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Synthesis and antimicrobial activity of novel bis-azaphenothiazines

Khushbu Kushwaha · Rajeev Sakhuja · Subhash C. Jain

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Abstract *N*-alkylation of azaphenothiazines using dibromoalkanes or dibromoalkenes did not result in the formation of bis-azaphenothiazines under known conditions such as refluxing, for more than 100 h, with $NaNH_2$/xylene or NaH/toluene. However, when the same reaction was tried with NaH/DMF at −5 °C to r.t., it yielded the desired product in 68–71 % yield. These novel bis-azaphenothiazines containing a suitable alkyl or alkenyl spacer were found to possess moderate to significant antimicrobial activities against three gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, and *Staphylococcus epidermis*), four gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, and *Klebsiella pneumoniae*) as well as four fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Candida albicans*). Compounds **2b**–**d** were found to exhibit strong antifungal activity, comparable to the *standard drug miconazole against A. niger and A. fumigatus.*

Keywords Azaphenothiazines · 10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazines · Alkyl/alkenyl linker · Bis-azaphenothiazines · Antimicrobial activity

Introduction

Azaphenothiazines have been known to possess numerous biological activities such as antidepressant (Clark *et al.*, 1965), antitumor (Krystian *et al.*, 2010), antiemetic (Gloria, 1968; Lenke *et al.*, 1971), antitussive (Gloria, 1968), analgesic (Lenke *et al.*, 1971), neuropharmacologic (Atkinson *et al.*, 1971), ganglioplegic (Giuliano *et al.*, 1965), and antihypertensive (Beschke and Schuler, 1964). Besides these, some 1-azaphenothiazine derivatives and their mesoionic compounds also showed antimicrobial activity (Swati *et al.*, 1996) against *Aspergillus flavus*, *Aspergillus niger*, *Candida lunata*, *Escherichia coli*, and *Staphylococcus aureus*. Despite these activities, work on various other derivatives of azaphenothiazines did not take off because of the problems associated with their synthesis as well as alkylation. It required harsh reaction conditions which normally lead to the formation of side products and hence poor yield of the final product. The above biological potential of azaphenothiazines prompted us to synthesize novel bis-azaphenothiazines as number of reports, in the literature, indicated the enhancement of biological activity of bis-heterocycles containing a suitable spacer over simple heterocyclic compounds (Brana *et al.*, 1997; Hernandez *et al.*, 1995; Nitiss *et al.*, 1998; Chaires *et al.*, 1997; Vennerstrom *et al.*, 1998; Yang *et al.*, 2011; May *et al.*, 2003). Since no report as yet is available on the bis-azaphenothiazines, we, therefore, took up their synthesis in order to understand the combined biological effects of the azaphenothiazines moieties containing a suitable alkyl or alkenyl spacer. We have now developed a facile and elegant synthesis of novel bis-azaphenothiazines and evaluate their minimum inhibitory concentration (MIC) and zone of inhibition for antimicrobial activity against various strains of bacteria and fungi, and the details are presented here.

Results and discussion

Chemistry

The synthesis of bis-azaphenothiazines was done by introducing an alkyl or alkenyl spacer between two

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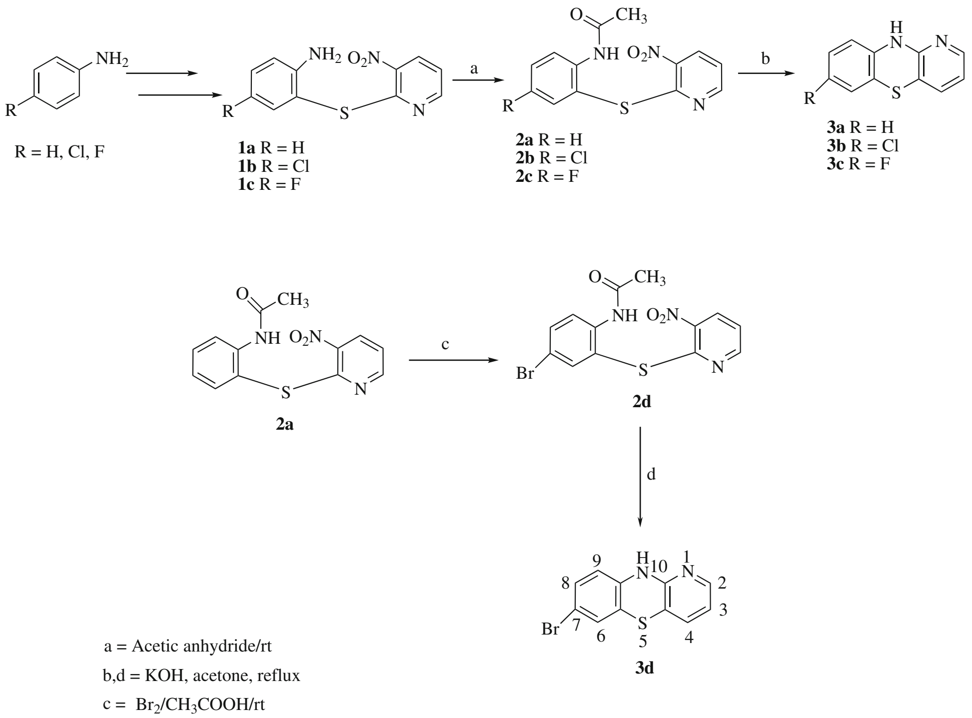
azaphenothiazine moieties using *N*-alkylation or *N*-alkenylation reaction. Earlier we have reported the synthesis of 7-substituted azaphenothiazines (Gupta *et al.*, 2007) and have now adopted an improved methodology for the synthesis of 7-bromo-10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazine (**3d**) (Scheme 1), starting from the previously synthesized intermediate **2a**. Bromination of **2a** gave exclusively **2d**, suggesting that the bromination had only occurred at para position in **2a**. Compound **2d** in KOH/acetone undergoes Smiles rearrangement (Yale and Sowinski, 1958) involving simultaneously inversion, cyclization, and deacetylation to give **3d** characterized as 7-bromo-10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazine on the basis of spectral studies.

Compound **2d** was purified as yellow colored solid in 70 % yield. Its IR spectrum showed absorption band at 1,688 cm^{-1} for acetyl carbonyl and characteristic absorption bands at 1,557 and 1,435 cm^{-1} for the presence of nitro group in the compound. The presence of acetyl group was also confirmed by a singlet at δ 2.09 for three protons in the ^{1}H NMR spectrum. This was further confirmed by the presence of peak at δ 168.9 for the carbonyl carbon and at δ 22.9 for the methyl group attached to the carbonyl carbon. A broad singlet at δ 7.96 (D_2O exchangeable) confirmed the presence of NH group. Multiplet at δ 8.56–8.53 integrating for two protons and doublet at δ 8.39 integrating for one proton were assigned to the protons of the pyridyl nucleus. The bromination that had occured in the phenyl ring was confirmed by the presence of three aromatic protons of the phenyl ring that appeared as singlet, doublet, and multiplet at δ 7.67, 7.65, and 7.32–7.30 each integrating for one proton. The compound showed (M^++2) peak at *m*/*z* 369 and M^+ peak at *m*/*z* 367 corresponding to molecular formula $C_{13}H_{10}N_3O_3SBr$ indicating that the monobromination has taken place. Thus compound **2d** was identified as 2-acetylamino-5-bromophenyl-(3-nitro-2-pyridyl)sulfide. The next step of the methodology was the inversion, cyclization, and deacetylation of **2d** in KOH-acetone via Smiles rearrangement to obtain **3d**. The structure of **3d** was assigned on the basis of detailed spectral studies. Its ^{1}H NMR spectra showed a mark deviation in the chemical shift values than those observed for compound **2d** along with the absence of the acetyl protons in the upfield region. It further showed a D_2O exchangeable peak for the NH proton at δ 7.09. The mass spectrum showed molecular ion peak M^+ at *m*/*z* 279 along with M^++2 peak at *m*/*z* 281 corresponding to molecular formula $C_{11}H_7BrN_2S$. Thus on the basis of above studies, compound **3d** was characterized 7-bromo-10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazine.

The next step of the synthesis involved alkylation at 10th position in **3a**–**d**. Earlier *N*-alkylation of 1-azaphenothiazine with different alkylbromides has been carried by heating the reactants with either $NaNH_2$ in xylene or NaH in toluene for 30–40 h (Ledochowski *et al.*, 1963; Beschke and Schuler, 1964). However, when we tried similar conditions for the synthesis of bis-azaphenothiazines using 1-azaphenothiazine and dibromoalkane or dibromoalkene in 2:1 ratio, we did not obtain the desired product. The bis-compounds could not also be obtained by varying reaction conditions such as using K_2CO_3 in acetone/THF/DMF or

Scheme 1 Synthesis of 7-substituted azaphenothiazines



1a R = H
1b R = Cl
1c R = F

2a R = H
2b R = Cl
2c R = F

3a R = H
3b R = Cl
3c R = F

2a

2d

3d

a = Acetic anhydride/rt
b,d = KOH, acetone, reflux
c = Br_2/CH_3COOH/rt

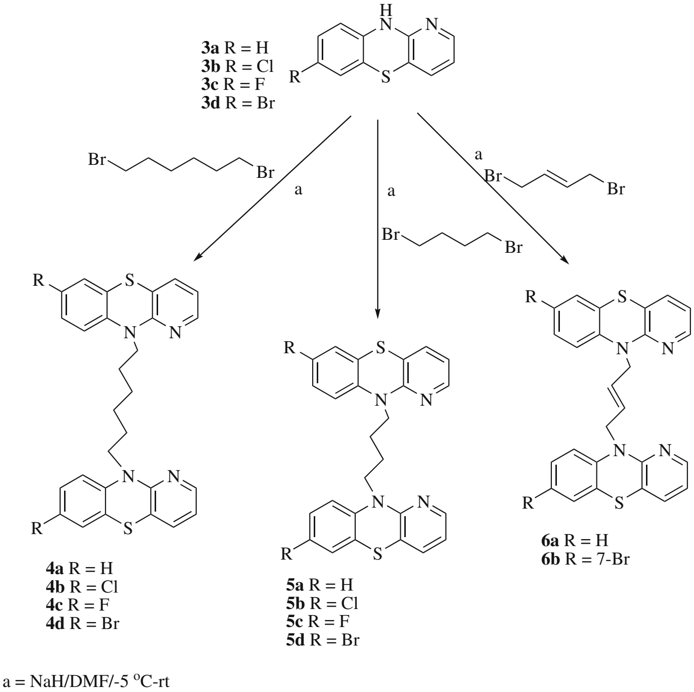
Et_3N in DMF/THF. Finally, *N*-alkylation of 1-azaphenothiazines **3a–d** using 1,6-dibromohexane proceeded in the presence of NaH at −5 °C in DMF and the desired bis-compounds **4a–d** were obtained in good yield. Using similar conditions, we have also synthesized compounds **5a–d** and **6a–b** using different alkyl/alkenyl dibromides (Scheme 2). All the synthesized compounds were characterized on the basis of their spectral studies. Compounds **1a–c**, **2a–c**, and **3a–c** were obtained by the literature procedures and characterized by comparing their m.p. and spectral data from those reported in the literature (Gupta *et al*., 2007).

We have also tried to repeat the simple *N*-alkylation of 1-azaphenothiazines under above mild conditions using various alkyl/alkenyl bromides and obtained the desired *N*-alkylated azaphenothiazines in good yield, without the formation of any side product. So novel method involving mild reaction conditions (instead of earlier harsh conditions) have been developed by us to obtain not only *N*-alkylated azaphenothiazines, but also bis-azaphenothiazines containing a suitable spacer of choice, in good yield.

Compounds **3a–d** were separately subjected to alkylation using 1,6-dibromohexane and 1,4-dibromobutane in the presence of NaH at −5 °C to obtain **4a–d** and **5a–d**, respectively, which were characterized on the basis of their complete spectral studies. The main evidence of the formation of compound **4a** came from its ^{1}H NMR spectrum that showed the presence of a triplet at δ 4.06, integrating for four protons for the methylenes attached to the two nitrogen atoms of the compound. The corresponding peak at δ 45.1 in ^{13}C NMR confirmed that the two nuclei have been linked in **4a** via 1,6 hexyl spacer. The rest of the methylene protons of hexyl spacer were observed as two multiplets at δ 1.81–1.77 and δ 1.56–1.49 each integrating for four protons. The aromatic protons were observed as double doublets, doublets at δ 7.98–7.96 ($J = 4.8$ and 1.5 Hz), δ 7.23–7.20 ($J = 7.4$ and 1.4 Hz), δ 7.03 ($J = 7.6$ Hz), and δ 6.82 ($J = 8.1$ Hz), respectively, each integrating for two protons; multiplet and triplet at δ 6.74–6.70 and 6.89 ($J = 7.2$ Hz) also integrating for two protons clearly showing that **4a** has been formed. The final evidence came from its EIMS mass spectrum that showed the molecular ion peak M^+ at *m*/*z* 482 corresponding to molecular formula $C_{28}H_{28}N_4S_2$. Thus on the basis of above studies **4a** has been assigned structure 1,6-bis(10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazin-10-yl]hexane.

Compounds **3a** and **3d** were also reacted with trans-1, 4-dibromobut-2-ene to yield **6a–b**. The main evidence of the formation of compound **6a** came from its ^{1}H NMR spectrum as it showed the presence of multiplet at δ 4.69–4.66, integrating for four protons for the methylenes attached to the two nitrogen atoms. The corresponding peak in ^{13}C NMR appeared at δ 50.3 that revealed that the two nuclei have been linked in **6a** via trans-1,4-but-2-enyl spacer. The olefinic protons of the linker were observed as multiplet at δ 5.93–5.91 integrating for two protons. The aromatic protons were observed as double doublets at δ 7.96–7.95 ($J = 4.5$ and 1.2 Hz) for two protons, doublets at δ 7.21 ($J = 7.2$ Hz), δ 6.99 ($J = 7.4$ Hz), and δ 6.94 ($J = 7.7$ Hz) each integrating for two protons, and multiplets at δ 6.87–6.82 and

Scheme 2 Synthesis of 7, 7-disubstituted bis-azaphenothiazines



δ 6.74–6.69 integrating for four and two protons, respectively. Its TOF mass spectrum showed the $M^{+}+1$ at *m/z* 453 corresponding to its molecular formula $C_{26}H_{20}N_4S_2$. Thus on the basis of above studies compound **6a** has been assigned structure 1,4-bis(10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazin-10-yl)but-2*E*-ene. Synthesized compounds **4a–d**, **5a–d**, and **6a–b** were found to be completely soluble in chloroform.

Biological activity

Compounds **2a–d**, **4a–d**, **5a–d**, and **6a–b** were screened for their invitro antimicrobial activities to determine zone of inhibition at 100 µg/ml against three gram positive bacteria (*Staphylococcus aureus* MTCC 096, *Bacillus subtilis* MTCC 441, and *Staphylococcus epidermis* MTCC 435), four gram negative bacteria (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhi* MTCC 733, and *Klebsiella pneumonia* MTCC 432) as well as four fungi (*Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus flavus* MTCC 277, and *Candida albicans* MTCC 227) using cup plate method (Chuickshank *et al.*, 1975). Inoculated Muller–Hilton agar for bacteria and sabouraud dextrose agar for fungi were poured separately into the sterilized petridishes (25–30 ml: each petri dish). The poured material was allowed to set (30 min) and thereafter the "CUPS" (08 mm diameter) were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. The test compound solution (0.1 ml) was placed with the help of a micro pipette into these cups. The plates were incubated at 37 °C for 16–18 h for bacteria and 28–30 h for fungi, and the results were recorded. The test solution was prepared using DMSO as solvent. Clinically antimicrobial drugs ciprofloxacin and miconazole were used as the positive control and DMSO was used for blank.

MIC is the minimum concentration of the compound, which inhibits the visible growth of bacteria or fungi. For the determination of zone of inhibition, inoculated MH agar for bacteria and SD agar for fungi were separately poured onto the sterilized petri dishes. The poured material was allowed to set and thereafter the "CUPS" (08 mm diameter) were made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. The test compound solution was added into these cups with the help of a sterile syringe. The plates were then incubated at 37 °C for 16 h for bacteria and 28–30 h for fungi. Ciprofloxacin and miconazole were used as standard drugs for bacteria and fungi, respectively. The experiments were repeated three times to reduce the error and the average value of antibacterial and antifungal activities are represented in Tables 3 and 4.

The results depicted in Tables 1, 2, 3, and 4 showed that most of the compounds possessed moderate activity against the bacterial strains. However, monoacetates **2b–d** have shown very good activity against *P. aeruginosa* which suggested that halogen substitution in the ring increases the biological activity. Among halo substituted compounds, bromo compound **2c** is the most active against *A. niger* and *A. fumigatus* and its antifungal activity was comparable to the standard drug miconazole at 100 µg/ml. Compound **4d** was found to possess significant activity against *S. epidermis*. It was also seen that azaphenothiazines **4a–d** which were separated via $–(CH_2)_6–$ linker gave better zone of inhibition as compared to the compounds **5a–d** and **6a–b** where azaphenothiazines were linked via $–(CH_2)_4–$ or –

Table 1 Antibacterial evaluation of **2a–d**, **4a–d**, **5a–d**, and **6a–b** (zone of inhibition in mm)

Compound	*S. aureus*	*B. subtilis*	*S. epidermis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*K. pneumoniae*
2a	11	**12**	11	10	**12**	**12**	11
2b	**12**	**12**	**13**	**12**	**14**	**12**	**13**
2c	**13**	**12**	**12**	**14**	**14**	**12**	11
2d	**12**	**12**	**13**	11	**14**	11	**13**
4a	**13**	**12**	**13**	**12**	11	**12**	**12**
4b	**12**	**12**	10	11	**12**	**12**	11
4c	**12**	**12**	**12**	**12**	**13**	**12**	11
4d	**12**	**13**	**14**	**12**	**13**	11	**12**
5a	11	10	**12**	11	**13**	**12**	**12**
5b	**12**	11	**12**	**12**	11	**12**	11
5c	11	**12**	**12**	11	10	**12**	11
5d	**12**	11	11	**12**	**12**	10	11
6a	**12**	10	10	11	**12**	11	11
6b	**12**	11	11	**12**	10	11	11
Ciprofloxacin	**19**	**18**	**16**	**20**	**18**	**19**	**17**

Bold values represent the zone of inhibition of compounds showing good to promising activity

Zone of inhibition at 100 µg/ml

Table 2 Antifungal evaluation of **2a–d**, **4a–d**, **5a–d**, and **6a–b** (Zone of inhibition in mm)

Compound	*A. niger*	*A. fumigatus*	*A. flavus*	*C. albicans*
2a	–	–	**12**	**12**
2b	**14**	**12**	**12**	**14**
2c	**18**	**16**	10	**14**
2d	**16**	**14**	**12**	**13**
4a	11	10	**12**	**12**
4b	–	**12**	10	**12**
4c	10	11	**12**	**13**
4d	11	11	**12**	11
5a	–	–	–	–
5b	10	11	**12**	11
5c	11	10	**12**	10
5d	11	**12**	10	11
6a	**12**	**13**	**12**	**12**
6b	–	**12**	11	10
Miconazole	**18**	**15**	**17**	**19**

Bold values represent the zone of inhibition of compounds showing good to promising activity

– Compounds which does not show any activity, zone of inhibition at 100 μg/ml

Table 4 Antifungal evaluation of **2a–d**, **4a–d**, **5a–d**, and **6a–b** (MIC in mm)

Compound	*A. niger*	*A. fumigatus*	*A. flavus*	*C. albicans*
2a	>50	>50	12.5	12.5
2b	6.25	12.5	25	6.25
2c	6.25	6.25	>50	6.25
2d	6.25	6.25	12.5	6.25
4a	25	>50	25	12.5
4b	>50	12.5	>50	12.5
4c	50	25	12.5	6.25
4d	>50	25	12.5	25
5a	>50	>50	>50	>50
5b	>50	50	12.5	25
5c	50	>50	25	>50
5d	50	12.5	>50	50
6a	12.5	6.25	12.5	12.5
6b	>50	12.5	>50	>50
Miconazole	6.25	6.25	12.5	6.25

(CH_2–CH=CH–CH_2)– linker, when tested on most of the bacterial strains. So optimum lipophilic chain length required for activity could be C_6 alkyl chain.

Against the fungal strains, monoacetates were found to possess even better activity as compared to bis-compounds. Monoacetates **2b–d** showed promising antifungal activity against *A. niger*, *A. fumigatus*, and *C. albicans*. Compounds **2c** and **2d** showed antifungal activity, comparable to that of standard drug miconazole, against *A. niger* and *A. fumigatus* whereas compounds **2b** and **2c** showed moderate activity against *C. albicans*. Among the final compounds, **6a–b** showed good activity against *A. fumigatus*.

Conclusions

In summary, we have developed a novel methodology for the *N*-alkylation of azaphenothiazines and used it to synthesize ten novel bis-azaphenothiazines in good yield. The structures of all the compounds were fully characterized on the basis of their detailed spectral studies. Antimicrobial

Table 3 Antibacterial evaluation of **2a–d**, **4a–d**, **5a–d**, and **6a–b** (MIC in mm)

Compound	*S. aureus*	*B. subtilis*	*S. epidermis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*K. pneumoniae*
2a	>50	25	25	>50	12.5	12.5	50
2b	25	25	6.25	12.5	6.25	12.5	6.25
2c	12.5	25	25	6.25	6.25	12.5	>50
2d	25	25	6.25	>50	6.25	50	6.25
4a	6.25	25	6.25	12.5	50	12.5	25
4b	25	25	>50	50	25	12.5	50
4c	25	25	12.5	12.5	6.25	25	50
4d	25	12.5	6.25	6.25	6.25	50	25
5a	50	>50	12.5	>50	6.25	12.5	25
5b	25	50	6.25	12.5	50	25	>50
5c	25	12.5	12.5	25	>50	12.5	50
5d	12.5	50	25	12.5	12.5	>50	50
6a	12.5	>50	>50	50	12.5	>50	50
6b	25	25	>50	12.5	>50	50	50
Ciprofloxacin	6.25	6.25	6.25	6.25	6.25	6.25	6.25

study on these compounds has indicated that compounds **4a–d**, **5a–d**, and **6a–b** possessed moderate to significant activities, while acetates **2b–d** were found to exhibit strong antifungal activity which was comparable to the standard drug miconazole at 100 μg/ml against two strains *A. niger* and *A. fumigatus*.

Experimental section

Melting points were determined on an electronic apparatus and are uncorrected. Infrared (FTIR) spectra were determined on a Perkin Elmer-2000 Spectrophotometer instrument. 1H NMR spectra were recorded on Bruker AC (300/400 MHz), and 1^{3}C NMR spectra were recorded on Bruker AC (75.47/100 MHz) using tetramethylsilane as an internal standard in $CDCl_3$. Elemental analyses were performed on a Perkin Elmer series 11, CHNS/O analyzer 2400. Mass spectra were recorded on Jeol-JMS-DX 303 mass spectrometer. Substituted azaphenothiazines were prepared by literature procedures (Gupta *et al*., 2007) starting from the corresponding anilines.

2-Acetylamino-5-bromophenyl-(3-nitro-2-pyridyl) sulfide (**2d**)

2-Acetylaminophenyl-(3-nitro-2-pyridyl)sulfide **2a** (1.00 g, 3.46 mmol) was dissolved in 20 ml of acetic acid under anhydrous conditions at room temperature. Bromine (0.5 ml) in 10 ml of acetic acid was added dropwise in 10 min. Reaction mixture was stirred for 30 min at the same temperature. The product that precipitated was filtered, washed with water, and dried and crystallized from chloroform/methanol to obtain **2d** in pure form as yellow colored solid. Yield 0.90 g (70 %); m.p. 184–186 °C; IR ν_{max} 3370 (–NH), 2923, 2852 (aromatic –CH stretchings), 1688 ($–COCH_3$), 1557 ($–NO_2$), 1435 ($–NO_2$), 1337, 1292 (–CN), 1122, 1067, 856 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.56–8.53 (m, 2H), 8.39 (d, 1H, $J = 7.9$ Hz), 7.96 (brs, 1H, NH, D_2O exchangeable), 7.67 (s, 1H), 7.65 (d, 1H, $J = 8.7$ Hz), 7.32–7.30 (m, 1H), 2.09 (s, 3H, $–COCH_3$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 168.9, 155.1, 150.6, 144.0, 139.8, 132.9, 128.6, 127.7, 124.1, 123.7, 119.0, 22.9; Mass Spectral data, EIMS *m*/*z* (%) 369 (M^++2, 29), 367 (M^+, 30), 353 (19), 325 (10), 288 (100), 246 (20).

7-Bromo-10*H*-benzo[*b*]pyrido[2,3-e][1,4]thiazine (**3d**)

To a stirred refluxing solution of **2d** (1.20 g, 3.26 mmol) in 50 ml of distilled acetone, powdered KOH (0.55 g, 9.8 mmol) was added in small portions in about 10 min. The mixture was refluxed for 4 h. The solvent was then distilled off and 50 ml of water was added to obtain light brown colored residue which was filtered and crystallized from petrol/ethylacetate to give **3d** as yellow solid. Yield 0.75 g (83 %); m.p. 204–206 °C; IR ν_{max} 3229 (–NH), 3056, 2986 (aromatic –CH strechings), 1513, 1428 (–C=C– strechings), 1313, 1283 (–CN), 1155, 1120, 1082, 959, 902, 851, 768 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (s, 1H), 7.17 (d, 1H, $J = 7.1$ Hz), 7.09 (brs, 1H, NH), 7.05 (s, 1H), 6.84 (s, 1H, D_2O exchangeable), 6.72–6.70 (m, 1H), 6.42 (d, 1H, $J = 7.8$ Hz); ^{13}C NMR (δ, $CDCl_3$, 75.47 MHz) 148.3, 145.9, 143.1, 134.9, 129.2, 128.3, 120.7, 118.8, 118.4, 115.4, 113.4; mass spectral data, EIMS *m*/*z* (%): 281 (M^++2, 89), 279 (M^+, 92), 204 (20), 200 (90), 126 (23).

General procedure

Synthesis of bis-azaphenothiazines ***4a–d****,* ***5a–d****, and* ***6a–b***

To a stirred solution of NaH (6.36 mmol) in 10 ml of dry DMF at −5 °C, a solution of azaphenothiazine (2.12 mmol) in DMF was added dropwise under inert atmosphere. To the deep brown colored suspension, a solution of dibromoalkane/dibromoalkene (1.06 mmol) in DMF was added dropwise at −5 °C. The reaction mixture was stirred at −5 °C for 30 min and then at room temperature for another 8–9 h. The course of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was quenched with ice cold water and then extracted with diethyl ether. The organic layer was separated, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude product was subjected to column chromatography in petroleum ether. Elutions with petroleum ether/ethyl acetate yielded pure compounds as white crystalline solids.

*1,6-bis(10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]hexane (**4a**)*

Yield 0.85 g (71 %); m.p. 160–162 °C; IR ν_{max} 2921, 2849, 2360, 2336 (aromatic –CH strechings), 1868, 1731, 1633, 1540 (–C=C– strechings), 1228 (–CN), 1148, 1047, 872, 818, 756 (C–S), 665 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.98–7.96 (dd, 2H, $J = 4.8$ and 1.5 Hz), 7.23–7.20 (dd, 2H, $J = 7.4$ and 1.4 Hz), 7.14–7.08 (m, 2H), 7.03 (d, 2H, $J = 7.6$ Hz), 6.89 (t, 2H, $J = 7.2$ Hz), 6.82 (d, 2H, $J = 8.1$ Hz), 6.74–6.70 (m, 2H), 4.06 (t, 4H, $2x > N–CH_2–$), 1.81–1.77 (m, 4H, $2x–CH_2–$), 1.56–1.49 (m, 4H, $2x–CH_2–$); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 145.4, 134.4, 127.9, 127.4, 123.1, 118.0, 115.8, 45.1, 30.0, 27.1; mass spectral data, EIMS *m*/*z* (%): 482 (M^+, 75), 283

(23), 267 (21), 259 (23), 239 (10), 225 (25), 213 (62), 200 (70), 136 (15); anal. calcd. for $C_{28}H_{26}N_4S_2$. Calculated: C 69.68 %, H 5.43 %, N 11.61 %, found: C 70.01 %, H 5.24 %, N 11.12 %.

*1,6-Bis(7-chloro-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]hexane (**4b**)*

Yield 0.82 g (70 %); m.p. 158–160 °C; IR ν_{max} 2925, 2857(aromatic –CH strechings), 1560, 1474, 1442 (–C=C– strechings), 1337, 1209 (–CN), 1159, 1126, 1113, 1047, 884, 798, 787 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.98–7.96 (dd, 2H, $J = 4.8$ and 1.5 Hz), 7.22–7.20 (dd, 2H, $J = 7.4$ and 1.3 Hz), 7.06–7.03 (dd, 2H, $J = 8.7$ and 2.3 Hz), 7.00 (d, 2H, $J = 2.2$ Hz), 6.74–6.73 (m, 2H), 6.71 (d, 2H, $J = 8.4$ Hz), 4.02 (t, 4H, $J = 7.2$ Hz, $2x >$ N–CH_2–), 1.77–1.72 (m, 4H, $2x$–CH_2–), 1.59–1.47 (m, 4H, $2x$–CH_2–); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 155.1, 145.6, 142.0, 134.6, 128.0, 127.6, 126.9, 123.6, 118.2, 116.7, 116.4, 45.1, 26.9, 26.7; mass spectral data, EIMS m/z (%): 554 (M^++4, 9), 552 (M^++2, 28), 550 (M^+, 88), 481 (15), 289 (30), 261 (21), 247 (48), 234 (51), 154 (100), 138 (45), 137 (62), 136 (78); anal. calcd. for $C_{28}H_{24}Cl_2N_4S_2$. Calculated: C 60.97 %, H 4.39 %, N 10.16 %, found: C 61.47 %, H 4.31 %, N 10.59 %.

*1,6-Bis(7-fluoro-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]hexane (**4c**)*

Yield 0.80 g (68 %); m.p. 152–154 °C; IR ν_{max} 2926, 2364, 2345(aromatic –CH strechings), 1576, 1418 (–C=C– strechings), 1257 (–CN), 1158, 1198, 1108, 885, 788 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.99 (d, 2H, $J = 4.1$ Hz), 7.23 (d, 2H, $J = 7.7$ Hz), 6.81–6.72 (m, 8H), 4.02 (t, 4H, $J = 7.2$ Hz, $2x >$ N–CH_2–), 1.77–1.72 (m, 4H, $2x$–CH_2–), 1.47–1.44 (m, 4H, $2x$–CH_2–); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 148.3, 145.9, 139.0, 134.9, 120.7, 115.4, 115.0, 113.1, 47.4, 27.5, 27.0; TOF ES + MS m/z 519 (M^++1); anal. calcd. for $C_{28}H_{24}F_2N_4S_2$. Calculated: C 64.84 %, H 4.66 %, N 10.80 %, found: C 63.89 %, H 4.66 %, N 10.67 %.

*1,6-Bis(7-bromo-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]hexane (**4d**)*

Yield 0.78 g (69 %); m.p. 152–154 °C; IR ν_{max} 2944, 2873, 2852 (aromatic –CH strechings), 1560, 1464, 1417 (–C=C– strechings), 1373, 1261, 1251, 1231(-CN), 1168 cm^{-1}; 1H NMR ($CDCl_3$, 300 MHz) δ 7.98 (d, 2H, $J = 4.3$ Hz), 7.22–7.20 (m, 2H), 7.18 (t, 2H, $J = 5.1$ Hz), 7.14 (s, 2H), 6.76–6.72 (m, 2H), 6.67–6.64 (m, 2H), 4.02 (t, 4H, $J = 6.7$ Hz, $2x >$ N–CH_2–), 1.76–1.72 (m, 4H, $2x$–CH_2–), 1.47–1.44 (m, 4H, $2x$–CH_2–); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 146.2, 135.2, 131.1, 130.2, 128.3, 120.7, 118.9, 117.4, 45.6, 27.5, 27.2; TOF ES + MS m/z 640 (M^++2); anal. calcd. for $C_{28}H_{24}Br_2N_4S_2$. Calculated: C 52.51 %, H 3.78 %, N 8.75 %, found: C 54.02 %, H 3.07 %, N 8.67 %.

*1,4-Bis(10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]butane (**5a**)*

Yield 0.79 g (70 %); m.p. 133 °C; IR ν_{max} 2929, 2852 (aromatic –CH strechings), 1589, 1448 (–C=C– strechings), 1411, 1372, 1259 (–CN), 1180, 1043, 776, 755 (C–S), 641 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.98–7.97 (dd, 2H, $J = 6.6$ and 1.4 Hz), 7.23–7.21 (dd, 2H, $J = 6.4$ and 1.4 Hz), 7.07 (d, 2H, $J = 7.6$ Hz) 7.03 (d, 2H, $J = 6.8$ Hz), 6.90 (d, 2H, $J = 6.4$ Hz), 6.82 (d, 2H, $J = 7.6$ Hz), 6.73 (m, 2H), 4.14 (t, 4H, $J = 7.2$ Hz, $2x >$ N–CH_2–), 1.87–1.83 (m, 4H, $2x$–CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 155.1, 145.0, 142.8, 134.1, 127.5, 127.0, 122.8, 121.5, 117.7, 117.3, 115.5, 44.4, 24.0; TOF ES + MS m/z 455 (M^++1). Anal. calcd. for $C_{26}H_{22}N_4S_2$. Calculated: C 68.69 %, H 4.88 %, N 12.32 %, found: C 70.24 %, H 4.34 %, N 11.45 %.

*1,4-Bis(7-chloro-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]butane (**5b**)*

Yield 0.75 g (68 %); m.p. 140–142 °C; IR ν_{max} 2925, 2854(aromatic –CH strechings), 1587, 1560 (–C=C– strechings), 1415, 1262 (–CN), 1111, 782, 802, 758 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.97–7.96 (dd, 2H, $J = 4.4$ and 2.2 Hz), 7.23–7.21 (dd, 2H, $J = 8.8$ and 1.4 Hz), 7.17–7.15 (dd, 2H, $J = 8.8$ and 2.3 Hz), 7.12 (d, 2H, $J = 2.2$ Hz), 6.75–6.74 (m, 2H), 6.62 (d, 2H, $J = 8.8$ Hz), 4.09 (t, 4H, $J = 6.5$ Hz, $2x$–NCH_2), 1.91–1.87 (m, 4H, $2x$–CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.8, 145.2, 141.8, 134.4, 130.2, 129.3, 123.9, 118.0, 116.7, 115.0, 44.1, 23.5; TOF ES + MS m/z 523 (M^++2); anal. calcd. for $C_{26}H_{20}Cl_2N_4S_2$. Calculated: C 59.65 %, H 3.85 %, N 10.70 %, found: C 61.84 %, H 2.97 %, N 10.61 %.

*1,4-Bis(7-fluoro-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]butane (**5c**)*

Yield 0.77 g (69 %); m.p. 139–141 °C; IR ν_{max} 2924 (aromatic –CH strechings), 2122, 1723, 1613 (–C=C–), 1398, 1265 (–CN), 1145, 1071, 847, 748, 705 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.98–7.97 (dd, 2H, $J = 6.6$ and 1.4 Hz), 7.21–7.20 (dd, 2H, $J = 7.3$ and 1.4 Hz), 7.00 (m, 4H), 6.74–6.72 (m, 4H), 4.04 (t, 4H, $J = 7.3$ Hz, 2*x*–NCH_2), 1.79–1.75 (m, 4H, 2*x*–CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 161.4, 160.4, 155.5, 152.7, 125.9, 114.5, 112.9, 112.7, 102.5, 56.3, 18.9; TOF ES + MS *m/z* 491 (M^++2); anal. calcd. for $C_{26}H_{20}F_2N_4S_2$. Calculated: C 63.65 %, H 4.11 %, N 11.42 %, found: C 65.51 %, H 3.98 %, N 11.45 %.

*1,4-Bis(7-bromo-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl]butane (**5d**)*

Yield 0.77 g (71 %); m.p. 120–122 °C; IR ν_{max} 2924, 2854 (aromatic –CH strechings), 1413, 1262 (–CN), 1183, 1132, 1111, 801, 758 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.97–7.96 (dd, 2H, $J = 4.8$ and 1.5 Hz), 7.23–7.21 (dd, 2H, $J = 7.2$ and 1.5 Hz), 7.17 (d, 2H, $J = 2.1$ Hz), 7.14–7.12 (dd, 2H, $J = 6.9$z and 2.4 Hz), 6.77–6.73 (m, 2H), 6.63 (d, 2H, $J = 8.7$ Hz), 4.09 (t, 4H, $J = 7.2$ Hz, 2*x*–NCH_2), 1.90–1.86 (m, 4H, 2*x*–CH_2);^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 154.8, 145.2, 141.8, 134.4, 130.2, 129.3, 123.9, 118.0, 116.7, 115.0, 44.1, 23.5; TOF ES + MS *m/z* 612 (M^++2); anal. calcd. for $C_{26}H_{20}Br_2N_4S_2$. Calculated: C 50.99 %, H 3.29 %, N 9.15 %, found: C 52.34 %, H 3.21 %, N 9.04 %.

*1,4-Bis(10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl)but-2E-ene (**6a**)*

Yield 0.76 g(68 %); m.p. 132–134 °C; IR ν_{max} 2925, 2854(aromatic –CH strechings), 1580 (–C=C–), 1412, 1364, 1256, 1219 (–CN), 1129, 1047, 779 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.96–7.95 (dd, 2H, $J = 4.5$ and 1.2 Hz), 7.21 (d, 2H, $J = 7.2$ Hz), 6.99 (d, 2H, $J = 7.4$ Hz), 6.94 (d, 2H, $J = 7.7$ Hz), 6.87–6.82 (m, 4H), 6.74–6.69 (m, 2H), 5.93–5.91 (m, 2H, –CH=CH–), 4.69–4.66 (m, 4H, 2*x*N–CH_2); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 148.3, 145.9, 143.4, 134.9, 130.2, 126.4, 117.5, 115.4, 113.8, 113.4, 50.3. TOF ES + MS *m/z* 453 (M^+ +1); anal.calcd. for $C_{26}H_{20}N_4S_2$. Calculated: C 69.00 %, H 4.45 %, N 12.38 %, found: C 68.89 %, H 4.41 %, N 13.25 %.

*1,4-Bis(7-bromo-10H-benzo[b]pyrido[2,3-e][1,4]thiazin-10-yl)but-2E-ene (**6b**)*

Yield 0.75 g(69 %); m.p. 128–130 °C; IR ν_{max} 2925 (aromatic –CH strechings), 1588 (–C=C–), 1478, 1414, 1357, 1263, 1219 (–CN), 1130, 867, 801, 757 (C–S) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.95 (d, 2H, $J = 4.8$ Hz), 7.24–7.21 (dd, 2H, $J = 8.7$ and 1.8 Hz), 7.12 (d, 2H, $J = 2.1$ Hz), 7.04–7.01 (d, 2H, $J = 8.9$ and 1.8 Hz), 6.78–6.74 (m, 2H), 6.61 (d, 2H, $J = 8.7$ Hz), 5.89–5.85 (m, 2H, –CH=CH–), 4.65–4.62 (m, 4H, 2*x*N–CH_2–); ^{13}C NMR ($CDCl_3$, 75.47 MHz) δ 154.2, 145.2, 141.9, 134.2, 130.0, 128.9, 123.2, 118.1, 117.2, 116.2, 115.1, 47.0 (N–CH_2). TOF ES + MS *m/z* 610 (M^+ +1); anal. calcd. for $C_{26}H_{18}Br_2N_4S_2$. Calculated: C 51.16 %, H 2.97 %, N 9.18 %, found: C 52.84 %, H 2.93 %, N 8.09 %.

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