Synthesis and Antitumor Evaluation of New Cyclohepta[*b*]Thiophene Analogs

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Abstract: New cyclohepta[*b*]thiophene analogs were designed and synthesized. Compounds **2-4**, **6** and **8a-c** were assessed for *in vitro* antitumor efficacy toward breast cancer (MCF-7), hepatocyte (HepG2) and colon adenocarcinoma (HT-29) cell lines. Compound **6** showed the highest efficacy toward the three tested cell lines. *In vivo* antitumor efficacy of compounds **2**, **6** and **8a**,**b** toward Ehrlich ascites carcinoma (EAC) in mice was also studied. The computational studies revealed that the explored compounds are fulfilling the optimal requirements for drug absorption with no predicted toxicity risks.
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Introduction

Cancer is considered as the main health problem with increasing global rate (Tavakkoli, 2012). Hence, recognizing new synthetic agents as lead compounds for exploring new antitumor therapeutics is critically desirable in order to improve survival rates and well-being. Molecular hybridization is an effective strategy that depends on combining pharmacophores of different bioactive moieties in the aim of giving access to lead compounds (Viegas-Junior, 2007). Following this strategy, new compounds were designed as potential antitumor agents.

2-Aminothiophene and cycloalkyl[*b*]thiophene derivatives appealed significant attention from medicinal chemists owing to their wide spectrum pharmacological activities, including anti-inflammatory (Madhusudana, 2012; Deka, 2012; Goncales, 2005), antiviral (Rashad, 2010; Stephens, 2001), antibacterial (Abbas, 2013; Behbehani, 2012; Dewal, 2012) and antitumor activities (Stephens, 2001; Wang, 2013; Titchennell, 2013; Perspicace, 2013; Kassab and Gedawy, 2013; Pinkerton, 2007; Galam, 2007). In addition, thiophene incorporating 2,5-dioxopyrrolidine moiety exhibited remarkable antitumor activity (Curtin, 2002; Romagnoli, 2014). Moreover, it was reported that [1,2,3]triazole hybridized with different heterocyclic rings demonstrated promising antitumor activity (Hupe, 1991; Duan, 2013; Pokhodylo, 2014; Pokhodylo, 2013). Likewise, literature survey revealed that 1,5-disubstituted tetrazoles have considerable antitumor activity against various cancer cell lines (Romagnoli, 2012; Arshad, 2014).

Herein, we are reporting the preparation of new cyclohepta[*b*]thiophene analogs incorporating 2,5-dioxopyrroline, 2,5-dioxopyrrolidine, 1,3-dioxoisoindoline, [1,2,3]triazole and 1,5-disubstituted tetrazole moieties and their *in vitro* antitumor evaluation toward breast cancer (MCF-7), hepatocyte (HepG2) and colon adenocarcinoma (HT-29) cell lines as well as their *in vivo* antitumor assay toward Ehrlich ascites carcinoma (EAC) in mice. Moreover, *In silico* studies were done to explore the molecular properties of the investigated compounds.

Experimental

Chemistry

Melting points (ºC) were determined on Fisher-Johns melting points apparatus. Microanalyses were done at the Microanalytical Unit, Cairo University, Egypt, and agreed with the expected structures. Using Unicam SP 1000 IR spectrometer (ν in cm^{-1}), infrared spectra (KBr disc) were measured at Faculty of Pharmacy, Mansoura University, Egypt. Bruker Avance 300 MHz spectrometer was used to record nuclear magnetic resonance (^1H and ^{13}C NMR) spectra in $CDCl_3$ or DMSO-d_6 and chemical shift values represented as ppm. Mass spectra were determined on JEOL JMS-600 H spectrometer (70 eV) at the Microanalytical Unit, Cairo University, Egypt. TLC plates precoated with silica gel 60 F254 (E. Merck) were used for monitoring reaction times, using UV for visualization of spots. *n*-Hexane: ethyl acetate (4:1) was used as an eluent. The synthesis of compound **1** was accomplished following the reported method (Perrissin, 1980).

*Synthesis of compounds **2-4**:*

Compound **1** (0.48 g, 2 mmol) and the acid anhydride (2 mmol) were refluxed in glacial acetic acid (10 mL) for 16-20 hours. The solvent was poured onto ice and the precipitate was filtered, dried and crystallized from ethanol.

Ethyl 2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**2**).

Yield 50%, m.p. 109-111 ºC. ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.30 (t, 3H, $CH_2C\underline{H_3}$), 1.55-1.79 (m, 6H, $3CH_2$), 2.68-2.93 (m, 4H, $2CH_2$), 4.16-4.29 (q, 2H, $C\underline{H_2}CH_3$), 6.61 (d, 2H, pyrroline-H). MS *m/z* (%): 321 (2.71, M^++2), 320 (2.39, M^++1), 319 (3.24, M^+), 193 (100.00). Anal. Calc. for $C_{16}H_{17}NO_4S$ (319.38): C, 60.17; H, 5.37; N, 4.39%. Found: C, 60.31; H, 5.52; N, 4.20%.

Ethyl 2-(2,5-dioxopyrrolidin-1-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**3**).

Yield 65%, m.p. 147-149 ºC. ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.31 (t, 3H, $CH_2C\underline{H_3}$), 1.57-1.79 (m, 6H, $3CH_2$), 2.55 (t, 4H, pyrrolidine-H), 2.66-2.96 (m, 4H, $2CH_2$), 4.26-4.40 (q, 2H, $C\underline{H_2}CH_3$). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 13.9, 26.6, 27.4, 27.5, 27.8, 28.7, 30.6, 60.4, 113.3, 130.1, 135.9, 142.4, 164.7, 173.4. MS *m/z* (%): 322 (0.13, M^++1), 321 (0.41, M^+), 193 (100.00). Anal. Calc. for $C_{16}H_{19}NO_4S$ (321.39): C, 59.79; H, 5.96; N, 4.36%. Found: C, 59.83; H, 5.93; N, 4.41%.

Ethyl 2-(1,3-dioxoisoindolin-2-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**4**).

Yield 70%, m.p. 84-86 ºC. ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.31 (t, 3H, $CH_2C\underline{H_3}$), 1.56-1.78 (m, 6H, $3CH_2$), 2.66-2.95 (m, 4H, $2CH_2$), 4.25-4.29 (q, 2H, $C\underline{H_2}CH_3$), 7.93-8.00 (m, 4H, Ar-H). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 13.9, 23.1, 26.6, 27.1, 27.8, 28.9, 60.2, 113.1, 123.7, 130.0, 131.2, 135.1, 135.8, 143.2, 164.7, 166.9. MS *m/z* (%): 370 (2.57, M^++1), 369 (6.84, M^+), 323 (100.00). Anal. Calc. for $C_{20}H_{19}NO_4S$ (369.43): C, 65.02; H, 5.18; N, 3.79%. Found: C, 65.10; H, 5.23; N, 3.86%.

Ethyl 2-azido-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**5**).

An aqueous solution of sodium nitrite (0.20 g, 3 mmol) was added to an ice-cooled solution of **1** (0.48 g, 2 mmol) in concentrated sulfuric acid (5 mL) and ice (5 g), followed by addition of sodium azide (0.13 g, 2 mmol) in water (5 mL). The mixture was stirred for 15 minutes. The solution was extracted with diethyl ether (3x10 mL) and the solvent was evaporated to give the corresponding azide analog that was immediately used in the next step without purification.

1-(3-Carboxy-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophen-2-yl)-5-methyl-1*H*-[1,2,3] triazole-4-carboxylic acid (**6**).

Compound **5** (0.26 g, 1 mmol) and ethyl acetoacetate (0.13 g, 1 mmol) were added to an ice-cooled solution of sodium metal (0.023 g, 1 mmol) in methanol (20 mL). The mixture was refluxed for 6 hours. The solvent was evaporated under vacuum and the remaining solid was triturated with 1 M hydrochloric acid, filtered, dried and crystallized from ethanol.

Yield 36%, m.p. 104-106 ºC. IR spectrum (KBr, *v*, cm^{-1}): 3448 (2CO$\underline{OH}$), 1716 (2$\underline{CO}$OH). ^{1}H NMR spectrum: (DMSO-d_6, δ ppm): 1.68-1.94 (m, 6H, $3CH_2$), 2.51 (s, 3H, CH_3), 2.86-3.04 (m, 4H, $2CH_2$). ^{13}C NMR spectrum: (DMSO-d_6, δ ppm): 9.6, 26.8, 27.5, 28.2, 29.8, 32.3, 52.0, 52.1, 130.7, 135.4, 141.0, 142.2, 161.9, 162.4. MS *m/z* (%): 322 (16.34, M^++1), 321 (76.09, M^+), 129 (100.00). Anal. Calc. for $C_{14}H_{15}N_3O_4S$ (321.35): C, 52.33; H, 4.70; N, 13.08%. Found: C, 52.48; H, 4.82; N, 12.91%.

*Synthesis of compounds **7a-c**:*

Compound **1** (0.48 g, 2 mmol) and the aromatic aldehyde (2 mmol) were refluxed in a mixture of glacial acetic acid and ethanol (1:1) (10 mL) for 12-18 hours. The solvent was poured onto ice and the precipitate was filtered, dried and crystallized from ethanol: water (3:1).

Ethyl 2-((thiophen-3-ylmethylene)amino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**7a**).

Yield %, m.p. 120-122 ^{o}C. ^{1}H NMR spectrum: ($CDCl_3$, δ ppm): 1.31 (t, 3H, $CH_2C\underline{H_3}$), 1.59-1.91 (m, 6H, $3CH_2$), 2.65-2.96 (m, 4H, $2CH_2$), 4.26-4.33 (q, 2H, $C\underline{H_2}CH_3$), 6.85-7.01 (m, 3H, Ar-H), 11.17 (s, 1H, CH=N). ^{13}C NMR spectrum: ($CDCl_3$, δ ppm): 14.2, 23.6, 26.9, 27.7, 28.2, 32.1, 60.6, 112.6, 112.7, 130.8, 130.9, 136.2, 145.5, 145.6, 166.7, 166.8. MS *m/z* (%): 333 (22.48, M^+), 55 (100.00). Anal. Calc. for $C_{17}H_{19}NO_2S_2$ (333.47): C, 61.23; H, 5.74; N, 4.20%. Found: C, 61.48; H, 5.90; N, 4.02%.

Ethyl 2-((3,4-dimethoxybenzylidene)amino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**7b**).

Yield 48%, m.p. 74-76 ^{o}C (http://www.ambinter.com/).

Ethyl 2-((4-bromobenzylidene)amino)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**7c**).

Yield %, m.p. 113-115 ^{o}C. ^{1}H NMR spectrum: ($CDCl_3$, δ ppm): 1.30 (t, 3H, $CH_2C\underline{H_3}$), 1.59-1.91 (m, 6H, $3CH_2$), 2.65-2.96 (m, 4H, $2CH_2$), 4.26-4.33 (q, 2H, $C\underline{H_2}CH_3$), 7.45 (d, 2H, Ar-H), 7.52 (d, 2H, Ar-H), 9.74 (s, 1H, CH=N). MS *m/z* (%): 408 (11.01, M^++2), 406 (11.21, M^+), 80 (100.00). Anal. Calc. for $C_{19}H_{20}BrNO_2S$ (406.34): C, 56.16; H, 4.96; N, 3.45%. Found: C, 56.09; H, 4.82; N, 3.38%.

*Synthesis of compounds **8a-c**:*

Compound **7a-c** (1 mmol) and sodium azide (0.06 g, 1 mmol) were refluxed in tetrahydrofuran (15 mL) for 24-36 hours. The solvent was evaporated under vacuum and the remaining solid was crystallized from ethanol: water (4:1).

Ethyl 2-(5-(thiophen-3-yl)-1*H*-tetrazol-1-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**8a**).

Yield 52%, m.p. 72-74 °C. ^{1}H NMR spectrum: ($CDCl_3$, δ ppm): 1.32 (t, 3H, $CH_2C\underline{H}_3$), 1.54-1.77 (m, 6H, $3CH_2$), 2.63-2.95 (m, 4H, $2CH_2$), 4.25-4.31 (q, 2H, $C\underline{H}_2CH_3$), 6.85-7.01 (m, 3H, thiophene-H). ^{13}C NMR spectrum: ($CDCl_3$, δ ppm): 13.9, 23.0, 26.6, 27.4, 30.4, 31.6, 60.4, 113.2, 124.8, 127.9, 130.0, 130.1, 135.8, 143.1, 152.6, 164.7, 166.9. MS *m/z* (%): 374 (0.92, M^+), 193 (100.00). Anal. Calc. for $C_{17}H_{18}N_4O_2S_2$ (374.48): C, 54.52; H, 4.84; N, 14.96%. Found: C, 54.60; H, 4.92; N, 14.86%.

Ethyl 2-(5-(3,4-dimethoxyphenyl)-1*H*-tetrazol-1-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**8b**).

Yield 48%, m.p. 74-76 °C. ^{1}H NMR spectrum: ($CDCl_3$, δ ppm): 1.31 (t, 3H, $CH_2C\underline{H}_3$), 1.59-1.91 (m, 6H, $3CH_2$), 2.65-2.96 (m, 4H, $2CH_2$), 3.54 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.26-4.33 (q, 2H, $C\underline{H}_2CH_3$), 6.55 (s, 1H, Ar-H), 6.67 (d, 1H, Ar-H), 6.85 (d, 1H, Ar-H). ^{13}C NMR spectrum: ($CDCl_3$, δ ppm): 14.2, 23.7, 26.9, 27.8, 28.5, 32.2, 55.2, 55.7, 60.6, 110.5, 110.6, 112.6, 121.1, 125.5, 126.9, 127.0, 130.8, 136.2, 145.6, 166.7, 166.8. MS *m/z* (%): 429 (0.54, M^+), 193 (100.00). Anal. Calc. for $C_{21}H_{24}N_4O_4S$ (428.50): C, 58.86; H, 5.65; N, 13.07%. Found: C, 58.94; H, 5.72; N, 12.98%.

Ethyl 2-(5-(4-bromophenyl)-1*H*-tetrazol-1-yl)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (**8c**).

Yield 26%, m.p. 64-66 °C. ^{1}H NMR spectrum: ($CDCl_3$, δ ppm): 1.30 (t, 3H, $CH_2C\underline{H}_3$), 1.59-1.91 (m, 6H, $3CH_2$), 2.65-2.96 (m, 4H, $2CH_2$), 4.26-4.33 (q, 2H, $C\underline{H}_2CH_3$), 7.45 (d, 2H, Ar-H), 7.52 (d, 2H, Ar-H). ^{13}C NMR spectrum: ($CDCl_3$, δ ppm): 14.2, 23.7, 26.9, 27.8, 28.6, 32.2, 60.6, 117.4, 129.8, 130.3, 130.9, 132.4, 135.0, 136.2, 136.3, 166.5, 191.0. MS *m/z* (%): 447 (1.32, M^+), 193 (100.00). Anal. Calc. for $C_{19}H_{19}BrN_4O_2S$ (447.35): C, 51.01; H, 4.28; N, 12.52%. Found: C, 51.12; H, 4.41; N, 12.42%.

Biological testing

In vitro antitumor screening

Compounds **2-4, 6** and **8a-c** were esteemed for *in vitro* antitumor efficacy following MTT assay (Mosmann, 1983; Denizot and Lang, 1986; Gerlier and Thomasset, 1986).

In vivo antitumor screening

Materials

Adult Swiss male albino mice (weighing 20-25 g) were acquired from Pharmacology Department, Mansoura University, Egypt.

NCI, Cairo, Egypt provided the Ehrlich ascites carcinoma (EAC) cells. After collecting and preparing the cells, the total count of viable cells was determined using trypan blue (Sheeja, 1997). Dilution of tumor cells with normal saline (0.9% NaCl) was done to reach the desired concentration (2 x 10^6 cells /0.2 mL).

Procedure

7 Groups of mice (n= 6) were used.

Group I: Negative control (no EAC cells) - received vehicle (normal saline).

Group II: Positive control (EAC cells) - received vehicle (normal saline).

Group III - EAC bearing mice inoculated with compound **2**.

Group IV - EAC bearing mice inoculated with compound **6**.

Group V - EAC bearing mice inoculated with compound **8a**.

Group VI - EAC bearing mice inoculated with compound **8b**.

Group VII - EAC bearing mice inoculated with 5-fluorouracil.

All groups (except group I) were injected intraperitoneally with 2 x 10^6 cells/mouse. After 24 hours, mice were injected with 0.5 mg of the tested compounds and 5-fluorouracil. The inoculation process was repeated for nine days.

100 µL of EAC cells (from three mice) in each group was collected after five days of treatment. The viable cell count was determined under microscope after dilution with saline to twenty-fold and staining by trypan blue.

Results and Discussion

Chemistry

Preparation of compounds **2-4**, **6** and **8a-c** was depicted in Scheme 1. Cyclohepta[*b*]thiophene derivative **1** (Perrissin, 1980) was used as a starting material.

Biological screening

In vitro antitumor efficacy of compounds **2-4**, **6** and **8a-c** toward breast cancer (MCF-7), hepatocyte (HepG2) and colon adenocarcinoma (HT-29) cell lines was evaluated following MTT assay and using 5-fluorouracil as a standard drug (Mosmann, 1983; Denizot and Lang, 1986; Gerlier and Thomasset, 1986). The compound concentration causes 50% inhibition of the activity (IC_{50}, µg/mL) was determined for the investigated compounds. Results (Table 1) indicated that compound **6** is the most active member, it showed broad spectrum antitumor activity.

Compounds **2**, **6** and **8a, b** (with the highest *in vitro* activity toward MCF-7 cell line) were tested for their *in vivo* antitumor efficacy toward EAC in mice utilizing 5-fluorouracil as a standard drug (Clarkson and Burchenal, 1965; Sheeja, 1997, Oberling and Guerin, 1954). % Increase in lifespan (% ILS) was calculated using the following equation: % ILS = [(MST of treated group/MST of positive control group)-1] x 100, where MST = days of each mouse in

a group/number of mice. Compounds **6** and **8b** showed the highest % ILS of mice (Table 2). Influence on blood profile: all tested compounds showed lower hemoglobin (Hb) and RBCs levels and higher WBCs count (Table 3). Viable tumor cell count was determined for the tested compounds. Compound **6** presented considerable decrease in viable EAC count (Table 4).

Structure-activity relationship (SAR) studies

Combination of 2,5-dioxopyrroline moiety with the cyclohepta[*b*]thiophene nucleus resulted in reasonable activity toward breast cancer (MCF-7) cell line (compound **2**). However, saturation of 2,3-dihydropyrroline ring resulted in inactive compound against the three tested cell lines (compound **3**). In an attempt to increase the hydrophobic characters, fusion of 2,5-dioxopyrrolidine with phenyl ring resulted in compound **4** with increased efficacy toward MCF-7 cell line compared to 2,5-dioxopyrrolidine analog **3**. Replacement of 2,5-dioxopyrrolidine with 5-methyl-[1,2,3]triazole-4-carboxylic acid moiety significantly increased the efficacy toward the three tested cell lines (compound **6** versus **3**). In addition, incorporation of 5-(thiophen-3-yl)-1*H*-tetrazole to the cyclohepta[*b*]thiophene scaffold displayed reasonable antitumor activity against MCF-7 and HepG2 cells (compound **8a**). However, replacing thiophen-3-yl in compound **8a** with 3,4-dimethoxyphenyl or 4-bromophenyl diminished activity (compounds **8b, c**).

Molecular properties and toxicity risks

Partition coefficient, solubility, molecular size, flexibility and other physicochemical properties of molecules affect their behavior in living organisms. Molecular diversity assessment is considered as an important element in drug design. As the balance between aqueous and lipid solubility can maintain good bioavailability, Lipinski's rule of five (Lipinski, 2001) and other molecular properties (Jarrahpour, 2012) were predicted for compounds **2-4**, **6** and **8a-c** (Table 5).

Molinspiration calculations

Lipinski's rule (Lipinski, 2001) and Veber's criteria (number of rotatable bonds (Nrotb) and topological polar surface area (TPSA)) (Veber, 2002) are key properties that influence oral absorption of drugs.

Molinspiration software was applied for anticipation of TPSA, Nrotb and Lipinski's rule for compounds **2-4**, **6** and **8a-c** (Table 5). TPSA is determined using the method of Ertl *et al.* (Ertl, 2000), this parameter describes drug absorption and bioavailability. All the analyzed compounds are compatible with Lipinski rule and Veber's criteria (Table 5).

Toxicity risks calculations

The methodology developed by Osiris was used to predict the toxicity risks (mutagenicity, tumorogenicity, irritancy and reproductive effects) of compounds **2-4**, **6** and **8a-c** (Jarrahpour, 2012). All the analyzed compounds were predicted to have no toxicity risks.

Conclusion

In a summary, all the investigated compounds came in accordance with the lipinski's rule and Veber's criteria and they are predicted to have good oral activity. In addition, compound **6** exhibited outstanding antitumor efficacy toward the three tested cell lines. Also, the same compound displayed the highest % ILS of mice inoculated with EAC as well as the lowest number of viable tumor cell count, thus; it can be used as a prototype for further modifications to develop new more active antitumor analogs.

Table 1. *In vitro* antitumor efficacy of compounds **2-4**, **6** and **8a-c** toward breast cancer (MCF-7), hepatocyte (HepG2) and colon adenocarcinoma (HT-29) cell lines

Comp. No.	**IC_{50} (µg/mL)**		
	MCF-7	**HepG2**	**HT-29**
2	67.6	>100	92.7
3	>100	>100	>100
4	79.8	>100	>100
6	**11.9**	**14.8**	**23.0**
8a	43.6	55.1	71.8
8b	72.2	>100	>100
8c	>100	>100	>100
5-Fluorouracil	5.7	6.9	4.6

Bold values indicate the preferable results.

Table 2. Effect of compounds **2**, **6** and **8a, b** on the survival time and % increase in lifespan of mice inoculated with EAC

Group	**Mean survival time (day)**	**% Increase in lifespan over control**
Normal	nd[a]	nd[a]
EAC only	16	nd[a]
5-Fluorouracil	45	178.1
2	31	93.7
6	**39**	**143.7**
8a	24	50
8b	35	118.7

[a] nd: not determined. Bold values indicate the preferable results.

Table 3. Effect of compounds **2**, **6** and **8a, b** on hematological parameters of mice inoculated with EAC

Group	Hb (g/dl)	RBC Count (10^6/mm^3)	Total WBC (10^3/mm^3)
Normal	13.24	5.92	5.89
EAC only	8.25	3.83	19.15
5-Fluorouracil	12.90	5.55	8.48
2	12.26	4.90	10.29
6	**12.73**	**5.14**	**9.64**
8a	10.09	4.19	13.89
8b	11.46	4.39	10.15

Bold values indicate the preferable results.

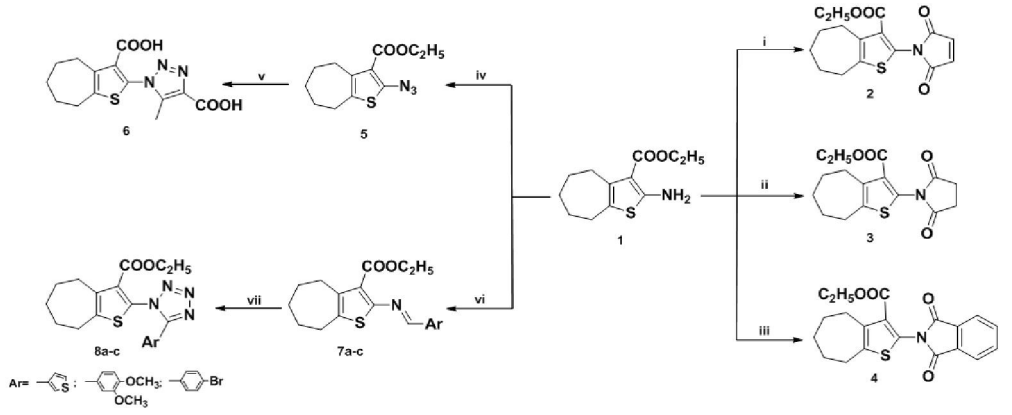
Table 4. Effect of compounds **2**, **6** and **8a, b** on tumor volume and viable tumor cell count of mice inoculated with EAC

Group	Tumor volume (mL)	Viable tumor cell count /100 µL
Normal	nd[a]	nd[a]
EAC only	8.47	75.13x10^6
5-Fluorouracil	1.47	18.44x10^6
2	**3.5**	**31.56x10^6**
6	**2.1**	**23.53x10^6**
8a	6.26	45.77x10^6
8b	4.3	39.47x10^6

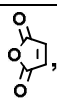
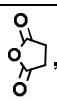
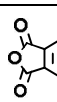
[a] nd: not determined. Bold values indicate the preferable results.

Table 5. TPSA, Nrotb and calculated Lipinski's rule for compounds **2-4**, **6** and **8a-c**

Comp. No.	TPSA	Nrotb	Lipinski's parameters				
			miLogP	OH-NH interact	O-N interact	M. wt.	No. of violations
2	65.38	4	3.08	0	5	319.38	0
3	63.69	4	1.76	0	5	321.40	0
4	65.38	4	4.82	0	5	369.44	0
6	105.32	3	2.55	2	7	321.36	0
8a	69.92	5	4.32	0	6	374.49	0
8b	88.38	7	4.38	0	8	428.51	0
8c	69.92	5	5.54	0	6	447.36	1
5-Fluorouracil	65.72	0	-0.59	2	4	130.08	0



Scheme 1. Synthesis of compounds **2-6**, **7a-c** and **8a-c**

i	 , glacial acetic acid	**ii**	 , glacial acetic acid	**iii**	 , glacial acetic acid
iv	1. H_2SO_4, $NaNO_2$ 2. NaN_3	**v**	$CH_3COCH_2COOC_2H_5$, $NaOCH_3$, methanol	**vi**	Ar—CHO, glacial acetic acid, ethanol
vii	NaN_3, tetrahydrofuran				

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