

## Synthesis of some tricyclic indeno [1, 2-d] pyrimidine derivatives as a new class of anti-breast cancer agents.

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### Abstract

As part of our search for anti-breast cancer agents a new series of tricyclic 4-substituted-2-yl)-2-thioxo-3, 4-dihydro-1*H*-indeno [1, 2-d] pyrimidin-5(2*H*)-one 4-9 were obtained in one pot synthesis by a modification of the Biginelli Reaction. The structures of the synthesized compounds were characterized by microanalyses, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and mass spectral data. All the synthesized compounds were evaluated for their *in vitro* anticancer activity against human breast cancer cell line (MCF7). Most of the screened compounds showed interesting cytotoxic activities compared to Doxorubicin as reference drug. Compounds 8, 5, 7 and 4 (IC<sub>50</sub> values 10.25, 23.48, 27.51 and 28.85 μM) revealed higher cytotoxic activities than the Doxorubicin as reference drug with IC<sub>50</sub> value (32.00 μM). Also, compound 9 is nearly as active as Doxorubicin with IC<sub>50</sub> values (33.55 μM). Compound 6 showed moderate activity.

**Keywords:** Synthesis, indenopyrimidines, anti-breast cancer activity.

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### Introduction

Most cancer patients are subjected to chemotherapy for the treatment of advanced cancers. However, most metastatic solid tumors eventually remain incurable even by treatment with recent anticancer drugs. Also, Cancer is a disease of striking significance in the world today. It is the second leading cause of death in the world after cardiovascular diseases and it is projected to beginning the primary cause of death there within the coming years [1, 2]. Cancer is a top killer of human beings. Thus, great urgency to develop highly efficacious and minimally toxic treatments for cancer. Although tremendous progress has been achieved in the development of novel cancer treatments, most of the current cancer drugs usually exhibit high toxicity and are severely resisted by tumor cells in the clinic. This dilemma is particularly true for DNA-damaging agents, the mainstay of cancer treatment [3]. Cancer is still continuing to be a major earth problem Worldwide. The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry as cancer causes about 13% of all the death [4].

Surpassing cardiovascular diseases, it is taking the position number one killer due to various factors [5]. Also the treatment of cancer is associated with various side effects

which include bone marrow depression, alopecia, drug-induced cancer, hepatotoxicity, and many more. Because of the need and value of anticancer drugs, many laboratories are intensively investigating the chemistry and biology of novel anticancer agents. Also the development of resistance against the existing anticancer drugs and cytotoxicity and genotoxicity of anticancer drugs to the normal cells are other major problems in cancer therapy, keeping research window open in search for newer anticancer molecules [6]. But the window passage has become narrower because it is rather hard to search a molecule that can selectively inhibit the proliferation of abnormal cells only with least or no affect on normal cells. Multicomponent condensation reactions (MCRs) have recently been discover to be a powerful method for the synthesis of organic compounds, since the products are formed in a single step and diversity can be achieved by simply varying each component [7-9].

Indenopyrimidine and their derivatives have been studied due to a variety of chemical and biological significance. The importance of indenopyrimidines as biologically active compounds includes their use as antibacterial [10-12], antiallergic[13], antitumor [11,12] [14, 15] antifolate [16], tyrosine kinase [17], antimicrobial [18], calcium channel antagonists [19], antibacterial [20-23], anti-

inflammatory, analgesic [24], antihypertensive [25], antileishmanial [26] tuberculostatic [27], anticonvulsants [28], diuretic, potassium sparing [29], and antiaggressive activities [30]. Also, indenopyrimidines were found to possess several pharmacological properties, including anticancer activity [31-35]. On the other hand, the pyrimidines constitute an important class of drug, with several types of pharmacological agents possessing anticancer activity [36-39] among others. A large number of structurally novel pyrimidines have ultimately been reported to show substantial anticancer activity in-vitro and in vivo [40]. Several mechanisms have been reported for anticancer activity of the pyrimidine compounds and the most prominent of these mechanisms was through the inhibition of the carbonic anhydrase [41-44]. The mechanism of tumor inhibition by pyrimidine carbonic anhydrase (CA) inhibitor was suggested by Chegwiddden and Spencer [45]. In continuation of our work it seemed of interest to design and synthesize some novel series of indeno [1, 2-*d*] pyrimidine derivatives to evaluate their anti-breast cancer activity.

## Experimental

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp, Southborough, UK). Recoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography, dichloromethane/methanol (9.5: 0.5 mL) mixture was used as a developing solvent system and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded in KBr discs using IR-470 Shimadzu spectrometer (Shimadzu, Tokyo, Japan). <sup>1</sup>H-NMR spectra (in DMSO-*d*<sub>6</sub>) were recorded on Bruker Ac-300 ultra-shield NMR spectrometer (Bruker, Flawil, Switzerland, δ ppm) at 300 MHz, using TMS as internal standard. Electron impact Mass Spectra were recorded on a Shimadzu Gc-Ms- Qp 5000 instrument (Shimadzu, Tokyo, Japan). Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus, Hanau, Germany). All compounds were within ± 0.4% of the theoretical values.

## Results

### General Procedure for the synthesis of compounds 4-9.

A mixture of thiourea (0.76g, 0.01 mole). Aldehydes (0.01 mole) and 1*H*-indene-1, 3(2*H*) - dione (1.46g, 0.01 mole) in absolute ethanol containing 37% HCl (4 drops) was heated under reflux for 3 h. The reaction mixture was allowed to cool, filtered off and recrystallized from dioxane to give compounds 4-9, respectively.

**Synthesis of 4-(5-methylfuran-2-yl)-2-thioxo-3, 4-dihydro-1*H*- indeno [1, 2-*d*] - pyrimidin-5(2*H*) - one (4).** Yield, 89%; m.p. 192.5 °C; IR (KBr, cm<sup>-1</sup>): 3468 (NH), 3057 (CH arom.), 2986, 2841 (CH aliph.), 1716 (C=O),

1240 (C=S). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 2.4 [s, 3H, CH<sub>3</sub>], 5.3 [s, 1H, CH-4], 6.1, 6.2 [2d, 2H, 2CH furan, *J* = 7.7 Hz], 7.1-8.1 [m, 4H, Ar-H], 9.3, 12.8 [2s, 2H, N<sub>1</sub>-H + N<sub>3</sub>-H, exchangeable with D<sub>2</sub>O]. <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): 12.7, 57.4, 110.3, 110.4, 111.3, 121.6, 124.8, 127.6, 131.9, 133.7, 134.5, 152.6, 152.8, 153.7, 172.1, 191.6. MS m/z (%): 296 [M<sup>+</sup>] (23.12), 81 (100). Anal. Calcd. for C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>O<sub>2</sub>S: C, 64.85; H, 4.08; N, 9.45. Found: C, 64.49; H, 4.37; N, 9.74.

### Synthesis of 4-(thiophen-2-yl)-2-thioxo-3, 4-dihydro-1*H*- indeno [1, 2-*d*] pyrimidin-5(2*H*)-one (5).

Yield, 79%; m.p. 162.6 °C; IR (KBr, cm<sup>-1</sup>): 3446 (NH), 3074 (CH arom.), 1722 (C=O), 1271 (C=S). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 5.2 [s, 1H, CH-4], 6.7-8.2 [m, 7H, Ar-H], 9.4, 12.8 [2s, 2H, N<sub>1</sub>-H + N<sub>3</sub>-H, exchangeable with D<sub>2</sub>O]. <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): 57.2, 108.6, 122.4, 123.5, 124.7, 125.9, 126.4, 127.7, 131.8, 133.6, 134.0, 137.3, 154.1, 175.6, 193.0. MS m/z (%): 298 [M<sup>+</sup>] (9.17), 92 (100). Anal. Calcd. for C<sub>15</sub>H<sub>10</sub>N<sub>2</sub>OS<sub>2</sub>: C, 60.38; H, 3.38; N, 9.39. Found: C, 60.07; H, 3.76; N, 9.78.

### Synthesis of 4-(styryl-2-thioxo-3, 4-dihydro-1*H*- indeno [1, 2-*d*] pyrimidin-5(2*H*)-one (6).

Yield, 70%; m.p. >320 °C; IR (KBr, cm<sup>-1</sup>): 3420 (NH), 3091 (CH arom.), 2922, 2838 (CH aliph.), 1714 (C=O), 1279 (C=S). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 4.5 [s, 1H, CH-4], 6.4, 6.6 [2d, 2H, CH=CH, *J* = 7.3 Hz], 7.1-8.2 [m, 9H, Ar-H], 9.5, 13.0 [2s, 2H, N<sub>1</sub>-H + N<sub>3</sub>-H, exchangeable with D<sub>2</sub>O]. <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): 58.3, 104.6, 120.7, 120.9, 125.5, 126.7, 127.2, 127.6 (2), 127.9 (2), 128.0, 132.6, 133.7, 134.9, 135.5, 153.0, 171.6, 190.8. MS m/z (%): 318 [M<sup>+</sup>] (3.78), 102 (100). Anal. Calcd. for C<sub>19</sub>H<sub>14</sub>N<sub>2</sub>OS: C, 71.67; H, 4.43; N, 8.80. Found: C, 71.39; H, 4.15; N, 9.11.

### Synthesis of 4-(4(dimethylamino) phenyl) - 2-thioxo-3, 4-dihydro-1*H*-I ndeno [1, 2-*d*] pyrimidin-5(2*H*) - one (7).

Yield, 92%; m.p. 239.8 °C; IR (KBr, cm<sup>-1</sup>): 3420, 3157 (NH), 3100 (CH arom.), 2955, 2876 (CH aliph.), 1706 (C=O), 1266 (C=S). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 3.2 [s, 6H, 2-N (CH<sub>3</sub>)], 4.9 [s, 1H, CH-4], 6.8, 8.2 [m, 8H, Ar-H], 9.1, 12.6 [2s, 2H, N<sub>1</sub>-H + N<sub>3</sub>-H, exchangeable with D<sub>2</sub>O]. <sup>13</sup>C-NMR (DMSO-*d*<sub>6</sub>): 42.7 (2), 55.8, 103.4, 110.6 (2), 119.9, 123.8 (2), 124.7, 126.8, 130.6, 132.8, 133.9, 134.1, 151.3, 157.0, 169.8, 189.3. MS m/z (%): 335 [M<sup>+</sup>] (33.54), 119 (100). Anal. Calcd. for C<sub>19</sub>H<sub>17</sub>N<sub>3</sub>OS: C, 68.03; H, 5.11; N, 12.53. Found: C, 68.32; H, 4.88; N, 12.21.

### Synthesis of 4-(4-hydroxy-3-methoxyphenyl) - 2-thioxo-3, 4-dihydro-1*H*-indeno [1, 2-*d*] pyrimidin-5(2*H*)-one (8).

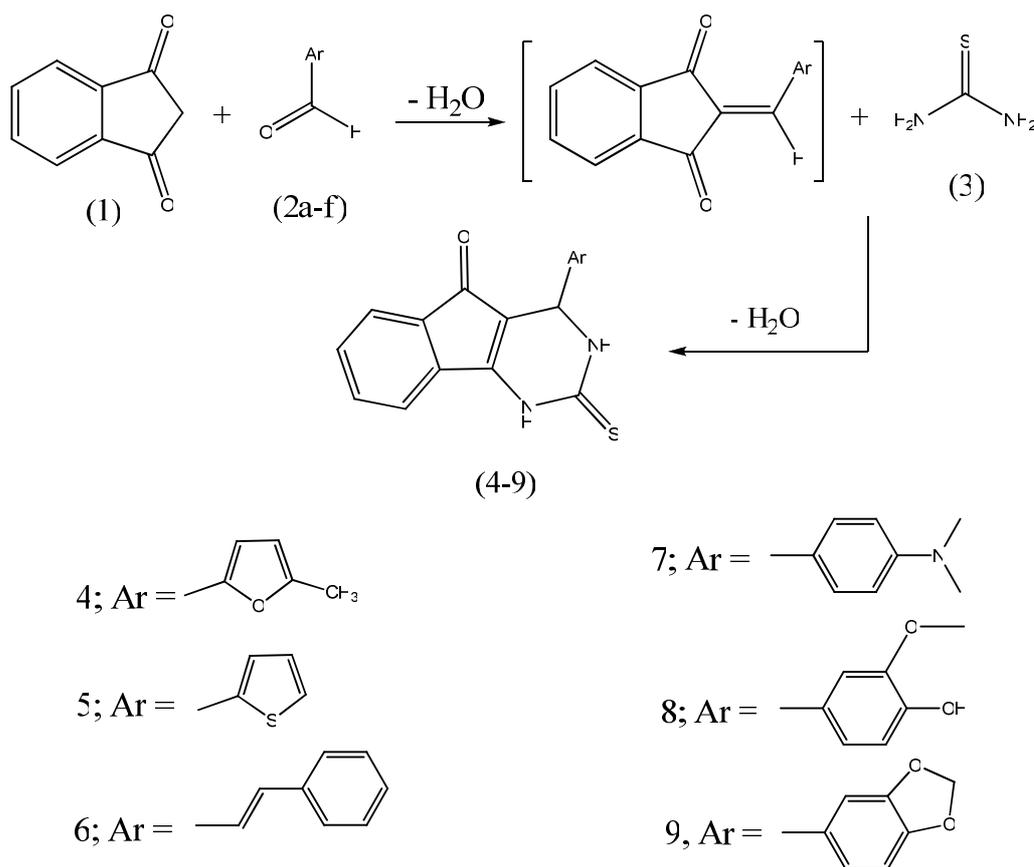
Yield, 69%; m.p. 218.9 °C; IR (KBr, cm<sup>-1</sup>): 3436 (OH), 3391 (NH), 3088 (CH arom.), 2966, 2839 (CH aliph.), 1720 (C=O), 1282 (C=S). <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ: 3.7 [s, 3H, OCH<sub>3</sub>], 4.8 [s, 1H, CH-4], 6.8, 8.3 [m, 8H, Ar-H], 9.4, 12.8 [2s, 2H, N<sub>1</sub>-H + N<sub>3</sub>-H, exchangeable with D<sub>2</sub>O]. <sup>13</sup>C-

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NMR (DMSO- $d_6$ ): 54.3, 56.7, 105.6, 110.1, 114.2, 117.6, 120.8, 124.7, 126.9, 131.7, 133.5, 134.2, 135.6, 143.9, 145.2, 157.3, 172.6, 189.7. MS m/z (%): 338 [M<sup>+</sup>] (2.78), 122 (100). Anal. Calcd. for C<sub>18</sub>H<sub>14</sub>N<sub>2</sub>O<sub>3</sub>S: C, 63.89; H, 4.17; N, 8.28. Found: C, 63.57; H, 4.49; N, 8.53.

**Synthesis of 4-(benzo[d][1,3]dioxol-5-yl)- 2-thio-3,4-dihydro-1H-indeno[1,2-d]pyrimidin-5(2H)-one (9).**  
Yield, 88%; m.p. 214.3 °C; IR (KBr, cm<sup>-1</sup>): 3438 (NH),

3081 (CH arom.), 2918, 2863 (CH aliph.), 1718 (C=O), 1237 (C=S). <sup>1</sup>H NMR (DMSO- $d_6$ )  $\delta$ : 5.1 [s, 1H, CH-4], 6.2 [s, 2H, CH<sub>2</sub>], 6.7-8.0 [m, 7H, Ar-H], 9.0, 12.3 [2s, 2H, N<sub>1</sub>-H + N<sub>3</sub>-H, exchangeable with D<sub>2</sub>O]. <sup>13</sup>C-NMR (DMSO- $d_6$ ): 55.3, 100.1, 104.3, 111.6, 111.8, 116.6, 121.4, 128.3, 128.4, 132.6, 133.1, 134.5, 134.7, 148.4, 148.7, 156.1, 176.0, 194.8. MS m/z (%): 336 [M<sup>+</sup>] (4.65), 74 (100). Anal. Calcd. for C<sub>18</sub>H<sub>12</sub>N<sub>2</sub>O<sub>3</sub>S: C, 64.27; H, 3.60; N, 8.33. Found: C, 64.54; H, 3.26; N, 8.02.



**Scheme 1:** Formation of compounds 4-9

**In vitro anticancer activity**

The cytotoxic activity was measured in vitro for the newly synthesized compounds using the SulfoRhodamine-B stain (SRB) assay using the method of [45]. The in vitro anticancer screening was done at the Pharmacology Unit, the National Cancer Institute, Cairo University. Cells were plated in 96- multiwell microtiter plate (10<sup>4</sup> cells/well) for 24h before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (10, 25, 50 and 100  $\mu$ M) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48h at 37 °C and in atmosphere of 5% CO<sub>2</sub>. After 48h, cells were fixed,

washed, and stained for 30 min. with 0.4% (W/V) with SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris -EDTA buffer. Color intensity was measured in an enzyme- linked immunosorbent assay ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line after the specified time [45]. The molar concentration required for 50% inhibition of cell viability (IC<sub>50</sub>) was calculated and the results are given in (Table 1). The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was IC<sub>50</sub> value, which corresponds to the concentration required for 50% inhibition of cell viability.

**In- vitro anti-breast cancer activity**

All the synthesized compounds were evaluated for their in vitro anticancer activity against human breast cancer cell line, MCF7. Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC<sub>50</sub> value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the in vitro cytotoxic activity of the synthesized compounds. Most of the tested compounds exhibited significant activity compared to the Doxorubicin as reference drug. From the results of Table 1, it was found that indenopyrimidine containing biologically active 3-

methoxy-4-hydroxyphenyl, 2-thioxo 8, 2-thienyl at 4-position with thioxo group at 2-position 5, *N*-dimethylphenyl at 4-position with thioxo moiety at 2-position 7 and 5-methylfuran at 4-position with thioxo moiety at 2-position 4 with IC<sub>50</sub> values (10.25, 23.48, 27.51, and 28.85 μM) exhibited more potent anti-breast cancer activity than the reference drug with IC<sub>50</sub> value (32.00 μM). Farther, indenopyrimidine bearing the biologically active pipronyl moiety at 4-position with thioxo group at 2-position 9 with IC<sub>50</sub> values (33.55 μM) is nearly as active as Doxorubicin as positive control. On the other hand, compounds 6 having styryl moiety at 4-position with thioxo group at 2-position revealed slightly lower activity of Doxorubicin with IC<sub>50</sub> value (than that 47.30 μM).

**Table 1.** In vitro anticancer screening of the synthesized compounds against human breast cancer cell line (MCF7).

Compound No.	Compound concentration (μM)				IC <sub>50</sub> (μM)
	10 μM	25 μM	50 μM	100 μM	
	Surviving fraction (Mean±S.E.)*				
Doxorubicin	0.551±0.026	0.480±0.003	0.139±0.005	0.130±0.016	32.00
4	0.845±0.013	0.515±0.021	0.380±0.003	0.443±0.017	28.85
5	0.693±0.023	0.503±0.033	0.377±0.010	0.391±0.013	23.48
6	0.807±0.080	0.723±0.031	0.293±0.010	0.133±0.014	47.30
7	0.810±0.022	0.550±0.019	0.331±0.013	0.350±0.015	27.51
8	0.541±0.003	0.323±0.020	0.360±0.018	0.460±0.015	10.25
9	0.825±0.013	0.668±0.021	0.307±0.007	0.271±0.017	33.55

Each value is the mean of three values ± Standard Error

**Discussion**

The aim of this work was the design and synthesis of some new series of tricyclic indeno [1, 2-d] pyrimidine 4-9 carrying a miscellaneous biologically active moiety at 4-position, thioxo group at 2-position and carbonyl group at 5-position (Scheme 1) and evaluation of their anticancer activity. The structure of compounds 4-9 was elucidated on the basis of microanalyses, IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and mass spectral data. The IR spectrum of compound 4 revealed the presence of bands for NH at 3468 cm<sup>-1</sup>, (C=O) at 1716 cm<sup>-1</sup>, (C=S) at 1240 cm<sup>-1</sup>. Also, <sup>1</sup>H-NMR spectrum indicated the presence of signals at 2.4 ppm which could be assigned to CH<sub>3</sub> group, 5.3 ppm attributed to CH-4 and two doublets at 6.1, 6.2 for furan ring. <sup>13</sup>C-NMR spectrum of compound 4 in (DMSO-d<sub>6</sub>) revealed signals at 172.1 ppm assigned to (C=S), 191.6 due to (C=O) group. IR spectrum of compound 5 exhibited the characteristic bands at 3446 cm<sup>-1</sup> (NH), 1722 cm<sup>-1</sup> (C=O), 1271 cm<sup>-1</sup> (C=S). <sup>1</sup>H-NMR spectrum of 5 showed signals at 5.2 ppm according to CH-4, 9.4, 12.8 ppm assigned to N<sub>1</sub>-H and N<sub>3</sub>-H. <sup>13</sup>C-NMR spectrum of compound 5 re-

vealed signals at 175.6 ppm due to (C=S) group and 193.0 ppm attributed to (C=O). IR spectrum of compound 6 showed the characteristic bands at 3420 cm<sup>-1</sup> (NH), 1714 cm<sup>-1</sup> (C=O), 1279 cm<sup>-1</sup> (C=S). <sup>1</sup>H-NMR spectrum of 6 exhibited signals at 4.5 ppm assigned to CH-4, two doublets appeared at 6.4, 6.6 ppm for CH=CH and 9.5, 13.0 ppm assigned to N<sub>1</sub>-H and N<sub>3</sub>-H. <sup>13</sup>C-NMR spectrum of compound 6 revealed signals at 171.6 ppm due to (C=S) group and 190.8 ppm attributed to (C=O) group. IR spectrum of compound 7 revealed the presence of bands for NH at 3420 cm<sup>-1</sup>, (C=O) at 1706 cm<sup>-1</sup>, (C=S) at 1266 cm<sup>-1</sup>. <sup>1</sup>H-NMR spectrum of compound 7 indicated the presence of signals at 3.2 ppm which could be assigned to N-(CH<sub>3</sub>)<sub>2</sub> group, 4.9 ppm attributed to CH-4. <sup>13</sup>C-NMR spectrum of compound 7 in (DMSO-d<sub>6</sub>) revealed signals at 169.8 ppm assigned to (C=S), 189.3 ppm due to (C=O) group. IR spectrum of compound 8 revealed the presence of bands for NH at 3436 cm<sup>-1</sup> (OH), 3391 cm<sup>-1</sup> (NH), 3088 cm<sup>-1</sup> (CH arom.), 2966, 2839 cm<sup>-1</sup> (CH aliph.), 1720 cm<sup>-1</sup> (C=O), 1282 cm<sup>-1</sup> (C=S). Also, <sup>1</sup>H-NMR spectrum indicated the presence of signals at 3.7 ppm which could be assigned to OCH<sub>3</sub> group, 4.8 ppm attrib-

uted to CH-4. <sup>13</sup>C-NMR spectrum of compound 8 in (DMSO-d<sub>6</sub>) revealed signals at 172.6 ppm assigned to (C=S), 189.7 attributed to (C=O) group. IR spectrum of compound 9 revealed the presence of bands for NH at 3438 cm<sup>-1</sup> (NH), 3081 cm<sup>-1</sup> (CH arom.), 2918, 2863 cm<sup>-1</sup> (CH aliph.), 1718 cm<sup>-1</sup> (C=O), 1237 cm<sup>-1</sup> (C=S). <sup>1</sup>H-NMR spectrum indicated the presence of signals at 6.2 ppm which could be assigned to CH<sub>2</sub> group of pipronyl moiety, 5.1 ppm attributed to CH-4 and two signals at 9.0, 12.3 ppm for 2NH groups. <sup>13</sup>C-NMR spectrum of compound 9 in (DMSO-d<sub>6</sub>) revealed signals at 176.0 ppm assigned to (C=S), 194.8 due to (C=O) group.

## Conclusion

The objective of the present study was to synthesize and investigate the anticancer activity of some indeno-pyrimidine derivatives carrying the biologically active thione moiety at 2-position. Compounds 8, 5, 7 and 4 showed promising anti-breast cancer activity higher than that of Doxorubicin as reference drug, while compounds 9 is nearly as active as Doxorubicin. In addition compound 6 revealed a moderate activity compared with the Doxorubicin as positive control.

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