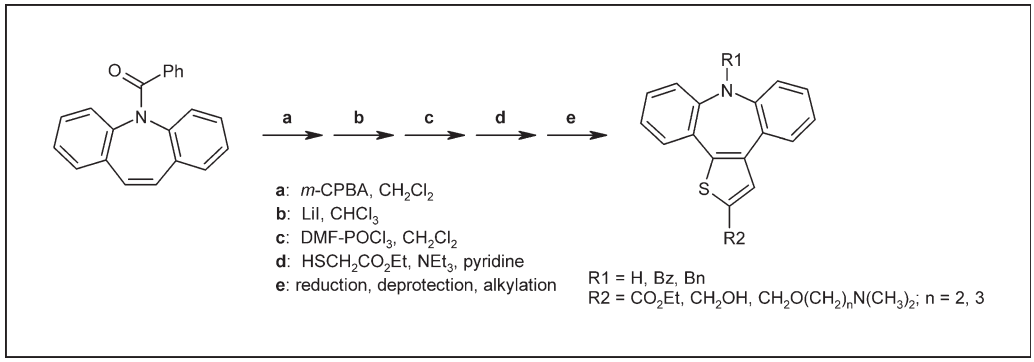
Ivana Ozimec Landek,* Dijana Pešić, Mladen Merćep [1], Barbara Stanić [1], and Milan Mesić

GlaxoSmithKline Research Centre Zagreb Limited, Prilaz baruna Filipovića 29, 10000 Zagreb, Croatia
*E-mail: ivana.o.ozimec-landek@gsk.com
Received April 21, 2010
DOI 10.1002/jhet.605
Published online 15 April 2011 in Wiley Online Library (wileyonlinelibrary.com).



Synthesis of a novel class of fused heterotetracyclic compounds, 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulenes (**VII**), is described. Starting *N*-benzoyl-protected 5*H*-dibenzo[*b*,*f*]azepine (**XI**, PG = Bz) was oxidized to 5-benzoyl-10,11-epoxy-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepine (**2**), which subsequently rearranged in Lewis acid-induced epoxide ring opening to give 5-benzoyl-5,11-dihydro-10*H*-dibenzo[*b*,*f*]azepin-10-one (**3**). Vilsmeier reaction of **3** provided β-chlorovinyl aldehyde **4** that readily cyclized with ethyl 2-mercaptoacetate to form dibenzazepino[4,5]-fused thiophene structure **5**. Further transformation of substituent at C-2 position of **5** and *N*-deprotection led to final aminoalkoxy derivatives **9**. All compounds with tetracyclic skeleton were tested *in vitro* for their anti-inflammatory activity.

J. Heterocyclic Chem., **48**, 856 (2011).

INTRODUCTION

Dibenzo[*b*,*f*]azepine framework is found within a number of medicinally important compounds, particularly those acting at central nervous system (for example, see refs. 2–4). Antidepressants imipramine (**Ia**, Y = H) and its 3-chloro analog clomipramine (**Ia**, Y = Cl), as well as antiepileptics carbamazepine (**Ib**) and oxcarbazepine (**Ic**) are examples of compounds bearing such tricyclic moiety that are well known as widely prescribed drugs (Fig. 1). Furthermore, there is a growing pool of evidence that tricyclic antidepressants-like imipramine and its derivatives also possess pronounced anti-inflammatory and analgesic properties (for example, see refs. 5–13).

On the other hand, some members of a series of 5-substituted 2,3-diaryl-thiophenes (**II**) were also reported to have significant anti-inflammatory activity through strong inhibition of necrosis factor alpha (TNF-α) production [14].

In our continuing efforts aimed toward synthesis and determination of anti-inflammatory activity of various heterocyclic dibenzo[*e*,*h*]azulenes, we recently reported on the synthesis, properties, structure determination, and preliminary biological results of dibenzo[*e*,*h*]azulenes characterized by furan **III** [15], pyrrole **IV** [16], thiophene **V** [17], and imidazole ring **VI** [18] annulated to the central oxepine or thiepine ring (Fig. 1). Preliminary data revealed the ability of these polycyclic systems to inhibit TNF-α production *in vitro*. Herein, we wish to report on the continuation of our project with the study of 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulenes [19], a series that embodies central azepine and [2,3]-fused thiophene ring **VII** (Fig. 1) and may be perceived as a combination of structural subunits **I** and **II**. Target molecules, both 8-*N*-substituted and 8-*N*-unsubstituted, bearing an alkoxy chain of variable length and a basic moiety at position C-2 are expected to possess significant anti-TNF-α activity [20] and desirable pharmacokinetic properties.

RESULTS AND DISCUSSION

Chemistry. The tetracyclic structure **VII** is formed by annulation of the thiophene ring onto the existing tricyclic dibenzo[*b*,*f*]azepine moiety, as is shown by the retrosynthetic sequence in Scheme 1. To enable this, the

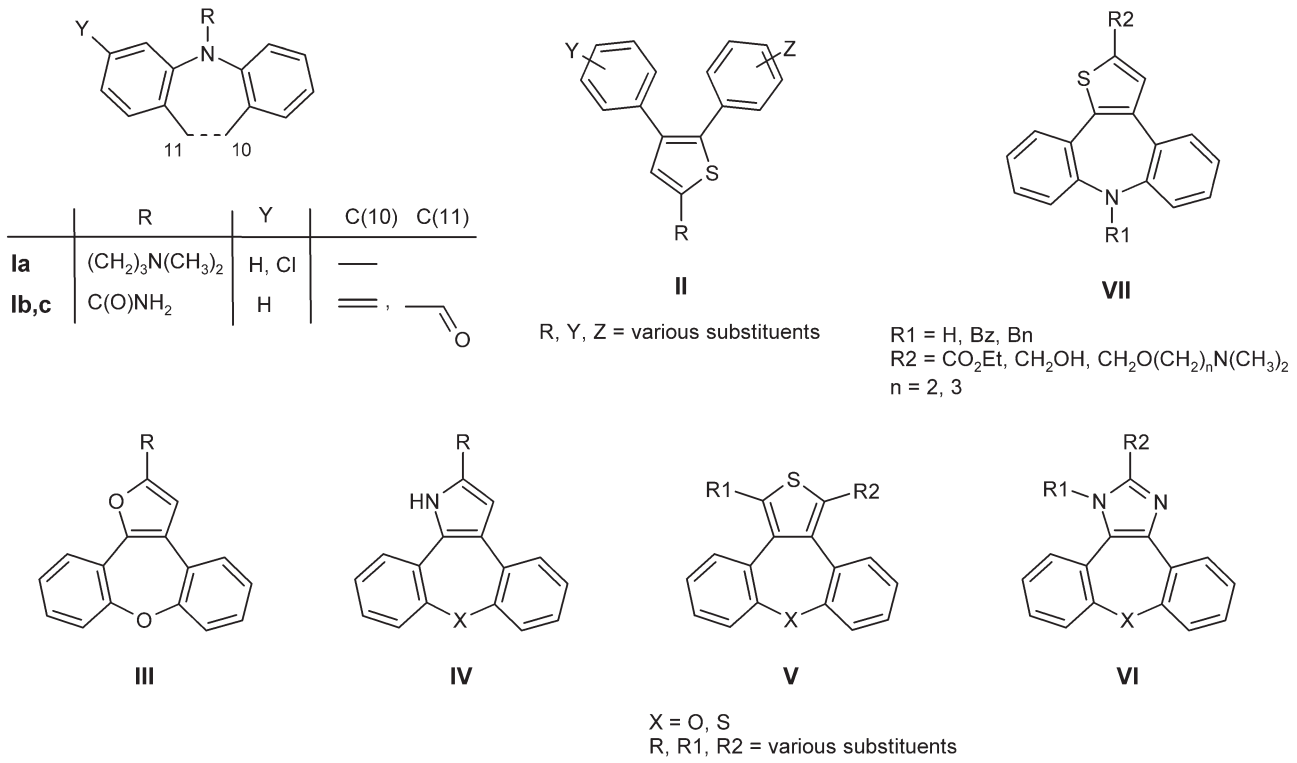
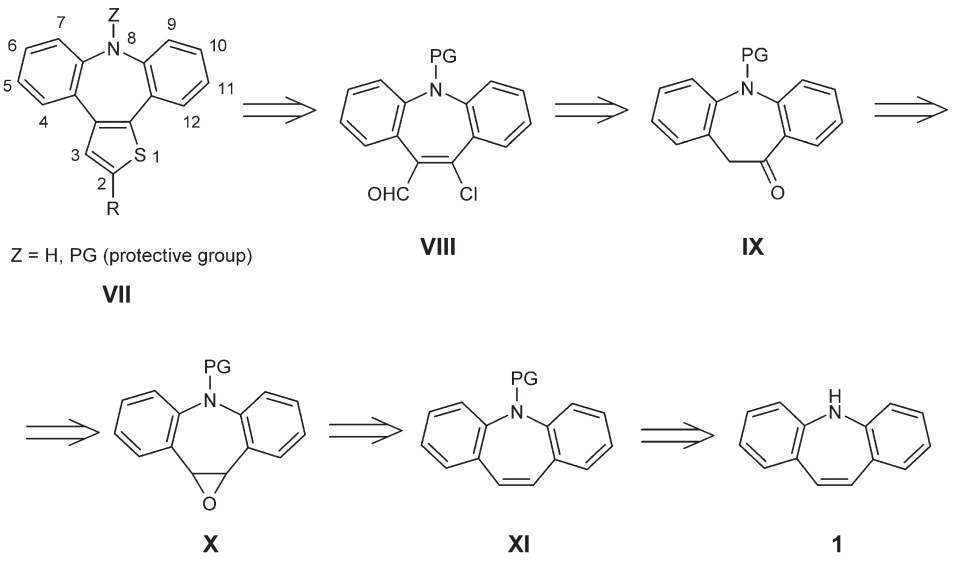


Figure 1. Structural combination into 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulenes and structures of known heterocyclic dibenzo[*e*,*h*]azulenes.

double bond of the commercially available 5*H*-dibenzo[*b*,*f*]azepine (iminostilbene, **1**) is converted in four steps into the β-chlorovinyl aldehyde functionality, a useful three carbon synthon for the efficient regiospecific annulation by the Fiesselmann reaction. According to the designed synthetic route that comprises peracid oxidation of the double bond and Vilsmeier reaction, selection of the appropriate protective group (PG) for iminostilbene nitrogen was of a key importance to assure reactivity of the intermediates and chemoselectivity in following steps, as is discussed below.

It is reported that chemical properties of the epoxy ring in **X** are dependent on the nature of the substituents at the nitrogen atom. Thus, when iminostilbene was

Scheme 1



reacted with organic peracids, 9-acridinecarbaldehyde and its *N*-oxide were obtained instead of 5*H*-dibenzo[*b*,*f*]-azepine-10,11-oxide (**X**, PG = H) [21]. Additionally, oxidation of the 10,11-double bond of *N*-alkyl- and *N*-benzyl-dibenzo[*b*,*f*]azepines presumably provides 10,11-oxides, but the oxirane ring is labile enough to facilitate further conversion of the compound to diphenylamine and acridone derivatives [22,23]. On the other hand, oxidation of *N*-acyl-dibenzo[*b*,*f*]azepines with *m*-chloroperbenzoic acid afforded the corresponding *N*-acyl-dibenzo[*b*,*f*]azepine-10,11-oxides (**X**, PG = acyl) that were stable [22,23]. Moreover, considering reactivity of *N*-acyl- and *N*-alkyl-dibenzo[*b*,*f*]azepines toward electrophiles, it is found that *N*-alkyl and *N*-benzyl derivatives readily react by introducing the substituent, including a formyl group by means of the Vilsmeier reaction, into the aromatic ring to form derivatives with *para*-substitution pattern to nitrogen [24]. On the contrary, *N*-acylated derivatives show different behavior toward electrophiles and from the observed deactivation of the aromatic rings by *N*-acylation, it is expected that electrophilic attack should occur preferentially at the 10(11)-position, i.e., at the ethano bridge [25,26]. Obviously, an acyl-type PG would assure desirable level of reactivity in oxidation and correct directing in Vilsmeier reaction. However, Vilsmeier reagent also reacts with an amide group adjacent to a potential C-nucleophile, as is the case with alkylacyl groups like, e.g., acetyl [27,28]. Hence, benzoyl group turned the best choice as PG since all anticipated issues regarding chemoselectivity during deprotection step were solved and it appeared as suitable for synthesis of both *N*-substituted and *N*-unsubstituted 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulenes.

Synthetic route to 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulene scaffold is depicted in Scheme 2. Benzoylation of the starting iminostilbene (**1**) and subsequent peracid oxidation of the double bond to provide 10,11-dihydro-10,11-epoxy-derivative **2** proceeded smoothly as previously described [22,25]. Lithium iodide induced ring opening and rearrangement [29] of oxirane **2** resulted in highly selective formation of isomeric 10-keto derivative **3** (67%).

As is shown by ^{1}H-NMR, methylene protons at C-11 in compound **3** are nonequivalent and form an AX spin system, which gives rise to a pair of doublets at $\delta = 3.96$ ppm and $\delta = 4.81$ ppm with $J = 15.0$ Hz. Most likely it is the effect of a hindered rotation about the *N*-acyl bond, which decreases the rate of ring inversion [30] thus keeping the central seven-membered ring in a conformation of a distorted symmetry with the two geminal protons subjected to different magnetic environments.

To enable annulation of thiophene ring in the succeeding step, tricyclic ketone **3** that contains active methylene group was reacted with *in situ* formed Vilsmeier reagent to give a key intermediate, β-chlorovinyl aldeyde **4** (58%). Reaction of **4** with ethyl 2-mercaptoacetate finally afforded novel heterotetracyclic, 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulene structure **5** (61%). Ester group at C-2 allows further transformation toward more functionalized final molecules. We were primarily interested in 8-*N*-unsubstituted derivatives with ω-aminoalkyl ether chains at the C-2 position (like **9c**), but 8-*N*-substituted compounds (like **9a** and **9b**) would be equally attractive targets considering potential difference in anti-TNF-α activity. Our initial attempts to reduce **5** with 1–2 equiv. of lithium aluminum hydride (LAH) in diethyl ether at room temperature led to complex mixture where **6**, with both ester and amide group reduced, was the main product, and **7**, with intact *N*-benzoyl group, isolated only in low yield and with insufficient purity. However, the use of 4 equiv. of LAH under the same conditions gave **6** almost exclusively. Nevertheless, in our hands, cleavage of *N*-benzyl bond in **6** failed under catalytic transfer hydrogenolysis [31], with ceric ammonium nitrate [32] and with hydrobromic acid [33]. With a large excess (60–100 equiv.) of sodium borohydride in methanol at the reflux temperature, we succeeded in selective reduction of ester bond obtaining **7** (69%), which on basic hydrolysis easily provided 8-*N*-deprotected **8** (69%). To avoid such a large excess of sodium borohydride, we also tried the reaction with more reactive lithium borohydride [34] in diethyl ether but besides **7**, a number of side products were obtained.

Finally, alcohols **6–8** were converted to their ω-(dimethyl)aminoalkyl ethers **9a–c** by a phase transfer-catalyzed alkylation with the appropriate ω-(dimethyl)-chloroalkylamine hydrochlorides in the presence of benzyl triethyl ammonium chloride as a catalyst [35]. Pure ω-aminoalkyl ethers were isolated by column chromatography as resins and biologically tested as free bases.

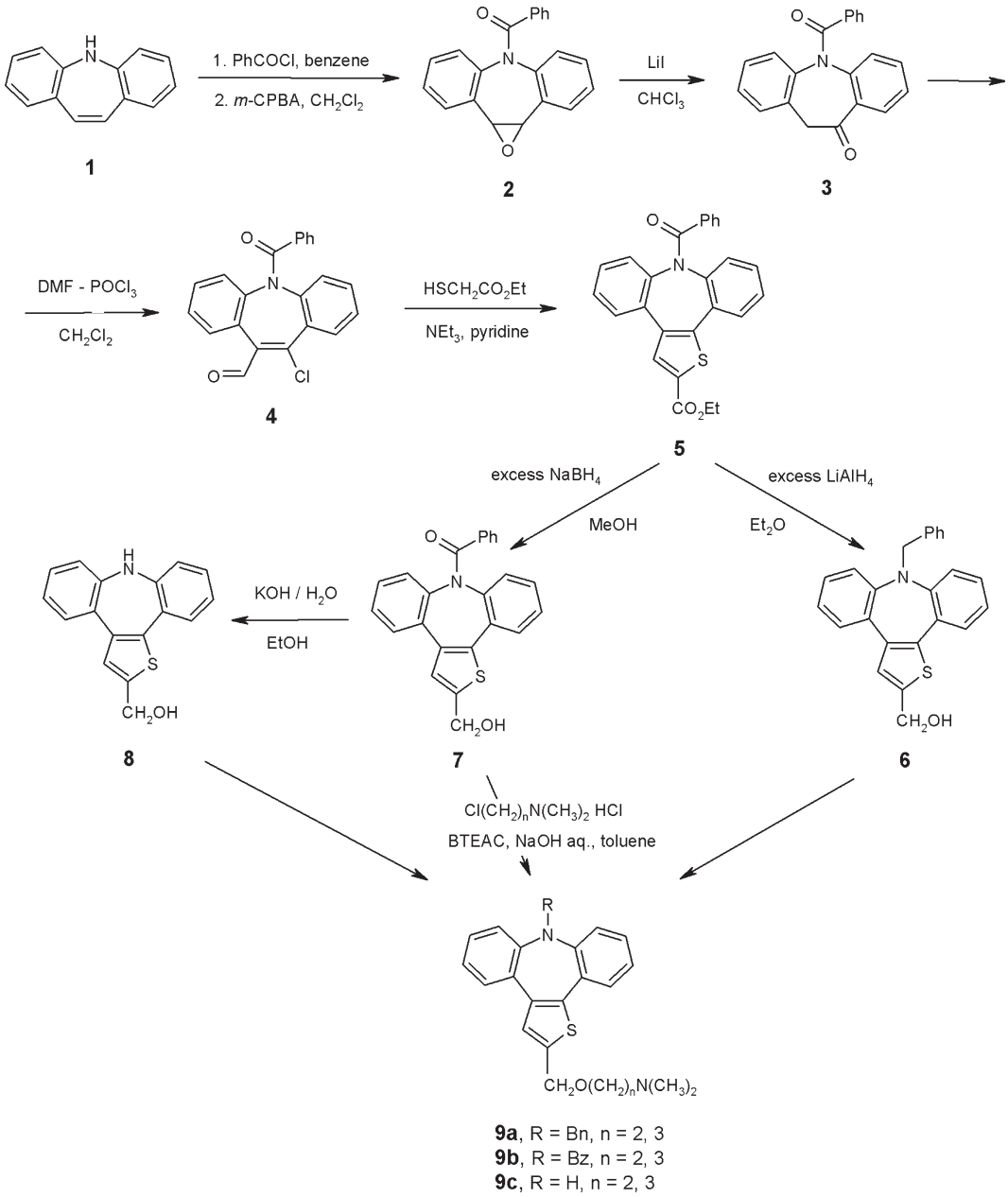
Anti-inflammatory activity. Novel tetracyclic compounds were tested for their ability to inhibit TNF-α secretion in lipopolysaccharide (LPS)-activated human peripheral blood mononuclear cells (hPBMC) assay [36,37]. Compounds belonging to series **9a–c**, possessing aminoalkoxy chain at C-2 position, showed potency to inhibit TNF-α production *in vitro* in low micromolar range with IC_{50} values for the most potent compounds in the range of 1–3 μM.

In conclusion, 8*H*-1-thia-8-aza-dibenzo[*e*,*h*]azulenes (**VII**), particularly derivatives with aminoalkoxy chain linked at thiophene ring, were recognized as a novel class of fused heterocyclic compounds showing anti-inflammatory activity through inhibition of TNF-α secretion.

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

Scheme 2



determined using Büchi Melting Point B-545 apparatus and are uncorrected. IR spectra were recorded on Nicolet Magna IR 760 FTIR spectrophotometer. NMR spectra were recorded at room temperature on Bruker Avance DPX 300 spectrometer at 300 MHz using tetramethylsilane as internal standard. Purity of the compounds was estimated by high pressure liquid chromatography (HPLC)-MS system Waters 2690 + Micromass Quattro Micro, by HPLC-UV system Waters 2690 + Waters 996 Photodiode Array Detector, or by GC-MS system Varian Chrompack Saturn 2000. High resolution mass spectrometry (HRMS) data were acquired using Q-TOF 2 Waters system. Preparative HPLC separations were done using Waters Mass Directed AutoPurification System. Microanalyses were performed using Perkin-Elmer 2400 CHNS 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).

5-Benzoyl-5H-dibenzo[b,f]azepine (XI, PG = Bz). Prepared according to refs. 22 and 25 from commercially available 5*H*-dibenzo[*b*,*f*]azepine (iminostilbene, **1**); crystallized from *n*-hexane; pale yellow crystals; 96%; mp 131–133°C; IR (potassium bromide): 3055, 3025, 1660 (C=O), 1619, 1598, 1567, 1489, 1460, 1436, 1343, 1304, 1271, 1130, 1117 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 7.20–7.56 ppm (m, 15H, arom. +

HC=CH); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 127.3 (CH), 127.4 (CH), 127.7 (CH), 128.5 (CH), 128.9 (CH), 129.4 (CH), 133.7 (C), 135.4 (C), 168.8 ppm (C=O); MS: *m/z* 298 (MH^+, 100%); HRMS: *m/z* calcd for $C_{21}H_{16}NO$: 298.1232 (MH^+), found: 298.1232.

5-Benzoyl-10,11-dihydro-10,11-epoxy-5H-dibenzo[b,f]-azepine (2). Prepared according to ref. 22 from **XI** (PG = Bz); recrystallized from ethyl acetate; pale yellow crystals; 55%; mp 188–190°C; IR (potassium bromide): 3064, 1661 (C=O), 1583, 1493, 1446, 1344, 1305 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 4.58 (s, 2H, 2 × CH epox.), 7.00–7.70 ppm (m, 13H, arom.); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 57.4 (CH epox.), 127.4 (CH), 127.6 (CH), 127.7 (CH), 129.2 (CH), 129.3 (CH), 129.7 (CH), 130.1 (C), 130.9 (CH), 136.0 (C), 168.0 ppm (C=O); MS: *m/z* 314 (MH^+, 100%); HRMS *m/z* calcd for $C_{21}H_{16}NO_2$: 314.1181 (MH^+), found: 314.1192.

5-Benzoyl-5,11-dihydro-10H-dibenzo[b,f]azepin-10-one (3). Lithium iodide (1.00 g, 0.0075 mol) was added to the solution of **2** (2.22 g, 0.0071 mol) in chloroform (30 mL). The reaction mixture was heated at reflux temperature for 1 h and then allowed to cool to room temperature. The mixture was washed with aq. sodium hydrogen sulfite (25 mL) and water (30 mL). The organic phase was dried (sodium sulfate) and solvent was evaporated. The crude product was purified by column chromatography (silica gel, eluent: dichloromethane/ethyl acetate 10:0.5) to give 1.50 g (67%) of **3** in the form of pale yellow crystals; mp 185–186.5°C; IR (potassium bromide): 3068, 3050, 1674 (C=O), 1657 (C=O), 1596, 1580, 1567, 1489, 1474, 1447, 1416, 1329, 1284, 1255, 1235, 1158, 1114, 1073, 1025 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 3.96 (d, 1H from CH_2, J = 15.0 Hz), 4.81 (d, 1H from CH_2, J = 15.0 Hz), 7.22–7.55 (m, 12H, arom.), 7.97 ppm (d, 1H, J = 7.6 Hz, arom.); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 48.9 (CH_2), 127.6 (CH), 127.9 (CH), 128.1 (CH), 128.3 (CH), 128.5 (CH), 128.7 (CH), 129.4 (CH), 129.8 (CH), 129.9 (CH), 130.2 (CH), 134.0 (CH), 129.3 (C), 132.4 (C), 135.4 (C), 142.1 (C), 144.0 (C), 168.6 (C=O amide), 192.4 ppm (C=O ketone); MS: *m/z* 314 (MH^+, 100%); HRMS *m/z* calcd for $C_{21}H_{16}NO_2$: 314.1181 (MH^+), found: 314.1189. Anal Calcd for $C_{21}H_{15}NO_2$: C, 80.49; H, 4.82; N, 4.47. Found: C, 80.56; H, 4.80; N, 4.27.

5-Benzoyl-11-chloro-5H-dibenzo[b,f]azepine-10-carbaldehyde (4). To *N,N*-dimethylformamide (0.82 mL, 10.6 mmol) previously cooled to 0°C, phosphorus(III) oxychloride (0.74 mL, 8.0 mmol) was added dropwise with continuous stirring and in an inert atmosphere, while maintaining the reaction temperature below 10°C. Dichloromethane (2.5 mL) was added and the resulting mixture was stirred at room temperature for 2 h. Then solution of **3** (1.66 g, 5.3 mmol) in dichloromethane (12 mL) was added dropwise and reaction mixture was stirred at room temperature for 2 days, when by TLC checking (eluent: dichloromethane/ethyl acetate 10:0.5), the reaction progress was not further noticeable. Crushed ice was added and the mixture was stirred until ice had melted. Then solid sodium acetate (3 g) was added and the mixture was stirred for additional 5 min. The organic phase was separated and the aqueous portion was extracted with dichloromethane (2 × 35 mL). The combined organic extracts were washed with sat. aq. sodium hydrogen carbonate and brine, dried (sodium sulfate), and solvent evaporated. The crude product was purified by column chromatography (silica gel, eluent: dichloromethane/ethyl acetate 10:0.5) to give 1.10 g (58 %) of **4** as pale yellow powder. The analytical sample is recrystallized from hexane/diethyl ether 5:1; mp 138.5–139.5°C; IR (potassium bromide): 3055, 2872, 1666 (C=O), 1596, 1580, 1544, 1495, 1480, 1453, 1441, 1346, 1313, 1284, 1132, 1069 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 7.20–7.80 (m, 12H, arom.), 7.93 (bd, 1H, J = 7.5 Hz, arom.), 10.65 ppm (s, 1H, CHO); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 126.8 (CH), 127.5 (CH), 127.9 (CH), 128.1 (CH), 129.4 (CH), 129.9 (CH), 130.2 (CH), 130.7 (CH), 132.7 (CH), 134.5 (2 × C), 167.9 (C=O amide), 190.8 ppm (CHO); MS: *m/z* 360, 362 (MH^+, 100%, 40%); HRMS: *m/z* calcd for $C_{22}H_{15}ClNO_2$: 360.0791 (MH^+), found: 360.0801. Anal Calcd for $C_{22}H_{14}ClNO_2$: C, 73.44; H, 3.92; N, 3.89. Found: C, 73.65; H, 4.02; N, 3.84.

Ethyl 8-benzoyl-8H-1-thia-8-aza-dibenzo[e,h]azulene-2-carboxylate (5). To the solution of **4** (0.87 g, 2.4 mmol) in pyridine (6 mL), ethyl 2-mercaptoacetate (0.76 g, 4.6 mmol) and triethylamine (2 mL) were added. The reaction mixture was stirred at 70°C for 1 h and then at reflux temperature for additional 2 h. Solvents were removed under reduced pressure and the residue was partitioned between water and ethyl acetate. The aqueous phase was extracted with ethyl acetate (2 × 25 mL). The combined organic extracts were washed with dil. HCl (5%) and water, boiled with decolorizing charcoal for 5 min, filtered and filtrate dried (sodium sulfate), and solvent evaporated. Recrystallization of thus obtained crude product from ethyl acetate/hexane 1:2 gave 0.62 g (61%) of **5** as colorless powder; mp 165–167.5°C; IR (potassium bromide): 1712 (C=O), 1667 (C=O), 1600, 1495, 1480, 1468, 1446, 1424, 1383, 1329, 1290, 1257, 1206, 1076 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 1.37 (t, 3H, CH_3), 4.40 (q, 2H, CH_2), 7.06–7.10 (m, 2H, arom.), 7.18–7.30 (m, 4H, arom.), 7.30–7.85 (m, 7H, arom.), 8.33 ppm (s, 1H, thioph.); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 14.1 (CH_3), 61.4 (CH_2), 127.3 (CH), 127.8 (CH), 128.2 (CH), 128.5 (CH), 128.6 (CH), 128.8 (CH), 128.9 (CH), 129.2 (CH), 129.7 (CH), 130.4 (CH), 133.9 (CH), 132.2 (C), 135.0 (C), 161.0 (C=O), 168.6 ppm (C=O); MS: *m/z* 426 (MH^+, 100%); HRMS: *m/z* calcd for $C_{26}H_{20}NO_3S$: 426.1164 (MH^+), found: 426.1174. Anal Calcd for $C_{26}H_{19}NO_3S$: C, 73.39; H, 4.50; N, 3.29; S, 7.54. Found: C, 72.93; H, 4.54; N, 3.58; S, 7.81.

(8-Benzyl-8H-1-thia-8-aza-dibenzo[e,h]azulen-2-yl)methanol (6). To the suspension of LAH (0.78 g, 20.0 mmol) in diethyl ether (50 mL), ester **5** (2.16 g, 5.1 mmol) was added in small portions. The mixture was stirred at room temperature for 2 h, when TLC (eluent: dichloromethane/ethyl acetate 10:0.5) showed total conversion. The excess of LAH was destroyed by dropwise addition of water/diethyl ether 1:1. The inorganic precipitate was filtered off and washed few times with ether. The filtrate was boiled with decolorizing charcoal for 5 min, filtered and filtrate dried (sodium sulfate), and solvent evaporated. The crude product was purified by column chromatography (eluent as for TLC) providing 1.25 g (67%) of **6** as yellowish foam; mp 83–86°C; IR (potassium bromide): 3600–3100 (broad, OH), 3060, 2925, 1489, 1452, 1370, 1326, 1237, 1135, 1028, 1008 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 4.78 (d, 2H, J = 5.7 Hz, CH_2OH), 5.00, 5.06 (2 irregular doublets, each 1H, benzylic CH_2, J = 14.9 Hz), 5.69 (t, 1H, J = 5.7 Hz, OH), 7.04–7.42 ppm (m, 14H, arom. + thioph.); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 54.1 (CH_2), 58.7 (CH_2), 121.1 (CH), 121.2 (CH), 124.2 (2 × CH), 125.1 (CH), 126.8 (CH),

127.8 (2 × CH), 127.9 (CH), 128.2 (CH), 128.4 (CH), 128.9 (CH), 130.2 (C), 131.7 (C), 137.6 (C), 138.0 (C), 138.2 (C), 145.8 (C), 149.9 (C), 150.2 ppm (C); MS: *m/z* 370 (MH^+, 100%); HRMS: *m/z* calcd for $C_{24}H_{20}NOS$: 370.1266 (MH^+), found: 370.1286. Anal Calcd for $C_{24}H_{19}NOS$: C, 78.02; H, 5.18; N, 3.79; S, 8.68. Found: C, 77.48; H, 5.30; N, 4.05; S, 8.55.

(8-Benzoyl-8H-1-thia-8-aza-dibenzo[e,h]azulen-2-yl)methanol (7). To the solution of **5** (0.56 g, 1.3 mmol) in methanol (30 mL), sodium borohydride (4.50 g, 0.12 mol) was added portionwise. The reaction mixture was heated at reflux temperature for 2 h. When TLC (eluent: dichloromethane/ethyl acetate 5:1) showed total conversion, water (50 mL) was added and product extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were dried (sodium sulfate) and solvent evaporated. The crude product was purified by column chromatography (eluent as for TLC) to give 0.40 g (69%) of **7** as colorless foam; mp 106–108°C; IR (potassium bromide): 3600–3100 (broad, OH), 3058, 2922, 1651 (C=O), 1598, 1574, 1494, 1476, 1446, 1343, 1133, 1027 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 4.82 (d, 2H, J = 5.7 Hz, CH_2), 5.79 (t, 1H, J = 5.7 Hz, OH), 7.07–7.70 ppm (m, 14H, arom. + thioph.); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 58.7 (CH_2), 127.5 (CH), 128.0 (CH), 128.2 (CH), 128.55 (CH), 128.58 (CH), 128.7 (CH), 129.0 (CH), 129.1 (CH), 129.9 (CH), 135.4 (C), 147.3 (C), 168.7 ppm (C=O); MS: *m/z* 384 (MH^+, 100%); HRMS: *m/z* calcd for $C_{24}H_{18}NO_2S$: 384.1058 (MH^+), found: 384.1062. Anal Calcd for $C_{24}H_{17}NO_2S$: C, 75.17; H, 4.47; N, 3.65; S, 8.36. Found: C, 74.88; H, 4.60; N, 3.89; S, 8.09.

(8H-1-Thia-8-aza-dibenzo[e,h]azulen-2-yl)methanol (8). To the solution of **7** (0.18 g, 0.46 mmol) in ethanol (2 mL), potassium hydroxide (1.0 g, 17.8 mmol) and water (1 mL) were added. The reaction mixture was heated at reflux temperature for 2 h, during which the product was separated at the flask wall. When the reaction completed, solvent was evaporated, water (10 mL) was added, and product was extracted with dichloromethane (2 × 10 mL). The combined organic extracts were dried (sodium sulfate) and solvent evaporated. Thus, obtained crude product was purified by column chromatography (eluent: dichloromethane/ethyl acetate 5:1) to give 90 mg (69%) of **8** in the form of yellow crystals; mp 181–183°C; IR (potassium bromide): 3303, 3252 (OH and NH stretching), 3053, 2941, 1579, 1473, 1426, 1296, 1236, 1157, 1135, 1016 cm^{-1}; ^{1}H-NMR (dimethyl sulfoxide d_6): δ 4.65 (d, 2H, J = 5.7 Hz, CH_2), 5.58 (t, 1H, J = 5.7 Hz, OH), 6.90–6.99 (m, 4H, arom.), 7.08 (s, 1H, thioph.), 7.12–7.21 (m, 3H, arom.), 7.25–7.28 (m, 1H, arom.), 7.31 ppm (s, 1H, NH); ^{13}C-NMR (dimethyl sulfoxide d_6): δ 58.6 (CH_2), 120.5 (CH), 120.7 (CH), 123.0 (CH), 123.1 (CH), 125.7 (CH), 128.2 (CH), 128.3 (CH), 128.7 (CH), 129.3 (CH), 126.7 (C), 137.7 (C), 146.1 (C), 149.4 (C), 149.9 ppm (C); MS: *m/z* 280 (MH^+, 100%); HRMS: *m/z* calcd for $C_{17}H_{14}NOS$: 280.0796 (MH^+), found: 280.0792. Anal Calcd for $C_{17}H_{13}NOS$: C, 73.09; H, 4.69; N, 5.01; S, 11.48. Found: C, 72.66; H, 4.71; N, 5.40; S, 11.34.

General procedure for the synthesis of ω-aminoalkyl ethers 9. To the solution of ω-chloroalkyl dialkyl ammonium chloride (5–14 equiv.) in 40% aq. NaOH (12 mL), benzyl triethyl ammonium chloride (0.45 equiv.) and solution of the alcohol **6–8** (1 equiv.) in toluene (10 mL) were added. The reaction mixture was heated under vigorous stirring and refluxing for 3 h (TLC control, eluent: dichloromethane/methanol/ammonia 90:10:1.5). Then, it was cooled to room temperature, diluted with water, and product was extracted with dichloromethane (2 × 25 mL). The combined organic extracts were washed with brine, dried (sodium sulfate), and solvent evaporated. Column chromatography (eluent: dichloromethane/methanol/ammonia) provided pure **9**.

[2-(8-Benzyl-8H-1-thia-8-aza-dibenzo[e,h]azulen-2-ylmethoxy)-ethyl]dimethylamine (9a, n = 2). This compound was obtained starting from **6** (0.20 g, 0.54 mmol) and 2-chloroethyl dimethyl ammonium chloride (1.10 g, 7.6 mmol) as described in procedure above; purified by column chromatography (eluent: dichloromethane/methanol/ammonia 91:8:1.5); yellow resin; 0.15 g (63%); IR (film): 3061, 3029, 2936, 2854, 2817, 2769, 1597, 1488, 1453, 1369, 1327, 1238, 1135, 1104 cm^{-1}; ^{1}H-NMR (deuteriochloroform): δ 2.42 (s, 6H, 2 × CH_3), 2.73 (t, 2H, J = 5.5 Hz, CH_2N), 3.77 (t, 2H, J = 5.5 Hz, OCH_2), 4.80 (s, 2H, Ar-CH_2), 4.96, 5.02 (2 irregular doublets, each 1H, benzylic CH_2, J = 14.6 Hz), 6.96–7.15 (m, 5H, arom.), 7.17–7.25 (m, 7H, arom.), 7.34–7.39 ppm (m, 2H, arom.); ^{13}C-NMR (deuteriochloroform): δ 45.1 (2 × CH_3), 54.7 (CH_2), 58.2 (CH_2), 67.0 (CH_2), 67.7 (CH_2), 120.3 (CH), 120.5 (CH), 123.6 (CH), 123.7 (CH), 126.4 (CH), 127.3 (CH), 127.5 (2 × CH), 127.7 (CH), 127.80 (2 × CH), 127.84 (CH), 128.35 (CH), 128.43 (CH), 130.4 (C), 131.8 (C), 137.5 (C), 138.0 (C), 139.2 (C), 139.7 (C), 149.8 (C), 150.1 ppm (C); MS: *m/z* 441 (MH^+, 55%), 352 ($[M\text{-}O(CH_2)_2N(CH_3)_2]^+$, 100%); HRMS: *m/z* calcd for $C_{28}H_{29}N_2OS$: 441.2001 (MH^+), found: 441.2000. Anal Calcd for $C_{28}H_{28}N_2OS$: C, 76.33; H, 6.41; N, 6.36; S, 7.28. Found: C, 76.24; H, 6.58; N, 6.89; S, 6.95.

[3-(8-Benzyl-8H-1-thia-8-aza-dibenzo[e,h]azulen-2-ylmethoxy)-propyl]dimethyl-amine (9a, n = 3). This compound was obtained starting from **6** (0.20 g, 0.54 mmol) and 3-chloropropyl dimethyl ammonium chloride (1.20 g, 7.6 mmol) as described in procedure above; purified by column chromatography (eluent: dichloromethane/methanol/ammonia 91:8:1.5); yellow resin; 0.15 g (61%); IR (film): 3061, 3029, 2941, 2855, 2814, 2764, 1597, 1488, 1453, 1372, 1326, 1238, 1210, 1135, 1096 cm^{-1}; ^{1}H-NMR (deuteriochloroform): δ 1.88–1.98 (m, 2H, $CH_2CH_2CH_2$), 2.35 (s, 6H, 2 × CH_3), 2.56 (t, 2H, J = 7.5 Hz, CH_2N), 3.68 (t, 2H, J = 6.4 Hz, OCH_2), 4.76 (s, 2H, Ar-CH_2), 4.96, 5.02 (2 irregular doublets, each 1H, benzylic CH_2, J = 14.6 Hz), 6.96–7.26 (m, 12H, arom.), 7.35–7.40 ppm (m, 2H, arom.); ^{13}C-NMR (deuteriochloroform): δ 27.1 (CH_2), 44.7 (2 × CH_3), 54.8 (CH_2), 56.2 (CH_2), 67.5 (CH_2), 68.0 (CH_2), 120.3 (CH), 120.5 (CH), 123.6 (CH), 123.7 (CH), 126.4 (CH), 126.9 (CH), 127.5 (2 × CH), 127.7 (CH), 127.8 (3 × CH), 128.35 (CH), 128.38 (CH), 130.4 (C), 131.9 (C), 137.5 (C), 137.9 (C), 139.5 (C), 139.6 (C), 149.8 (C), 150.1 ppm (C); MS: *m/z* 455 (MH^+, 50%), 352 ($[M\text{-}O(CH_2)_3N(CH_3)_2]^+$, 100%); HRMS: *m/z* calcd for $C_{29}H_{31}N_2OS$: 455.2157 (MH^+), found: 455.2163. Anal Calcd for $C_{29}H_{30}N_2OS$: C, 76.61; H, 6.65; N, 6.16; S, 7.05. Found: C, 76.69; H, 6.69; N, 6.46; S, 7.57.

[2-(8-Benzoyl-8H-1-thia-8-aza-dibenzo[e,h]azulen-2-ylmethoxy)-ethyl]dimethylamine (9b, n = 2). This compound was obtained starting from **7** (75 mg, 0.20 mmol) and 2-chloroethyl dimethyl ammonium chloride (0.14 g, 1.0 mmol) as described in procedure above; reaction duration of about 2 h; purified by column chromatography (eluent: dichloromethane/methanol/ammonia 100:5:1); separated from *N*-debenzoyl-derivative **9c**, n = 2, by preparative HPLC; yellow resin; 20 mg (23%); IR

(film): 3058, 2926, 2855, 1657 (C=O), 1494, 1474, 1344, 1130, 1100 cm^{-1}; ^{1}H-NMR (deuteriochloroform): δ 2.52 (s, 6H, 2 × CH_3), 2.85 (t, 2H, J = 5.3 Hz, CH_2N), 3.85 (t, 2H, J = 5.3 Hz, OCH_2), 4.83 (s, 2H, Ar-CH_2), 7.09–7.65 ppm (m, 14H, arom. + thioph.); ^{13}C-NMR (deuteriochloroform): δ 44.8 (2 × CH_3), 57.9 (CH_2), 66.7 (CH_2), 67.6 (CH_2), 127.4 (CH), 127.6 (CH), 127.7 (CH), 127.8 (CH), 127.9 (CH), 128.2 (CH), 128.4 (CH), 128.6 (CH), 128.7 (CH), 129.4 (CH), 134.9 (C), 140.3 (C), 169.3 ppm (C=O); MS: *m/z* 455 (MH^+, 100%), 366 ($[M\text{-}O(CH_2)_2N(CH_3)_2]^+$, 10%); HRMS: *m/z* calcd for $C_{28}H_{27}N_2O_2S$: 455.1793 (MH^+), found: 455.1807. Anal Calcd for $C_{28}H_{26}N_2O_2S$: C, 73.98; H, 5.76; N, 6.16; S, 7.05. Found: C, 74.32; H, 5.95; N, 6.56; S, 7.17.

[3-(8-Benzoyl-8H-1-thia-8-aza-dibenzo[e,h]azulen-2-ylmethoxy)-propyl]dimethylamine (9b, n = 3). This compound was obtained starting from **7** (75 mg, 0.20 mmol) and 3-chloropropyl dimethyl ammonium chloride (0.15 g, 1.0 mmol) as described in procedure above; reaction duration of about 2 h; purified by column chromatography (eluent: dichloromethane/methanol/ammonia 100:5:1); separated from *N*-debenzoyl-derivative **9c**, n = 3, by preparative HPLC; yellow resin; 25 mg (27%); IR (film): 3059, 2941, 2858, 2816, 2767, 1659 (C=O), 1494, 1475, 1342, 1157, 1133, 1096, 844 cm^{-1}; ^{1}H-NMR (deuteriochloroform): δ 1.82–2.00 (m, 2H, $CH_2CH_2CH_2$), 2.33 (s, 6H, 2 × CH_3), 2.50–2.55 (m, 2H, CH_2N), 3.68 (t, 2H, J = 6.4 Hz, OCH_2), 4.78 (s, 2H, Ar-CH_2), 7.13–7.60 ppm (m, 14H, arom. + thioph.); ^{13}C-NMR (deuteriochloroform): δ 27.2 (CH_2), 44.8 (2 × CH_3), 56.1 (CH_2), 67.4 (CH_2), 68.3 (CH_2), 127.4 (CH), 127.5 (CH), 127.7 (CH), 127.8 (CH), 127.9 (CH), 128.1 (CH), 128.4 (CH), 128.6 (CH), 129.4 (CH), 134.9 (C), 141.1 (C), 169.3 ppm (C=O); MS: *m/z* 469 (MH^+, 100%), 366 ($[M\text{-}O(CH_2)_3N(CH_3)_2]^+$, 20%); HRMS: *m/z* calcd for $C_{29}H_{29}N_2O_2S$: 469.1950 (MH^+), found: 469.1963. Anal Calcd for $C_{29}H_{28}N_2O_2S$: C, 74.33; H, 6.02; N, 5.98; S, 6.84. Found: C, 74.02; H, 6.42; N, 6.36; S, 7.15.

Dimethyl[2-(8H-1-thia-8-aza-dibenzo[e,h]azulen-2-ylmethoxy)-ethyl]amine (9c, n = 2). This compound was obtained starting from **8** (50 mg, 0.18 mmol) and 2-chloroethyl dimethyl ammonium chloride (0.13 g, 0.90 mmol) as described in procedure above; purified by column chromatography (eluent: dichloromethane/methanol/ammonia 100:5:1); yellow resin; 50 mg (80%); IR (film): 3260 (N—H stretching), 3055, 2938, 2858, 1580, 1472, 1428, 1301, 1248, 1158, 1128, 1101 cm^{-1}; ^{1}H-NMR (deuteriochloroform): δ 2.47 (s, 6H, 2 × CH_3), 2.77 (t, 2H, J = 5.5 Hz, CH_2N), 3.76 (t, 2H, J = 5.5 Hz, OCH_2), 4.71 (s, 2H, Ar-CH_2), 5.27 (s, 1H, NH), 6.79–6.83 (m, 2H, arom.), 6.95–7.05 (m, 2H, arom.), 7.10 (s, 1H, thioph.), 7.13–7.19 (m, 2H, arom.), 7.30–7.35 ppm (m, 2H, arom.); ^{13}C-NMR (deuteriochloroform): δ 44.2 (2 × CH_3), 57.6 (CH_2), 66.0 (CH_2), 67.1 (CH_2), 119.9 (CH), 120.0 (CH), 122.9 (CH), 123.0 (CH), 127.84 (CH), 127.88 (CH), 128.1 (CH), 128.2 (CH), 128.9 (CH), 127.1 (C), 128.0 (C), 138.2 (C), 139.4 (C), 147.5 (C), 148.4 (C), 148.9 ppm (C); MS: *m/z* 351 (MH^+, 100%), 262 ($[M\text{-}O(CH_2)_2N(CH_3)_2]^+$, 97%); HRMS: *m/z* calcd for $C_{21}H_{23}N_2OS$: 351.1531 (MH^+), found: 351.1538. Anal Calcd for $C_{21}H_{22}N_2OS$: C, 71.97; H, 6.33; N, 7.99; S, 9.15. Found: C, 72.25; H, 6.69; N, 7.46; S, 9.17.

Dimethyl-[3-(8H-1-thia-8-aza-dibenzo[e,h]azulen-2-ylmethoxy)-propyl]amine (9c, n = 3). This compound was obtained starting from **8** (50 mg, 0.18 mmol) and 3-chloropropyl dimethyl ammonium chloride (0.24 g, 1.51 mmol) as described in procedure above; purified by column chromatography (eluent: dichloromethane/methanol/ammonia 100:5:1); yellow resin; 50 mg (77%); IR (film): 3215 (N—H stretching), 3056, 2943, 2859, 1579, 1472, 1429, 1374, 1301, 1250, 1158, 1128, 1092 cm^{-1}; ^{1}H-NMR (deuteriochloroform): 1.94–2.03 (m, 2H, $CH_2CH_2CH_2$), 2.49 (s, 6H, 2 × CH_3), 2.70–2.74 (m, 2H, CH_2N), 3.63 (t, 2H, J = 6.1 Hz, OCH_2), 4.68 (s, 2H, Ar-CH_2), 5.24 (s, 1H, NH), 6.79–6.82 (m, 2H, arom.), 6.96–7.06 (m, 2H, arom.), 7.09 (s, 1H, thioph.), 7.13–7.20 (m, 2H, arom.), 7.31–7.35 ppm (m, 2H, arom.); ^{13}C-NMR (deuteriochloroform): δ 26.6 (CH_2), 43.8 (2 × CH_3), 55.9 (CH_2), 66.8 (CH_2), 67.4 (CH_2), 119.7 (CH), 119.9 (CH), 122.7 (CH), 122.8 (CH), 127.4 (CH), 127.7 (CH), 127.9 (CH), 128.1 (CH), 128.7 (CH), 125.3 (C), 126.6 (C), 137.6 (C), 140.1 (C), 147.8 (C), 148.5 (C), 149.1 ppm (C); MS: *m/z* 365 (MH^+, 100%), 262 ($[M\text{-}O(CH_2)_3N(CH_3)_2]^+$, 85%); HRMS: *m/z* calcd for $C_{22}H_{25}N_2OS$: 365.1688 (MH^+), found: 365.1685. Anal Calcd for $C_{22}H_{24}N_2OS$: C, 72.49; H, 6.64; N, 7.69; S, 8.80. Found: C, 72.69; H, 6.28; N, 7.46; S, 9.14.

Biology—isolation of PBMCs, PBMC culture, and TNF-α quantification. 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 FicollPaque™ Plus and centrifuged at 400 × *g* 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 μL 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-α production with 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 10–0.3 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 IC_{50} 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 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.

Acknowledgments. The authors express their gratitude to Mr. Željko Osman and Mr. Genadij Razdorov for the mass spectroscopy support, Mrs. Biserka Metelko for the NMR spectroscopy, and Mrs. Štefica Flegar for the IR spectra and elemental analysis data.

REFERENCES AND NOTES

[1] Present address: Children's Hospital Srebrnjak, Srebrnjak 100, 10000 Zagreb, Croatia.

[2] Mula, M.; Schmitz, B.; Sander, J. W. Expert Opin Pharmacother 2008, 9, 3159.

[3] Onali, P.; Dedoni, S.; Olianas, M. C. J Pharmacol Exp Ther 2010, 332, 255.

[4] Carril, M.; SanMartin, R.; Dominguez, E. Curr Org Chem 2007, 11, 1385.

[5] Chavant, F.; Deguil, J.; Pain, S.; Ingrand, I.; Milin, S.; Fauconneau, B.; Pérault-Pochat, M.-C.; Lafay-Chebassier, C. J Pharmacol Exp Ther 2010, 332, 505.

[6] Hwang, J.; Zheng, L. T.; Ock, J.; Lee, M. G.; Kim, S.-H.; Lee, H.-W.; Lee, W.-H.; Park, H.-C.; Suk, K. Neuropharmacology 2008, 55, 826.

[7] Perrot, S.; Javier, R.-M.; Marty, M.; Le Jeunne, C.; Laroche, F. Rheumatology 2008, 47, 1117.

[8] Hashioka, S.; McGeer, P. L.; Monji, A.; Kanba, S. Cent Nerv Syst Agents Med Chem 2009, 9, 12.

[9] Bianchi, M.; Sacerdote, P.; Panerai, A. E. Pharmacol Biochem Behav 1994, 48, 1037.

[10] Abdel-Salam, O. M. E.; Nofal, S. M.; El-Shenawy, S. M. Pharmacol Res 2003, 48, 157.

[11] Sacerdote, P.; Bianchi, M.; Panerai, A. E. Eur J Pharmacol 1997, 319, 287.

[12] Butler, S. H.; Weil-Fugazza, J.; Godefroy, F.; Besson, J.-M. Pain 1985, 23, 159.

[13] Gupta, A. K.; Singh, H.; Singh, G.; Ahluwalia, K. S. Indian J Pharmacol 1978, 10, 35.

[14] Tsuji, K.; Nakamura, K.; Ogino, T.; Konishi, N.; Tojo, T.; Ochi, T.; Seki, N.; Matsuo, M. Chem Pharm Bull 1998, 46, 279.

[15] Pešić, D.; Ozimec Landek, I.; Merćep, M.; Mesić, M. J Heterocycl Chem 2006, 43, 749.

[16] Pešić, D.; Ozimec Landek, I.; Čikoš, A.; Metelko, B.; Gabelica, V.; Stanić, B.; Merćep, M.; Mesić, M. J Heterocycl Chem 2007, 44, 1129.

[17] Ozimec Landek, I.; Pešić, D.; Novak, P.; Stanić, B.; Nujić, K.; Merćep, M.; Mesić, M. Heterocycles 2009, 78, 2489.

[18] Rupčić, R.; Modrić, M.; Hutinec, A.; Čikoš, A.; Stanić, B.; Mesić, M.; Pešić, D.; Merćep, M. J Heterocycl Chem 2010, 47, 640.

[19] The substitutive nomenclature is used here for this class of compounds but the correct IUPAC defined name is 8*H*-dibenzo[*b*,*f*]-thieno[2,3-d]azepine.

[20] Merćep, M.; Mesić, M.; Pešić, D.; Županović, Ž.; Hrvačić, B. US Patent 2,003,153,750, 2003; Chem Abstr 2008, 148, 472014.

[21] Kawashima, K.; Ishiguro, T. Chem Pharm Bull 1978, 26, 951.

[22] Ohta, T.; Miyata, N.; Hirobe, M. Chem Pharm Bull 1981, 29, 1221.

[23] Ohta, T.; Miyata, N.; Hirobe, M. Chem Pharm Bull 1984, 32, 3857.

[24] Kricka, L. J.; Ledwith, A. Chem Rev 1974, 74, 101.

[25] Kricka, L. J.; Lambert, M. H.; Ledwith, A. J Chem Soc Perkin Trans 1 1974, 52.

[26] Kricka, L. J.; Ledwith, A. J Chem Soc Perkin Trans 1 1973, 859.

[27] Marson, C. M. Tetrahedron 1992, 48, 3659.

[28] Paul, S.; Gupta, M.; Gupta, R. Synlett 2000, 1115.

[29] Haász, F.; Galamb, V. J Chem Res (S) 1993, 494.

[30] Abraham, R. J.; Kricka, L. J.; Ledwith, A. J Chem Soc Perkin Trans 2 1974, 1648.

[31] Bajwa, J. S.; Slade, J.; Repiè, O. Tetrahedron Lett 2000, 41, 6025.

[32] Bull, S. D.; Davies, S. G.; Fenton, G.; Mulvaney, A. W.; Shyam Prasad, R.; Smith, A. D. J Chem Soc Perkin Trans 1 2000, 3765.

[33] Schindler, W.; Blattner, H. Swiss Pat. 405,319, 1966; Chem Abstr 1966, 65, 90602.

[34] Brown, H. C.; Krishnamurthy, S. Tetrahedron 1979, 35, 567.

[35] Menozzi, G.; Mosti, L.; Fossa, P.; Mattioli, F.; Ghia, M. J Heterocycl Chem 1997, 34, 963.

[36] Corral, L. G.; Haslett, A. J.; Muller, G. W.; Chen, R.; Wong, L. M.; Ocampo, C. J.; Paterson, R. T.; Stirling, D. I.; Kaplan, G. J Immunol 1999, 163, 380.

[37] Muller, G. W.; Corral, L. G.; Shire, M. G.; Wang, H.; Moreira, A.; Kaplan, G.; Stirling, D. I. J Med Chem 1996, 39, 3238.