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Laboratory note

6-Thienyl and 6-phenylimidazo[2,1-b]thiazoles as inhibitors of mitochondrial NADH dehydrogenase[§]

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Abstract – Starting from the potent inhibitory effect of the previously described 2-methyl-6-(2-thienyl)imidazo[2,1-b]thiazole on mitochondrial complex I, we prepared a series of derivatives in order to study the effect of a different substitution at the positions 2, 5 and 6. The replacement of the thienyl group at position 6 with a phenyl group does not modify the biological behaviour of the lead compound, whereas the shift of the methyl group from position 2 to position 5 yields a compound devoid of inhibitory effects. In both the 6-thienyl and 6-phenyl series, the lengthening of the chain at position 2 has provided useful information to outline the structural determinants of the ubiquinone antagonist action in imidazothiazole derivatives. © 1999 Éditions scientifiques et médicales Elsevier SAS

imidazo[2,1-b]thiazole / mitochondrial complex I / ubiquinone / rotenone

1. Introduction

NADH-ubiquinone reductase, commonly known as complex I, is the most complicated and least understood of the respiratory complexes of mitochondria and bacteria [1–3].

The interest in this intricate enzyme complex is increasing due to its possible involvement in the pathogenesis of human neurodegenerative diseases such as Alzheimer's [4], Parkinson's [5], diabetes [6] and the fact that complex I is becoming a preferred target of commercial pesticides, especially acaricides [7]. There is a plethora of complex I inhibitors that act as antagonists of the hydrophobic substrate ubiquinone, and generally differ in their action depending upon which quinone intermediate they preferentially antagonize (quinone, semiquinone or quinol) [8]. Because the chemical determinants of the different antagonistic actions are unclear [1, 8, 9], the study of a series of derivatives in which the chemical structures have been systematically modified is useful to elucidate how complex I inhibitors interact with the enzyme [10].

Recently, we have undertaken a systematic work to study the structure-activity relationships in complex I inhibitors bearing an indole [11, 12] and imidazothiazole moiety [13]. 2-Methyl-6-(2-thienyl)imidazo[2,1-b]thiazole **1** *(figure 1)* was the most potent compound and was found to have a mode of action overlapping that of the classical inhibitor rotenone as well as that of the product-like inhibitor myxothiazol [8, 13]. Using **1** as the lead compound, we have evaluated first the effect of the substitution of the thienyl group in position 6 with a phenyl group (**2**) and the shift of the methyl group from position 2 to 5 (**3**). The results indicated that the thienyl group may be replaced by a phenyl group without loss of potency, whereas the position of the methyl group is critical (compound **3** is inactive). We next planned the synthesis of 6-thienyl and 6-phenyl derivatives with

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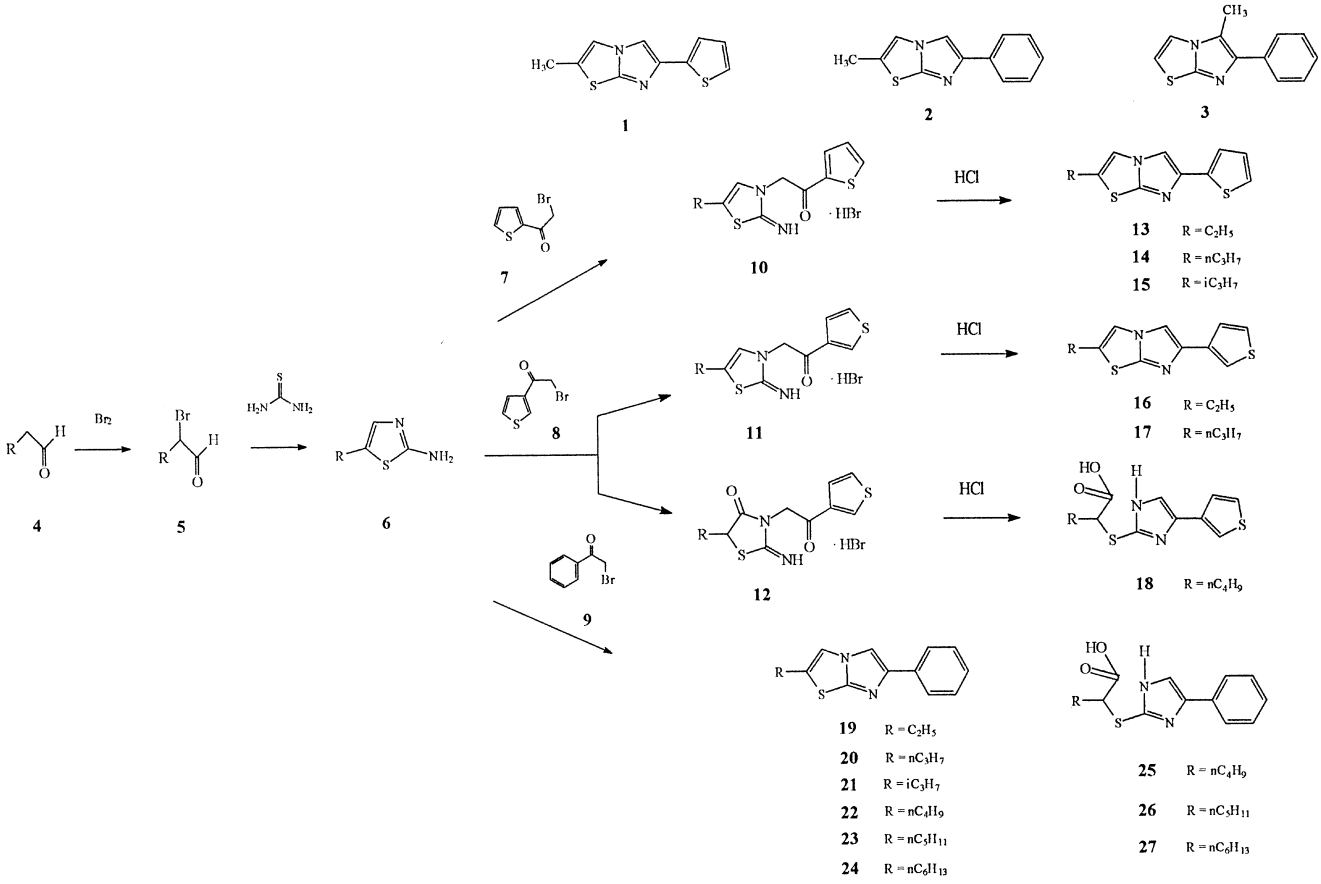

Figure 1. Synthesis of 6-thienyl and 6-phenylimidazo[2,1-b]thiazoles.

various substituents at position 2, to identify whether this position in the imidazothiazole ring corresponded to the attachment point for mimics of the polyisoprenoid side chain of ubiquinone, whose length is known to be critical for Complex I activity [14, 15].

If this were the case, 2-substituents with increasing hydrophobicity (**13–17** and **19–24**) were expected to have an enhanced inhibitory potency, proportional to their hydrophobicity as previously reported for 2-substituted acridones [16]. However, the structure-activity results indicated that, in imidazothiazoles, position 2 is sterically critical for complex I inhibition, but is unlikely to be the junction for mimics of the ubiquinone side chain. During the synthesis, some unexpected compounds were isolated (**18** and **25–27**) and two of them were tested (**26** and **27**).

2. Chemistry

The 5-alkyl-2-aminothiazoles **6** *(figure 1)* were prepared from the appropriate aldehydes **4** which were brominated at the α-position (**5**) and treated with thiourea. When compounds **6** (R = ethyl, propyl) were treated with the bromoacetylthiophens **7–8**, white solids were isolated, corresponding to the intermediate salts **10–11** which were characterized only on the basis of their IR spectrum ($\nu_{C=O} \approx 1\,670\ cm^{-1}$). These compounds were refluxed with dilute hydrochloric acid in order to obtain the imidazothiazoles **13–17**, whose spectroscopic data *(table I)* are in agreement with the assigned structures. Under the same experimental conditions, starting from **6** (R = butyl) and 3-(bromoacetyl)thiophene **8**, the resulting precipitate had a structure different from **10–11** since the

Table I. Imidazothiazoles **13**–**27**.

Compound	Formula(mw)	M.p., °C	ν_{max}, cm^{-1}	δ (ppm); *J* (Hz) in DMSO-d_6 [a]
13 [b]	$C_{11}H_{10}N_2S_2$ (234.3)	111–113	1 265, 850, 725, 685	1.25 (3H, t, CH_3, *J* = 7.5) 2.77 (2H, q, CH_2, *J* = 7.5) 7.06 (1H, m, T) 7.35 (1H, m, T) 7.41 (1H, m, T) 7.70 (1H, s, th) 8.01 (1H, s, im)
14 [c]	$C_{12}H_{12}N_2S_2$ (248.4)	105–106	1 270, 850, 720, 680	0.95 (3H, t, CH_3, *J* = 7) 1.64 (2H, sex, CH_2, *J* = 7) 2.73 (2H, t, CH_2, *J* = 7) 7.07 (1H, m, T) 7.36 (1H, m, T) 7.41 (1H, m, T) 7.71 (1H, s, th) 8.02 (1H, s, im)
15	$C_{12}H_{12}N_2S_2$ (248.4)	108–112	1 660, 1 620, 1 560, 1 060	1.29 (6H, d, CH_3, *J* = 7) 3.12 (1H, sep, CH, *J* = 7) 7.07 (1H, m, T) 7.36 (1H, m, T) 7.41 (1H, m, T) 7.71 (1H, s, th) 8.00 (1H, s, im)
16	$C_{11}H_{10}N_2S_2$ (234.3)	103–105	1 270, 1 070, 780, 715	1.25 (3H, t, CH_3, *J* = 7.5) 2.76 (2H, q, CH_2, *J* = 7.5) 7.46 (1H, m, T) 7.55 (1H, m, T) 7.69 (1H, m, T) 7.71 (1H, s, th) 7.98 (1H, s, im)
17	$C_{12}H_{12}N_2S_2$ (248.4)	126–130	1 270, 1 260, 780, 725	0.95 (3H, t, CH_3, *J* = 7.5) 1.63 (2H, sex, CH_2, *J* = 7.5) 2.72 (2H, t, CH_2, *J* = 7.5) 7.46 (1H, m, T) 7.56 (1H, m, T) 7.69 (1H, m, T) 7.71 (1H, s, th) 7.98 (1H, s, im)
18	$C_{13}H_{16}N_2O_2S_2$ (296.4)	176–180	3 500–2 200, 1 620, 1 590, 1 070, 780	0.85 (3H, t, CH_3, *J* = 7) 1.32 (4H, m, CH_2) 1.76 (2H, m, CH_2) 3.94 (1H, t, CH, *J* = 7) 7.44 (1H, m, T) 7.56 (1H, s, im) 7.58 (1H, m, T) 7.67 (1H, m, T)
19 [d]	$C_{13}H_{12}N_2S$ (228.3)	136–140	1 600, 1 195, 1 065, 720	1.26 (3H, t, CH_3, *J* = 7.5) 2.78 (2H, q, CH_2, *J* = 7.5) 7.24 (1H, t, ar, *J* = 8) 7.38 (2H, t, ar, *J* = 8) 7.72 (1H, s, th) 7.81 (2H, d, ar, *J* = 8) 8.13 (1H, s, im)
20	$C_{14}H_{14}N_2S$ (242.3)	142–145	1 600, 1 260, 1 065, 720	0.94 (3H, t, CH_3, *J* = 7.5) 1.63 (2H, sex, CH_2, *J* = 7.5) 2.72 (2H, t, CH_2, *J* = 7.5) 7.23 (1H, t, ar, *J* = 8) 7.37 (2H, t, ar, *J* = 8) 7.71 (1H, s, th) 7.80 (2H, d, ar, *J* = 8) 8.12 (1H, s, im)
21	$C_{14}H_{14}N_2S$ (242.3)	136–140	1 595, 1 180, 770, 710	1.29 (6H, d, CH_3, *J* = 7) 3.12 (1H, sep, CH, *J* = 7) 7.24 (1H, t, ar, *J* = 8) 7.38 (2H, t, ar, *J* = 8) 7.71 (1H, s, th) 7.81 (2H, d, ar, *J* = 8) 8.11 (1H, s, im)
22	$C_{15}H_{16}N_2S$ (256.4)	128–130	1 595, 1 535, 1 260, 720	0.89 (3H, t, CH_3, *J* = 7.5) 1.34 (2H, sex, CH_2, *J* = 7.5) 1.58 (2H, qui, CH_2, *J* = 7.5) 2.73 (2H, t, CH_2, *J* = 7.5) 7.22 (1H, t, ar, *J* = 8) 7.36 (2H, t, ar, *J* = 8) 7.71 (1H, s, th) 7.79 (2H, d, ar, *J* = 8) 8.12 (1H, s, im)
23	$C_{16}H_{18}N_2S$ (270.4)	130–133	1 590, 1 250, 1 055, 710	0.88 (3H, t, CH_3, *J* = 7) 1.32 (4H, m, CH_2) 1.62 (2H, qui, CH_2, *J* = 7) 2.75 (2H, t, CH_2, *J* = 7) 7.24 (1H, t, ar, *J* = 8) 7.38 (2H, t, ar, *J* = 8) 7.73 (1H, s, th) 7.81 (2H, d, ar, *J* = 8) 8.13 (1H, s, im)
24	$C_{17}H_{20}N_2S$ (284.4)	109–111	1 590, 1 255, 760, 715	0.86 (3H, t, CH_3, *J* = 7) 1.29 (6H, m, CH_2) 1.60 (2H, qui, CH_2, *J* = 7) 2.74 (2H, t, CH_2, *J* = 7) 7.23 (1H, t, ar, *J* = 8) 7.38 (2H, t, ar, *J* = 8) 7.71 (1H, s, th) 7.80 (2H, d, ar, *J* = 8) 8.13 (1H, s, im)
25	$C_{15}H_{18}N_2O_2S$ (290.4)	176–179	3 500–2 200, 1 615, 1 595, 1 060, 750	0.85 (3H, t, CH_3, *J* = 7) 1.33 (4H, m, CH_2) 1.77 (2H, m, CH_2) 3.93 (1H, t, CH, *J* = 7) 7.20 (1H, t, ar, *J* = 8) 7.35 (2H, t, ar, *J* = 8) 7.65 (1H, s, im) 7.72 (2H, d, ar, *J* = 8)
26	$C_{16}H_{20}N_2O_2S$ (304.4)	185–187	3 500–2 200, 1 610, 1 590, 1 055, 750	0.84 (3H, t, CH_3, *J* = 7) 1.26 (4H, m, CH_2) 1.38 (2H, m, CH_2) 1.77 (2H, m, CH_2) 3.94 (1H, t, CH, *J* = 7) 7.20 (1H, t, ar, *J* = 8) 7.36 (2H, t, ar, *J* = 8) 7.64 (1H, s, im) 7.75 (2H, d, ar, *J* = 8)
27	$C_{17}H_{22}N_2O_2S$ (318.4)	185–188	3 500–2 200, 1 610, 1 595, 1 060, 750	0.83 (3H, t, CH_3, *J* = 7) 1.23 (6H, s, CH_2) 1.38 (2H, t, CH_2, *J* = 7) 1.79 (2H, m, CH_2) 3.94 (1H, t, CH, *J* = 7) 7.20 (1H, t, ar, *J* = 8) 7.36 (2H, t, ar, *J* = 8) 7.64 (1H, s, im) 7.73 (2H, d, ar, *J* = 8)

[a]Abbreviations: T = thiophene, th = thiazole, im = imidazole, ar = aromatic. [b]Ref. [24], m.p. 116–118. [c]Ref. [23], m.p. not reported. [d]Ref. [24, 25], m.p. 125–127.

IR showed an additional C=O stretching absorption. This compound was considered as the iminothiazolone **12** which, after refluxing with dilute hydrochloric acid, gave the imidazole **18**. The structure of this new derivative was confirmed by IR, ^{1}H-NMR *(table I)* and ^{13}C-NMR, MS (see Experimental section).

The same reaction was repeated with 2-bromoacetophenone **9** and similar behaviour was observed: when R was ethyl or propyl, the intermediate salt was analogous to **11**, leading to compounds **19–21**, whereas, when R was a longer chain, the precipitate was analogous to **12** (it led to compounds **25–27**) and the filtrate

contained the intermediate analogous to **11** which, after the usual treatment with dilute hydrochloric acid, gave the imidazothiazoles **22–24**.

For a deeper insight into the structure of the carboxylic acids **18** and **25–27**, one of these, 2-(4-phenyl-2-imidazolylsulfanyl)heptanoic acid **26**, was subjected to DEPT, HETCOR, MS (see Experimental section) and to crystallographic studies. Its crystal structure shows that the imidazole and the phenyl ring are almost planar as observed in analogous compounds [17, 18], whereas the orientation of the carboxylic group is almost perpendicular to them. Further crystallographic details will be given elsewhere.

3. Biological results

Table II reports the biological activity of the reference compounds (**1–3**) and of the newly synthesized imidazothiazole derivatives (**13–17** and **19–24**). Among the unexpected imidazole by-products, compounds **26–27** were quite inactive. Consequently, the analogues **18** and **25** were not tested.

The increase of hydrophobicity at position 2 in both 6-(2-thienyl) (**13–15**) and 6-(3-thienyl) derivatives (**16** and **17**) decreased the inhibitory potency on complex I activity in comparison with the 2-methyl derivative **1**. Therefore, position 2 seems to be very critical for the potency of complex I inhibition.

Table II. Biological activity of compounds **1**–**3**, **13**–**17**, **19**–**24** and **26**–**27**.

Compound	Residual activity of NADH:UBQ reductase (%) [a]
1	12
2	24
3	80
13	63
14	91
15	63
16	63
17	100
19	82
20	17
21	100
22	73
23	46
24	0
26	114
27	107

[a]The numbers are the average of at least three separate experiments. The assay conditions are described in the experimental section. UBQ (30 μM) was used as electron acceptor. The final concentration of the compounds was 0.25 mM.

Contrary to 6-thienyl derivatives, 6-phenyl derivatives with 2-substituents of increasing hydrophobicity either maintained or reduced the inhibitory capacity of the lead compound. There was no direct correlation between the increase in length and hydrophobicity of the substituent and inhibition, since the 2-ethyl (**19**) and the 2-butyl (**22**) derivatives had a markedly reduced potency with respect to the 2-propyl derivative (**20**). Nevertheless, 2-pentyl (**23**) and mostly 2-esyl (**24**) derivatives showed an increased inhibitory effect, thus suggesting that the length of the chain is important for the interaction with the complex I binding site.

The different behaviour of the thienyl or phenyl derivatives indicated a slightly different interaction as inhibitors of complex I. In previous work we studied in detail the inhibitory properties of compound **1**, which appeared to act as a quinol antagonist [13]. In order to clarify the relationship between the new phenyl derivatives and quinone interaction in complex I, compounds **2** and **20** were tested with two different substrates, namely the hydrophilic ubiquinone-1 (Q_1) and the hydrophobic undecyl-benzoquinone (UBQ). The potency of **2** was higher with Q_1 (I_{50} = 75 μM) than with UBQ (I_{50} = 130 μM), similarly to compound **1** [13]. Conversely, compound **20** showed the same I_{50} for both Q_1 and UBQ (120 μM). Thus, the inhibitory effect of the more hydrophobic **20** could depend on some internal reaction steps that are not extensively influenced by the nature of the quinone substrate for complex I activity. Indeed, compound **20** acted as a classical non-competitive inhibitor with respect to both Q_1 and UBQ, similarly to rotenone *(figure 2)* [8], while **2** showed mixed competition with UBQ, as previously reported for **1** [13]. Taken together, the biological data suggest that the substitution of a thienyl group with a bulkier phenyl group at position 6 could shift the inhibitory action of imidazothiazole compounds from antagonists of the quinol product to antagonists of the semiquinone intermediate. The latter would correspond to the action of rotenone in complex I [8].

4. Discussion

Structurally, most complex I inhibitors have a head-tail module that is critical for the antagonist action versus the ubiquinone substrate [8, 10]. The results of the present study clarify that complex I inhibition is strictly dependent on the substitutions at the extreme positions 2 and 6 of the imidazothiazole ring. The different potency of the substituents at the 2 position, when position 6 is occupied by either a thienyl or a phenyl group, indicates that steric constraints in the binding site limit the length and bulkiness of imidazothiazoles for their optimal interac-

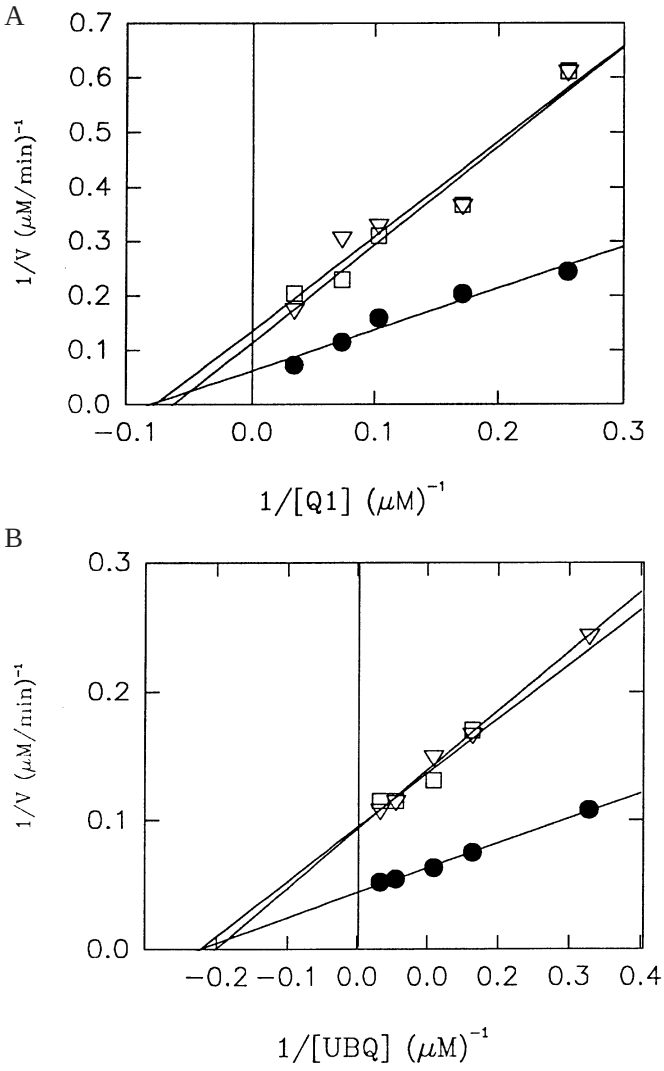

Figure 2. Effect of compound **2** and **20** on the Q_1 (A) and UBQ (B) titration of the NADH:Q reductase activity. (●) Control. (▽) In the presence of 0.18 mM compound **2**. (□) In the presence of 0.18 mM compound **20**.

tion with complex I. In phenyl derivatives, the aliphatic chain substituents in position 2 might mimic the hydrophobic tail of ubiquinone. A further lengthening of the chain which resembles the polyprenil tail of ubiquinone should better clarify if position 2 corresponds to the attachment point for hydrophobic substituents.

Future studies with systematic substitutions at other positions such as nitrogen 7 will define the determinants to optimize the ubiquinone antagonist action of imidazothiazoles and produce extremely potent inhibitors of complex I.

5. Experimental

5.1. Chemistry

Compounds **2–3** have been previously described [19]. The aldehydes **4** (butyraldehyde, valeraldehyde, hexanal, heptanal, octanal) and 2-bromoacetophenone **9** are commercially available. 2-(Bromoacetyl)thiophene **7** [20] and 3-(bromoacetyl)thiophene **8** [21] were prepared according to the literature. The bromoaldehydes **5** were prepared under experimental conditions analogous to those reported for the synthesis of α-bromopropionaldehyde [22]. The imidazo[2,1-b]thiazoles **13** [23, 24], **14** [23] and **19** [25] were already reported in the literature.

The melting points are uncorrected. Analyses (C, H, N) were within ± 0.4% of the theoretical values. TLC was performed on Bakerflex plates (Silica gel IB2-F): the eluent was a mixture of petroleum ether/acetone in various proportions. The IR spectra *(table I)* were recorded in nujol on a Perkin-Elmer 683. The ^{1}H-NMR *(table I)* and the ^{13}C-NMR spectra were recorded on a Varian Gemini (300 MHz), and were referenced to solvent signals (DMSO). The EI mass spectra were recorded on a VG 7070E. X-ray crystallography: the data were collected on a Siemens P4 four-circle diffractometer.

5.1.1. 5-Alkyl-2-aminothiazoles **6**

The appropriate bromoaldehyde **5** (100 mmol) was treated with 80 mmol of thiourea and stirred at 90–100 °C for 2 h. After cooling, the reaction mixture was treated with 2 N NaOH until basic and extracted with chloroform. Compounds **6** thus obtained were used, without further purification, in the following step.

5.1.2. Reaction of the 5-alkyl-2-aminothiazoles **6** *with the bromoacetylthiophenes* **7–8** *(synthesis of* **13–18***)*

The appropriate compound **6** (45 mmol) was dissolved in acetone (100 mL) and treated with 2-(bromoacetyl)thiophene **7** or 3-(bromoacetyl)thiophene **8** (45 mmol). The reaction mixture was refluxed for 3–6 h (according to a TLC test) and the resulting salt **10–12** was treated, without further purification, with 200 mL of 2 N HCl. After 1 h reflux, the solution was cautiously basified by adding dropwise 15% NH_4OH. The resulting base (**13–18**) was collected by filtration and crystallized from ethanol with a yield of 35–40% *(table I)*.

The following spectra were also recorded:

1) as an example of compound **10**, R = isopropyl was chosen, ^{1}H-NMR: 1.21 (6H, d, CH_3, J = 7); 3.01 (1H, sep, CH, J = 7); 5.69 (2H, s, CH_2); 7.20 (1H, s, th); 7.38 (1H, m, T); 8.11 (1H, m, T); 8.18 (1H, m, T); 9.59 (1H, s, NH).

2) compound **12,** IR: 3 500–2 200, 1 760, 1 685, 1 640, 1 560 cm^{-1}. ^{1}H-NMR: 0.87 (3H, t, CH_3, *J* = 7); 1.40 (4H, m, CH_2); 2.05 (2H, m, CH_2); 4.90 (1H, t, CH, *J* = 7); 5.37 (2H, s, CH_2); 7.58 (1H, m, T); 7.74 (1H, m, T); 8.78 (1H, m, T). MS: 296 ($M^{+\cdot}$, 11), 278 (M-H_2O, 4), 268 (M-CO, 9), 124 (C_6H_4SO, 30), 111 (C_5H_3SO, 100).

3) Compound **18,** MS: 296 ($M^{+\cdot}$, 13), 278 (M-H_2O, 100), 235 (278-C_3H_7, 85), 207 (235-CO, 40), 182 (49), 123 (26), 109 (27), 55 (34).

5.1.3. Reaction of the 5-alkyl-2-aminothiazoles **6** *with 2-bromoacetophenone* **9** *(synthesis of* **19–27***)*

The appropriate compound **6** (10 mmol) was dissolved in acetone (50 mL) and treated with 10 mmol of 2-bromoacetophenone **9**. The mixture was refluxed for 2–5 h (according to a TLC test) and worked up with two procedures. For compounds **19–21,** acetone was evaporated under reduced pressure and the resulting residue was refluxed for 1 h with 100 mL of 2 N HCl. The solution thus obtained was then basified with 15% NH_4OH and the resulting base **19–21** was crystallized from ethanol with a yield of 30–35% *(table I)*. For Compounds **22–27** the precipitate was collected by filtration and worked up as above. Compounds **25–27** were crystallized from ethanol with a yield of 15%. The filtrate, evaporated and worked up in the same manner, gave compounds **22–24** which were crystallized from ethanol with a yield of 3%. The following data were also recorded for compound **26**: ^{13}C-NMR (with DEPT and HETCOR): 13.80 (CH_3), 21.90 (CH_2), 26.13 (CH_2), 30.81 (CH_2), 31.83 (CH_2), 50.29 (S-CH), 118.04 (im-5), 124.34 (ar-2 + 6), 126.56 (ar-4), 128.59 (ar-3 + 5), 133.13 (im-4), 138.09 (ar-1), 139.67 (im-2), 172.43 (COOH). MS: 304 ($M^{+\cdot}$, 45), 286 (M-H_2O, 8), 260 (M-CO_2, 16), 229 (286-C_4H_9, 15), 203 (48), 190 (70), 176 (100), 117 (56). Crystal data: $C_{16}H_{20}N_2O_2S$, *M* = 304.4, Monoclinic, *a* = 17.305 (2), *b* = 8.5690 (10), *c* = 10.8620 (10) Å, β= 99.67 (1)°, *V* = 1587.8 (3) $Å^3$ (by least-squares refinement on diffractometer angles for 43 randomly selected and automatically centred reflections), space group $P2_1/c$ (n. 14), Z = 4, F (000) = 648, D_c = 1.27 g cm^{-3}, μ (Mo-Kα) = 0.21 mm^{-1}.

5.2. Biology

5.2.1. Mitochondrial preparation

Mitochondria from beef heart were prepared according to standard procedures. They were used after at least two cycles of freezing and thawing in order to brake down membranes.

5.2.2. Biochemical assay

Submitochondrial particles from beef heart were prepared essentially as described by Hansen and Smith [26]. The redox activity of NADH:ubiquinone oxidoreductase was measured by using Q_1 or UBQ as electron acceptor. The test compound, dissolved in DMSO, was added to 0.4 mg/mL of mitochondrial protein in 2 mL of 50 mM K_2HPO_4 buffer containing 1 mM EDTA, 2 mM KCN (pH 7.4) and 0.1 mM NADH at room temperature. The reaction was started by quinone and followed by decrease in absorbance of NADH in the dual wavelength mode at 350 minus 410 nm with an extinction of 5.5 mM^{-1} cm^{-1} as previously described [27].

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References

[1] Walker J.E., Q. Rev. Biophys. 25 (1992) 253–324.

[2] Videira A., Biochim. Biophys. Acta 1364 (1998) 89–100.

[3] Yagi T., Yano T., Di Bernardo S., Matsuno-Yagi A., Biochim. Biophys. Acta 1364 (1998) 125–133.

[4] Weyer G., Babej-Dolle R.M., Hadler D., Hofmann S., Hermann W.M., Neuropsychobiology 36 (1997) 73–82.

[5] Schapira A.H.V., Biochim. Biophys. Acta 1364 (1998) 261–270.

[6] Degli Esposti M., Ngo A., Mayers M.A., Diabetes 45 (1996) 1531–1534.

[7] Lummen P., Biochim. Biophys. Acta 1364 (1998) 287–296.

[8] Degli Esposti M., Biochim. Biophys. Acta 1364 (1998) 222–235.

[9] Degli Esposti M., Ghelli A., Ratta M., Cortes D., Estornell E., Biochem. J. 301 (1994) 161–167.

[10] Miyoshi H., Biochim. Biophys. Acta 1364 (1998) 236–244.

[11] Andreani A., Rambaldi M., Locatelli A., Andreani F., Poglayen G., Degli Esposti M., Eur. J. Med. Chem. 27 (1992) 729–734.

[12] Andreani A., Rambaldi M., Locatelli A., Leoni A., Ghelli A., Degli Esposti M., Pharm. Acta Helv. 69 (1994) 15–20.

[13] Andreani A., Rambaldi M., Leoni A., Locatelli A., Ghelli A., Ratta M., Benelli B., Degli Esposti M., J. Med. Chem. 38 (1995) 1090–1097.

[14] Lenaz G., Biochim. Biophys. Acta 1364 (1998) 207–221.

[15] Degli Esposti M., Ngo A., McMullen G.L., Ghelli A., Sparla F., Benelli B., Ratta M., Linnane A.W., Biochem. J. 313 (1996) 327–334.

[16] Oettmeier W., Masson K., Soll M., Reil E., Biochem. Soc. Trans. 22 (1994) 213–216.

[17] Shilcrat S.C., Hill D.T., Bender P.E., Griswold D.E., Baures P.W., Eggleston D.S., Lantos I., Pridgen L.N., J. Heterocycl. Chem. 28 (1991) 1181–1187.

[18] Jain P.C., Acta Crystallogr. Sect. C: Cryst. Struct. Commun. 43 (1987) 2415–2418.

[19] Pyl T., Giebelmann R., Beyer H., Liebigs Ann. Chem. 643 (1961) 145–153.

[20] Kipnis F., Soloway H., Ornfelt J., J. Amer. Chem. Soc. 71 (1949) 10–11.

[21] MacDowell D.W.H., Greenwood T.D., J. Heterocycl. Chem. 2 (1965) 44–48.

[22] Reichel L., Jahns H.J., Liebigs Ann. Chem. 751 (1971) 69–76.

[23] Tanaka M., Otsuka K., Obata M., Tanabe T., Saida T., Nomura K., Amemija K., Saga K., Kano S., Japan Kokai 74 70, 986; Chem. Abstr. 81 (1974) 136142c.

[24] Meakins G.D., Musk S.R.R., Robertson C.A., Woodhouse L.S., J. Chem. Soc. Perkin Trans. I (1989) 643–648.

[25] O'Daly M.A., Hopkinson C.P., Meakins G.D., Raybould A.J., J. Chem. Soc. Perkin Trans. I (1991) 855–860.

[26] Hansen M., Smith A.L., Biochim. Biophys. Acta 81 (1964) 214–222.

[27] Degli Esposti M., Ghelli A., Crimi M., Estornell E., Fato R., Lenaz G., Biochem. Biophys. Res. Commun. 190 (1993) 1090–1096.