

# Facile microwave synthesis of a novel phenothiazine derivative and its cytotoxic activity

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**Abstract:** Herein, a facile procedure for microwave synthesis and NMR characterization of phenothiazine derivatives 10-(3-hydroxypropyl)-2-(methylsulphanyl)-10*H*-phenothiazine (**3**) (25% yield) and 1-[3-(2-methylsulfonyl-10*H*-phenothiazine-10-yl)-propyl]pyrimidine-2,4-(1*H*,3*H*)-dione (**6**) (67% yield) is described. After successful microwave synthesis steps, MTT cytotoxicity experiments gave rise to greater cytotoxic effect of compound **6** in MCF-7 human breast adenocarcinoma cell line (IC<sub>50</sub>= 1.35 μM) as compared to literature values for tamoxifen (IC<sub>50</sub>= 8.3 μM) and doxorubicin (IC<sub>50</sub>= 27 μM).

**Keywords:** Microwave synthesis; phenothiazine derivatives; 1-(3-(2-methylsulfonyl-10*H*-phenothiazine-10-yl)-prop-1-yl)pyrimidine-2,4(1*H*,3*H*)-dione; NMR; IC<sub>50</sub>. ©2020 ACG Publication. All right reserved.

## 1. Introduction

Phenothiazine derivatives with a propyl bridge are known to be antagonists of D<sub>2</sub>-type (D<sub>2</sub> and D<sub>3</sub>) dopaminergic receptors, generating both antipsychotic<sup>1</sup> and anticancer effects.<sup>2</sup> The anticancer effect of phenothiazine derivatives such as thioridazine<sup>2</sup> is through the inhibition of D<sub>2</sub>-type dopaminergic receptors in certain cancer stem cells.<sup>2</sup>

Phenothiazine type antipsychotic drugs contain a phenothiazine ring (2 in Scheme 1), a basic heterocyclic ring with an amine group, and a propyl group that bridges the basic heterocyclic group to the phenothiazine ring at position 10, such as 6 and 7 in Scheme 1. In drug discovery, an electronegative substituent like -S-CH<sub>3</sub>, -CF<sub>3</sub>, -Cl, or -CN at position 2 of the phenothiazine ring (2 in Scheme 1) increases the neuroleptic effect of the corresponding psychotic drug.<sup>1</sup> Furthermore, any reduction or increase in the carbon chain of the propyl bridge will significantly decrease the neuroleptic effect of phenothiazine type antipsychotic drugs.<sup>1</sup> For instance; promethazine with a two-carbon bridge possesses antihistaminic effect<sup>3</sup> rather than neuroleptic effect.

In general, the synthesis of phenothiazine derivatives with a propyl bridge involves *N*-alkylation reactions in toluene using a very strong base such as NaNH<sub>2</sub> (sodium amide).<sup>4,5</sup> Although reaction product yields for the *N*-alkylation of the phenothiazine ring using NaNH<sub>2</sub> is reported to vary between 80-90%,<sup>5</sup> some alkene by products are likely to form by the reaction of NaNH<sub>2</sub> (as a very strong base) with alkyl halides and also the import of NaNH<sub>2</sub> is banned at the customs of some countries (including Turkey) as NaNH<sub>2</sub> is used in making explosives. Alternatively, *N*-propionyl conjugation of 3-chloropropionyl chloride on phenothiazine followed by reduction of the *N*-propionyl group with borane (BH<sub>3</sub>) was reported in literature.<sup>6</sup> However, the latter reactions increase the number of reaction steps as well as the cost to produce phenothiazine derivatives.<sup>7</sup>

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*N*-alkylation reactions of phenothiazine derivatives are the most critical steps in terms of cost, time and yield. Aside from amine alkylation reactions under basic conditions, modern green chemistry methods utilizing microwave (MW) irradiation for rapid synthesis of alkylated amines in solvent<sup>7,8</sup> and solvent-free<sup>9</sup> systems have attracted much attention. For instance, step-wise alkylation of secondary amines in basic water solution by MW irradiation was reported to give rise to tertiary amines only.<sup>7</sup> However, MW reactions in solvent systems commonly face some problems due to solubility (of the solute) and volatility (of the solvent) issues as well as temperature related limitations arising from the boiling point of the solvent used. Interestingly, Sarmiento et al.<sup>8</sup> synthesized (in ~60% yield) a *N*-propionyl derivative of phenothiazine in DMF catalyzed by MW irradiation, which could then be reduced by diborane (B<sub>2</sub>H<sub>6</sub>) to yield a *N*-propyl linked phenothiazine derivative. However, Sarmiento et al. work requires additional steps and work-up to obtain the final phenothiazine product with a propyl linker, which may be costly and time consuming. Using solvent-free conditions, a microwave irradiation procedure for the *N*-alkylation of an amine compound (isatin) using alkyl halides and Na<sub>2</sub>CO<sub>3</sub> (as base) was previously published by Shmidt et al.<sup>9</sup>

Herein, this paper presents a facile microwave irradiation method under solvent-free conditions to synthesize a novel phenothiazine derivative with a propyl linker, 1-(3-(2-methylsulfonyl-10*H*-phenothiazine-10-yl)-prop-1-yl)pyrimidine-2,4-(1*H*,3*H*)-dione, which was initially designed *in silico* as an antagonist of human D<sub>2</sub> dopaminergic receptor.

## 2. Experimental

### 2.1. Chemical Material and Instrumentation

Glassware were dried at 120 °C over 24 hours before using for chemical reactions. For reactions, 5 mL, 25 mL, 50 mL round-bottom flasks were used as needed. Reaction systems were sealed with rubber-septums. All reactions were performed under N<sub>2</sub> atmosphere. NaH used in the reaction was washed with CH<sub>2</sub>Cl<sub>2</sub> four times before use. Fluorescent silicagel on Al Foils (F: 254 nm) (Sigma-Aldrich, Milwaukee, WI, USA) was used for thin layer chromatography. Silicagel with a pore size of 60 Å was used in column chromatography as the solid phase. The required solvents were all chemical grade and used without purification as purchased from companies. 2-(methylsulphonyl)-10*H*-phenothiazine was purchased from Sigma-Aldrich (Milwaukee, WI, USA).

1D and 2D NMR spectra were recorded on a 400 MHz Varian NMR instrument at the Advanced Technology and Research Institute at Konya Selcuk University, Turkey. Deuterated chloroform (CDCl<sub>3</sub>, δ=7.23 ppm) was used as the solvent in NMR experiments. NMR Fourier Induction Decay (FID) data were processed and converted into spectra using VNMRJ4.2 Software. <sup>1</sup>H, <sup>1</sup>H-ROESY NMR FID data were collected between -1 ppm and 14 ppm with 1024 data points in F1 dimension and 1024 data points in F2 dimension. <sup>1</sup>H, <sup>1</sup>H-ROESY NMR FID data were zero-filled and linear-predicted to 2K data points in both dimensions. The FID data were then apodized in both dimensions using 90° Sine and 0.07 Hz Gaussian functions in both dimensions. Apodized ROESY FID data were Fourier transformed in inverse and quadrature modes along t<sub>2</sub> dimension and in inverse and quadrature modes using the hypercomplex protocol in t<sub>1</sub> dimension. ROESY spectra were displayed and analyzed in phase sensitive mode.

Perkin Elmer Spectrum 100 Infrared Spectrometer (Waltham, MA, USA) was used to collect FT-IR spectra. After the samples were pelleted with KBr, FT-IR spectra were recorded in the spectral range 650-3800 cm<sup>-1</sup>. Mass spectroscopy experiments were implemented in ESI<sup>+</sup> mode by ESI<sup>+</sup>-LCMS (*m/z* %) on Waters Micromass ZQ Mass Spectrometer (Milford, MA, USA). Microwave synthesis of compounds 3 and 6 were carried by MARS 5 Microwave Accelerated Reaction System (CEM Corporation, North Carolina, USA).

### 2.2. Biological Materials and Apparatus

Human breast ductal adenocarcinoma cell line [MCF-7 (ER<sup>+</sup>/PR<sup>+</sup>)] was purchased from ATCC (American Type Culture Collection, Gaithersburg, Maryland, USA). These cells were cultured in flasks (at 37 °C and under 5% CO<sub>2</sub> atmosphere) using RPMI medium (Biochrome, Germany) containing 10% FBS (Fetal Bovine Serum) (Biochrome, Germany) and 1% penicillin/streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin) (Biochrome, Germany). After cells filled out 80% of the culture flasks, they were then washed out with 1xPBS (Gibco, USA) and detached with treatment of 0.25% of Trypsin-

EDTA (Biochrome, Germany) enzymatic solution. Required amount of detached cells were used for IC<sub>50</sub> studies, while the rest was treated with 10% DMSO in RPMI medium and stored at -80 °C for later use.

### 2.3. Chemistry

#### 2.3.1. Microwave Synthesis of Compound 3

Into an explosion proof 15 mL teflon chamber, 1750 mg 2-(methylsulphanyl)-10H-phenothiazine (7.13 mmol), 907 mg Na<sub>2</sub>CO<sub>3</sub> (8.55 mmol), 715  $\mu$ L 3-chloropropanol (d: 1.131 g/mL; 8.55 mmol) and 15  $\mu$ L of dry *N,N*-dimethylformamide (DMF) was added and mixed gently by a thin spatula. The reaction vessel was then sealed tightly and mounted into MARS 5 Microwave Accelerated Reaction System (CEM Corporation, North Carolina, USA). 800 watts of microwave energy was applied for 15 minutes followed by monitoring by TLC ( $R_f$  = 0.4, Hexane/Ethylacetate, 7:3). Fifteen minutes of microwave irradiation of the reaction was repeated 3 more times (a total of 1 hour) to confirm the completion of the reaction by TLC. The desired compound was purified by silicagel column chromatography (Hexane/Ethylacetate, 7:3). Yield: 542 mg (25%, 1.78 mmol). ESI<sup>+</sup>-LCMS ( $m/z$  %) MW: 303.29; computed MW: 304 Da. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm) = 6.80-7.20 (7H, m), 4.78 (1H, s), 4.02 (1H, t,  $J$  = 6.40 Hz and 6.00 Hz), 3.78 (1H, t,  $J$  = 5.60 Hz), 2.04 (1H, m), 1.60 (3H, s). FT-IR (KBr, cm<sup>-1</sup>): 3328, 2944, 2878, 1566, 1458, 1300, 1248, 1034, 932, 748.

#### 2.3.2. One-pot Microwave Synthesis of Compound 6

100 mg of **3** (0.32 mmol, 1 eq) previously dissolved in dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>, 4 mL) was transferred via a dry syringe into a dry 25 mL round-bottom flask sealed with a rubber septum under N<sub>2</sub> atmosphere. Then, 67  $\mu$ L of triethylamine (48.6 mg, 48 mmol, 1.5 eq) and 35  $\mu$ L of PBr<sub>3</sub> (0.35 mmol, 1.2 eq) were micropipetted into the reaction vessel, respectively. The reaction was stirred under N<sub>2</sub> atmosphere for 2 hours. The completion of the synthesis of the intermediary product, 10-(3-bromoprop-1-yl)-2-(methylsulfonyl)-10H-phenothiazine (**4** in Scheme 1), was monitored by TLC ( $R_f$  = 0.38-0.85, Hexane/Ethylacetate, 9:1). Then, solvent of the mixture was evaporated under vacuum and the residue of **4** was transferred into an explosion proof 15 mL teflon chamber for microwave synthesis. Into the teflon chamber, Na<sub>2</sub>CO<sub>3</sub> (407 mg, 3.84 mmol, 1.2 eq), pyrimidine-2,4-(1*H*,3*H*)-dione (uracil, 51 mg, 0.45 mmol, 1.4 eq) and 15  $\mu$ L of dry *N,N*-dimethylformamide (DMF) were added respectively, and then mixed gently by a thin spatula. The reaction vessel was then sealed tightly and mounted into MARS 5 Microwave Accelerated Reaction System (CEM Corporation, North Carolina, USA). 800 watts of microwave irradiation energy was applied for one hour to complete the reaction, which was confirmed by TLC ( $R_f$  = 0.57, Hexane/Ethylacetate, 1:1). Compound **6** was isolated by silicagel column chromatography (Hexane/Ethylacetate, 1:1). Off-white solid (83 mg, 0.21 mmol, 67% isolated yield). ESI<sup>+</sup>-LCMS ( $m/z$  %): 397.87 Da (calculated M.W. 397.51 Da). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>, ppm):  $\delta$  8.76 (1H, s), 7.22-6.76 (7H, m), 7.05 (1H, d, <sup>3</sup> $J_{HH}$  7.20 Hz), 5.29 (1H, d, <sup>3</sup> $J_{HH}$  5.60 Hz), 3.91 (1H, t, <sup>3</sup> $J_{HH}$  5.60 Hz), 3.79 (1H, t, <sup>3</sup> $J_{HH}$  6.40 Hz), 2.23 (1H, p), 1.25 (1H, s).

### 2.4. Biological Assay

#### 2.4.1. MTT Citotoxicity Assay

MCF-7 cancer cell inhibition assay (IC<sub>50</sub>) was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide] kit by Promega (Madison, WI, USA).<sup>10-12</sup> A certain number of cells were transferred into the inner wells of 96-well plates and incubated for 24 hours. Plate wells were added a fixed concentration of MTT solution and incubated for 2.5 hours at 37 °C under 5% CO<sub>2</sub> atmosphere. 200  $\mu$ L of solution from each well was transferred to the inner wells of 96-well plate for reading absorbances at 490 nm by Elisa Plate Reader. Increasing concentrations of compound **6** were added into the wells (three determinations for each concentration scale) and incubated for 72 hours at 37 °C under 5% CO<sub>2</sub> atmosphere. Cells in the wells were then treated with MTT solution for

reading absorbance values at 490 nm by Elisa Plate Reader. Percent inhibition was then computed as follows:

$$\text{Inhibition \%} = [1 - (\text{avg-OD}^{490}_{\text{compound+MCF-7}} / \text{avg-OD}^{490}_{\text{MCF-7}})] \times 100 \quad \text{EQ.1}$$

where  $\text{avg-OD}^{490}_{\text{compound+MCF-7}}$  is the average of absorbance values at 490 nm for MCF-7 cells treated with compound 6 and  $\text{avg-OD}^{490}_{\text{MCF-7}}$  is the average of absorbance values at 490 nm for intact MCF-7 cells.

### 2.5. Quantum Mechanics Computational Studies

3D structures of compounds 6 and 7 were determined by GAMESS US quantum mechanics software v.2010.R2.<sup>13</sup> The computations were implemented in the gas phase using the density functional theory (DFT) at the B3LYP/6-31+G(d,p) basis set. Starting structure coordinates and geometry optimization input parameters (gradient convergence tolerance =  $10^{-4}$ , density convergence =  $10^{-5}$ , linear dependence threshold =  $10^{-6}$ , self-consistent field = RHF) for GAMESS US were prepared and visualized by Avogadro Software v.1.2.0.<sup>14</sup>

## 3. Results and Discussion

### 3.1. Chemistry

A synthetic route to synthesize compound **3** and **6** by microwave irradiation method is given in Scheme 1. Starting from 2-methylthio-10*H*-phenothiazine, 3-(2-methylthio-10*H*-phenothiazine-10-yl-propyl)-pyrimidine-2,4-(1*H*,3*H*)-dione (**6** in Scheme 1) was synthesized in two steps by microwave irradiation and characterized by NMR, FT-IR and mass spectroscopy techniques.

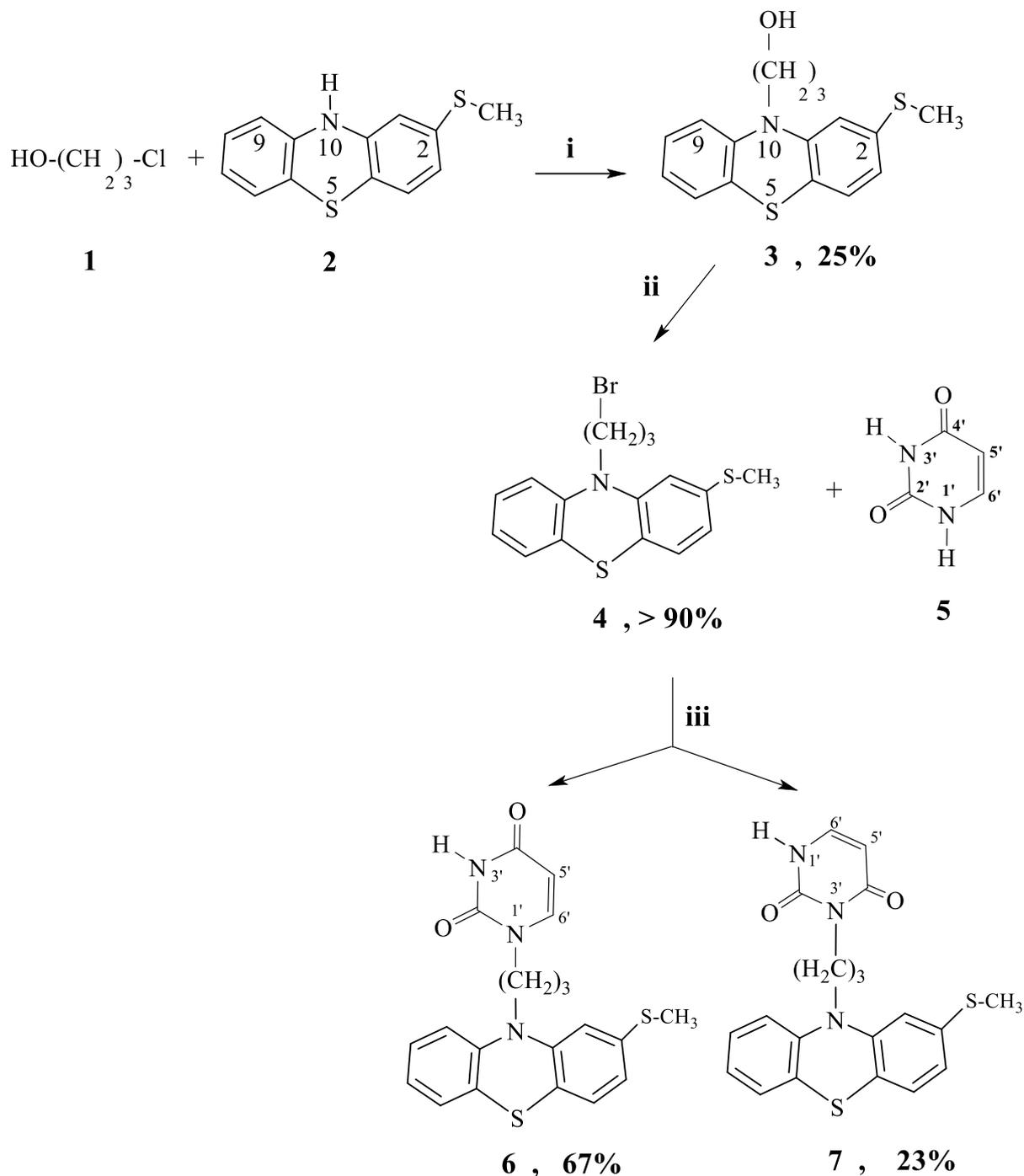
For solvent-free synthesis of *N*-alkylation reactions by MW irradiation, Shmidt et al.<sup>9</sup> reported that optimal conditions for the *N*-alkylation reaction was obtained in presence of  $\text{Na}_2\text{CO}_3$  and a few drops of DMF. In similar to Shmidt et al.<sup>9</sup> work,  $\text{Na}_2\text{CO}_3$  was initially added to carry out microwave synthesis of reactions **i** and **iii** (Scheme 1) and was found out that the addition of  $\text{Na}_2\text{CO}_3$  was not sufficient to reach high yields in *N*-alkylation. After several more trials, it was found that addition of a tiny amount of DMF (*N,N*-dimethylformamide) along with  $\text{Na}_2\text{CO}_3$  into reactions **i** and **iii** significantly improved the yields for the synthesis of compounds **3** and **6** (Scheme 1), respectively, which was later noticed to be consistent with Shmidt et al. work.<sup>9</sup>

Microwave irradiation of reaction **i** (Scheme 1), which uses 2-methylthio-10*H*-phenothiazine and 3-chloropropanol, gave rise to compound **3** [ESI<sup>+</sup>-LCMS (*m/z* %): 303.29; calculated MW: 304 Da] (Figure S1) with relatively an affordable yield, 25%. Although several other MW reaction conditions (i.e.; doubling  $\text{Na}_2\text{CO}_3$  concentration, increasing DMF concentration upto 200  $\mu\text{L}$ ) were tried, the reaction yield of compound **3** could not be optimized for an unknown reason. <sup>1</sup>H-NMR (Figures S2 and S3) and FT-IR (Figure S4) spectra confirmed compound **3** in high purity. As seen in Figure S2, multiplet signals around 6.8-7.2 ppm are assigned to the phenothiazine protons, a broad peak at 4.78 ppm belongs to the -OH proton. The triplet signals at  $\delta_{\text{H}}$  4.02 and 3.78 and the multiplet signal at  $\delta_{\text{H}}$  2.04 belong to the methylene (-CH<sub>2</sub>-) protons (Figure S3). Also the singlet signal at  $\delta_{\text{H}}$  1.60 matches -S-CH<sub>3</sub> methyl protons (Figure S3).

FT-IR spectrum in Figure S4 reveals a broad peak at 3328  $\text{cm}^{-1}$  for -OH stretching. Characteristic vibrations pertaining to the aliphatic C-H stretching of the -CH<sub>2</sub>- groups were observed at 2944-2878  $\text{cm}^{-1}$  in FT-IR spectrum of compound **3** (Figure S4). Characteristic aromatic -C=C stretching were observed at 1566 and 1458  $\text{cm}^{-1}$ . Aromatic and aliphatic -C-N- stretching were observed at 1300 and 1248  $\text{cm}^{-1}$ , respectively. Moreover aromatic -C-S-, aliphatic -C-C(OH), and aliphatic -S-C(H<sub>3</sub>) stretchings were observed at 1034  $\text{cm}^{-1}$ , 932  $\text{cm}^{-1}$  and 748  $\text{cm}^{-1}$ , respectively.

In general, preparation of phenothiazine derivatives with a propyl bridge involves *N*-alkylation reactions in toluene using a very strong base such as  $\text{NaNH}_2$  (sodium amide).<sup>3</sup> Instead of  $\text{NaNH}_2$ , synthesis of compound **3** was tried using several other bases including NaH, 4-dimethylaminopyridine (DMAP),  $\text{Na}_2\text{CO}_3$ , and triethylamine to carry out reaction **i** in Scheme 1 at varying high temperatures. Unfortunately, attempts to synthesize compound **3** under different basic conditions in organic solvents

failed. Therefore, these laboratory results indicate that *N*-alkylation reactions by microwave irradiation is a good choice of method as a replacement of using NaNH<sub>2</sub> in the preparation of phenothiazine derivatives.



**Scheme 1.** Microwave synthesis of a derivative of 2-methylsulfanyl-10H-phenothiazine. Reaction conditions, **i**: Na<sub>2</sub>CO<sub>3</sub>/DMF/microwave (800 watts)/1 h; **ii**: PBr<sub>3</sub>/TEA/CH<sub>2</sub>Cl<sub>2</sub>/25 °C/1 h; **iii**: Na<sub>2</sub>CO<sub>3</sub>/DMF/microwave (800 watt)/1 h. (DMF: Dimethylformamide; TEA: triethylamine).

In an attempt to synthesize compound 3 using NaH, five different reactions were carried at various temperatures including 25 °C, 50 °C, 70 °C, 100 °C, and 120 °C. As a general reaction procedure,

to a solution of 515 mg of 2-(methylsulfanyl)-10*H*-phenothiazine (2 mmol) in 13 mL of xylene and 7 mL of CH<sub>2</sub>Cl<sub>2</sub> in a 50 mL round-bottom flask was added 120 mg of 60% NaH (3 mmol) and 208 mg of 3-chloropropanol (2.2 mmol) and stirred under N<sub>2</sub> atmosphere for 4 days at the aforementioned temperatures. The course of the reactions were monitored many times by TLC (Hexane:Ethylacetate 75:25). The reactions were terminated after four days by addition of 20 mL of ethylacetate into the reactions vessels, which were then transferred into separate extraction funnels. HCl formed in the reactions was neutralized by addition of 10 mL of 10% NaHCO<sub>3</sub> solutions into the extraction funnels. After extractions, the upper organic phase was separated and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. In all cases, the reactions did not give rise to high yields for a possible new product as determined by TLC (Hexane:Ethylacetate 75:25). The best TLC result was obtained for the reaction setup at 110 °C, which gave rise to two new weakly spots at R<sub>f</sub>=0.26 (spot-1) and R<sub>f</sub>=0.15 (spot-2) for the organic phase. Silicagel column chromatography purification (Hexane:Ethylacetate 75:25) of the organic extraction phase led to very low yields for the new products, ~25 mg (spot-1) and ~15 mg (spot-2), respectively. Unfortunately, <sup>1</sup>H-NMR spectra for the unknown products did not match the <sup>1</sup>H-NMR spectrum of **3** obtained by microwave synthesis (Figure S5), proving that using NaH as a base did not lead to the synthesis of compound **3**.

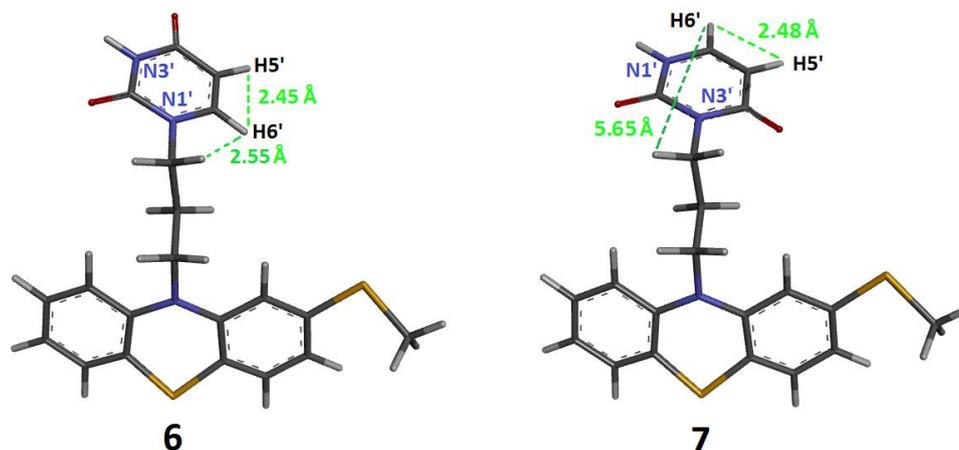
Using the same procedure above, the base NaH was replaced with Na<sub>2</sub>CO<sub>3</sub> (2.2 mmol), DMAP (4-dimethylaminopyridine, 2.2 mmol, pK<sub>a</sub> = 9.6), and TEA (triethylamine, 2.2 mmol, pK<sub>a</sub> = 10.75), respectively. Unfortunately, the last three bases did not give rise to new products at all as determined by TLC. Therefore, the microwave synthesis of compound **3** is currently the best method in the laboratory scale as compared to the synthetic procedure described above using NaH, Na<sub>2</sub>CO<sub>3</sub>, DMAP, and TEA as bases.

Compound **6** was obtained after a two-step reaction carried out in one pot. In the first reaction step 10-(3-bromoprop-1-yl)-2-(methylsulfanyl)-10*H*-phenothiazine (compound **4**) was synthesized by reacting compound **3** with PBr<sub>3</sub>. In the second reaction step, compound **6** was synthesized by reacting compound **4** with uracil in presence of Na<sub>2</sub>CO<sub>3</sub> and tiny amount of DMF under microwave irradiation. After work-up and purification by silicagel chromatography (Hexane/Ethylacetate, 1:1), compound **6** was obtained in high yield (67%). As seen in Scheme 1, compounds **6** [1'-(2-methylthio-10*H*-phenothiazine-10-yl-propyl)-pyrimidine-2',4'-(1'*H*,3'*H*)-dione] and **7** [3'-(2-methylthio-10*H*-phenothiazine-10-yl-propyl)-pyrimidine-2',4'-(1'*H*,3'*H*)-dione] are isomers with the same molecular weight (calculated M.W. 397.87 Da). Therefore, compound **6** was first confirmed by ES<sup>+</sup>-LCMS (*m/z* %: 397.87 Da; calculated MW. 397.51 Da) (Figure S6) and then <sup>1</sup>H-NMR (Figure S7) and ROESY NMR data (Figure S8).

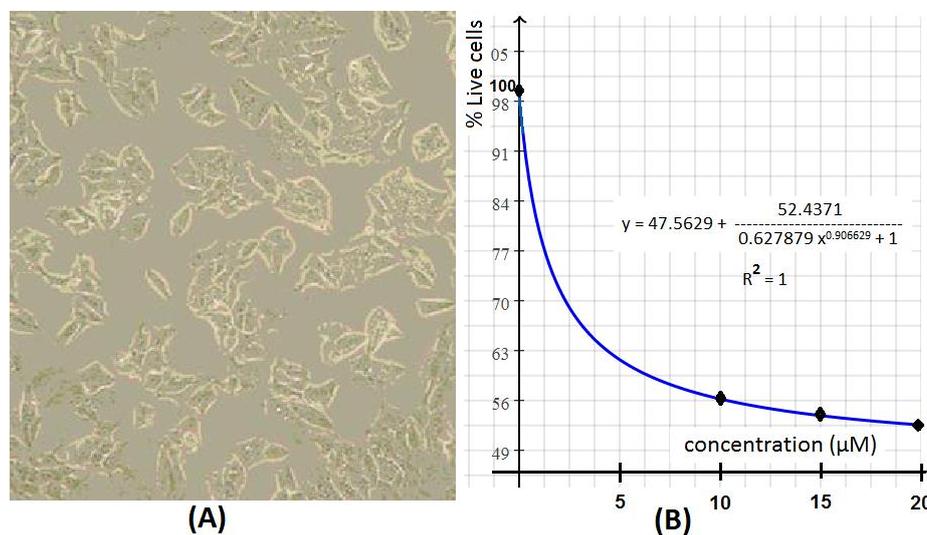
Assignment of the methylene proton peaks belonging to the propyl bridge requires more attention. The N-CH<sub>2</sub>- proton peaks appear as triplets at 3.91 ppm (<sup>3</sup>J<sub>HH</sub> 5.60 Hz) and 3.79 ppm (<sup>3</sup>J<sub>HH</sub> 6.40 Hz) due to their neighboring -CH<sub>2</sub>- methylene protons which appears as a pentate peak at 2.23 ppm (Figure S8). NOE cross-peak intensities were correlated to proton-proton distances in three ranges: 1.8-2.8 Å for strong NOEs, 2.8-3.3 Å for medium NOEs and 3.3-5.0 Å for weak NOEs.<sup>15</sup> Density functional theory QM computations revealed that a reference distance between the C5'H-C6'H protons of the pyrimidine-2,4-dione (uracil) ring is about 2.45 Å for compound **6** (Figure 1), which leads to a strong ROESY NOE cross-peak at 5.29 ppm (set to -1000 integral units for negative NOE), see Figure S8. A strong negative NOE cross-peak (with -993 integral units) that appears at 3.79 ppm spatially connects the N-CH<sub>2</sub>- methylene protons of the propyl bridge to the C6'H proton of the uracil ring (Figure S8). According to the Integrated Spin-Pair Approximation (ISPA) method<sup>16</sup> formulated as  $r_{ij} = r_{ref} (a_{ref} / a_{ij})^{1/6}$  (where  $r_{ref}$ =2.45 Å,  $a_{ref}$ = -1000 iu, and  $a_{ij}$ = -993 iu) to measure through-space proton-proton distances, the  $r_{ij}$  distance (for the C5'H-C6'H proton-proton system) is found to be roughly 2.50 Å, which is highly comparable to the C5'H-C6'H proton-proton distance (2.55 Å) of compound **6** as determined by quantum mechanics computations (Figure 1). Such a strong NOE connectivity is not expected for compound **7** as C5'H-C6'H proton-proton distance was computed to be more than 5 Å, which is way far from the limits of NOE detection by NMR (< 5 Å). Thus, the ROESY spectrum shown in Figure S8 strongly proves that the desired product is compound **6**.

One-pot synthesis of compound **6** was also tried by using NaNH<sub>2</sub> (sodium amide) as the base in the last step of the reaction pathway (Scheme 1). After the bromination reaction of **3** was complete to synthesize **4** as described in section 2.3.2, the solvent (CH<sub>2</sub>Cl<sub>2</sub>) of the reaction mixture was evaporated under vacuum. The remnant was then dissolved in 15 mL of anhydrous *N,N*-dimethylformamide (DMF)

under N<sub>2</sub> atmosphere. To the mixture of **4** was then added 46 mg of **5** (uracil, 0.41 mmol, 1.4 eq) and 18 mg of NaNH<sub>2</sub> (0.46 mmol, 1.6 eq) followed by magnetically stirring under N<sub>2</sub> atmosphere at room temperature for 16 hours. Completion of the reaction was confirmed by TLC (Hexane:Ethylacetate, 1:1). The reaction product **6** was purified by silicagel column chromatography (Hexane/Ethylacetate, 1:1). Off-white solid (76 mg, 0.19 mmol, 59% isolated yield). Comparison of <sup>1</sup>H-NMR spectrum with that of **6** obtained by microwave synthesis reaction confirms product **6** (Figure S9).



**Figure 1.** Quantum mechanics structures of compounds **6** and **7**.



**Figure 2.** (A) Cultured MCF-7 human breast adenocarcinoma cells, (B) line-fitting for % live cells plotted versus increasing concentrations of compound **6**

### 3.2. MTT Cytotoxicity Assays

Cytotoxic activity of 3-(2-methylthio-10*H*-phenothiazine-10-yl-propyl)-pyrimidine-2,4-(1*H*,3*H*)-dione (**6**) was determined by the MTT cytotoxicity assay against the MCF-7 cancer cell line (Figure 2A). Percent inhibition determined by EQ.1 in section 2.4.1 was converted to percent live cells by % live-cells = 100 - % inhibition, which were plotted against concentration as seen in Figure 2B. The graph was then line-fitted to determine an IC<sub>50</sub> value of 1.35 μM at 76% live cells, which is the average of upper and lower bounds for % live cells.

Indeed, the IC<sub>50</sub> value of compound **6** is considerably better as compared to prescribed chemotherapy agents applied for the treatment of breast cancer, i.e.; doxorubicin (IC<sub>50</sub> = 27 μM in MCF-7 cells)<sup>17</sup> and tamoxifen (IC<sub>50</sub> = 8.3 μM in MCF-7 cells).<sup>18</sup>

## 4. Conclusion

Herein, compound **6** was successfully synthesized in two steps by microwave irradiation method, purified and characterized by NMR, FT-IR and MS techniques. Our laboratory results indicate that the solvent-free microwave synthesis of compound **6** is about 5-10 % more efficient than that of the reaction catalyzed by NaNH<sub>2</sub>. In addition, the MW reactions applied in this work are less costly as the reaction is carried out under solvent-free conditions. Compound **6** was found to possess greater cytotoxic activity (IC<sub>50</sub> = 1.35 μM) as compared to some other prescribed chemotherapy agents (doxorubicin and tamoxifen) used in the treatment of breast cancer.

To the best of our knowledge, solvent-free microwave synthesis of phenothiazine type neuroleptics has not been reported before. Therefore, the solvent-free microwave synthesis of compounds **3** and **6** is likely to pave the ways to establish a general procedure for the synthesis of other phenothiazine derivatives possessing a propyl bridge.

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For its novelty and great cytotoxic activity, a Turkish patent was received for 3-(2-methylthio-10*H*-phenothiazine-10-yl-propyl)-pyrimidine-2,4-(1*H*,3*H*)-dione (Turkish Patent #: 2013/139199).

## Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/organic-communications>

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