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2,8-DITHIA-DIBENZO[*e,h*]AZULENES AND THEIR 8-OXA ANALOGS. SYNTHESIS AND ANTI-INFLAMMATORY ACTIVITY

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Abstract - Synthesis of 2,8-dithia-dibenzo[*e,h*]azulenes (**III**, X = S) and their 8-oxa analogs (**III**, X = O),[1] two novel classes of fused heterocyclic compounds, is described. Starting 11*H*-dibenzo[*b,f*]thiepin-10-one, 11*H*-dibenzo[*b,f*]oxepin-10-one and its 2-chloro derivative (**1a-c**) were oxidized to 1,2-diketones (**2a-c**) which subsequently reacted with 2,2'-dimethyl thiodiglycolate to form fused thiophene ring by Hinsberg cyclization reaction. Substituents at positions C(1) and C(3) were then further transformed in order to obtain aminoalkoxy derivatives **8-11**. Structures of regioisomers **6c** and **6d** were elucidated using two-dimensional NMR techniques. All compounds with tetracyclic skeleton were tested *in vitro* for their anti-inflammatory activity.

INTRODUCTION

A variety of compounds containing dibenzo[*b,f*]oxepine and dibenzo[*b,f*]thiepine framework have attracted considerable attention because of their unique biological activity. Bermoprofen (**Ia**) and zaltoprofen (**Ib**), Figure 1, are examples of compounds bearing such tricyclic moiety, which have found extensive use as non-steroid anti-inflammatory agents.[2,3] On the other hand, diaryl substituted heterocycles are known as compounds with significant anti-inflammatory activity.[4-8] More specifically, some 3,4-diarylthiophenes (**II**) have been identified as selective cyclooxygenase-2 (COX-2) inhibitors.[9,10] Combining of structural subunits **I** and **II** into a new hetero-polycyclic class, 2-thia-dibenzo[*e,h*]azulenes **III**, is expected to afford novel compounds with potential anti-inflammatory activity. Target molecules, bearing an alkoxy chain of variable length and a basic moiety at positions C(1) or C(3), were envisaged in order to test the influence of this additional functionality on anti-inflammatory properties.

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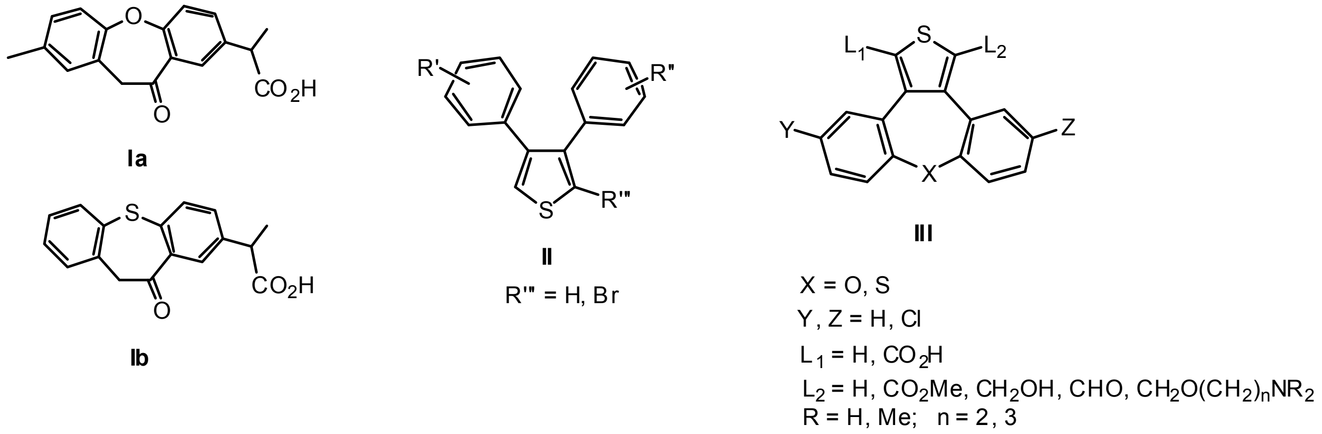
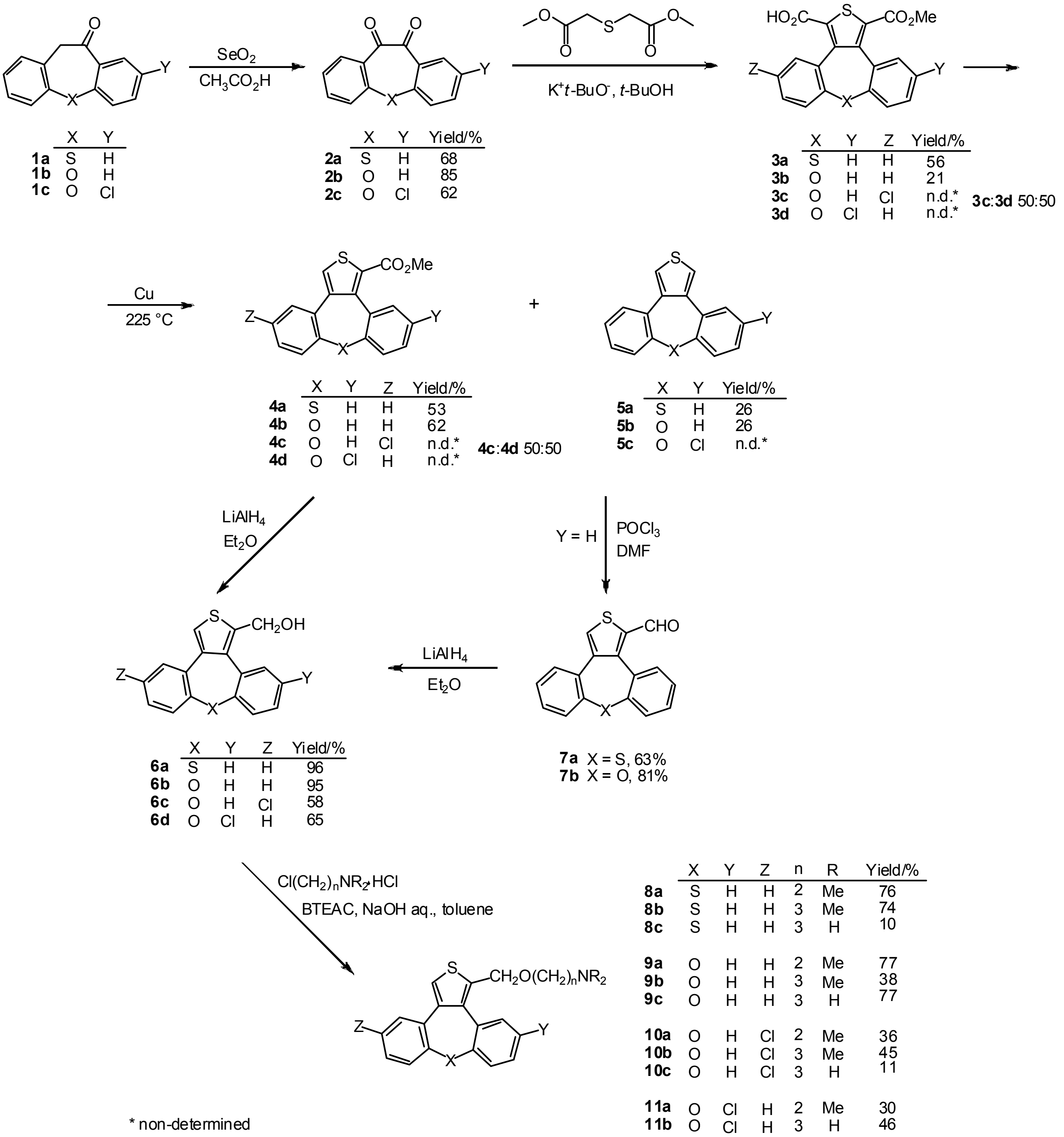


Figure 1

RESULTS AND DISCUSSION

Synthesis. - Synthetic route to 2-thia-dibenzo[*e*,*h*]azulene scaffold is depicted in Scheme 1, and follows known synthesis of 3,4-diarylthiophenes.[11-13] Starting ketones **1a-c**[14-16] were oxidized in good yields (62-85%) to corresponding 1,2-diketones **2a-c** with selenium dioxide. Condensation of 1,2-diketones with dimethyl 2,2'-thiodiacetate in the presence of potassium *tert*-butoxide in *tert*-butanol, known as Hinsberg thiophene synthesis,[12] provided novel tetracyclic, 1,3-disubstituted 2-thia-dibenzo[*e*,*h*]azulenic structures **3a-d**. When symmetrical α-diketones **2a** and **2b** were submitted to this Stobbe-type condensation with thiodiacetate, half-esters of thiophene dicarboxylic acids, **3a** and **3b**, were obtained in 56% and 21% yield, respectively. On the other hand, reaction of chloro-substituted, unsymmetrical **2c** with thiodiacetate resulted in approximately 50:50 mixture of regioisomers **3c** and **3d**, which was a promising platform for preparation of two regioisomeric final compounds. All efforts to separate products **3c** and **3d** by crystallization failed. Chromatographic separation and purification of regioisomers appeared tedious and provided pure **3c** and **3d** in minimal quantities, while both were eluted as inseparable mixture. Due to the ineffective isolation of regioisomers, the mixture of **3c** and **3d** was submitted to the next reaction step, affording less polar products **4c** and **4d**, amenable to separation by column chromatography. Consequently, in order to prepare target compounds with thiophene ring bearing only one substituent, compounds **3a-d** were subjected to copper promoted decarboxylation of the free acid by heating at above 200 °C.[11] This reaction afforded the expected decarboxylated esters **4a-d** besides their decarbomethoxylated derivatives **5a-c**. Apart from providing analytical samples, two products were generally not separated at this stage. Instead, the mixture was subjected to reduction with lithium aluminum hydride (LAH), providing the expected alcohols **6a-d,** which were separated from **5a-c** by column chromatography. Compounds **5a** and **5b** proved valuable starting materials for alternative preparation of alcohols **6a** and **6b** *via* Vilsmeier formylation of thiophene ring,[17] which led to carbaldehydes **7a** and **7b,** 63 and 81% respectively after crystallization, followed by reduction with LAH. Finally, alcohols **6a-d** were converted to their ω-(dialkyl)aminoalkyl ethers **8-11,** by a phase transfer catalyzed alkylation with the appropriate *ω*-(dialkyl)chloroalkylamine hydrochlorides in the presence of benzyltriethylammonium chloride as a catalyst, in toluene / 40% NaOH.[18] Pure aminoethers were isolated by column chromatography as oils and biologically tested as free bases.



	X	Y
1a	S	H
1b	O	H
1c	O	Cl

	X	Y	Yield/%
2a	S	H	68
2b	O	H	85
2c	O	Cl	62

	X	Y	Z	Yield/%
3a	S	H	H	56
3b	O	H	H	21
3c	O	H	Cl	n.d.*
3d	O	Cl	H	n.d.*

3c:**3d** 50:50

	X	Y	Z	Yield/%
4a	S	H	H	53
4b	O	H	H	62
4c	O	H	Cl	n.d.*
4d	O	Cl	H	n.d.*

4c:**4d** 50:50

	X	Y	Yield/%
5a	S	H	26
5b	O	H	26
5c	O	Cl	n.d.*

	X	Y	Z	Yield/%
6a	S	H	H	96
6b	O	H	H	95
6c	O	H	Cl	58
6d	O	Cl	H	65

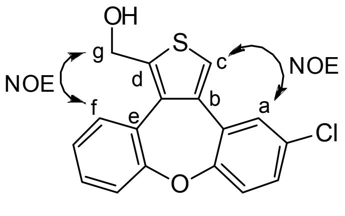
7a X = S, 63%
7b X = O, 81%

	X	Y	Z	n	R	Yield/%
8a	S	H	H	2	Me	76
8b	S	H	H	3	Me	74
8c	S	H	H	3	H	10
9a	O	H	H	2	Me	77
9b	O	H	H	3	Me	38
9c	O	H	H	3	H	77
10a	O	H	Cl	2	Me	36
10b	O	H	Cl	3	Me	45
10c	O	H	Cl	3	H	11
11a	O	Cl	H	2	Me	30
11b	O	Cl	H	3	H	46

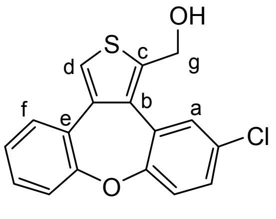
* non-determined

Scheme 1

Structure determination of c and d series of compounds. - To determine relative structures of non-symmetric regioisomeric compounds in the **c** and **d** series, combined use of one- and two-dimensional NMR spectra of **6c** and **6d** (Figure 2 and 3) proved useful, enabling structure determination of all chloro-substituted analogs by synthetic methods. Position of the hydroxymethyl group at the thiophene ring was deduced from heteronuclear multiple bond correlation (HMBC) spectra and nuclear Overhauser effect spectroscopy (NOESY). For example, in **6d**, methylene protons H-g at 4.93 ppm showed a three-bond correlation peak to carbon C-b at 132.9 ppm, as well as the aromatic proton H-a at 7.83 ppm. This correlation pattern was not observed in **6c**, since the CH_2 protons at 4.93 ppm are four bonds away from C-b at 131.0 ppm and no correlation peak was found. Similarly, proton H-d at the thiophene ring of **6d** at 7.73 ppm gave connectivity to C-e at 129.6 ppm, whereas no such correlation was observed for **6c** between H-c at 7.82 ppm and C-e at 128.6 ppm. In addition, according to the NOE cross-peak analysis, methylene protons H-g in **6c** were found to be in a close spatial contact to the aromatic proton H-f at 7.70 ppm, thus corroborating the position of the hydroxymethyl group, and NOE contact between H-a at 7.67 ppm and H-c at 7.82 ppm in **6c** supports the overall structure assignment.



6c



6d

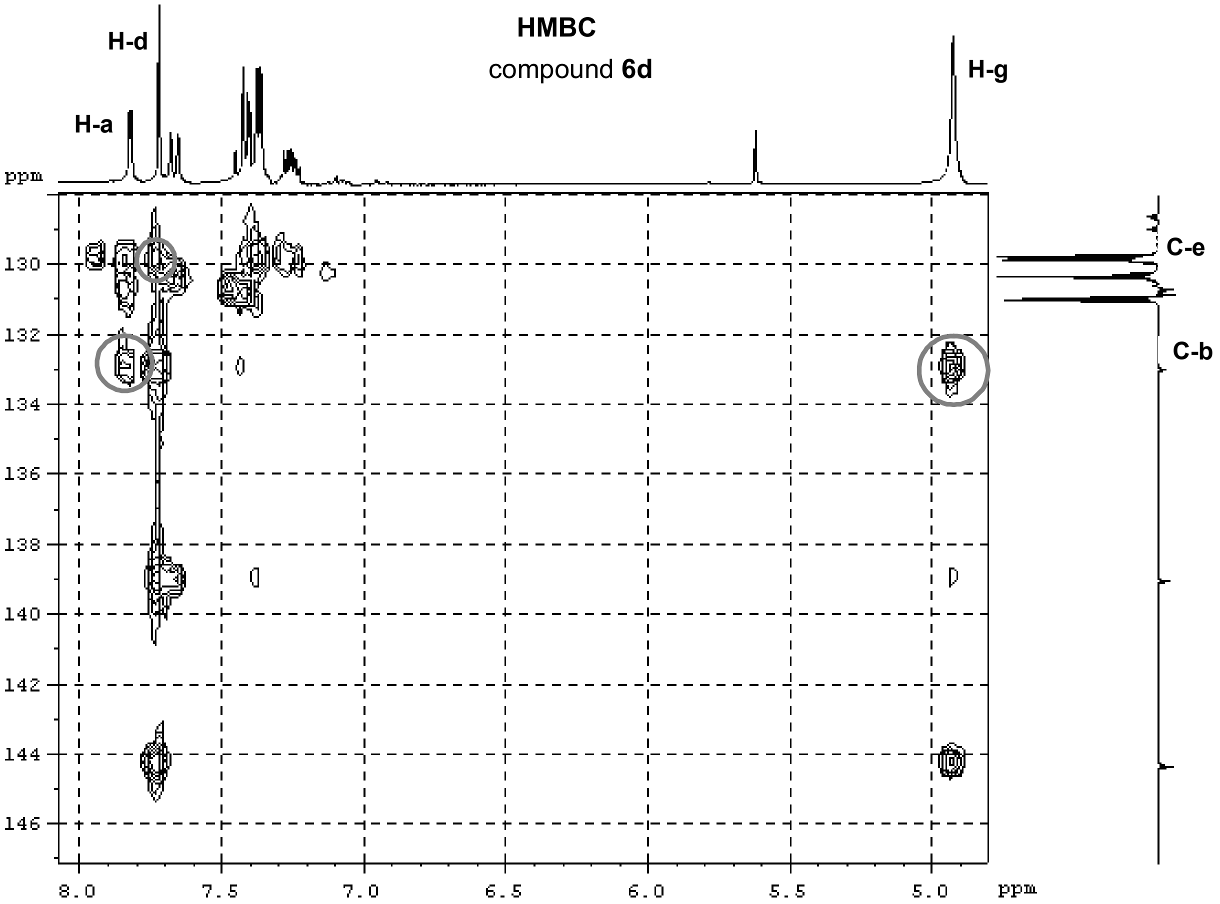


Figure 2

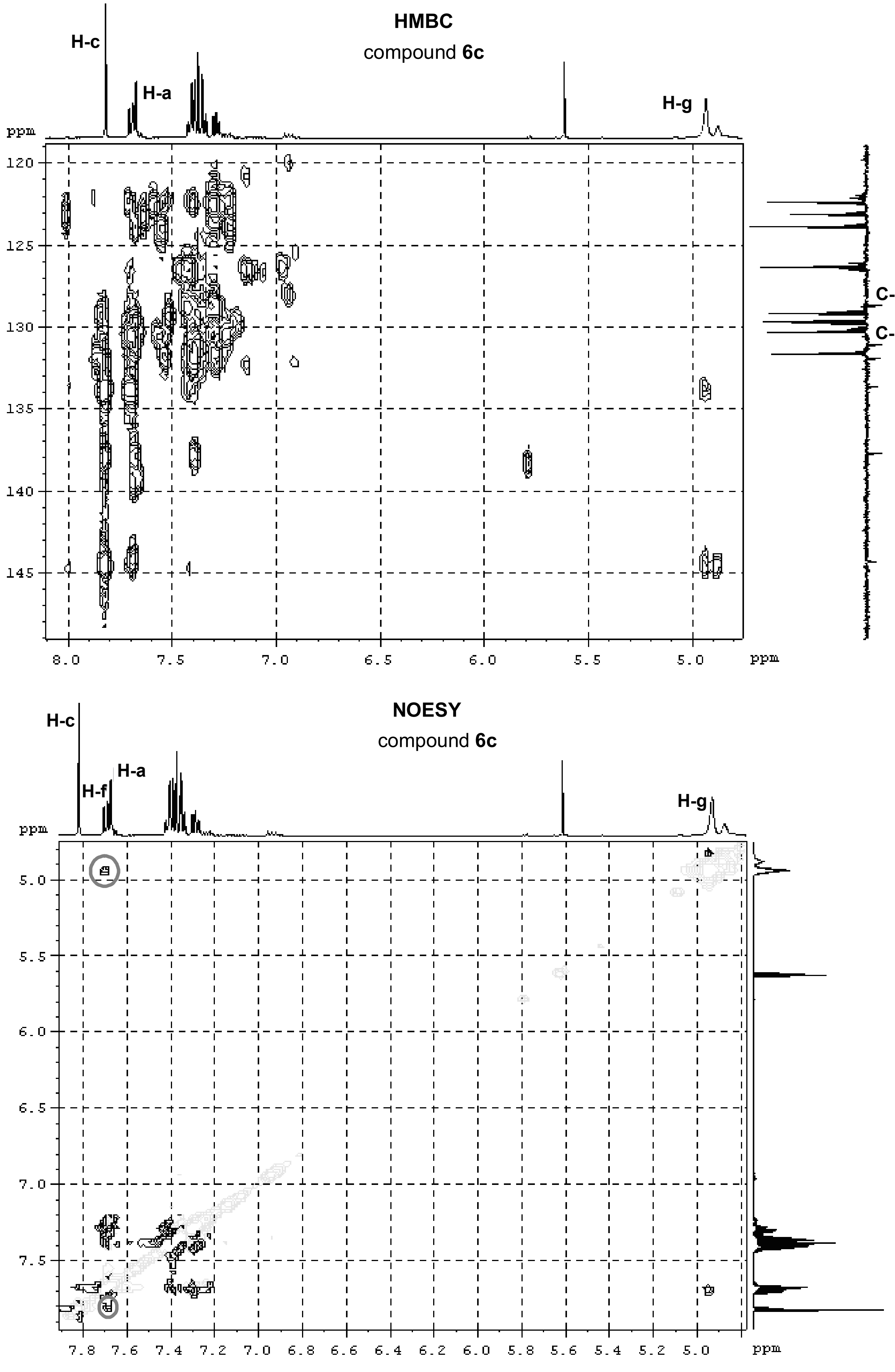

HMBC
compound **6c**
H-c
H-a
H-g
C-e
C-b
ppm
120
125
130
135
140
145
8.0
7.5
7.0
6.5
6.0
5.5
5.0
ppm
NOESY
compound **6c**
H-c
H-f
H-a
H-g
ppm
5.0
5.5
6.0
6.5
7.0
7.5
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
ppm

Figure 3

All above data converge to **6c** having CH_2OH group on the opposite side of Cl atom in the molecule and **6d** on the same side. According to the synthetic sequence, the **c** series possesses the two substituents on the opposite side of the molecular vertical plane of “symmetry”, and **d** series on the same side of this plane. Structures of regioisomeric precursors **3c-d** and **4c-d** can be deduced from synthetic sequence, only for **3c-d** decarboxylation was completed on the small scale (5 mg) of the separated isomers **3c** and **3d**, and then R_f values (eluent toluene) of the obtained products **4c** and **4d** respectively were compared to R_f values of the same compounds, which structure was elucidated previously.

Anti-inflammatory activity. - Inhibition of biomolecules crucial in mechanism of inflammation has been proposed as a disease modifying approach in the treatment of inflammatory disorders. Cytokine tumor necrosis factor alpha (TNF-α), p38 MAP kinase (p38) and COX-2 enzyme are recognized as the most relevant proinflammatory players in studies *in vitro*.[19-21] Therefore, prepared tetracyclic compounds were tested for their ability to inhibit TNF-α secretion in LPS-activated human peripheral blood mononuclear cells (hPBMC) assay.[22,23] Compounds belonging to series **8**, **9**, **10** and **11**, possessing aminoalkoxy chain at thiophene ring, showed potency to inhibit TNF-α production *in vitro* in low micromolar range with IC50 values for the most potent compounds in the range of 1-3 μM. However, none of the tested compounds showed significant inhibition of p38 kinase or COX-2 enzyme.

In conclusion, 2,8-dithia-dibenzo[*e,h*]azulenes and their 8-oxa analogs (**III**), particularly derivatives with aminoalkoxy chain linked at thiophene ring, were recognised as a novel class of fused heterocyclic compounds with potential to emerge as a new class of anti-inflammatory agents with specific TNF-α inhibitory activity.

EXPERIMENTAL

Chemistry – general methods. Commercial reagents were used as received without additional purification. All used chemicals and solvents were p.a. purity. Melting points were determined using Büchi Melting Point B-545 apparatus and are uncorrected. IR spectra were recorded as potassium bromide pastilles or as a film on a sodium chloride plate, on Nicolet Magna IR 760 FT IR-spectrophotometer. One- and two-dimensional spectra were recorded at room temperature on Bruker Avance DPX 300 spectrometer at 300 MHz. Deuterated chloroform ($CDCl_3$), deuterated dimethyl sulfoxide (DMSO-d_6) or deuterated acetone (acetone-d_6) were used as solvent and tetramethylsilane (TMS) as internal standard. For NOE contact analysis NOESY spectra were recorded with mixing time set to 200 ms. Purity of the compounds was estimated on HPLC-MS system Waters 2690 + Micromass Quattro Micro, on HPLC-UV system Waters 2690 + Waters 996 Photodiode Array Detector and on GC-MS system Varian Chrompack Saturn 2000. HRMS data were acquired using Q-TOF 2 Waters system. Microanalyses were performed using Perkin-Elmer 2400 C H N S analyzer. Thin layer chromatography (TLC) was performed on aluminum plates Merck Silica gel 60 F_{254} with UV light detection at 254 nm and/or 365 nm. Proportions of solvents used for TLC are by volume. Column chromatography was performed on silica gel 60 (Merck, 0.063-0.200 nm). Compounds **1a-c** were synthesized as described before.[14-16]

Dibenzo[*b*,*f*]thiepine-10,11-dione (2a). Compound **1a** (10.0 g, 0.044 mol) was added to the suspension of SeO_2 (5.90 g, 0.053 mol) in glacial acetic acid (170 mL). Reaction mixture was then heated at 110 °C for 2.5 h (TLC control, hexane/EtOAc 3:1). Black precipitate was filtered off and filtrate evaporated under reduced pressure. Crude residue was dissolved in EtOAc (250 mL) and then washed with std. aq. $NaHCO_3$ (100 mL) and NaCl (100 mL). The combined aqueous layers were extracted once with EtOAc (50 mL). The combined organic layers were dried (Na_2SO_4), worked-up with charcoal and evaporated. Crystallization from EtOAc afforded pure product as bright yellow crystals; 7.2 g (68%); mp 127-129 °C (lit.,[24] 124-126 °C). IR (KBr) ν/cm^{-1}: 1681 (C=O); 1583, 1462, 1434, 1280, 1260, 1220, 1061, 991. ^{1}H NMR (DMSO-d_6) δ/ppm: 7.55-7.59 (m, 1H, arom.); 7.59-7.62 (m, 1H, arom.); 7.71-7.73 (m, 1H, arom); 7.75-7.77 (m, 1H, arom.). ^{13}C NMR (DMSO-d_6) δ/ppm: 130.2, 131.9, 132.6, 133.8 (C-H arom.); 133.6, 139.9 (C arom.); 190.0 (C=O). MS (CI) *m/z*: 241 (MH$^+$).

Dibenzo[*b*,*f*]oxepine-10,11-dione (2b). This compound was obtained starting from **1b** (3.70 g, 0.018 mol) as described in procedure above; yellow crystals, 2.75 g; second crop: 0.60 g; total yield 85%; mp 118-120 °C. IR (KBr) ν/cm^{-1}: 1673 (C=O); 1601, 1570, 1470, 1447, 1299, 1282, 1220, 1109, 998, 957, 926. ^{1}H NMR (DMSO-d_6) δ/ppm: 7.41-7.46 (m, 1H, arom.); 7.56-7.59 (dd, 1H, arom.); 7.75-7.81 (m, 1H, arom); 7.88-7.91 (dd, 1H, arom.). ^{13}C NMR (DMSO-d_6) δ/ppm: 121.8, 125.9, 131.7, 136.1 (C-H arom.); 126.9, 156.5 (C arom.); 184.6 (C=O). MS (CI) *m/z*: 225 (MH$^+$, 100%); 197 ([M-CO]H$^+$, 95%). Anal. Calcd for $C_{14}H_8O_3$: C, 75.00; H, 3.60. Found: C, 75.20; H. 3.20.

2-Chloro-dibenzo[*b*,*f*]oxepin-10,11-dione (2c). This compound was obtained starting from **1c** (2.00 g, 0.0082 mol) as described in procedure above; crystallization from EtOAc/hexane; yellow crystals, 1.32 g (62%); mp 104-107 °C. IR (KBr) ν/cm^{-1}: 3098, 3075, 3042 (C-H); 1693, 1673 (C=O); 1601, 1467, 1449, 1403, 1291, 1267, 1224, 1119, 940, 841, 763. ^{1}H NMR (DMSO-d_6) δ/ppm: 7.41-7.47 (m, 1H, arom.); 7.55-7.62 (m, 2H, arom.); 7.74-7.83 (m, 3H, arom); 7.89 (dd, 1H, arom.). ^{13}C NMR (DMSO-d_6) δ/ppm: 121.6, 124.0, 125.8, 130.4, 131.7, 135.3, 135.9 (C-H arom.); 126.3, 128.0, 129.4, 155.0, 155.9 (C arom.); 182.6, 183.1 (C=O). MS (CI) *m/z*: 259, 261 (MH$^+$, 100%, 35%). Anal. Calcd for $C_{14}H_7ClO_3$: C, 65.01; H, 2.73. Found: C, 65.29; H. 2.53.

Monomethyl 2,8-dithia-dibenzo[*e*,*h*]azulene-1,3-dicarboxylate (3a). A mixture of dibenzo[*b*,*f*]thiepine-10,11- dione (**2a**) (6.2 g, 0.026 mol) and dimethyl 2,2'-thiodiacetate[10] (8.84 g, 0.050 mol) was added as rapidly as possible to a suspension of potassium *tert*-butoxide (8.90 g, 0.079 mol) in *t*-BuOH (100 mL), which was stirred at 60 °C. The reaction mixture almost immediately turned dark and stirring was continued for 45 min. The mixture was cooled to rt and then acidified with 5 M HCl (70 mL). Most of the solvent was removed at 30 °C under reduced pressure. Water (100 mL) was added to the residue and the organic material was extracted with Et_2O (2×100 mL). The combined organic layers were extracted with 2 M NH_4OH (5×50 mL). Acidification with conc. HCl afforded brown oil, which was extracted with CH_2Cl_2 (3×50 mL). The solvent was dried (Na_2SO_4) and evaporated. Crystallization of the residue from Et_2O/hexane 1:1 gave pure product as colorless powder; 2.90 g (30%); mp 258-261 °C (decomp); second crop, less pure: 2.50 g (total yield 56%). IR (KBr) ν/cm^{-1}: 3400-2200 (broad, COOH); 3057, 2949, 2869; 1733 (C=O); 1687 (C=O); 1656, 1546, 1526, 1442, 1427, 1298, 1244, 1197. ^{1}H NMR (DMSO-d_6) δ/ppm: 3.74 (s, 3H, CH_3); 7.30-7.40 (m, 4H, arom.); 7.54-7.59 (m, 2H, arom.); 7.61-7.67 (m, 2H, arom.); 13.5 (b, 1H, COOH). ^{13}C NMR (DMSO-d_6) δ/ppm: 52.7 (CH_3); 128.2 (2×CH); 129.56 (CH); 129.65 (CH); 132.50 (CH); 132.56 (CH); 133.18 (CH); 133.37 (CH); 130.4 (C); 133.4 (C); 135.60 (C); 135.66 (C); 136.66 (C); 136.78 (C); 145.1 (C); 145.9 (C); 161.5 (C=O); 162.4 (C=O). MS (ES$^+$) *m/z*: 369 (MH$^+$, 75%); 351 ([M-OH]$^+$, 20%); 337 ([M-OCH$_3$]$^+$, 100%). Anal. Calcd for $C_{19}H_{12}O_4S_2$: C, 61.94; H, 3.28; S, 17.41. Found: C, 61.92; H. 3.01; S, 17.03.

Monomethyl 2-thia-8-oxa-dibenzo[*e*,*h*]azulene-1,3-dicarboxylate (3b). This compound was obtained starting from **2b** (8.40 g, 0.038 mol) as described in procedure above; by crystallization from CH_2Cl_2 non-identified by-product was separated (2.0 g); filtrate was worked up with charcoal, evaporated and by crystallization from EtOAc/hexane 1:1 the expected pure product was obtained as light pink solid; 2.75 g (21%); mp 216-219 °C (decomp). IR (KBr) ν/cm^{-1}: 3500-2200 (broad, COOH); 3061, 2952; 1740 (C=O); 1693 (C=O); 1605, 1573, 1532, 1445, 1427, 1370, 1306, 1266, 1250, 1210. ^{1}H NMR (DMSO-d_6) δ/ppm: 3.81 (s, 3H, CH_3); 7.16-7.22 (m, 2H, arom.); 7.42-7.46 (m, 4H, arom.); 7.62-7.67 (m, 2H, arom.); 13.0 (b, 1H, COOH). ^{13}C NMR (DMSO-d_6) δ/ppm: 52.6 (CH_3); 120.56 (CH); 120.65 (CH); 124.5 (2×CH); 130.9 (CH); 131.0 (CH); 132.7 (CH); 133.0 (CH); 125.2 (C); 125.4 (C); 130.1 (C); 133.1 (C); 141.1 (C); 141.9 (C); 157.9 (C); 158.0 (C); 161.4 (C=O); 162.2 (C=O). MS (ES$^+$) *m/z*: 353 (MH$^+$, 50%); 335 ([M-OH]$^+$, 100%). MS (ES$^-$) *m/z*: 307 ([M-CO$_2$H]$^-$, 100%); 249 ([M-CO$_2$H-CO$_2$CH$_3$]$^-$, 90%). Anal. Calcd for $C_{19}H_{12}O_5S$: C, 64.76; H, 3.43; S, 9.10. Found: C, 64.69; H. 3.58; S, 9.31.

1-Methyl 5-chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1,3-dicarboxylate (3c) and 3-methyl 5-chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1,3-dicarboxylate (3d). These compounds were obtained starting from **2c** (3.00 g, 0.0116 mol) as described in procedure above; purifications of crude products mixture by repeated column chromatography (eluent DCM/MeOH 9:1) provided small quantities of pure compounds. The majority was eluted as inseparable mixture. The first eluted compound was **3c**, colorless solid, 80 mg, mp 274.5-276 °C (decomp). IR (KBr) *ν*/cm^{-1}: 3500-2200 (broad, COOH); 1736 (C=O); 1687 (C=O); 1661, 1530, 1493, 1447, 1431, 1413, 1287, 1260, 1245, 1206. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 3.81 (s, 3H, CH_3); 7.20-7.26 (m, 1H, arom.); 7.46-7.49 (m, 4H, arom.); 7.63-7.67 (m, 1H, arom.); 14.0 (b, 1H, COOH). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 52.6 (CH_3); 120.6 (CH); 122.3 (CH); 124.7 (CH); 130.5 (CH); 131.2 (CH); 132.5 (CH); 132.8 (CH); 125.0 (C); 126.7 (C); 128.4 (C); 130.4 (C); 133.9 (C); 139.4 (C); 141.6 (C); 156.7 (C); 157.7 (C); 161.2 (C=O); 161.9 (C=O). MS (ES$^-$) *m/z*: 385, 387 (M-H, 5%, 2%); 341, 343 ($[M-CO_2H]^-$, 100%, 37%). Anal. Calcd for $C_{19}H_{11}ClO_5S$: C, 59.00; H, 2.87; S, 8.29. Found: C, 58.75; H. 2.69; S, 8.14.

Compound **3d** was eluted second, light yellow solid, 100 mg, mp 217-220 °C (decomp). IR (KBr) *ν*/cm^{-1}: 3500-2300 (broad, COOH); 3067, 2953, 2929; 1726 (C=O); 1707 (C=O); 1606, 1572, 1529, 1449, 1432, 1370, 1298, 1256, 1214, 1138, 1087. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 3.79 (s, 3H, CH_3); 7.13-7.19 (m, 2H, arom.); 7.36-7.47 (m, 4H, arom.); 7.70-7.73 (m, 2H, arom.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 52.3 (CH_3); 120.1 (CH); 122.3 (CH); 124.4 (CH); 130.0 (CH); 130.2 (CH); 132.3 (CH); 133.1 (CH); 125.9 (C); 127.4 (C); 128.2 (C); 128.4 (C); 140.2 (C); 156.7 (C); 157.3 (C); 161.5 (C); 163.0 (C); 168.7 (C=O); 170.8 (C=O). MS (ES$^-$) *m/z*: 385, 387 (M-H, 20%, 7%); 341, 343 ($[M-CO_2H]^-$, 100%, 35%). Anal. Calcd for $C_{19}H_{11}ClO_5S$: C, 59.00; H, 2.87; S, 8.29. Found: C, 58.85; H. 3.19; S, 8.55.

Methyl 2,8-dithia-dibenzo[*e*,*h*]azulene-1-carboxylate (4a) and 2,8-dithia-dibenzo[*e*,*h*]azulene (5a). An intimate mixture of **3a** (5.40 g, 0.015 mol) and Cu powder (2.50 g, 0.039 mol) was heated gradually to 225 °C and then for 30-45 min at this temperature. The mixture was cooled to rt and organic material was dissolved in EtOAc. The inorganic precipitate was filtered off and filtrate concentrated. Two major products were separated by column chromatography (eluent hexane/EtOAc 3:1). The first eluted compound was **5a**, light yellow solid, 1.0 g (26%), mp 131-133 °C. IR (KBr) *ν*/cm^{-1}: 3097, 3049, 2962, 1556, 1520, 1484, 1444, 1360, 1261, 1247, 1096, 1060, 1031, 949. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 7.32-7.38 (m, 1H, arom.); 7.40-7.46 (m, 1H, arom.); 7.60-7.65 (m, 2H, arom.); 7.85 (s, 1H, thioph.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 125.0 (CH); 128.4 (CH); 129.2 (CH); 130.0 (CH); 132.5 (CH); 134.1 (C); 138.8 (C); 140.5 (C). MS (CI) *m/z*: 267 (MH^+, 100%). Anal. Calcd for $C_{16}H_{10}S_2$: C, 72.14; H, 3.78; S, 24.08. Found: C, 71.79; H. 3.76; S, 24.56.

Compound **4a** was eluted second, colorless powder, 2.50 g (53%); mp 174-175.5 °C. IR (KBr) *ν*/cm^{-1}: 3065, 2992, 2947; 1716 (C=O); 1566, 1522, 1454, 1435, 1415, 1358, 1291, 1255, 1213, 1086, 1053. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 3.73 (s, 3H, CH_3); 7.32-7.47 (m, 4H, arom.); 7.51-7.55 (m, 1H, arom.); 7.63-7.68 (m, 3H, arom.); 8.13 (s, 1H, thioph.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 52.0 (CH_3); 128.0 (CH); 128.8 (CH); 129.2 (CH); 129.3 (CH); 129.9 (CH); 130.0 (CH); 132.2 (CH); 132.7 (CH); 133.1 (CH); 135.0 (C); 135.6 (C); 136.6 (C); 137 (C); 137.6 (C); 142.8 (C); 144.7 (C); 161.6 (C=O). MS (CI) *m/z*: 325

(MH^+, 70%); 293 ($[M-OCH_3]^+$, 100%). Anal. Calcd for $C_{18}H_{12}O_2S_2$: C, 66.64; H, 3.73; S, 19.77. Found: C, 66.25; H. 3.46; S, 19.45.

Methyl 2-thia-8-oxa-dibenzo[*e*,*h*]azulene-1-carboxylate (4b) and 2-thia-8-oxa-dibenzo[*e*,*h*]azulene (5b). These compounds were obtained starting from **3b** (2.75 g, 0.0078 mol) as described in procedure above; two major products were separated by column chromatography (eluent hexane/EtOAc 3.5:1). The first eluted compound was **5b**, light yellow crystals, 0.50 g, (26%), mp 149.5-150.5 °C. IR (KBr) ν/cm^{-1}: 3104, 3055, 1572, 1534, 1503, 1466, 1443, 1356, 1287, 1236, 1199, 1171, 1103, 880, 863. ^{1}H NMR (DMSO-d_6) δ/ppm: 7.24-7.30 (m, 1H, arom.); 7.37-7.41 (m, 2H, arom.); 7.66-7.69 (m, 1H, arom.); 7.97 (s, 1H, thioph.). ^{13}C NMR (DMSO-d_6) δ/ppm: 121.4 (CH); 123.9 (CH); 125.6 (CH); 128.1 (C); 129.0 (CH); 129.4 (CH); 136.7 (C); 156.1 (C). MS (CI) *m/z*: 251 (MH^+, 100%). Anal. Calcd for $C_{16}H_{10}OS$: C, 76.77; H, 4.03; S, 12.81. Found: C, 76.65; H. 3.82; S, 12.47.

Compound **4b** was eluted second, colorless crystals, 1.50 g (62%), mp 127-130 °C. IR (film) ν/cm^{-1}: 3094, 3061, 2950; 1722 (C=O); 1607, 1574, 1534, 1461, 1435, 1367, 1296, 1256, 1226, 1207, 1110, 1084, 1066. ^{1}H NMR (DMSO-d_6) δ/ppm: 3.80 (s, 3H, CH_3); 7.18-7.24 (m, 1H, arom.); 7.26-7.31 (m, 1H, arom.); 7.41-7.46 (m, 4H, arom.); 7.62-7.65 (m, 1H, arom.); 7.69-7.72 (m, 1H, arom.); 8.26 (s, 1H, thioph.). ^{13}C NMR (DMSO-d_6) δ/ppm: 52.1 (CH_3); 121.01 (CH); 121.03 (CH); 124.6 (CH); 125.8 (CH); 128.9 (2×CH); 130.0 (CH); 130.7 (CH); 132.9 (CH); 126.1 (C); 127.3 (C); 127.9 (C); 139.0 (C); 141.0 (C); 156.8 (C); 157.2 (C); 161.7 (C=O). MS (CI) *m/z*: 308 (M^+, 50%); 277 ($[M-OCH_3]^+$, 100%). Anal. Calcd for $C_{18}H_{12}O_3S$: C, 70.11; H, 3.92; S, 10.40. Found: C, 70.32; H. 3.82; S, 10.12.

Methyl 5-chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-carboxylate (4c), methyl 11-chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-carboxylate (4d) and 5-chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulene (5c). These compounds were obtained starting from mixture of isomers **3c** and **3d** (4.35 g) as described in procedure above; products were separated by column chromatography (eluent toluene). The first eluted compound was **5c**, light yellow solid, 0.5 g, mp 70-73 °C. IR (film) ν/cm^{-1}: 3101, 3062, 2964, 2924, 1600, 1575, 1532, 1501, 1456, 1435, 1353, 1240, 1197, 1167, 1092, 1033. ^{1}H NMR (DMSO-d_6) δ/ppm: 7.26-7.32 (m, 1H, arom.); 7.40-7.47 (m, 4H, arom.); 7.67-7.77 (m, 1H, arom.); 7.75-7.76 (m, 1H, arom.); 8.01 (d, J = 3.2 Hz, 1H, thioph.); 8.13 (d, J = 3.2 Hz, 1H, thioph.). ^{13}C NMR (DMSO-d_6) δ/ppm: 121.5, 123.5, 124.3, 125.3, 126.1, 128.5, 129.1, 129.2, 129.7 (CH arom.); 128.0, 129.74, 130.1, 135.4, 136.6, 154.9, 155.9 (C arom.). MS (CI) *m/z*: 284, 286 (M^+, 100%, 35%). Anal. Calcd for $C_{16}H_9ClOS$: C, 67.49; H, 3.19; S, 11.26. Found: C, 67.58; H. 2.99; S, 10.98.

The second eluted compound was **4d**, yellowish solid, 0.25 g, mp 129-132 °C. IR (KBr) ν/cm^{-1}: 3093, 2951, 1721 (C=O); 1572, 1532, 1445, 1430, 1400, 1353, 1289, 1259, 1226, 1209, 1093. ^{1}H NMR (DMSO-d_6) δ/ppm: 3.82 (s, 3H, CH_3); 7.28-7.34 (m, 1H, arom.); 7.43-7.46 (m, 2H, arom.); 7.48-7.50 (m, 2H, arom.); 7.70-7.73 (m, 1H, arom.); 7.76 (dd, 1H, arom.); 8.29 (s, 1H, thioph.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 52.3 (CH_3); 120.9 (CH); 122.8 (CH); 126.0 (CH); 129.0 (CH); 129.3 (CH); 130.2 (CH); 130.3 (CH); 132.3 (CH); 127.1 (C); 127.7 (C); 128.5 (C); 128.7 (C); 138.8 (C); 156.0 (C); 156.6 (C); 161.3 (C); 175.0 (C=O). MS (CI) *m/z*: 342, 344 (M^+, 100%, 35%). Anal. Calcd for $C_{18}H_{11}ClO_3S$: C, 63.07; H, 3.23; S, 9.35. Found: C, 63.28; H. 3.29; S, 9.25.

The third eluted compound was **4c**, colorless solid, crystallized from Et_2O/hexane, 0.25 g; mp 185-188 °C. IR (KBr) *ν*/cm^{-1}: 3091, 3008, 2952, 1726 (C=O); 1599, 1531, 1497, 1436, 1397, 1350, 1280, 1254, 1229, 1209, 1191, 1120, 1083, 1063. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 3.79 (s, 3H, CH_3); 7.18-7.26 (m, 1H, arom.); 7.44-7.48 (m, 4H, arom.); 7.63-7.66 (m, 1H, arom.); 7.80 (bs, 1H, arom.); 8.39 (s, 1H, thioph.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 52.2 (CH_3); 121.0 (CH); 122.9 (CH); 124.8 (CH); 128.4 (CH); 129.6 (CH); 130.0 (CH); 130.9 (CH); 132.9 (CH); 125.8 (C); 129.0 (C); 129.7 (C); 131.6 (C); 137.5 (C); 140.7 (C); 155.5 (C); 156.9 (C); 161.6 (C=O). MS (CI) *m/z*: 342, 344 (M^+, 100%, 35%). Anal. Calcd for $C_{18}H_{11}ClO_3S$: C, 63.07; H, 3.23; S, 9.35. Found: C, 62.84; H. 3.07; S, 8.98.

(2,8-Dithia-dibenzo[*e*,*h*]azulen-1-yl)methanol (6a). To the suspension of $LiAlH_4$ (0.4 g, 0.011 mol) in Et_2O (20 mL) a solution of **4a** (2.4 g, 7.4 mmol) in THF (10 mL) was added. Reaction mixture was stirred at rt for 1 h (TLC control, hexane/EtOAc 3:1). Excess of $LiAlH_4$ was destroyed with dropwise addition of 1:1 mixture of H_2O and Et_2O. The resulting inorganic precipitate was filtered off and washed several times with portions of Et_2O. The filtrate was concentrated and subjected to column chromatography (eluent hexane/EtOAc 3:1) to afford pure **6a** as light yellow foam, 2.10 g (96%), mp 133-136 °C. **6a** can be also prepared from **7a** according to this procedure. IR (KBr) *ν*/cm^{-1}: 3600-3100 (broad, O-H); 3053, 2927; 1587, 1564, 1531, 1487, 1456, 1428, 1372, 1248, 1235, 1142, 1094, 1059, 1035, 1006, 980. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 4.60 (irregular dd, 1H from CH_2, *J* = 13.6, 4.7 Hz); 4.77 (irregular dd, 1H from CH_2, *J* = 13.6, 6.2 Hz); 5.74 (m, 1H, OH); 7.30-7.45 (m, 4H, arom.); 7.52-7.63 (m, 3H, arom.); 7.67-7.70 (m, 1H, arom.); 7.69 (s, 1H, thioph.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 57.4 (CH_2); 122.3 (CH); 128.18 (CH); 128.24 (CH); 128.6 (CH); 129.2 (CH); 129.8 (CH); 131.1 (CH); 132.3 (CH); 133.0 (CH); 134.8 (C); 135.5 (C); 135.6 (C); 137.3 (C); 139.1 (C); 141.2 (C); 143.8 (C). MS (CI) *m/z*: 295 (M-H, 100%). Anal. Calcd for $C_{17}H_{12}OS_2$: C, 68.89; H, 4.08; S, 21.64. Found: C, 68.88; H. 4.14; S, 21.56.

(2-Thia-8-oxa-dibenzo[*e*,*h*]azulen-1-yl)methanol (6b). This compound was obtained starting from **4b** (1.50 g, 4.8 mmol) or from **7b** as described in procedure above, light yellow foam, 1.30 g (95%), mp 112-115 °C. IR (KBr) *ν*/cm^{-1}: 3600-3100 (broad, O-H); 3060, 2962; 1573, 1539, 1503, 1470, 1443, 1261, 1243, 1203, 1100, 1035, 786. ^{1}H NMR (DMSO-*d*$_6$) *δ*/ppm: 4.77 (b, 2H, CH_2); 5.79 (t, 1H, OH); 7.23-7.34 (m, 2H, arom.); 7.36-7.45 (m, 4H, arom.); 7.56-7.59 (m, 1H, arom.); 7.63-7.66 (m, 1H, arom.), 7.81 (s, 1H, thioph.). ^{13}C NMR (DMSO-*d*$_6$) *δ*/ppm: 57.3 (CH_2); 121.1 (CH); 121.3 (CH); 121.4 (CH); 125.2 (CH);

125.6 (CH); 128.7 (CH); 129.21 (CH); 129.23 (CH); 130.3 (CH); 127.3 (C); 128.6 (C); 131.7 (C); 137.3 (C); 143.7 (C); 156.5 (C); 157.0 (C). MS (CI) *m/z*: 279 (M-H, 100%). Anal. Calcd for $C_{17}H_{12}O_2S$: C, 72.83; H, 4.31; S, 11.44. Found: C, 73.03; H. 4.38; S, 11.28.

(5-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-yl)methanol (6c). This compound was obtained starting from **4c** (0.30 g, 0.88 mmol) as described in procedure above, light yellow foam, 0.16 g (58%), mp 142-145 °C. IR (KBr) ν/cm^{-1}: 3500-3100 (broad, O-H); 3099, 3057, 2924, 1574, 1537, 1439, 1350, 1244, 1205, 1137, 1121, 1093, 1006. ^{1}H NMR (acetone-d_6) δ/ppm: 4.93 (bs, 2H, CH_2); 7.27-7.31 (m, 1H, arom.); 7.34-7.43 (m, 4H, arom.); 7.66-7.71 (m, 2H, arom.); 7.82 (s, 1H, thioph.). ^{13}C NMR (acetone-d_6) δ/ppm: 58.8 (CH_2); 122.3 (CH); 123.0 (CH); 123.8 (CH); 126.3 (CH); 128.6 (C); 129.1 (CH); 129.7 (CH); 130.3 (CH); 131.0 (C); 131.6 (CH); 131.9 (C); 133.6 (C); 137.7 (C); 144.3 (C); 156.8 (C); 158.5 (C). MS (CI) *m/z*: 314, 316 (M^+, 100%, 35%). Anal. Calcd for $C_{17}H_{11}ClO_2S$: C, 64.86; H, 3.52; S, 10.19. Found: C, 65.01; H. 3.39; S, 10.00.

(11-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-yl)methanol (6d). This compound was obtained starting from **4d** (0.25 g, 0.73 mmol) as described in procedure above, yellowish foam, 0.15 g (65%), mp 160-163 °C. IR (KBr) ν/cm^{-1}: 3500-3100 (broad, O-H); 3063, 2924, 1574, 1538, 1455, 1414, 1245, 1204, 1105, 1037. ^{1}H NMR (acetone-d_6) δ/ppm: 4.93 (bs, 2H, CH_2); 7.23-7.29 (m, 1H, arom.); 7.36-7.38 (m, 2H, arom.); 7.40-7.43 (m, 2H, arom.); 7.66-7.68 (m, 1H, arom.); 7.73 (s, 1H, thioph.); 7.83 (d, $J = 2.0$ Hz, 1H, arom.). ^{13}C NMR (acetone-d_6) δ/ppm: 58.4 (CH_2); 122.0 (CH); 122.3 (CH); 124.0 (CH); 126.7 (CH); 129.6 (C); 129.7 (CH); 129.8 (CH); 130.3 (CH); 130.6 (C); 130.8 (C); 130.9 (CH); 132.9 (C); 139.0 (C); 144.3 (C); 157.4 (C); 157.9 (C). MS (CI) *m/z*: 314, 316 (M^+, 75%, 26%); 298, 300 ($[M-OH]H^+$, 100%, 37%). Anal. Calcd for $C_{17}H_{11}ClO_2S$: C, 64.86; H, 3.52; S, 10.19. Found: C, 64.57; H. 3.33; S, 9.97.

2-Thia-8-oxa-dibenzo[*e*,*h*]azulene-1-carbaldehyde (7b). To the ice-cold DMF (0.47 mL, 6.0 mmol) $POCl_3$ (0.37 mL, 4.0 mmol) was added at such a rate that temperature was maintained below 5 °C. The solidified product was allowed to stand at rt for 15 min. Solution of **5b** (0.10 g, 0.4 mmol) in DMF (1.5 mL) was then added. The reaction mixture was heated at 100 °C for 6 h (TLC control, hexane/EtOAc 3:1). When the reaction was completed, dark-brown mixture was cooled to rt and std. aq. AcONa (50 mL) was added cautiously (vigorous reaction may take place!). The product was extracted with CH_2Cl_2 (3×25 mL). The combined CH_2Cl_2 extracts were washed with std. aq. $NaHCO_3$ and std. NaCl, dried (Na_2SO_4) and solvent was evaporated. Crude product was purified by column chromatography (eluent hexane/EtOAc 3:1) providing **7b** as a light yellow powder, 0.09 g (81%), mp 107-109 °C. IR (KBr) ν/cm^{-1}: 3087, 3059, 2963, 2843; 1657 (C=O); 1571, 1530, 1443, 1430, 1385, 1354, 1293, 1243, 1208, 1172, 1102. ^{1}H NMR (DMSO-d_6) δ/ppm: 7.29-7.38 (m, 2H, arom.); 7.44-7.48 (m, 2H, arom.); 7.52-7.59 (m, 2H, arom.);

7.72-7.75 (m, 2H, arom.); 8.49 (d, J = 1.1 Hz, 1H, thioph.); 9.94 (d, J = 1.1 Hz, 1H, CHO). ^{13}C NMR (DMSO-d_6) δ/ppm: 121.3 (CH); 121.8 (CH); 125.8 (CH); 126.0 (CH); 128.9 (CH); 130.2 (CH); 131.5 (CH); 132.0 (CH); 132.9 (CH); 125.4 (C); 126.9 (C); 139.0 (C); 140.0 (C); 143.5 (C); 156.5 (C); 157.7 (C); 183.9 (CHO). MS (CI) *m/z*: 279 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{10}O_2S$: C, 73.36; H, 3.62; S, 11.52. Found: C, 73.01; H. 3.63; S, 11.87.

2,8-Dithia-dibenzo[*e,h*]azulene-1-carbaldehyde (7a). This compound was obtained starting from **5a** (0.10 g, 3.8 mmol) as described in procedure above, crystallization from EtOAc/hexane, light yellow crystals, 0.07 g (63%), mp 158-160.5 °C. IR (KBr) ν/cm^{-1}: 3094, 2925, 2849; 1653 (C=O); 1522, 1421, 1382, 1348, 1287, 1241, 1181, 1097, 1027, 760, 737. ^{1}H NMR ($CDCl_3$) δ/ppm: 7.29-7.44 (m, 5H, arom.); 7.53-7.56 (m, 1H, arom.); 7.65-7.68 (m, 1H, arom.); 7.71-7.75 (m, 1H, arom.); 7.82 (d, J = 1.2 Hz, 1H, thioph.); 9.84 (d, J = 1.2 Hz, 1H, CHO). ^{13}C NMR ($CDCl_3$) δ/ppm: 127.6 (CH); 127.8 (CH); 128.1 (CH); 128.5 (CH); 128.9 (CH); 130.5 (CH); 131.9 (CH); 132.1 (CH); 132.8 (CH); 134.7 (C); 134.9 (C); 136.1 (C); 136.8 (C); 139.4 (C); 142.9 (C); 147.5 (C); 183.5 (CHO). MS (CI) *m/z*: 295 (MH$^+$, 100%). Anal. Calcd for $C_{17}H_{10}OS_2$: C, 69.36; H, 3.42; S, 21.78. Found: C, 69.0; H. 3.22; S, 21.44.

[2-(2,8-Dithia-dibenzo[*e,h*]azulen-1-ylmethoxy)ethyl]dimethylamine (8a). To a solution of (2-chloro-ethyl)dimethylammonium chloride (3.10 g, 0.021 mol) in 40% aq. NaOH (35 mL), benzyltriethylammonium chloride (0.2 g, 0.88 mmol) and solution of the alcohol **6a** (1.27 g, 4.28 mmol) in toluene (35 mL) were added. The reaction mixture was heated under vigorous stirring and refluxing for 3 h (TLC control, CH_2Cl_2/MeOH/NH_4OH 90:10:1.5). Then it was cooled to rt, diluted with water and product extracted with CH_2Cl_2 (3×40 mL). The combined organic extracts were washed with brine, dried (Na_2SO_4) and solvent evaporated. Column chromatography (CH_2Cl_2/MeOH/NH_4OH 100:5:1.5) provided pure **8a** as light yellow very viscous oil, 1.20 g (76%). IR (film) ν/cm^{-1}: 3053, 2934, 2856, 1487, 1456, 1348, 1157, 1103, 1036, 953, 759. ^{1}H NMR ($CDCl_3$) δ/ppm: 2.35 (s, 6H, 2×CH_3); 2.61-2.67 (m, 2H, CH_2N); 3.59-3.77 (dm, 2H, OCH_2); 4.65, 4.75 (2 irregular d, each 1H, Ar-CH_2, J = 12.3 Hz); 7.21-7.36 (m, 4H, arom.); 7.39 (s, 1H, thioph.); 7.46-7.52 (m, 2H, arom.); 7.60-7.63 (m, 1H, arom.); 7.67-7.70 (m, 1H, arom.). ^{13}C NMR ($CDCl_3$) δ/ppm: 45.6 (2×CH_3); 58.6 (CH_2); 66.7 (CH_2); 67.9 (CH_2); 122.6 (CH); 128.1 (CH); 128.3 (CH); 128.4 (CH); 128.8 (CH); 129.6 (CH); 131.3 (CH); 132.6 (CH); 133.3 (CH); 136.0 (C); 136.8 (C); 137.5 (C); 137.7 (C); 139.2 (C); 139.6 (C); 142.4 (C). MS (ES$^+$) *m/z*: 368 (MH$^+$, 100%); 279 ([M - $O(CH_2)_2N(CH_3)_2$]$^+$, 85%). HRMS (ES$^+$): *m/z* calc. for $C_{21}H_{22}NOS_2$: 368.1143 (MH$^+$); found: 368.1131. Anal. Calcd for $C_{21}H_{21}NOS_2$: C, 68.63; H, 5.76; N, 3.81; S, 17.45. Found: C, 68.78; H. 5.56; N, 4.02; S, 17.05.

[3-(2,8-Dithia-dibenzo[*e,h*]azulen-1-ylmethoxy)propyl]dimethylamine (8b). This compound was obtained starting from **6a** (0.2 g, 0.67 mmol) and (3-chloro-propyl)dimethylammonium chloride (1.07 g, 6.77 mmol) as described in procedure above, light yellow viscous oil, 0.19 g (74%). IR (film) ν/cm^{-1}: 3053, 2942, 2856, 2815, 2765, 1486, 1461, 1352, 1264, 1235, 1155, 1098, 1035, 760, 738. ^{1}H NMR ($CDCl_3$) δ/ppm: 2.08-2.18 (m, 2H, CH_2); 2.71 (s, 6H, 2×CH_3); 2.96-3.04 (m, 2H, CH_2N); 3.59-3.65 (m, 2H, OCH_2); 4.66, 4.72 (2 irregular d, each 1H, Ar-CH_2, J = 12.3 Hz); 7.25-7.37 (m, 4H, arom.); 7.40 (s, 1H, thioph.); 7.40-7.44 (m, 1H, arom.); 7.50-7.53 (m, 1H, arom.); 7.61-7.64 (m, 1H, arom.); 7.68-7.71 (m, 1H, arom.). ^{13}C NMR ($CDCl_3$) δ/ppm: 25.4 (CH_2); 43.4 (2×CH_3); 56.1 (CH_2); 66.7 (CH_2); 67.1 (CH_2); 122.5 (CH); 128.2 (CH); 128.4 (CH); 128.5 (CH); 128.9 (CH); 129.6 (CH); 131.0 (CH); 132.7 (CH); 133.5 (CH); 135.9 (C); 136.8 (C); 137.1 (C); 137.6 (C); 139.3 (C); 139.4 (C); 142.6 (C). MS (ES$^+$) m/z: 382 (MH$^+$, 100%); 279 ($[M - O(CH_2)_3N(CH_3)_2]^+$, 85%). HRMS (ES$^+$): m/z calc. for $C_{22}H_{24}NOS_2$: 382.1299 (MH$^+$); found: 382.1274. Anal. Calcd for $C_{22}H_{23}NOS_2$: C, 69.25; H, 6.08; N, 3.67; S, 16.81. Found: C, 69.44; H. 5.86; N, 4.00; S, 17.05.

3-(2,8-Dithia-dibenzo[*e*,*h*]azulen-1-ylmethoxy)propylamine (8c). This compound was obtained starting from **6a** (0.08 g, 0.27 mmol) and 3-chloro-propylammonium chloride (0.35 g, 2.7 mmol) as described in procedure above, light yellow very viscous oil, 10 mg (10%). IR (film) ν/cm^{-1}: 3440 (broad, N-H); 2962, 2923, 2854, 1486, 1456, 1429, 1349, 1262, 1096, 1028, 800, 758. MS (ES$^+$) m/z: 354 (MH$^+$, 10%); 279 ($[M - O(CH_2)_3NH_2]^+$, 100%). HRMS (ES$^+$): m/z calc. for $C_{20}H_{20}NOS_2$: 354.0986 (MH$^+$); found: 354.1015.

Dimethyl-[2-(2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)ethyl]amine (9a). This compound was obtained starting from **6b** (1.25 g, 4.46 mmol) and (2-chloro-ethyl)dimethylammonium chloride (3.21 g, 0.022 mol) as described in procedure above, light yellow very viscous oil, 1.20 g (77%). IR (film) ν/cm^{-1}: 3060, 2924, 2854; 1573, 1539, 1505, 1470, 1443, 1243, 1204, 1102, 1038. ^{1}H NMR ($CDCl_3$) δ/ppm: 2.40 (s, 6H, 2×CH_3); 2.72 (b, 2H, CH_2N); 3.7-3.8 (b, 2H, OCH_2); 4.74, 4.83 (2b, each 1H, Ar-CH_2); 7.15-7.25 (m, 2H, arom.); 7.29-7.31 (m, 2H, arom.); 7.32-7.36 (m, 2H, arom.); 7.45 (s, 1H, thioph.); 7.52-7.55 (m, 2H, arom.). ^{13}C NMR ($CDCl_3$) δ/ppm: 45.5 (2×CH_3); 58.5 (CH_2); 66.7 (CH_2); 67.8 (CH_2); 121.3 (CH); 121.5 (CH); 121.6 (CH); 125.2 (CH); 125.5 (CH); 128.6 (CH); 129.2 (CH); 129.3 (CH); 130.7 (CH); 127.8 (C); 129.1 (C); 135.3 (C); 137.3 (C); 138.6 (C); 157.3 (C); 158.0 (C). MS (ES$^+$) m/z: 352 (MH$^+$, 100%); 263 ($[M - O(CH_2)_2N(CH_3)_2]^+$, 85%). HRMS (ES$^+$): m/z calc. for $C_{21}H_{22}NO_2S$: 352.1371 (MH$^+$); found: 352.1378. Anal. Calcd for $C_{21}H_{21}NO_2S$: C, 71.76; H, 6.02; N, 3.99; S, 9.12. Found: C, 72.03; H. 5.89; N, 3.67; S, 9.54.

Dimethyl-[3-(2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)propyl]amine (9b). This compound was obtained starting from **6b** (70 mg, 0.25 mmol) and (3-chloro-propyl)dimethylammonium chloride (0.4 g, 2.53 mmol) as described in procedure above, light yellow viscous oil, 35 mg (38%). IR (film) *ν*/cm^{-1}: 2941, 2857, 2815, 2765, 1573, 1539, 1504, 1469, 1443, 1354, 1243, 1205, 1101, 1037, 789, 748. ^{1}H NMR ($CDCl_3$) *δ*/ppm: 2.02-2.11 (m, 2H, CH_2); 2.56 (s, 6H, 2×CH_3); 2.83 (bt, 2H, CH_2N); 3.68 (bt, 2H, OCH_2); 4.74, 4.77 (2b, each 1H, Ar-CH_2); 7.16-7.25 (m, 2H, arom.); 7.30-7.32 (m, 2H, arom.); 7.33-7.37 (m, 2H, arom.); 7.46 (s, 1H, thioph.); 7.48-7.51 (m, 1H, arom.); 7.52-7.57 (m, 1H, arom.). ^{13}C NMR ($CDCl_3$) *δ*/ppm: 26.2 (CH_2); 44.0 (2×CH_3); 56.3 (CH_2); 66.6 (CH_2); 67.7 (CH_2); 121.3 (CH); 121.4 (CH); 121.7 (CH); 125.2 (CH); 125.5 (CH); 128.6 (CH); 129.2 (CH); 129.4 (CH); 130.4 (CH); 127.8 (C); 129.0 (C); 135.3 (C); 137.3 (C); 138.7 (C); 157.3 (C); 158.0 (C). MS (ES$^+$) *m/z*: 366 (MH$^+$, 100%); 263 ([M - $O(CH_2)_3N(CH_3)_2$]$^+$, 90%). Anal. Calcd for $C_{22}H_{23}NO_2S$: C, 72.30; H, 6.34; N, 3.83; S, 8.77. Found: C, 72.54; H. 5.99; N, 3.92; S, 9.02.

3-(2-Thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)propylamine (9c). This compound was obtained starting from **6b** (70 mg, 0.25 mmol) and 3-chloro-propylammonium chloride (0.32 g, 2.46 mmol) as described in procedure above, light yellow viscous oil, 65 mg (77%). IR (film) *ν*/cm^{-1}: 3431 (broad, N-H); 2925, 2859, 1573, 1538, 1504, 1471, 1442, 1352, 1243, 1204, 1089, 789, 747, 734. ^{1}H NMR ($CDCl_3$) *δ*/ppm: 2.03-2.11 (b, 2H, CH_2); 3.03-3.11 (b, 2H, CH_2N); 3.59-3.68 (b, 2H, OCH_2); 4.70, 4.79 (2b, each 1H, Ar-CH_2); 5.0-5.5 (b, 2H, NH_2); 7.11-7.33 (m, 6H, arom.); 7.40 (s, 1H, thioph.); 7.44-7.51 (m, 2H, arom.). ^{13}C NMR ($CDCl_3$) *δ*/ppm: 28.9 (CH_2); 38.2 (CH_2); 66.3 (CH_2); 67.6 (CH_2); 120.9 (CH); 121.0 (CH); 121.3 (CH); 124.9 (CH); 125.2 (CH); 128.3 (CH); 128.8 (CH); 129.0 (CH); 130.2 (CH); 127.4 (C); 128.7 (C); 134.7 (C); 137.1 (C); 138.2 (C); 156.9 (C); 157.5 (C). MS (ES$^+$) *m/z*: 338 (MH$^+$, 50%); 263 ([M - $O(CH_2)_3NH_2$]$^+$, 100%). HRMS (ES$^+$): *m/z* calc. for $C_{20}H_{20}NO_2S$: 338.1215 (MH$^+$); found: 338.1239. Anal. Calcd for $C_{20}H_{19}NO_2S$: C, 71.19; H, 5.68; N, 4.15; S, 9.50. Found: C, 70.93; H. 5.65; N, 4.08; S, 9.21.

[2-(5-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)ethyl]dimethylamine (10a). This compound was obtained starting from **6c** (90 mg, 0.28 mmol) and (2-chloro-ethyl)dimethylammonium chloride (0.41 g, 2.86 mmol) as described in procedure above, light yellow very viscous oil, 40 mg (36%). IR (film) *ν*/cm^{-1}: 2940, 2860, 2818, 2769, 1539, 1502, 1467, 1440, 1350, 1244, 1203, 1101, 1039, 851, 782. ^{1}H NMR ($CDCl_3$) *δ*/ppm: 2.63 (s, 6H, 2×CH_3); 2.99 (bt, 2H, CH_2N); 3.94 (t, *J* = 5.1 Hz, 2H, OCH_2); 4.79, 4.84 (2b, each 1H, Ar-CH_2); 7.21-7.28 (m, 3H, arom.); 7.32-7.36 (m, 2H, arom.); 7.43-7.46 (m, 1H, arom.); 7.48 (s, 1H, thioph.); 7.50-7.51 (m, 1H, arom.). ^{13}C NMR ($CDCl_3$) *δ*/ppm: 44.7 (2×CH_3); 57.8 (CH_2); 66.5 (CH_2); 66.8 (CH_2); 121.6 (CH); 122.3 (CH); 122.7 (CH); 125.6 (CH); 128.3 (CH); 129.0 (CH); 129.7 (CH); 130.5 (CH); 127.4 (C); 129.5 (C); 130.7 (C); 135.1 (C); 137.1 (C); 137.4 (C); 156.1 (C); 157.8 (C). MS (ES$^+$) *m/z*: 386, 388 (MH$^+$, 37%, 13%); 297, 299 ([M - $O(CH_2)_2N(CH_3)_2$]$^+$, 100%, 35%); 263 ([M - $O(CH_2)_2N(CH_3)_2$ - Cl]H$^+$, 70%). HRMS (ES$^+$): *m/z* calc. for $C_{21}H_{21}NO_2SCl$: 386.0982

(MH$^+$); found: 386.0973.

[3-(5-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)propyl]dimethylamine (10b). This compound was obtained starting from **6c** (70 mg, 0.22 mmol) and (3-chloro-propyl)dimethylammonium chloride (0.35 g, 2.21 mmol) as described in procedure above, light yellow very viscous oil, 40 mg (45%). IR (film) *ν*/cm^{-1}: 2943, 2857, 2815, 2765, 1539, 1502, 1466, 1439, 1353, 1244, 1203, 1093, 1038, 851, 782. MS (ES$^+$) *m/z*: 400, 402 (MH$^+$, 55%, 20%); 297, 299 ([M - O(CH$_2$)$_3$N(CH$_3$)$_2$]$^+$, 100%, 37%); 263 ([M - O(CH$_2$)$_3$N(CH$_3$)$_2$ - Cl]H$^+$, 45%). HRMS (ES$^+$): *m/z* calc. for $C_{22}H_{23}NO_2SCl$: 400.1138 (MH$^+$); found: 400.1121. Anal. Calcd for $C_{22}H_{22}ClNO_2S$: C, 66.07; H, 5.54; N, 3.50; S, 8.02. Found: C, 66.42; H. 5.64; N, 3.87; S, 7.99.

3-(5-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)propylamine (10c). This compound was obtained starting from **6c** (70 mg, 0.22 mmol) and 3-chloro-propylammonium chloride (0.29 g, 2.23 mmol) as described in procedure above, light yellow very viscous oil, 9 mg (11%). IR (film) *ν*/cm^{-1}: 3417 (broad, N-H); 2956, 2925, 2854, 1538, 1504, 1468, 1440, 1367, 1261, 1244, 1203, 1092, 1023, 798. ^{1}H NMR (CDCl$_3$) *δ*/ppm: 1.6-1.9 (b, 2H, NH$_2$); 2.02-2.13 (b, 2H, CH$_2$); 3.11-3.23 (b, 2H, CH$_2$N); 3.60-3.71 (b, 2H, OCH$_2$); 4.70, 4.78 (2b, each 1H, Ar-CH$_2$); 7.22-7.39 (m, 5H, arom); 7.49 (s, 1H, thioph.); 7.46-7.60 (m, 2H, arom). ^{13}C NMR (CDCl$_3$) *δ*/ppm: 29.7 (CH$_2$); 38.5 (CH$_2$); 66.6 (CH$_2$); 66.8 (CH$_2$); 121.6 (CH); 122.2 (CH); 122.6 (CH); 125.5 (CH); 128.3 (CH); 128.9 (CH); 129.6 (CH); 130.5 (CH); 127.3 (C); 130.6 (C); 132.1 (C); 135.3 (C); 137.2 (C); 137.6 (C); 155.9 (C); 157.6 (C). MS (ES$^+$) *m/z*: 372, 374 (MH$^+$, 6%, 2%); 297, 299 ([M - O(CH$_2$)$_3$NH$_2$]$^+$, 100%, 38%); 263 ([M - O(CH$_2$)$_3$NH$_2$ - Cl]H$^+$, 45%). HRMS (ES$^+$): *m/z* calc. for $C_{20}H_{19}NO_2SCl$: 372.0825 (MH$^+$); found: 372.0845.

[2-(11-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)ethyl]dimethylamine (11a). This compound was obtained starting from **6d** (55 mg, 0.18 mmol) and (2-chloro-ethyl)dimethylammonium chloride (0.25 g, 1.75 mmol) as described in procedure above, light yellow viscous oil, 20 mg (30%). IR (film) *ν*/cm^{-1}: 3063, 2964, 2860, 2818, 2769, 1538, 1504, 1464, 1455, 1417, 1351, 1261, 1246, 1206, 1035, 801. ^{1}H NMR (CDCl$_3$) *δ*/ppm: 2.80 (s, 6H, 2×CH$_3$); 3.21 (t, *J* = 4.6 Hz, 2H, CH$_2$N); 4.10 (b, 2H, OCH$_2$); 4.77 (s, 2H, Ar-CH$_2$); 7.18-7.24 (m, 1H, arom.); 7.26-7.35 (m, 4H, arom.); 7.48 (s, 1H, thioph.); 7.50-7.52 (m, 1H, arom.); 7.53-7.56 (m, 1H, arom.). ^{13}C NMR (CDCl$_3$) *δ*/ppm: 43.0 (2×CH$_3$); 56.3 (CH2); 64.4 (CH$_2$); 65.6 (CH$_2$); 120.2 (CH); 121.1 (CH); 122.1 (CH); 124.8 (CH); 127.5 (C); 127.7 (CH); 128.1 (C); 128.2 (CH); 128.5 (CH); 128.9 (CH); 129.2 (C); 133.7 (C); 135.3 (C); 137.6 (C); 155.5 (C); 156.0 (C). MS (ES$^+$) *m/z*: 386, 388 (MH$^+$, 100%, 36%); 297, 299 ([M - O(CH$_2$)$_2$N(CH$_3$)$_2$]$^+$, 50%, 19%). HRMS (ES$^+$): *m/z* calc. for $C_{21}H_{21}NO_2SCl$: 386.0982 (MH$^+$); found: 386.0951.

3-(11-Chloro-2-thia-8-oxa-dibenzo[*e*,*h*]azulen-1-ylmethoxy)propylamine (11b). This compound was obtained starting from **6d** (55 mg, 0.18 mmol) and 3-chloro-propylammonium chloride (0.23 g, 1.75 mmol) as described in procedure above, light yellow very viscous oil, 30 mg (46%). ^{1}H NMR (acetone-d_6) δ/ppm: 1.8-1.9 (b, 2H, NH_2); 1.95 (p, J = 6.9 Hz, 2H, CH_2); 3.36 (t, J = 6.9 Hz, 2H, CH_2N); 3.76 (b, 2H, OCH_2); 4.74 (b, 2H, Ar-CH_2); 7.24-7.46 (m, 5H, arom.); 7.64-7.68 (m, 1H, arom.); 7.74-7.80 (m, 1H, arom.); 7.78 (s, 1H, thioph.). MS (ES$^+$) *m/z*: 372, 374 (MH$^+$, 100%, 36%); 297, 299 ([M - $O(CH_2)_3NH_2]^+$, 58%, 19%). Anal. Calcd for $C_{20}H_{18}ClNO_2S$: C, 64.59; H, 4.88; N, 3.77; S, 8.62. Found: C, 64.72; H. 5.09; N, 3.56; S, 8.59.

Biology - isolation of PBMCs, PBMC culture and TNF-α quantification. Peripheral blood mononuclear cells (PBMCs) were isolated from the peripheral blood of healthy volunteers using density gradient centrifugation. Freshly taken, heparinized whole blood was mixed with the same volume of sterile saline. Diluted blood samples were layered over FicollPaqueTM Plus and centrifuged at 400g for 30 min. PBMCs were collected from the interface between the plasma and the density gradient solution. After washing in RPMI 1640, cells were resuspended in RPMI 1640 containing 10% of heat inactivated (56 °C, 30 min) fetal bovine serum (Biowhittaker). PBMCs (3.5×104) were cultured in 200 μL volumes in 96-well cell culture plates with flat bottom at 37 °C in humidified atmosphere containing 5% CO2. Cells were stimulated on TNF-α production with lipopolysaccharide (LPS, serotype 0111:B4, Sigma) at final concentration of 1 ng/mL or left unstimulated (cultured in medium alone). Initially, compounds were dissolved in DMSO as 10 mM stock solutions. Final 10 μM and 3 μM (primary screening) or 10 μM - 0.3 μM range concentrations made in cell culture medium were tested when added together with LPS. Final DMSO volume ratio in all assays did not exceed 0.1% and had no significant inhibitory effect. Standard p38 inhibitor VX745 served as internal TNF-α inhibitor in this test, with average IC50 value ≈ 0.2 μM. Negative and LPS control samples were prepared in sextaplicates while testing compound samples in triplicates. Cell free supernatants were harvested after overnight period and quantified for TNF-α content by enzyme linked immunosorbent assay (ELISA) specific for human TNF-α. To ensure the detection specificity and sensitivity, assay was performed according to manufacturer instructions (R&D) using suggested pair of antibodies specific for human TNF-α. Test sensitivity was 5 pg/mL. TNF-α content in unknown samples was calculated by extrapolation from the standard curve made for recombinant TNF-α in serial dilutions of known start concentration.

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REFERENCES AND NOTES

1. The substitutive nomenclature is used here for these two classes of compounds, but the correct IUPAC defined names are dibenzo[*b*,*f*]thieno[3,4-*d*]thiepines for **III** when X = S and dibenzo[*b*,*f*]thieno[3,4-*d*]oxepines for **III** when X = O.
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