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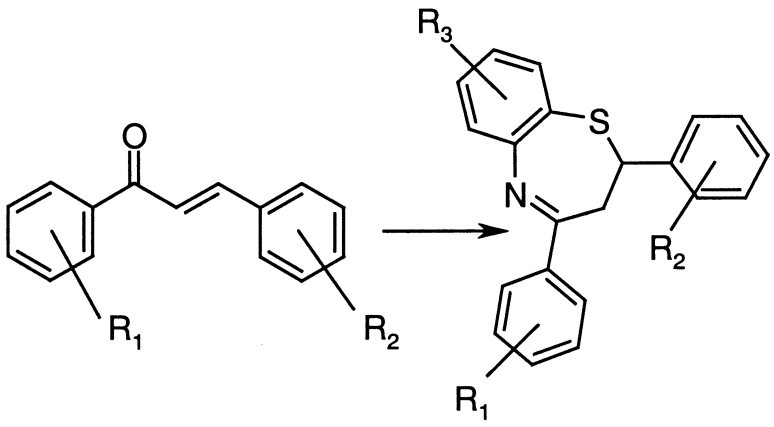

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A Combinatorial Approach to [1,5]Benzothiazepine Derivatives as Potential Antibacterial Agents

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[1,5]Benzothiazepines are widely used in a number of different therapeutic areas and therefore represent an interesting scaffold for de novo exploration. Recent literature reports suggest their value as antibacterial agents. The present paper reports the exploration of this scaffold for the generation of combinatorial libraries both in solution and on solid phase.

Introduction

The [1,5]benzothiazepine scaffold is extremely versatile and features in a great number of famous drugs. Currently [1,5]benzothiazepin-2-ones are being used as coronary vasodilators (e.g., Diltiazem), as calcium antagonists (e.g., Clentiazem), and as antidepressants (e.g., Thiazesim).

Among the various therapeutic applications reported for these derivatives, some authors shed light on the possibility to elicit antibacterial properties within this class.[1]

Combinatorial chemistry has recently been highlighted as an important tool for drug discovery activities[2] both for library production on solid phase and for array synthesis in solution phase during the lead optimization phase.

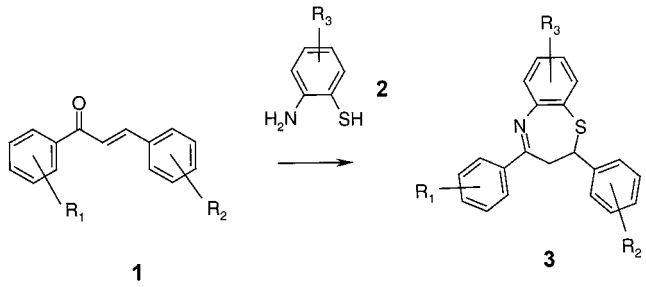
As a part of our continuing efforts in the identification of new chemical entities (NCE) endowed with pharmacological activities, we considered the possibility to exploit a novel combinatorial approach to the [1,5]benzothiazepine scaffold in order to investigate its above cited antibacterial properties. We therefore decided to plan a library of rationally designed derivatives for an "in vitro" antimicrobial screening.

A number of literature reports[3] suggested the synthetic value of chalcones **1** as key intermediates for the combinatorial assembly of a number of different heterocyclic scaffolds.

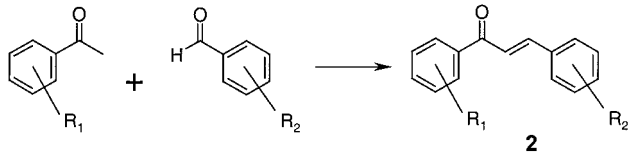
Here we discuss the syntheses and the conversion of the chalcones **1** into [1,5]benzothiazepines upon cyclocondensation with *o*-aminothiophenol as reported in Scheme 1.

Diversity is obviously the driver to explore compounds potentially endowed with pharmacological activity, and the [1,5]benzothiazepine scaffold affords the possibility to introduce three points of diversity quite readily as depicted in Scheme 1.

Scheme 1



Scheme 2



Results and Discussion

Solution Phase Synthesis Approach: Chemistry Assessment. To set up a first solution phase library route, the two steps for the formation of the [1,5]benzothiazepine derivatives were analyzed and optimized.

As illustrated in Scheme 2, the chalcones **1** were readily prepared by crotonic condensation of a number of aryl aldehydes with substituted acetophenones. Under the synthetic conditions set up during the chemical assessment for the library production, these intermediates were forced to precipitate from the reaction media, undergoing an "in situ" purification. This was effected by increasing the molarity of the reaction medium until a 1 M solution was reached. Actually, the assessment started from a 0.1 M mixture of equimolar amounts of the ketone and the aldehyde in EtOH/H_2O (4:1), using 2 equiv of potassium hydroxide at 40 °C, and eventually led to a 1 M reaction mixture at room temperature, using 5 equiv of the same base. The reaction yields increased accordingly from less than 30% to almost quantitative, and the desired intermediates could be recovered by a simple filtration.

The generation of the chalcone intermediates in good yield and high purity was, in the event, critical for the success of

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our project because we observed that the use of impure intermediates was detrimental to the [1,5]benzothiazepine synthesis.

The chalcone intermediates **1** were subsequently reacted with 2-aminothiophenol **2** in MeOH/AcOH to give the desired cyclized products **3** as depicted in Scheme 1.

A careful optimization of the reaction conditions was also performed for this step.

A slight excess of the desired thiol (20%) was fundamental to speed up the reaction, as well as an exact amount (0.5 equiv) of the acidic catalyst (AcOH). Best yields were obtained maintaining the reactants in refluxing methanol for about 5 h.

A number of alternative and well-known synthetic methods[4] for chalcone preparation were also examined. Unfortunately, in our hands and according to our experimental protocol, they resulted as unsuitable for array synthesis because of the need for extensive chromatographic purification of the target compounds from the side products obtained.

Another problem that we faced during the chemical assessment of the scaffold preparation was related to the storage conditions of the *o*-aminothiophenol (*o*-ATP) intermediates.

These products, when conserved under a normal air atmosphere, rapidly converted into the corresponding *o*-aminophenyl disulfides, even if they were stored at low temperature. This, obviously, affected the purity of the reactants for the formation of the desired scaffold and increased the number of the side products. This degradation increased linearly with time and led to complete failure of the desired reactions after a couple of weeks from the first opening of the reactant bottle.

Therefore, the *o*-ATP intermediates were stored under a nitrogen or, preferably, argon atmosphere and dispensed immediately before the reaction. This simple experimental procedure reduced the above-mentioned oxidation to acceptable limits.

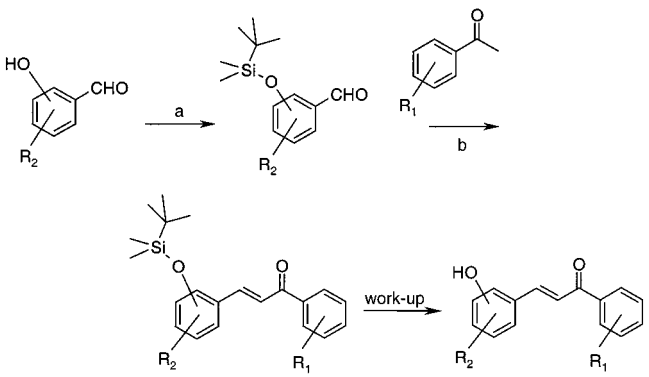
As far as the *o*-ATP oxidation problem was concerned, we also investigated the possibility to add mild reducing agents to the reaction mixture to reconvert the disulfides to the corresponding amino-thiophenols during the cyclization step. Despite some encouraging preliminary results, we decided to abandon this alternative because of the increased operational complexity.

Another interesting observation was made during the set up process. The analytical results showed that the desired chalcones were easily synthesized in solution phase in high yields and good purities as long as the aromatic aldehyde used did not contain any phenolic residues.

The presence of free phenolic hydroxyl groups on the aldehyde counterpart led to poor or negligible yields. Consequently, we were compelled to identify an appropriate protecting group for the phenolic moiety, and we investigated the possibility to mask it as either a TBDMS- or allyl-ether. Once these residues were protected, we verified that the aldehydes now reacted according to the normal experimental conditions to furnish the desired intermediates.

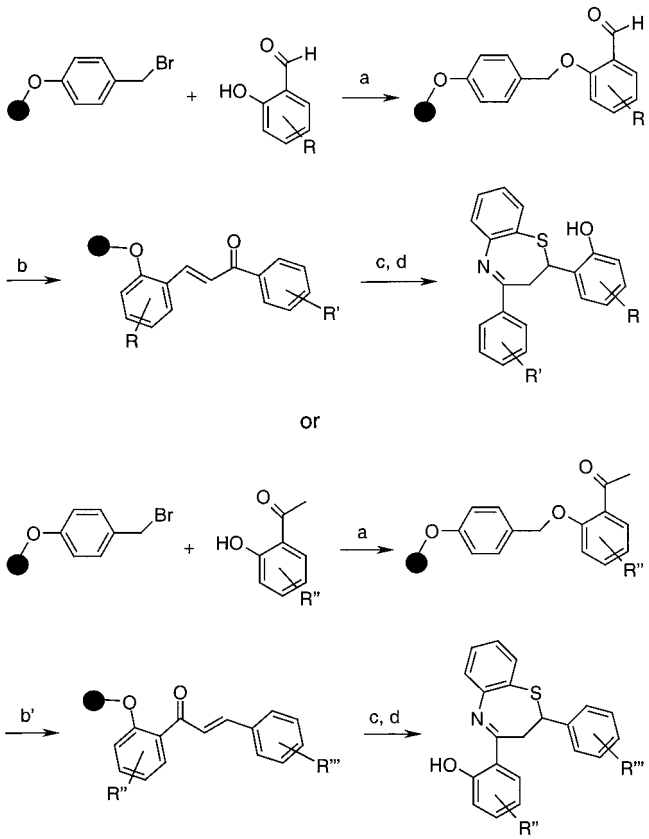
The TBDMS group was chosen as our preferred protecting group because it proved labile to the optimized workup conditions, thus the unprotected chalcones were isolated directly without any need of a specific deprotection step as depicted in Scheme 3.

Scheme 3[a]



[a] Reagents: (a) TBDMSCl, TEA, CH_2Cl_2; (b) MeOH/AcOH.

Scheme 4[a]



[a] Reagents and conditions: (a) CS_2CO_3, NaI, DMF, 50 °C; (b) R′-COMe, NaOMe, MeOH, THF; (b′) R‴-CHO, NaOMe, MeOH; (c) THF or DMF, cat. AcOH, 60 °C, 5 h and then rt overnight; (d) TFA, rt, 3 h.

Solid Phase Synthesis Approach: Preliminary Studies. We also envisaged the possibility to develop a solid phase synthesis approach as an alternative to the use of protecting groups to mask a phenolic hydroxyl-group on the aldehyde or on the ketone. This functional group, actually, could be used as an anchor point to an appropriate resin. The solid phase approach would guarantee a greater purity of the chalcone intermediates as well as the possibility of obtaining benzothiazepines bearing phenolic groups without the need of introducing protecting groups as depicted in Scheme 4. Moreover, the solid phase approach, once optimized, would

also allow the preparation of larger libraries with respect to the solution phase ones.

Some interesting preliminary results are here reported, while the final set up of the solid phase approach is currently in progress.

In a first attempt, we used Wang resin to anchor the aldehydes or the ketones via a Mitsunobu reaction (PPh_3/DEAD/THF), but the methodology proved to be disappointing in our hands because of the poor loading obtained. We therefore moved toward chloro- or bromo-Wang resin using alkylating conditions. The bromo-Wang system proved to be optimal for our purposes and we achieved the coupling of the desired reactant in an almost quantitative fashion using Cs_2CO_3/NaI in DMF at 40 °C. The formation of the chalcone in the next step was readily achieved by adding an excess of the desired ketone to the anchored aldehyde (or vice versa) in THF/MeOH and using freshly prepared sodium methoxide as a base at room temperature. Finally, the resin carrying the obtained chalcone was suspended in ethanol or THF and the *o*-ATP added together with a few drops of acetic acid. The reaction was kept at 60 °C for 5 h and underwent additional overnight stirring at room temperature. TFA cleavage allowed the recovery of the desired benzothiazepine, which was obtained in acceptable yields over four steps.

Monomer Selection and Solution Phase Library Preparation. After having set up the reaction conditions for the solution library using array synthesis, we applied computational methods to identify the commercially available monomers able to cover the maximum diversity in chemical space within the limits of our 20 × 20 planned library. Some monomers, selected with the below reported procedures, are represented in Figure 2.

Using EasyFilter[5] on the ACD database,[6] 51 acetophenones and 69 aryl-aldehydes were selected using appropriate chemical filters. The 2-amino-thiophenol derivatives were limited to the only two commercially available monomers. The consequent virtual library was fully enumerated, resulting in 2 × 51 × 69 = 7038 compounds. Successively VOLGA,[7] a program for library optimization, was used in order to find a sublibrary of size equal to 1 × 20 × 20 = 400 compounds containing the maximum number of molecules satisfying the following cutoffs:[8] MW < 650; ClogP < 7; rotatable bonds < 10; aromatic rings < 4; 1 < HB donors < 6; HB acceptors < 10. After 4000 iterative steps VOLGA was able to elaborate a library containing 92% of "desired" molecules compared with the initial 13% obtained by a random selection of monomers as reported in Figure 1 (see also Figure 2).

In the meantime, in order to have a fast and reliable characterization of the library obtained, a high throughput analytical method was also set up in addition to the HPLC system used for assessment of the reaction conditions. We decided to move from a HPLC system using UV diode array detection to a HPLC/MS system using MS detection. Using the conditions set up and reported in the experimental conditions, we were able to identify the formation of the desired intermediates and determine the percentages of the starting materials still present in each well.

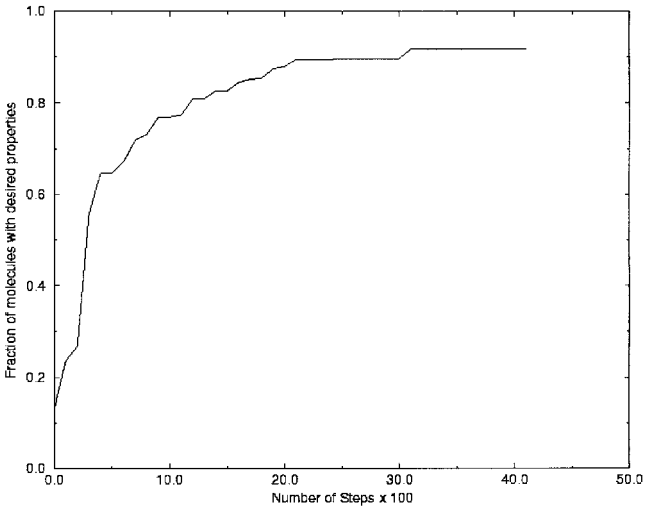


Figure 1. VOLGA optimization of the best 1 × 20 × 20 sublibrary from a 2 × 51 × 69 virtually accessible one. The best library after 4000 steps had a fraction of molecules satisfying the cutoffs equal to 0.92.

Finally, at this stage of the work, 160 derivatives (two plates out of five) of the projected 400 derivatives composing the library were obtained in solution. We randomly sampled these plates, and we found that the selected derivatives were endowed with an acceptable degree of purity (greater than 80%). The spectral data of eight of these products are reported in the Experimental Section material. Once the library is completed, it will be submitted to a primary MIC test on three selected microbial strains (*Streptomyces aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae*) to test our original hypothesis.

Experimental Section

Infrared spectra were recorded on a Bruker IFS 48 spectrometer. ^{1}H NMR spectra were recorded on a Varian Unity 400 (400 MHz); the data are reported as follows: chemical shift in ppm from the Me_4Si signal as external standard, multiplicity (b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), and coupling constants.

Flash chromatography was carried out on Merck silica gel 60 (230−400 mesh). Mass spectra were performed on a triple quadrupole (VG-4 Fison Instrument, U.K.) equipped with fast atom bombardment (FAB) ionization. Anhydrous DMF was purchased from Aldrich; THF was used after distillation over K/benzophenone; CH_2Cl_2 and CH_3CN were used after distillation over P_2O_5. Reactions were monitored by analytical thin-layer chromatography (TLC) using Merck silica gel 60 F-254 glass plates (0.25 mm). Resins used are available from Novabiochem.

Analytical Method. All the HPLC-MS data were obtained using a HP1100 liquid chromatography system equipped with a diode array detector (Hewlett-Packard, Germany) coupled with a Platform II mass spectrometer (Micromass Ltd., U.K.). The autosampler was a Gilson 233XL (Gilson, France). All samples were analyzed by flow injection mass spectrometry and by liquid chromatography mass spectrometry (HPLC/MS), both performed with the same equipment. Flow injection analyses were obtained infusing 20 μL of each

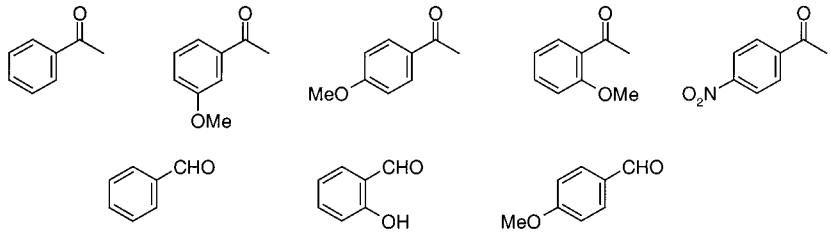


Figure 2. Some selected monomers.

sample into the mass spectrometer by autosampler. The mass spectrometer worked in positive electrospray ionization mode (ES+). The chromatographic separations were obtained using a Supelcosil ABZ+ Plus column (Supelco, USA) 3.3 × 0.46 cm, 3 μm. The mobile phase was a mixture of water (A) and acetonitrile (B) from 20% to 90% of B in 8 min, and then 5 min with 90% of B. The flow rate was 0.8 mL/min. The injection volume was 20 μL. Diode array chromatograms were collected using a large bandwidth (from 220 to 350 nm).

Solution Phase Synthesis. Synthesis of Chalcones. General Procedure. To a solution of acetophenone (0.5 mmol) and benzaldehyde (0.5 mmol) in dry methanol (0.4 mL) was added KOH (2.5 mmol) in distilled water (0.1 mL). After standing at room temperature overnight (ca. 20 h), water was added to the mixture and the precipitated collected. The crude product was used without further purification.

If, after water addition, no precipitation was detected in the majority of the wells, then neutralization with 1 M HCl and extraction with CH_2Cl_2 followed. The two phases were separated with a filter plate containing polypropylene support. The crude product was used without further purification also in this case.

Synthesis of [1,5]Benzothiazepines. General Procedure. A mixture of chalcone (0.5 mmol), glacial acetic acid (0.25 mmol), and *o*-aminothiophenol (0.6 mmol) in dry methanol was refluxed for 5 h. The solvent was removed in vacuo. The crude was analyzed and tested as such.

Solid Phase Synthesis. Supporting of Hydroxy-aldehydes or Hydroxy-ketones. General Procedure. A mixture of Wang bromide resin (1.10 mmol/g) (0.55 mmol), aldehyde or ketone (2.75 mmol), Cs_2CO_3 (1.65 mmol), and NaI (0.55 mmol) in DMF (8 mL) was stirred at 50 °C for 5 h. The resin was filtered and washed with 2:1 DMF/H_2O, 9:1 DMF/H_2O, DMF (×2), and alternating MeOH and DCM (×4). After drying under vacuum overnight, the supported reactant on Wang resin was obtained. After cleavage of a small amount (90 mg) of the resin so obtained, more than 60% of the aldehyde or of the ketone was recovered. The alkylation was also checked by nanoprobe.

Synthesis of Chalcone. General Procedure. Twelve equivalents of ketone or aldehyde were added to the resin (500 mg), preswelled in THF (2.5 mL) at room temperature. Then a solution of MeONa (0.5 M in MeOH) (2.5 mL) was added slowly. The mixture was stirred at room temperature overnight.

The resin was washed with alternating MeOH and DCM (×5) and dried under vacuum (50 °C) overnight. After cleavage of a small amount (30 mg) of the resin so obtained, more than 70% of the chalcone was recovered. The formation of the chalcone was also checked by nanoprobe.

Synthesis of [1,5]Benzothiazepines. General Procedure. The supported chalcone (230 mg) was suspended in THF. A few drops of acetic acid were added, and the mixture was heated to 60 °C for 5 h.

The resin was washed with alternating MeOH and DCM (×5) and dried under vacuum (50 °C) overnight. After cleavage was carried out in TFA/DCM 50% at room temperature for 1 h, the [1,5]benzothiazepine was obtained in a total yield of more than 60%.

Characterization of Selected [1,5]Benzothiazepines. The purity of the below reported products is to be considered greater than 80%.

2,3-Dihydro-2-(2-methoxyphenyl)-4-(2-hydroxyphenyl)-[1,5]benzothiazepine. MS: *m/z* 362 [MH]. ^{1}H NMR: 3.93 (s, 3H), 2.90 (t, 1H), 3.48 (dd, 1H), 5.59 (dd, 1H), 6.92 (d, 1H), 6.95 (m, 2H), 7.11 (d, 1H), 7.23 (dt, 1H), 7.28 (dt, 1H), 7.33 (dd, 1H), 7.43 (dt, 1H), 7.48 (dt, 1H), 7.56 (dd, 1H), 7.74 (dd, 1H), 7.83 (dd, 1H), 14.6 (bs, 1H). Yield = 44%.

2,3-Dihydro-2-(4-methoxyphenyl)-4-(2-hydroxyphenyl)-[1,5]benzothiazepine. MS: *m/z* 362 [MH], 242, 228. ^{1}H NMR: 3.07 (t, 1H), 3.39 (dd, 1H), 3.82 (s, 3H), 5.06 (dd, 1H), 6.87 (d, 2H), 6.92 (dt, 1H), 7.09 (dd, 1H), 7.23 (dt, 1H), 7.25 (d, 2H), 7.33 (dd, 1H), 7.42 (dt, 1H), 7.50 (dt, 1H), 7.59 (dd, 1H), 7.66 (dd, 1H), 14.6 (bs, 1H). Yield = 35%.

2,3-Dihydro-2-phenyl-4-(2-hydroxyphenyl)-[1,5]benzothiazepine. MS: *m/z* 332 [MH], 228. ^{1}H NMR: 3.12 (t, 1H), 3.46 (dd, 1H), 5.09 (dd, 1H), 6.94 (m, 1H), 7.20 (bm, 1H), 7.26 (m, 1H), 7.3−7.4 (m, 7H), 7.46 (m, 1H), 7.53 (m, 1H), 7.61 (m, 1H), 7.69 (m, 1H), 14.0 (broad, 1H). Yield = 80%.

2,3-Dihydro-2-(4-nitrophenyl)-4-phenyl-[1,5]benzothiazepine. MS: *m/z* 361 [MH], 212, 254. ^{1}H NMR: 3.08 (t, 1H), 3.35 (dd, 1H), 5.05 (dd, 1H), 7.20 (m, 1H), 7.37 (m, 1H), 7.49 (d, 2H), 7.5−76 (m, 4H), 7.61 (m, 1H), 8.08 (d, 2H), 8.19 (d, 2H). Yield = 85%.

2,3-Dihydro-2,4-diphenyl-[1,5]benzothiazepine. MS: *m/z* 316 [MH], 212. ^{1}H NMR: 3.08 (t, 1H), 3.33 (dd, 1H), 5.00 (dd, 1H), 7.16 (t, 1H), 7.35 (d, 1H), 7.26−7.50 (m, 5H), 7.46−7.54 (m, 4H), 7.63 (dd 1H), 8.07 (dd, 2H). Yield = 60%.

2,3-Dihydro-4-(4-methoxyphenyl)-2-phenyl-[1,5]benzothiazepine. MS: *m/z* 346 [MH], 242. ^{1}H NMR: 3.08 (t, 1H), 3.32 (bd, 1H), 3.91 (s, 3H), 4.98 (dd, 1H), 7.02 (m, 2H), 7.16 (bm, 1H), 7.30 (m, 6H), 7.48 (m, 1H), 7.63 (d, 1H), 8.07 (bs, 2H). Yield = 74%.

2,3-Dihydro-2-(3-methoxyphenyl)-4-(2-hydroxyphenyl)-[1,5]benzothiazepine. MS: *m/z* 362 [MH]. ^{1}H NMR: 3.08

(t, 3H), 3.41 (dd, 1H), 3.77 (s, 3H), 5.03 (dd, 1H), 6.83 (dd, 1H), 6.89 (m, 3H), 7.08 (d, 1H), 7.23 (m, 2H), 7.33 (dd, 1H), 7.42 (dt, 1H), 7.50 (dt, 1H), 7.57 (bd, 1H), 7.67 (dd, 1H). Yield = 60%.

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