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),† two novel classes of fused heterocyclic compounds, is described. Starting 11H-dibenzo[b,f]thiepin-10-one, 11H-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 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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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-diaryltiophenes. Starting ketones 1a-c 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, 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. 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, 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
omega-(dialkyl)chloroalkylamine hydrochlorides in the presence of benzyltriethylammonium chloride as a catalyst, in toluene / 40% NaOH. Pure aminoethers were isolated by column chromatography as oils and biologically tested as free bases.

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\begin{array}{llll}
1a & S & H & \text{Yield} \% \\
1b & O & H & \\
1c & O & Cl & \\
2a & S & H & 68 \\
2b & O & H & 85 \\
2c & O & Cl & 62 \\
3a & S & H & H & 56 \\
3b & O & H & H & 21 \\
3c & O & H & Cl & n.d. \\
3d & O & Cl & H & n.d. \\
4a & S & H & H & 53 \\
4b & O & H & H & 62 \\
4c & O & H & Cl & n.d. \\
4d & O & Cl & H & n.d. \\
5a & S & H & 26 \\
5b & O & H & 26 \\
5c & O & Cl & n.d. \\
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\% \\
7c & & & & \\
8a & S & H & 2 & Me & 76 \\
8b & S & H & 3 & Me & 74 \\
8c & S & H & 3 & H & 10 \\
9a & O & H & 2 & Me & 77 \\
9b & O & H & 3 & Me & 38 \\
9c & O & H & 3 & H & 77 \\
10a & O & Cl & 2 & Me & 36 \\
10b & O & Cl & 3 & Me & 45 \\
10c & O & Cl & 3 & H & 11 \\
11a & O & Cl & 2 & Me & 30 \\
11b & O & Cl & 3 & H & 46 \\
\end{array}
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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.

Figure 2
Figure 3
All above data converge to 6c having CH$_2$OH 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-dibeno[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.\textsuperscript{14-16}

**Dibenzo[bf]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$_2$SO$_4$), 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., 124-126 °C). IR (KBr) $\nu$/cm$^{-1}$: 1681 (C=O); 1583, 1462, 1434, 1280, 1260, 1220, 1061, 991. $^1$H NMR (DMSO-$d_6$) $\delta$/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-$d_6$) $\delta$/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[bf]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) $\nu$/cm$^{-1}$: 1673 (C=O); 1583, 1462, 1434, 1280, 1260, 1220, 1109, 998, 957, 926. $^1$H NMR (DMSO-$d_6$) $\delta$/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-$d_6$) $\delta$/ppm: 121.8, 125.9, 131.7, 136.1 (C-H arom.); 126.9, 156.5 (C arom.); 184.6 (C=O). MS (Cl) $m$/z: 225 (MH$^+$, 100%); 197 ([M-CO]H$^+$, 95%). Anal. Calcd for C$_{14}$H$_8$O$_3$: C, 75.00; H, 3.60. Found: C, 75.20; H, 3.20.

**2-Chloro-dibenzo[bf]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) $\nu$/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-$d_6$) $\delta$/ppm: 7.41-7.46 (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-$d_6$) $\delta$/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 (Cl) $m$/z: 259, 261 (MH$^+$, 100%, 35%). Anal. Calcd for C$_{14}$H$_7$ClO$_3$: 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
Dibenzob[f]thiepine-10,11-dione (2a) (6.2 g, 0.026 mol) and dimethyl 2,2'-thiodiacetate\textsuperscript{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\textsubscript{2}O (2×100 mL). The combined organic layers were extracted with 2 M NH\textsubscript{4}OH (5×50 mL). Acidification with conc. HCl afforded brown oil, which was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3×50 mL). The solvent was dried (Na\textsubscript{2}SO\textsubscript{4}) and evaporated. Crystallization of the residue from Et\textsubscript{2}O/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\textsuperscript{-1}: 3400-2200 (broad, COOH); 3057, 2949, 2869; 1733 (C=O); 1687 (C=O); 1656, 1546, 1526, 1442, 1427, 1298, 1244, 1197. \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) δ/ppm: 3.74 (s, 3H, CH\textsubscript{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). \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}) δ/ppm: 52.7 (CH\textsubscript{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); 136.66 (C); 136.78 (C); 141.5 (C); 145.9 (C); 161.5 (C=O); 162.4 (C=O). MS (ES\textsuperscript{+}) m/z: 369 (MH\textsuperscript{+}, 75%); 351 ([M-OH]\textsuperscript{+}, 20%); 337 ([M-OCH\textsubscript{3}]\textsuperscript{+}, 100%). Anal. Calcd for C\textsubscript{19}H\textsubscript{12}O\textsubscript{4}S\textsubscript{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\textsubscript{2}Cl\textsubscript{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\textsuperscript{-1}: 3500-2200 (broad, COOH); 3061, 2952; 1740 (C=O); 1693 (C=O); 1605, 1573, 1532, 1445, 1427, 1370, 1306, 1266, 1250, 1210. \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}) δ/ppm: 3.81 (s, 3H, CH\textsubscript{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). \textsuperscript{13}C NMR (DMSO-d\textsubscript{6}) δ/ppm: 52.6 (CH\textsubscript{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\textsuperscript{+}) m/z: 353 (MH\textsuperscript{+}, 50%); 335 ([M-OH]\textsuperscript{+}, 100%). MS (ES\textsuperscript{-}) m/z: 307 ([M-CO\textsubscript{2}H\textsubscript{3}]\textsuperscript{-}, 100%); 249 ([M-CO\textsubscript{2}H-CO\textsubscript{2}CH\textsubscript{3}]\textsuperscript{-}, 90%). Anal. Calcd for C\textsubscript{19}H\textsubscript{12}O\textsubscript{5}: 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]azulene-1,3-dicarboxylate (3c) and 3-methyl 5-chloro-2-thia-8-oxa-dibenzo[e,h]azulene-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); 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); 161.1 (C=O); 161.9 (C=O). MS (ES\(^-\)) m/z: 385, 387 (M-H, 5%, 2%); 341, 343 ([M-CO\(_2\)\(_2\)H]-, 100%, 37%). Anal. Calcd for C\(_{19}\)H\(_{11}\)ClO\(_5\)S: 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\(_2\)\(_2\)H]-, 100%, 35%). Anal. Calcd for C\(_{19}\)H\(_{11}\)ClO\(_5\)S: 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.). \(^{13}\)C NMR (DMSO-\(d_6\)) δ/ppm: 125.0 (CH); 128.4 (CH); 129.2 (CH); 130.0 (CH); 132.5 (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: 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\(_3\)); 7.32-7.37 (m, 1H, arom.); 7.40-7.46 (m, 1H, arom.); 7.60-7.65 (m, 2H, arom.). \(^{13}\)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 (Cl) 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.
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. ¹H NMR (DMSO-d₆) 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.). ¹³C NMR (DMSO-d₆) 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 C₁₈H₁₂O₂S₂: C, 66.64; H, 3.73; S, 19.77. Found: C, 66.25; H, 3.46; S, 19.45.

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. ¹H NMR (DMSO-d₆) δ/ppm: 3.80 (s, 3H, CH₃); 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.). ¹³C NMR (DMSO-d₆) δ/ppm: 52.1 (CH₃); 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-OCH₃]⁺, 100%). Anal. Calcd for C₁₈H₁₆O₃S: C, 70.77; H, 4.03; S, 12.81. Found: C, 70.32; H, 3.82; S, 10.12.

Methyl 5-chloro-2-thia-8-oxa-dibenzo[e,h]azulene-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. ¹H NMR (DMSO-d₆) 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.). ¹³C NMR (DMSO-d₆) δ/ppm: 121.5, 123.5, 124.3, 125.3, 126.1, 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 C₁₆H₉ClO₃S: 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. ¹H NMR (DMSO-d₆) δ/ppm: 3.82 (s, 3H, CH₃); 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$_6$) $\delta$/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$_3$S: 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$_2$O/hexane, 0.25 g; mp 185-188 °C. IR (KBr) $\nu$/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$_6$) $\delta$/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$_6$) $\delta$/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); 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$_3$S: 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$_2$O (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$_2$O and Et$_2$O. The resulting inorganic precipitate was filtered off and washed several times with portions of EtO. 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) $\nu$/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$_6$) $\delta$/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, 4H, arom.); 7.67-7.70 (m, 1H, arom.); 7.69 (s, 1H, thioph.). $^{13}$C NMR (DMSO-d$_6$) $\delta$/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) $\nu$/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$_6$) $\delta$/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$_6$) $\delta$/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 (Cl) m/z: 279 (M-H, 100%). Anal. Calcd for C_{17}H_{12}O_{2}S: 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 4e (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. ¹H NMR (acetone-d₆) δ/ppm: 4.93 (bs, 2H, CH₂); 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.). ¹³C NMR (acetone-d₆) δ/ppm: 58.8 (CH₂); 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 (Cl) m/z: 314, 316 (M⁺, 100%, 35%). Anal. Calcd for C_{17}H_{11}ClO₂S: 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. ¹H NMR (acetone-d₆) δ/ppm: 4.93 (bs, 2H, CH₂); 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.). ¹³C NMR (acetone-d₆) δ/ppm: 58.4 (CH₂); 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 (Cl) m/z: 314, 316 (M⁺, 75%, 26%), 298, 300 ([M-OH]H⁺, 100%, 37%). Anal. Calcd for C_{17}H_{11}ClO₂S: 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) POCl₃ (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 CH₂Cl₂ (3×25 mL). The combined CH₂Cl₂ extracts were washed with std. aq. NaHCO₃ and std. NaCl, dried (Na₂SO₄) 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. ¹H NMR (DMSO-d₆) 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-$d_6$) $\delta$/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$_2$S: 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) $\nu$/cm$^{-1}$: 3094, 2925, 2849; 1653 (C=O); 1522, 1421, 1382, 1348, 1287, 1241, 1181, 1097, 1027, 760, 737. $^1$H NMR (CDCl$_3$) $\delta$/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$_3$) $\delta$/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: 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$_2$Cl$_2$/MeOH/NH$_4$OH 90:10:1.5). Then it was cooled to rt, diluted with water and product extracted with CH$_2$Cl$_2$ (3×40 mL). The combined organic extracts were washed with brine, dried (Na$_2$SO$_4$) and solvent evaporated. Column chromatography (CH$_2$Cl$_2$/MeOH/NH$_4$OH 100:5:1.5) provided pure 8a as light yellow very viscous oil, 1.20 g (76%). IR (film) $\nu$/cm$^{-1}$: 3053, 2934, 2856, 1487, 1456, 1348, 1157, 1103, 1036, 953, 759. $^1$H NMR (CDCl$_3$) $\delta$/ppm: 2.35 (s, 6H, 2×CH$_3$); 2.61-2.67 (m, 2H, CH$_2$N); 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$_3$) $\delta$/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$_2$)$_2$N(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$_{22}$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\(_2\)N); 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\(_2\)\(_3\))N(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\(_2\)\(_3\))NH\(_2\)]\(^+\), 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\(_2\)N); 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\(_2\)\(_3\))NH\(_2\)]\(^+\), 85%). HRMS (ES\(^+\)): m/z calc. for C\(_{21}\)H\(_{21}\)NO\(_2\)S: 352.1371 (MH\(^+\)); found: 352.1378. Anal. Calcd for C\(_{21}\)H\(_{21}\)NO\(_2\)S: 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. ¹H NMR (CDCl₃) δ/ppm: 2.02-2.11 (m, 2H, CH₂); 2.56 (s, 6H, 2×CH₃); 2.83 (bt, 2H, CH₂N); 4.74, 4.77 (bt, each 1H, Ar-CH₂); 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.); IR (film) ν/cm⁻¹: 2941, 2857, 2815, 2765, 1573, 1539, 1504, 1469, 1443, 1354, 1243, 1205, 1101, 1037, 789, 748. ¹H NMR (CDCl₃) δ/ppm: 2.02-2.11 (m, 2H, CH₂); 2.56 (s, 6H, 2×CH₃); 2.83 (bt, 2H, CH₂N); 4.74, 4.77 (bt, each 1H, Ar-CH₂); 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.). ¹³C NMR (CDCl₃) δ/ppm: 26.2 (CH₂); 44.0 (2×CH₃); 66.6 (CH₂); 67.7 (CH₂); 121.3 (CH); 121.4 (CH); 121.7 (CH); 125.2 (CH); 125.5 (CH); 128.5 (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); 158.0 (C). MS (ES⁺) m/z: 366 (MH⁺, 100%); 263 ([M - O(CH₂)₃N(CH₃)₂]⁺, 90%). Anal. Calcd for C₂₂H₂₃NO₂S: 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. ¹H NMR (CDCl₃) δ/ppm: 2.03-2.11 (b, 2H, CH₂); 3.03-3.11 (b, 2H, CH₂N); 3.59-3.68 (b, 2H, OCH₂); 4.70, 4.79 (2b, each 1H, Ar-CH₂); 5.0-5.5 (b, 2H, NH₂); 7.11-7.33 (m, 6H, arom.); 7.40 (s, 1H, thioph.); 7.44-7.51 (m, 1H, arom.). ¹³C NMR (CDCl₃) δ/ppm: 28.9 (CH₂); 38.2 (CH₂); 66.3 (CH₂); 67.6 (CH₂); 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); MS (ES⁺) m/z: 338 (MH⁺, 50%); 263 ([M - O(CH₂)₃NH₂]⁺, 100%). HRMS (ES⁺): m/z calc. for C₂₀H₁₉NO₂S: 338.1215 (MH⁺); found: 338.1239. Anal. Calcd for C₂₀H₁₉NO₂S: 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. ¹H NMR (CDCl₃) δ/ppm: 2.63 (s, 6H, 2×CH₃); 2.99 (bt, 2H, CH₂N); 4.79, 4.84 (2b, each 1H, Ar-CH₂); 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.). ¹³C NMR (CDCl₃) δ/ppm: 44.7 (2×CH₃); 57.8 (CH₂); 66.5 (CH₂); 66.8 (CH₂); 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); 137.3 (C); 137.4 (C); 156.1 (C); 157.8 (C). MS (ES⁺) m/z: 386, 388 (MH⁺, 37%, 13%); 297, 299 ([M - O(CH₂)₂N(CH₃)₂ - Cl]H⁺, 70%). HRMS (ES⁺): m/z calc. for C₂₁H₂₁NO₂SCl: 386.0982.
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(CH₂)₃N(CH₃)₂]⁺, 100%, 37%); 263 ([M - O(CH₂)₃N(CH₃)₂ · Cl]H⁺, 45%). HRMS (ES⁺): m/z calc. for C₂₂H₂₃NO₂SCl: 400.1138 (MH⁺); found: 400.1121. Anal. Calcd for C₂₂H₂₂ClNO₂S: 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. ¹H NMR (CDCl₃) δ/ppm: 1.6-1.9 (b, 2H, NH₂); 2.02-2.13 (b, 2H, CH₂); 3.11-3.23 (b, 2H, CH₂N); 3.60-3.71 (b, 2H, OCH₂); 4.70, 4.78 (2b, each 1H, Ar-CH₂); 7.22-7.39 (m, 5H, arom); 7.49 (s, 1H, thioph.); 7.46-7.60 (m, 2H, arom). ¹³C NMR (CDCl₃) δ/ppm: 29.7 (CH₂); 38.5 (CH₂); 64.4 (CH₂); 65.6 (CH₂); 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: 372, 374 (MH⁺, 6%, 2%); 297, 299 ([M - O(CH₂)₃NH₂]⁺, 100%, 38%); 263 ([M - O(CH₂)₃NH₂ - Cl]H⁺, 45%). HRMS (ES⁺): m/z calc. for C₂₀H₁₉NO₂SCl: 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. ¹H NMR (CDCl₃) δ/ppm: 2.80 (s, 6H, 2×CH₃); 3.21 (t, J = 4.6 Hz, 2H, CH₂N); 4.10 (b, 2H, OCH₂); 4.77 (s, 2H, Ar-CH₂); 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.). ¹³C NMR (CDCl₃) δ/ppm: 43.0 (2×CH₃); 56.3 (CH₂); 64.4 (CH₂); 65.6 (CH₂); 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(CH₂)₃N(CH₃)₂]⁺, 50%, 19%). HRMS (ES⁺): m/z calc. for C₂₁H₂₁NO₂SCl: 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%). 

**1H NMR** (acetone-
\[\text{d}_6\]) \(\delta/\text{ppm}\): 1.8-1.9 (b, 2H, NH\(_2\)); 1.95 (p, \(J = 6.9 \text{ Hz}, 2\text{H}, \text{CH}_2\)); 3.36 (t, \(J = 6.9 \text{ Hz}, 2\text{H}, \text{CH}_2\text{N}\)); 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\(_2\)\(_3\)NH\(_2\)]\(^+\), 58%, 19%). Anal. Calcd for C\(_{20}\)H\(_{18}\)ClNO\(_2\)S: 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-\(\alpha\) 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 (3.5×10\(^4\)) were cultured in 200 \(\mu\)L volumes in 96-well cell culture plates with flat bottom at 37 °C in humidified atmosphere containing 5% CO\(_2\). Cells were stimulated on TNF-\(\alpha\) 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 \(\mu\)M and 3 \(\mu\)M (primary screening) or 10 \(\mu\)M - 0.3 \(\mu\)M 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-\(\alpha\) inhibitor in this test, with average IC\(50\) value \(\approx 0.2\) \(\mu\)M. 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-\(\alpha\) content by enzyme linked immunosorbert assay (ELISA) specific for human TNF-\(\alpha\). 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-\(\alpha\). Test sensitivity was 5 pg/mL. TNF-\(\alpha\) content in unknown samples was calculated by extrapolation from the standard curve made for recombinant TNF-\(\alpha\) 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[bf]thieno[3,4-d]thiepines for III when X = S and dibenzo[bf]thieno[3,4-d]oxepines for III when X = O.
