13.10	3.04	5.30
1.36	capsaicin	1.15	8.71	2.49	4.02
1.62	myxothiazol	1.25	2.93	2.87	4.88

[a] For mutually exclusive inhibitors, the experimental value of I/A in the presence of both inhibitors should approach the sum of the individual values of I/A.[20] The numbers underlined are the theoretical values which best approach the experimental values. [b] For mutually nonexclusive inhibitors, i.e., inhibitors interacting with sites that do not overlap each other, the theoretical value of I/A in the presence of both inhibitors should approach a number resulting from the sum of individual I/A plus the factor $I1I2/A1A2$.[20] In other words, the experimental value of I/A is much higher for the two nonexclusive inhibitors than for the two exclusive inhibitors as the expression of the increased additivity of their inhibitory power on the enzymatic activity. The numbers underlined are the theoretical values which best approach the experimental values.

be in agreement with the concept of two rotenone and piericidin binding sites in the Q junction of NADH dehydrogenase.[4] Recent results with analogs of 4-methylphenylpyridinium confirmed the existence of two different binding sites also for these chemically simple compounds.[4,21]

We suggest that compound **10** and, presumably, the other 6-thienyl derivatives interact specifically with one of the two rotenone binding sites, in accordance with previous suggestions regarding benzothiadiazoles.[23] Our data (Table 5) indicate that this site overlaps the interaction site of myxothiazol, an antibiotic originally discovered as a potent inhibitor of ubiquinol:cytochrome *c* reductase and now found to inhibit also NADH dehydrogenase.[7,10,24] That both myxothiazol and compound **10** displace rotenone (Table 5 and refs 7 and 10) further supports our suggestion that these inhibitors share a common interaction site in mitochondrial NADH dehydrogenase.

Mutual exclusivity studies[20] revealed that compound *E*-**5**·HBr was nonexclusive with all inhibitors of NADH dehydrogenase we could test, including rotenone, rolliniastatin-1, meperidine, capsaicin, and, remarkably, also **10** (data not shown). This suggests that compound *E*-**5**·HBr has a different action site from that of compound **10**, despite their common backbone. The data shown in Figure 3 support this hypothesis since they indicate that the methylation at position 7 of the imidazothiazole ring in compound *E*-**5** does not affect greatly its inhibitory action with respect to *E*-**5**·HBr, whereas the same position is clearly crucial for the potency of compound **10** because the methyl iodide loses the inhibiting action (Figure 2). Another indication of a diversity in the mode of action of the 6-thienyl derivatives from that of the 5-thienylvinyl derivatives is given by their different inhibition of the NADH:UBQ and NADH:UBQ:DCIP reductase activity (Figure 1 and Table 3). Whereas compound **10** and its analogs inhibit the two activities with similar potency, the methyl

iodides of *E*-**3**−**5** are much more potent in inhibiting the UBQ reductase activity than the DCIP reductase activity (Table 3 and Figure 1), suggesting that these compounds deviate the electron transfer from NADH to DCIP before the site of full reduction of UBQ. The 5-thienylvinyl compounds could therefore act also before the Q interaction site in NADH dehydrogenase, thereby explaining their lack of mutual competition with inhibitors such as rotenone which specifically act at the Q site.

The compounds of the two series described here are different at positions 5 and 6 of the common imidazothiazole ring. Clearly, these molecular differences determine a different interaction with mitochondrial NADH dehydrogenase because the methylation of the nitrogen at position 7 affects only the inhibitory properties of the 6-thienyl series (Figures 2 and 3). Further studies with analogs having various substituents in key positions of the imidazothiazole ring system will facilitate a more accurate location of the interaction site of the present compounds and, hopefully, clarify the unusual mode of action of compounds *E*-**3**−*E*-**5**.

Experimental Section

Chemistry. The melting points are uncorrected. Elemental analyses of compounds **3**−**15** (C, H, N) were within ±0.4% of the theoretical values. The IR spectra were recorded in nujol on a Perkin-Elmer 298 spectrometer and the ^{1}H-NMR on a Varian Gemini spectrometer (300 MHz). Bakerflex plates (silica gel IB2-F) were used for TLC and Kieselgel 60 (Merck) for column chromatography: the eluent was a mixture of petroleum ether/acetone, 80/20.

6-Substituted 5-(2-Thienylvinyl)imidazo[2,1-*b*]thiazoles 3−**5.** (2-Thienylmethyl)triphenylphosphonium chloride (**17**)[1] (25 mmol) was stirred at 40 °C for 15 min under nitrogen with a solution of sodium ethoxide (from Na, 70 mmol, and absolute ethanol, 30 mL). The appropriate 6-substituted imidazo[2,1-*b*]thiazole-5-carboxaldehyde **16**[12] (25 mmol), dissolved in absolute ethanol (150 mL), was added to the mixture which was refluxed under nitrogen for 3 h. The solvent was evaporated under reduced pressure, and the residue was treated with water and extracted with chloroform. After the usual procedures, the resulting product was separated by column chromatography and crystallized from ethanol with a yield of 40−50% (*E* isomer); in the case of compound **5** this yield includes both the *E* and *Z* isomers which were present in the proportion of ~50/50.

6-Thienylimidazo[2,1-*b*]thiazoles 6−**15.** The appropriate 2-aminothiazole or -thiazoline **18** (45 mmol) was dissolved in acetone (100 mL) and refluxed for 30 min with the equivalent of 2-(bromoacetyl)thiophene[13] or 3-(bromoacetyl)thiophene.[14] After cooling, the resulting salt **20** was collected ($\nu_{C=O}$ ~ 1670 cm^{-1}) and refluxed with 500 mL of 2 N HBr. When the solution was complete, it was cautiously basified by dropping 15% NH_4OH. The resulting base (**6**−**15**) was collected by filtration and crystallized from ethanol with a yield of 35−45%.

Hydrobromides 3·HBr−5·HBr and 10·HBr. The appropriate imidazo[2,1-*b*]thiazole (5 mmol) was stirred at room temperature for 7 h with 10 mL of 48% hydrobromic acid. The mixture was evaporated under reduced pressure, and the resulting salt was washed with acetone. The yield was almost quantitative.

7-Methylimidazo[2,1-*b*]thiazolium Iodides 3·CH$_3$I−5·CH$_3$I and 10·CH$_3$I. The appropriate imidazo[2,1-*b*]thiazole (5 mmol) was stirred at room temperature for 16 h with 5 mL of methyl iodide. The resulting salt was collected by filtration and washed with acetone; the yield was almost quantitative.

Biochemistry. Mitochondria from various animal tissues and whole organisms of the horse parasitic worm *Parascaris equorum* were prepared as described previously.[1] Submitochondrial particles from beef heart and *Parascaris* mitochondria were obtained essentially as described by Hansen and Smith.[22] Several enzymatic assays at room temperature were employed for determining the action of inhibitors. NADH:undecylbenzoquinone (UBQ) reductase was the standard assay for the quinone reductase function of mitochondrial NADH dehydrogenase and was undertaken as described recently in detail.[9] In some cases dichlorophenolindophenol (DCIP) was used as the final electron acceptor from NADH, generally in conjunction with 30 μM UBQ[2] to accelerate the rates and render them more specific for NADH dehydrogenase activity.[18] In a few experiments, ubiquinone-**1** or other synthetic quinones were employed as acceptors at approximately 30 μM as in the case of UBQ.[9] NADH oxidase activity was measured usually in the same buffer of the NADH:quinone reductase assay but without the addition of complex III inhibitors and KCN.[9] NADH:cytochrome *c* reductase activity was measured without complex III inhibitors but with 2 mM KCN and 20 μM horse heart cytochrome *c*.[9] The NADH:ferricyanide (2 mM) reductase activity was measured at 30 °C also for determining the content of active NADH dehydrogenase.[9] The concentration of NADH was always 0.15 mM in all the enzymatic assays. See the legends to the figures and tables for further experimental details. Mutual exclusivity of inhibitors was evaluated on the NADH:UBQ reductase activity of beef heart particles by using the method of Chou and Talalay,[20] as described previously.[9,10]

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