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PRACTICAL SYNTHESES OF THE ADHESION MOLECULE INHIBITOR ER-49890 AND ITS STEREOISOMER

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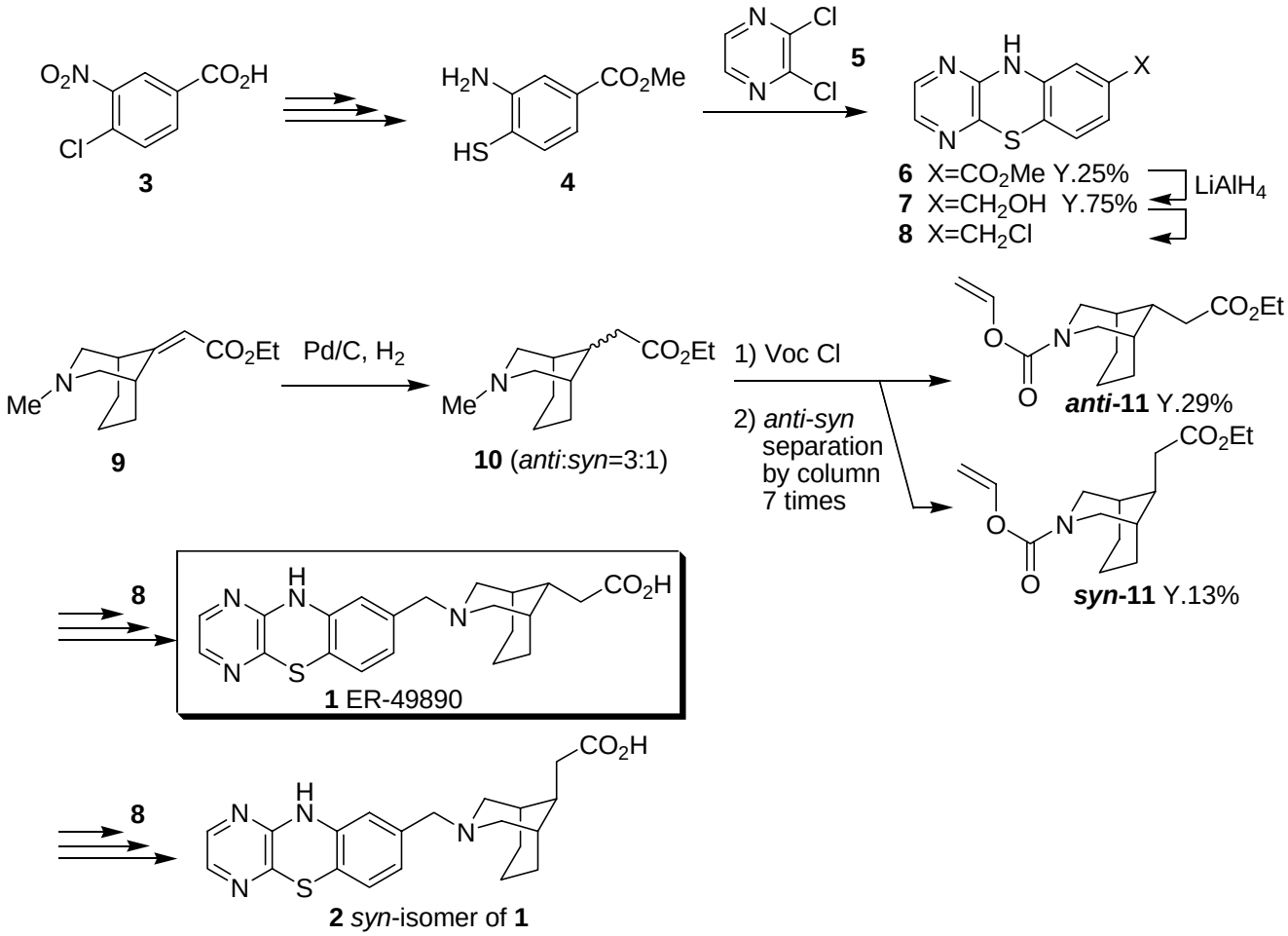
Abstract – Practical synthetic routes to *anti*-[3-(10*H*-pyrazino[2,3-*b*][1,4]-benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-yl]acetic acid (ER-49890, **1**) and its *syn*-isomer are reported. The *anti*-(3-azabicyclo[3.3.1]non-9-yl)acetic acid side chain (***anti*-12**) was synthesized stereoselectively using Pd/C hydrogenation in the presence of HCl. The *syn*-isomer (***syn*-12**) was concisely obtained by crystallization as its oxalic acid salt from a 1:1 mixture of *anti*- and *syn*-isomers. The 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine core (**7**) was prepared from commercially available 4-chloro-3-nitrobenzyl alcohol (**13**) in four steps and good yield.

INTRODUCTION

Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1 (VCAM-1) have been implicated in inflammatory disorders including rheumatoid arthritis, colitis, psoriasis and multiple sclerosis, and offer an attractive target for the design of novel therapeutic drugs.[1] We have discovered that *anti*-[3-(10*H*-pyrazino[2,3-*b*][1,4]benzothiazin-8-ylmethyl)-3-azabicyclo[3.3.1]non-9-yl]acetic acid (ER-49890, **1**), is a novel, orally-active inhibitor of the upregulation of such adhesion molecules.[2] During the course of our studies, we needed a practical route for the scale-up synthesis of both **1** and its *syn*-stereoisomer (**2**) in order to evaluate their detailed pharmacological profiles. However, as shown in Scheme 1 our previously reported synthetic route to **1**

and **2**, required the separation of the *anti*- and *syn*-isomers using repeated column chromatography as the step forming the mixture of acetates (**10**) proceeded in low selectivity. Two additional problems in using the reported route for scale-up synthesis are the low stability of aminothiophenol intermediate (**4**), and the step involving reduction of ester (**6**) using lithium aluminum hydride.[3] We therefore set out to find improved preparations of the three key intermediates, *anti*- and *syn*-(3-azabicyclo[3.3.1]non-9-yl)acetate (**10**), and the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine core (**7**).

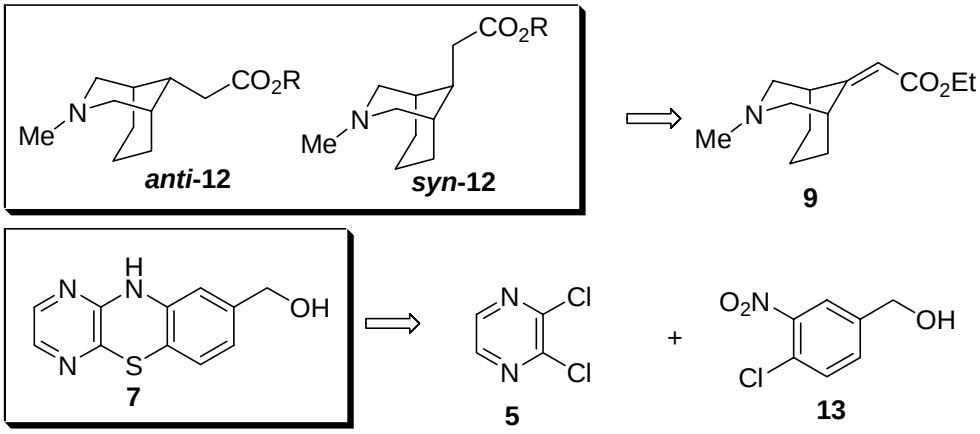


Scheme 1 Previously Reported Synthetic Routes to ER-49890 (**1**) and Its *Syn*-Isomer (**2**)

RESULTS AND DISCUSSION

As shown in Scheme 2, we planned to prepare ***anti*-** and ***syn*-12** stereoselectively from a common starting compound (**9**). We selected 4-chloro-3-nitrobenzyl alcohol (**13**) as the starting material for the synthesis of 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine core **(7**), in order to avoid having to use unstable aminothiophenol (**4**) as an intermediate.

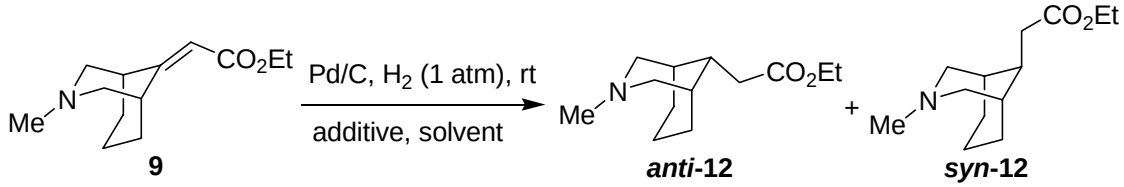
First, the *anti*-selective formation of the (3-azabicyclo[3.3.1]non-9-yl)acetic acid side chain (***anti*-12**), for which a stereoselective synthesis has not been reported, was investigated. As previously described,[2] hydrogenation of unsaturated ester (**9**)[4] using palladium on carbon (Pd/C) provided an *anti*-*syn* mixture of



Scheme 2 Retrosynthesis of Desired Intermediates

12 in the ratio of 3:1. We examined the effect of solvent and additives on this ratio as calculated from the integrated intensity of the equatorial protons at C-2 and C-4 of the 3-azabicyclo[3.3.1]nonane ring at 2.90 ppm (*anti*) and 2.62 ppm (*syn*) respectively (Table 1).

Table 1 The Effect of Solvent and Additive on the Hydrogenation of **9**

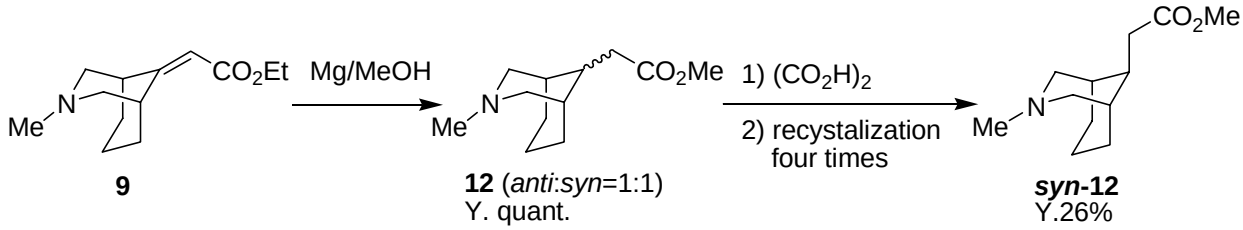


Run	Solvent	Additive[a)]	*Anti* : *Syn*[b)]
1	EtOH	—	3 : 1
2	Hexane	—	4 : 1
3	EtOAc	—	2 : 1
4	EtOH	Pyridine	3 : 1
5	EtOH	AcOH	4 : 1
6	EtOH	MsOH	5 : 1
7	EtOH	TsOH	6 : 1
8	EtOH, H_2O	TsOH	6 : 1
9	EtOH	concd HCl	29 : 1

a) additive (1.5 eq.). b) Ratios were calculated from ^{1}H-NMR spectra.

Use of less-polar solvents such as hexane, AcOEt or pyridine-containing ethanol gave no improvement, neither did addition of weak acid such as acetic acid (AcOH). Use of a stronger bulky acid such as *p*-toluenesulfonic acid (TsOH) gave a 2-fold improvement in selectivity, a similar result being obtained with methanesulfonic acid (MsOH). On the other hand, a dramatic improvement in *anti*-selectivity was achieved on addition of concd HCl with the ratio increasing to 29:1. We postulated that the difference between TsOH and concd HCl might be due to water, but adding water under the TsOH conditions to the

same concentration as for concd HCl had no significant effect. Despite this result, we had established a selective synthesis of ***anti*-12** using Pd/C hydrogenation in the presence of concd HCl.



Scheme 3 Practical Synthetic Route to ***Syn*-12**

On the other hand, *syn* selective hydrogenation could not be achieved, and so we attempted to obtain the *syn*-stereoisomer (***syn*-12**) by crystallization, which it is known can be useful for the scale-up separation of a single stereoisomer from a mixture. A 1:1 *anti*:*syn* mixture was obtained by magnesium-methanol reduction[5] of α,β-unsaturated ester (**9**). Fortunately, **12** could be obtained as its *syn*-rich oxalic acid salt from ethanol (isomer ratio 4:1). After a total of four recrystallizations, pure *syn*-isomer (***syn*-12**) was obtained in a yield of 26%. The structures of ***anti*-12** and ***syn*-12** were deduced based on NOE experiments as shown in Figure 1. In ***anti*-12** an NOE was detected between H-2*ax*, H-4*ax* and H-9, and between H-6*ax*, H-8*ax* and H-10. In ***syn*-12** an NOE was observed between H-2*ax*, H-4*ax* and H-10, and between H-6*ax*, H-8*ax* and H-9.

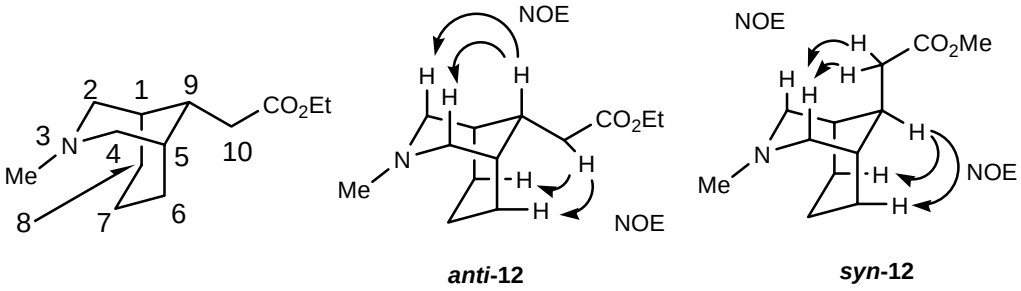
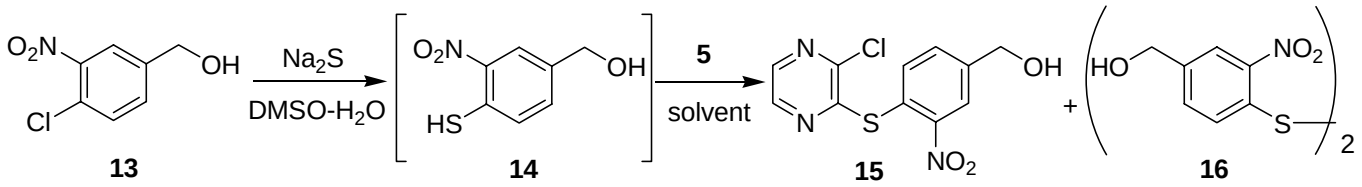


Figure 1 NOE Correlation of ***Anti*-12** and ***Syn*-12**

The 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine core (**7**) was synthesized as shown in Schemes 4 and 5. Displacement of the chloride of **13** with sodium sulfide (Na_2S) in DMSO-water solvent afforded **14** in more than 90% yield as evaluated by HPLC. Extraction of **14** followed by condensation with 2,3-dichloropyrazine (**5**) in acetonitrile-water solvent afforded **15** (Y.38%) and the dimer (**16**) (Y.10%) as shown in Run 1. As it has been reported that triphenylphosphine reduces a disulfide to the corresponding thiol,[6] addition of a small amount of triphenylphosphine was examined. This was successful, and the yield of **15** rose to 48%. Reduction of the nitro group of **15** using iron powder, followed by cyclization under

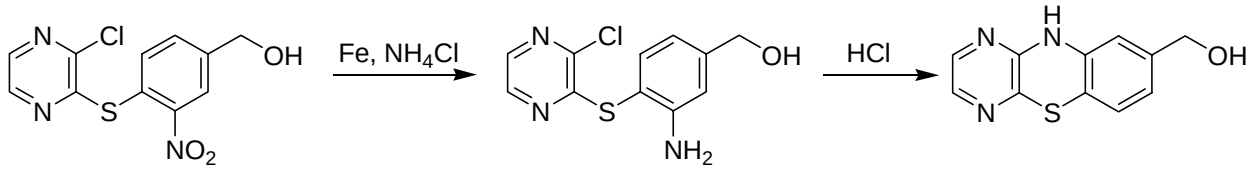
acidic conditions afforded the key intermediate (**7**). The yield of this synthesis of the core was 48% starting from alcohol (**13**), a 2.5-fold improvement over that of the previous route (Y.19%).



13 **14** **15** **16**

Run	Solvent	Condition	**15** (Y.%)	**16** (Y.%)
1	MeCN-H_2O	90 Ž, 3 h	38	10
2	MeCN-H_2O	0.05 eq. PPh_3 90 Ž, 3 h	48	—

Scheme 4 Synthetic Route to Intermediate (**15**)



15 **17** Y.100% **7** Y.99%

Scheme 5 New Synthetic Route for **7**

In conclusion, we have established practical synthetic routes to ER-49890 (**1**) and its *syn*-isomer (**2**) involving an *anti*-selective hydrogenation of ethyl (3-azabicyclo[3.3.1]non-9-ylidene)acetate (**9**), and *syn*-selective crystallization of methyl (3-azabicyclo[3.3.1]non-9-yl)acetate (**12**), respectively, and a new synthesis of the 10*H*-pyrazino[2,3-*b*][1,4]benzothiazine core (**7**). These simple stereoselective methods offer a useful stereo-controlled route to *anti*- and *syn*-(3-azabicyclo[3.3.1]non-9-yl)acetate as possible bioisosteres of piperidine in various bioactive molecules.

EXPERIMENTAL

Melting points were measured using a Yanako melting-point apparatus and are uncorrected. IR spectra were measured with a JASCO FT/IR-620 spectrophotometer. ^{1}H-NMR spectra were recorded on a Varian Unity 400 (400 MHz) spectrometer, and chemical shifts are expressed in ppm downfield from TMS as an internal reference. MS spectra were obtained on a Thermo Quest SSQ700 mass spectrometer. Elemental analysis was performed at the Analytical Research Laboratories in Eisai. Materials were used as bought without any special purification. All organic extracts were dried over anhydrous $MgSO_4$, and solvents were removed with a rotary evaporator under reduced pressure.

Ethyl *anti*-(3-methyl-3-azabicyclo[3.3.1]non-9-yl)acetate (*anti*-12)

To a solution of ethyl (3-methyl-3-azabicyclo[3.3.1]non-9-ylidene)acetate **(9)**[4] (70 g, 0.31 mol) in ethanol (EtOH; 550 mL) containing concd hydrochloric acid (concd HCl; 42 mL) was added 10% palladium on carbon (50% water by weight, Pd/C; 21 g). The mixture was stirred vigorously at rt under a hydrogen atmosphere for 12 h and was then filtered through celite. The solvent was evaporated under reduced pressure, and the residue was dissolved in water (1 L). This solution was washed with AcOEt, and then made alkaline with 33% aqueous ammonia (500 mL). This mixture was extracted with AcOEt, and the combined extracts were washed with saturated brine, dried and evaporated to afford 65 g (93%) of ***anti*-12** as a colorless oil. IR (film, cm^{-1}) 2907, 1736, 1276, 1162. ^{1}H-NMR ($CDCl_3$) δ: 1.25 (3H, t, *J*=7.2 Hz), 1.37-1.46 (1H, m), 1.50-1.58 (2H, m), 1.62 (2H, br s), 1.68-1.80 (2H, m), 1.97 (1H, t, *J*= 7.6 Hz), 2.13 (3H, s), 2.18-2.25 (2H, m), 2.36-2.50 (1H, m), 2.48 (2H, d, *J*= 7.6 Hz), 2.90 (2H, br d, *J*= 11.2 Hz), 4.12 (2H, q, *J*=7.2 Hz). ESI-MS *m/z*: 226 $(M+H)^+$. *Anal*. Calcd for $C_{13}H_{23}NO_2 \cdot C_2H_2O_4 \cdot 0.1H_2O$: C, 56.80; H, 8.01; N, 4.42. Found: C, 56.74; H, 7.82; N, 4.31.

Methyl *syn*-(3-methyl-3-azabicyclo[3.3.1]non-yl)acetate (*syn*-12)

To a solution of ethyl (3-methyl-3-azabicyclo[3.3.1]non-9-ylidene)acetate **(9)**[4] (21.4 g, 0.096 mol) in methanol (500 mL) was added magnesium shavings (13.0 g, 0.57 mol) with stirring over a period of 1 h under a nitrogen atmosphere. The internal temperature rose to 35-40 °C after the reaction initiated, and cooling was continued until it fell back to rt. The reaction mixture was stirred for a total of 22 h, then the solvent was evaporated under reduced pressure. The residue was poured into saturated aqueous ammonium chloride, and this solution was extracted with AcOEt. The extract was dried and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (50% AcOEt-heptane) to give 18.0 g (89%) of a mixture of ***anti*-12** and ***syn*-12** as a pale yellow oil. This mixture was dissolved in EtOH (185 mL) and oxalic acid (7.7 g, 86 mmol) was added. The mixture was stirred at rt for 12 h, then the precipitate was collected by filtration to give 22.3 g of a mixture of ***syn*-12** and ***anti*-12** in a 4:1 ratio. This solid was recrystallized three times from EtOH (550 mL), EtOH (500 mL) and EtOH (200 mL) to give 6.6 g (26%) of the oxalic acid salt of the desired ***syn*-12** as a colorless solid. This salt (2.0 g, 6.6 mmol) was dissolved in water (10 mL) and this solution was treated with 33% aqueous ammonia (5 mL), and then extracted with AcOEt. The extract was washed with saturated brine, dried, and concentrated *in vacuo* to give 1.4 g (quant.) of ***syn*-12** as a pale yellow oil. IR (film, cm^{-1}) 2905, 1738, 1262, 1148. ^{1}H-NMR ($CDCl_3$) δ: 1.42-1.50 (1H, m), 1.60-1.76 (4H, m), 1.79-1.87 (2H, m), 2.00 (1H, t, *J*=8.0 Hz), 2.13 (3H, s), 2.34 (2H, br d, *J*=12.0 Hz), 2.43 (2H, d, *J*=8.0 Hz), 2.40-2.54 (1H, m), 2.62 (2H, br d, *J*= 11.2 Hz), 3.66 (3H, s). ESI-MS *m/z*: 212 $(M+H)^+$. *Anal*. Calcd for $C_{12}H_{21}NO_2 \cdot C_2H_2O_4$: C, 55.80; H, 7.69; N, 4.65. Found: C, 55.44; H, 7.52; N, 4.61.

4-[(3-Chloropyrazin-2-yl)thio]-3-nitrobenzyl alcohol (15)

To a solution of sodium sulfide nonahydrate (7.7 g, 32 mmol) in water (25 mL), a solution of 4-chloro-3-nitrobenzyl alcohol **(13)** (3.0 g, 16 mmol) in DMSO (20 mL) was added over 1 min at rt and then the reaction mixture was stirred at 40-50 °C for 2 h under a nitrogen atmosphere. The reaction mixture was poured into a suspension of triphenylphosphine (PPh_3; 0.20 g, 0.80 mmol) in water (50 mL)-AcOEt (50 mL) and the AcOEt layer was separated. The aqueous phase was added to a suspension of PPh_3 (0.20 g, 0.80 mmol) in AcOEt (50 mL)-1N HCl (40 mL), and extracted with AcOEt. The extract was washed with saturated brine. The organic layer was extracted with 1N NaOH (50 mL). To this aqueous solution was added a solution of 2,3-dichloropyrazine **(5)** (2.2 g, 15 mmol) and PPh_3 (0.20 g, 0.80 mmol) in acetonitrile (30 mL), and the resulting mixture was stirred at 90 °C for 3 h under a nitrogen atmosphere. After cooling of the reaction mixture to rt, the mixture was poured into ice-water, and extracted with AcOEt. The extract was washed with saturated brine, dried, and evaporated. The residue was purified by silica gel column chromatography (70% AcOEt-heptane) to give 2.3 g (48%) of **15** as a yellow solid. mp 113-114 °C (recrystalised from AcOEt). IR (KBr, cm^{-1}) 3244, 1521, 1051, 855, 795. ^{1}H-NMR ($CDCl_3$) δ: 1.94 (1H, t, *J*=6.0 Hz), 4.87 (2H, d, *J*=6.0 Hz), 7.60-7.66 (1H, m), 7.66 (1H, d, *J*=8.0 Hz), 8.08-8.12 (1H, m), 8.11 (1H, d, *J*=2.4 Hz), 8.15 (1H, d, *J*=2.4 Hz). ESI-MS *m/z*: 320 $(M+Na)^+$. *Anal*. Calcd for $C_{11}H_8N_3O_3ClS$: C, 44.38; H, 2.71; N, 14.11. Found: C, 44.41; H, 2.73; N, 14.02.

4,4'-Dithiobis(3-nitrobenzyl alcohol) (16)

mp 147-149 °C (recrystalised from AcOEt). IR (KBr, cm^{-1}) 3343, 1519, 1330, 805. ^{1}H-NMR (CD_3OD) δ: 4.67 (4H, s), 7.61 (2H, d, *J*=8.0 Hz), 7.82 (2H, d, *J*=8.0 Hz), 8.34 (2H, s). HR-MS (ESI) *m/z*: Calcd for $C_{14}H_{12}N_2O_6S_2$ $(M)^+$: 368.0137. Found: 368.0134.

3-Amino-4-[(3-chloropyrazin-2-yl)thio]benzyl alcohol (17)

To a solution of 4-[(3-chloropyrazin-2-yl)thio]-3-nitrobenzyl alcohol **(15)** (1.6 g, 5.4 mmol) and ammonium chloride (0.16 g, 3.0 mmol) in THF (20 mL), isopropyl alcohol (20 mL) and water (7 mL) were added iron powder (7.5 g, 0.13 mol) portionwise. The reaction mixture was refluxed for 1.5 h, then filtered. The organic phase was evaporated to give 1.5 g (quant.) of **17** as a yellow solid. mp 160-161 °C (recrystalised from AcOEt). IR (KBr, cm^{-1}) 3305, 1336, 1052, 850, 672. ^{1}H-NMR (DMSO-d_6) δ: 4.40 (2H, d, *J*=5.6 Hz), 5.15 (1H, t, *J*=5.6 Hz), 5.37 (2H, s), 6.49 (1H, d, *J*=8.0 Hz), 6.75 (1H, s), 7.17 (1H, d, *J*=8.0 Hz), 8.16 (1H, d, *J*=2.4 Hz), 8.33 (1H, d, *J*=2.4 Hz). ESI-MS *m/z*: 268 $(M+H)^+$. *Anal*. Calcd for $C_{11}H_{10}N_3OClS$: C, 49.35; H, 3.76; N, 15.69. Found: C, 49.43; H, 3.86; N, 15.36.

10*H*-Pyrazino[2,3-*b*][1,4]benzothiazine-8-methanol (7)

A solution of 3-amino-4-[(3-chloropyrazin-2-yl)thio]benzyl alcohol **(17)** (1.5 g, 5.4 mmol) and concd HCl (0.45 mL, 5.4 mmol) in methanol (20 mL) was refluxed for 0.5 h. After addition of ice-cooled aqueous 10% ammonia, the precipitate was collected, washed with water and dried to afford 1.2 g (99%)

of **7** as a yellow solid. mp 187-189 °C. Spectral date for this compound were identical to those of an authentic sample obtained by the known method.[3]

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