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Synthesis of Some Novel Urea, Thiourea and Amide Derivatives through Three Components one pot Reaction and their Anti-tumor Activity

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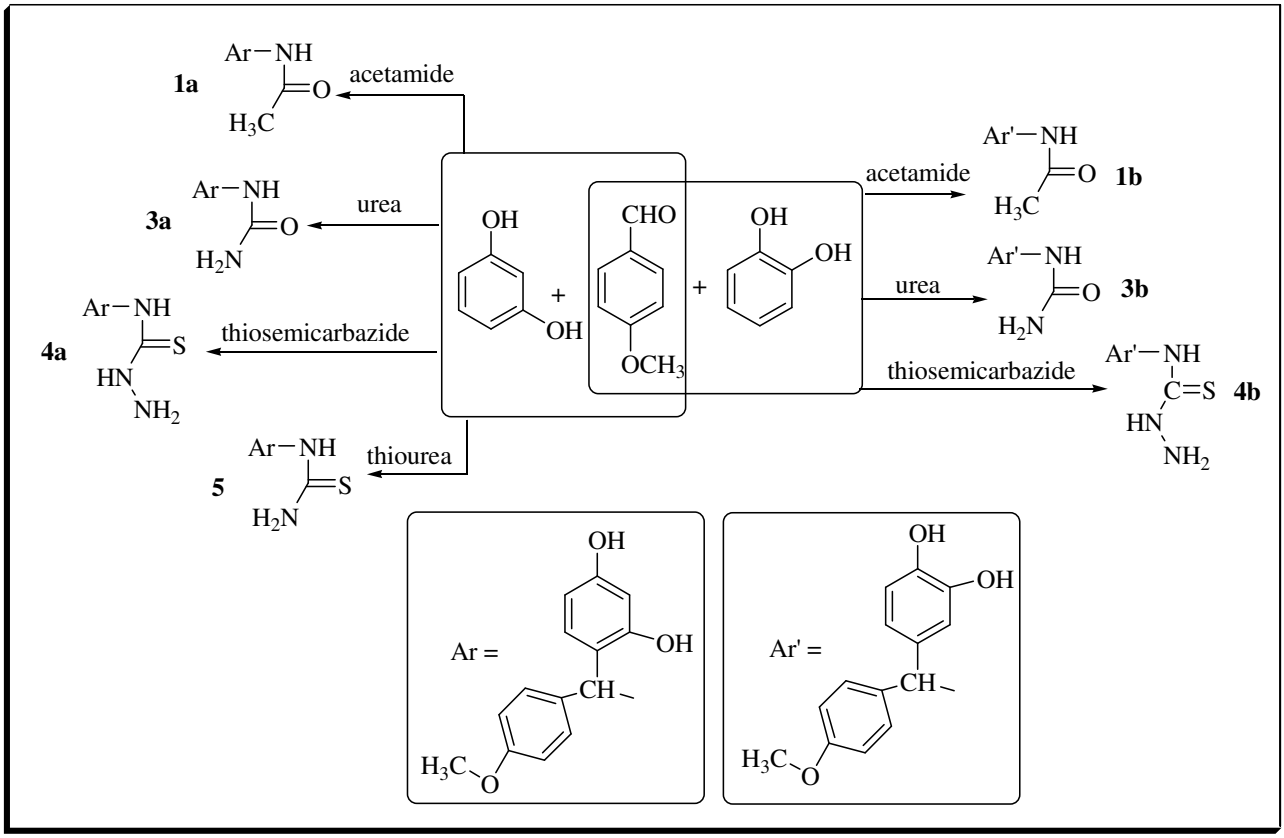
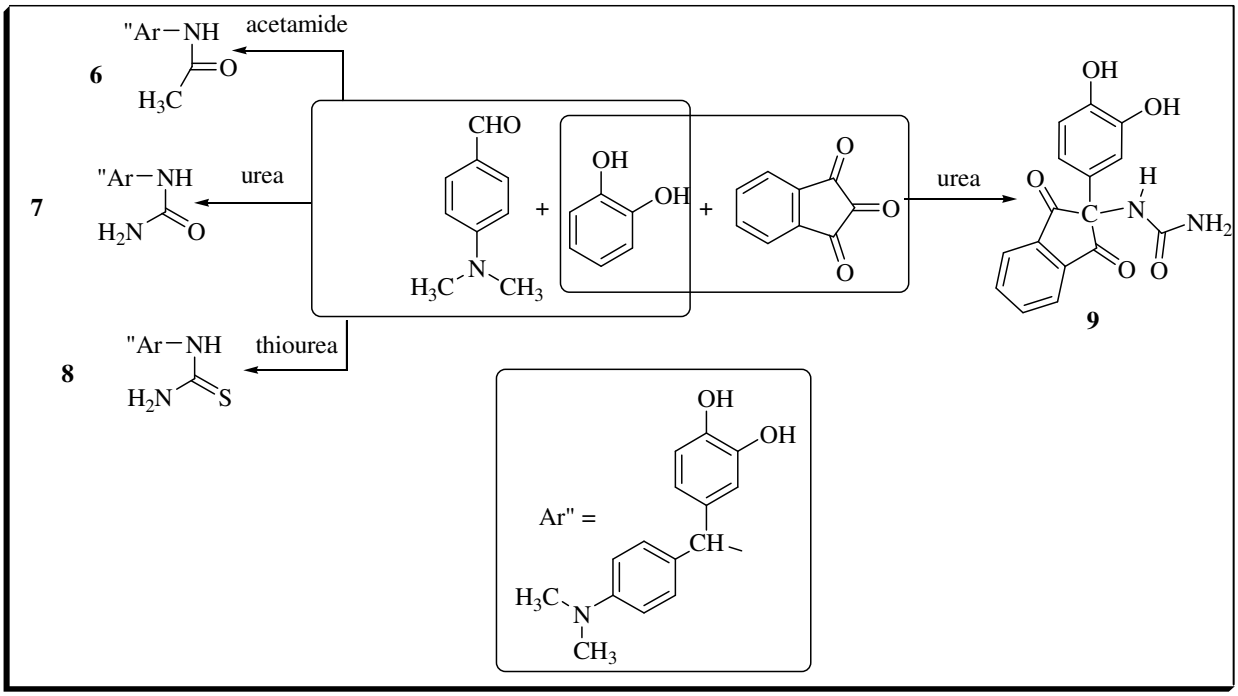




Abstract

The one-pot three component condensation of catechol and resorcinol with aromatic aldehydes and amides, thioamides such as urea, thiourea, thiosemicarbazide and acetamide under solvent free and neutral conditions to afford urea, thiourea and amide derivatives in high yields. The antitumor activity of some of the synthesized products was tested.

Keywords: Three component condensation; 4H-benzo[e]-[1,3]oxazin-7-ol; Dioxoindenylurea; Antitumor activity.

1. Introduction

Multi-component condensation reactions have a wide range of applicability in the field of synthetic organic chemistry and they constitute an especially attractive synthetic strategy because they provide easy and rapid access to large libraries of organic compounds with diverse substitution patterns. These are one pot reactions, they are easier to carry out than multistep syntheses, and their products are formed in a single step. Diversity can be achieved simply by varying each component (Khazaei *et al.* 2013; Verma *et al.* 2012; Shirini *et al.* 2013; Kouznetsov *et al.* 2012), In the past few years, combinatorial methods using multi-component reactions have been closely examined as fast and convenient solutions for the synthesis of diverse classes of compounds. Recently, this strategy became important in drug discovery in the context of synthesis of biologically active compounds. This method increases the efficiency of the reactions and decreases the number of laboratory operations along with solvents and chemicals used. Also, it reduces reaction time and facilitates the yield of products more than the normal multistep methods.

Also, a number of reports have recently highlighted diarylureas as potential antiproliferative agents against melanoma cell line. Sorafenib (Nexavar) is a diarylurea derivative that has been exclusively used in clinical trials (El Gamal *et al.* 2011), Sorafenib was subsequently recognized as exhibiting useful receptor tyrosine kinase poly-pharmacology, particularly inhibition of VEGF receptor, PDGF receptor, c-KIT and FLT3 kinases (El Gamal *et al.* 2011), Leading to drug approval for the treatment of renal cell cancers. N-(4-tert-Butylphenyl)-N'-(2-chloroethyl) urea shows potent anticancer activity (Mounetou *et al.* 2001; Mounetou *et al.* 2003), Urea derivative such as diuron is used as herbicides (Proctor *et al.* 2002), Thienopyridines including their 1,3-diarylurea derivatives have shown different biological activities namely as antitumor agents (Hayakawa *et al.* 2004), and receptor tyrosine kinase inhibitors (Heyman *et al.* 2007).

These reports Encouraged us to synthesis a novel urea, thiourea and amide derivatives through three component one pot reactions to study the antitumor activity of some of the synthesized products.

2. Results and discussion

The new derivatives were prepared according to the reaction sequences depicted in Schemes 1-4. Fusion of equimolar amounts of anisaldehyde, resorcinol or catechol and acetamide at 150-180°C afforded N-[(2,4-dihydroxyphenyl)(4-methoxyphenyl)methyl]acetamide **(1a)**, N-[(3,4-dihydroxyphenyl)(4-methoxyphenyl)methyl]acetamide **(1b)**, respectively. However fusion of anisaldehyde, resorcinol and acetamide in the presence of $POCl_3$ gave 4-(4-methoxyphenyl)-2-methyl-4H-benzo[e]-[1,3]oxazin-7-ol **(2)**.

Interestingly, compound **(2)** can also be prepared through an alternative route via the reaction of **1a** with $POCl_3$, which can be proved by m.mp and I.R.

The formation of **(1a)** and **(2)** can be explained according to the following proposed mechanism, **Figure 1**.

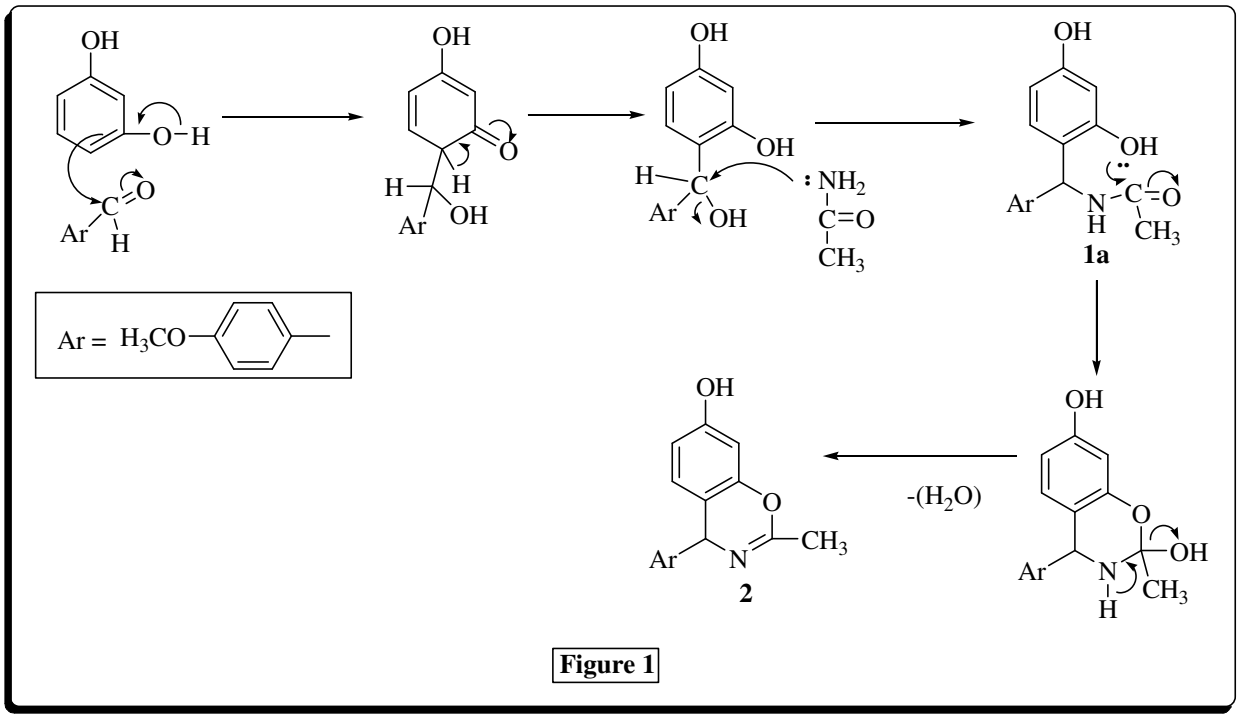
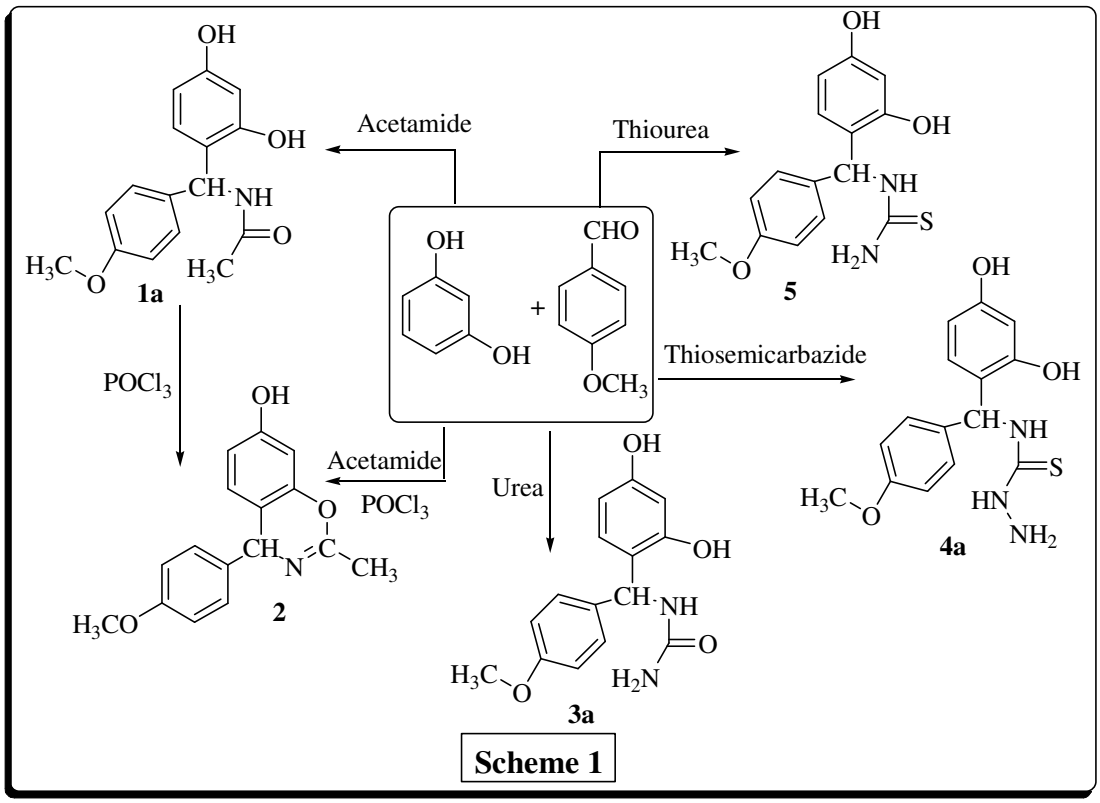


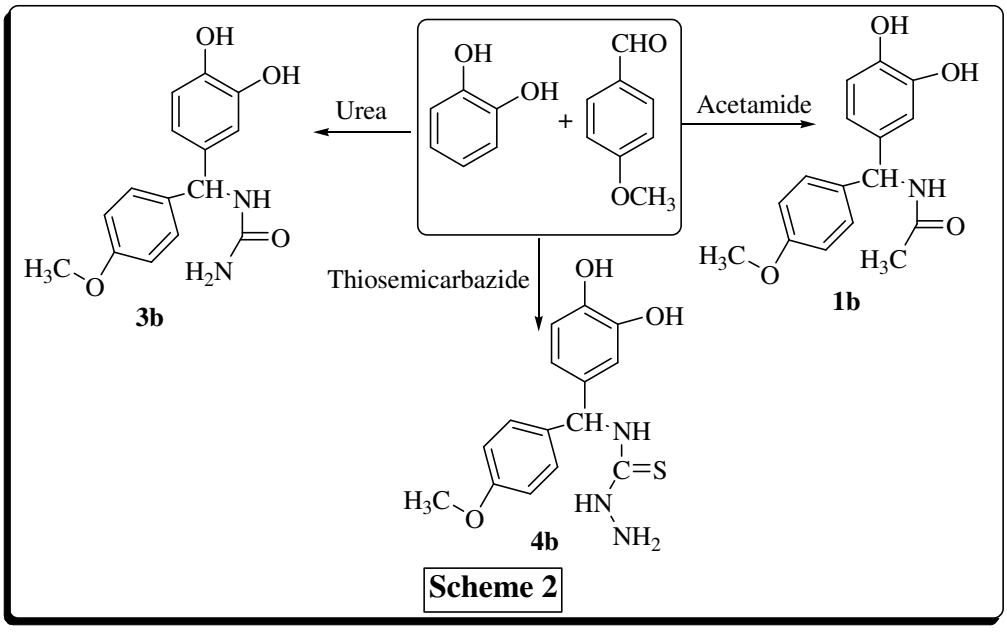
Figure 1

With a similar mechanism fusion of anisaldehyde, resorcinol or catechol and urea gave 1-[(2,4-dihydroxyphenyl)(4-methoxyphenyl)methyl]urea **(3a)**, 1-[(3,4-dihydroxyphenyl)(4-methoxyphenyl)methyl]urea **(3b)**, respectively.

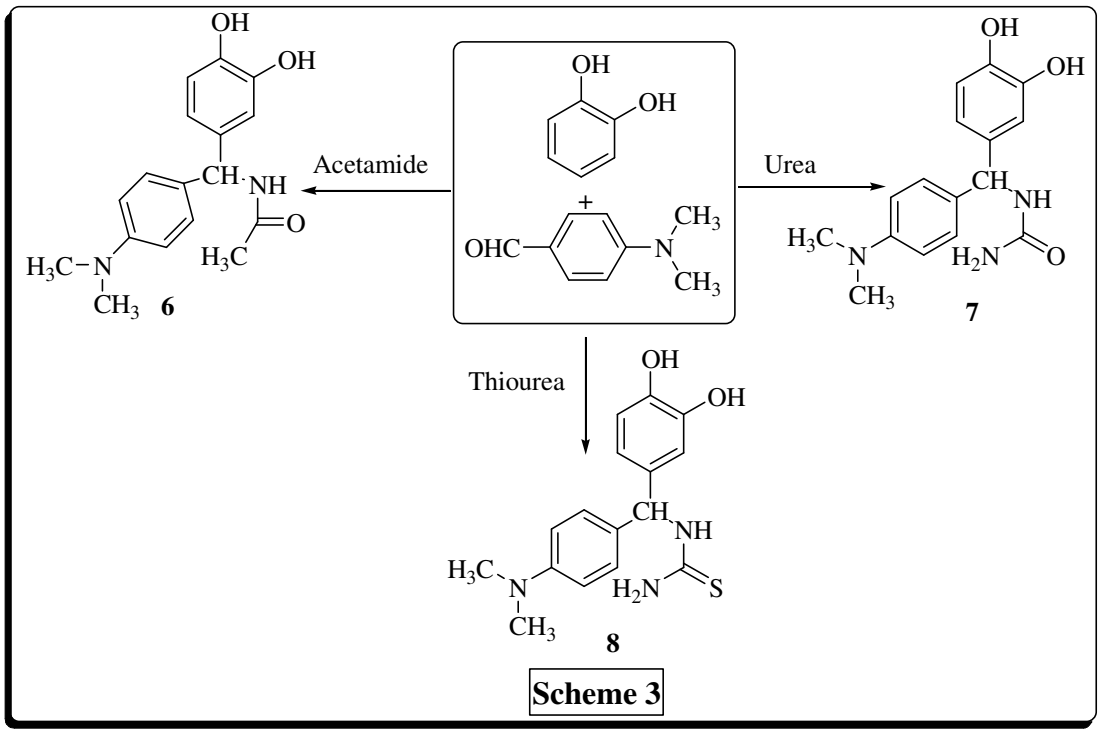
However, fusion of anisaldehyde, resorcinol and thiosemicarbazide gave 4-[(2,4-dihydroxyphenyl)(4-methoxyphenyl)methyl]thiosemicarbazide **(4a)**. However reflux of anisaldehyde, catechol and thiosemicarbazide in $AlCl_3$/ethanol gave 4-[(3,4-dihydroxy-phenyl)(4-methoxyphenyl)methyl]thiosemicarbazide **(4b)**.

On the other hand, fusion of anisaldehyde, catechol and thiourea gave1-[(2,4-dihydroxy-phenyl)(4-methoxyphenyl)methyl]thiourea **(5)**.

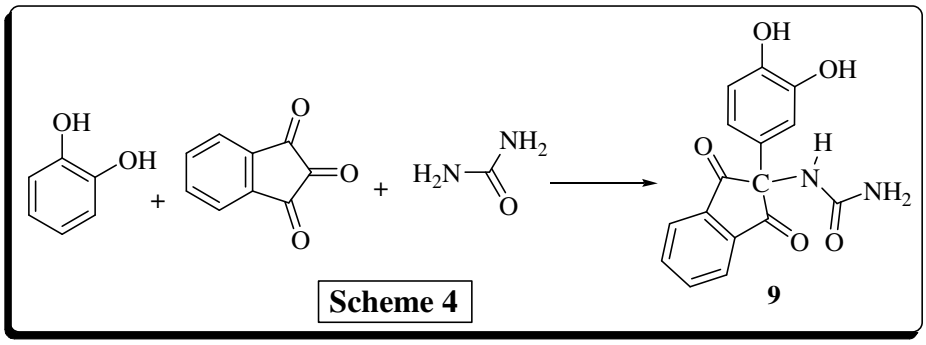




Interestingly, fusion of catechol, N,N-dimethylbenzaldehyde with acaetamide gave N-[(3,4dihydroxyphenyl)(4-(dimethylamino)phenyl)methyl]acetamide **(6),** with urea gave 1-[(3,4-dihydroxyphenyl)(4-(dimethylamino)phenyl)methyl]urea **(7)**, while with thiourea gave 1-[(3,4-dihydroxyphenyl)(4-(dimethylamino)phenyl)methyl]thiourea **(8)**, respectively.



Finally, fusion of catechol, urea with ninhydrin at 160°C gave 1-[2-(3,4-dihydroxyphenyl)-1,3-dioxo-2,3-dihydro-1H-inden-2-yl]urea(**9**).



3. Experimental

All Melting points were determined on an electrothermal (9100) apparatus and are uncorrected. The infrared spectra (ν in cm^{-1}) were recorded using KBr disks on a Pye Unicam SP-3-300 infrared spectrophotometer in the Central laboratory of Ain Shams University. Mass spectra were recorded on a GC-MS 2010 Shimadzu in Cairo University. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer in DMSO-d_6 as solvent and TMS as internal standard in Laboratoire Chimie et Procédés, DCSO, UMR 7652, Ecole Nationale Supérieure de Techniques Avancées (ENSTA), Paris, France, chemical shifts are quoted in δ (ppm). TLC was carried out for monitoring of the progress of all reactions and homogenity of the synthesized compounds. TLC was determined using TLC aluminium sheets silica gel F254 (Merck).

3.1 General procedure for the synthesis of compounds ***(1-9)***

Resorcinol and catechol (0.01mol), anisaldehyde, N,N-dimethylbenzaldehyde and ninhydrin (0.01mol) and acetamide, urea, thiosemicarbazide and thiourea (0.01mol), were mixed in dry flask. The reaction mixture was fused at 150-180°C in an oil bath for 4-6 hr. The products were scratched by water or boiling in ethanol and then isolated by filtration. The products were washed by boiling ethanol (2x50 ml) and then by boiling dioxane (2x50 ml), filtered and dried.

3.1.1 N-[(2,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]acetamide ***(1a)***

Red crystals; yield (75%), mp: >300°C; I.R: 1605 (CO), 3291-3365 (NH, OHs), MS: m/z, 287(M^{+}, 0.50%), 288(M+1, 0.35%), 57(100%). Anal.Calcd. for $C_{16}H_{17}NO_4$ (287.31): C, 66.89; H, 5.96; N, 4.88. Found: C, 67.22; H, 5.84; N, 4.72.

3.1.2 N-[(3,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]acetamide ***(1b)***

Brown powder; yield (59%), mp: >300°C; I.R: 1608 (CO), 3201-3475 (NH, OHs);), ^{1}H-NMR: 8.65 (s, 2H, OH), 7.4 (s, 1H, NH), 7.25-6.6 (m, 7H, ArH), 6.3 (s, 1H, CH), 3.65 (s, 3H, OCH_3), 2.5 (s, 3H, $CO\underline{CH_3}$), MS: m/z, 287(M^{+}, 72.45%), 288(M+1, 64.29%), 87(100%). Anal.Calcd. for $C_{16}H_{17}NO_4$ (287.31): C, 66.89; H, 5.96; N, 4.88. Found: C, 66.68; H, 6.02; N, 4.71.

3.1.3 1-[(2,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]urea ***(3a)***

Reddish brown powder; yield (52%), mp: >300°C; I.R: 1608 (CO), 3221-3399 (NH, OHs, NH_2), MS: m/z, 288(M^{+}, 14.4%), 289(M+1, 73.3%), 141(100%). Anal.Calcd. for $C_{15}H_{16}N_2O_4$ (288.3): C, 62.49; H, 5.59; N, 9.72. Found: C, 62.21; H, 5.72; N, 9.87.

3.1.4 1-[(3,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]urea ***(3b)***

Reddish brown powder; yield (66%), mp: 158°C; I.R: 1663 (CO), 3267-3473 (NH, OHs, NH_2), ^{1}H-NMR: 8.6 (s, 2H, OH), 7.9 (s, 1H, NH), 7.4-6.25 (m, 7H, ArH), 5.45 (s, 1H, CH), 5.2 (s, 2H, NH_2), 3.4 (s, 3H, OCH_3), MS: m/z, 288(M^{+}, 48.82%), 289(M+1, 47.24%), 69(100%). Anal.Calcd. for $C_{15}H_{16}N_2O_4$ (288.3): C, 62.49; H, 5.59; N, 9.72. Found: C, 62.32; H, 5.51; N, 9.59.

3.1.5 4-[(2,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]thiosemicarbazide ***(4a)***

Red powder; yield (56%), mp: > 300°C; I.R: 1202- 3430 (NH, OHs, NH_2), ^{1}H-NMR: 11.35 (s, 2H, OH), 8.5&8.4 (s & t, 2H, NH), 8.4-6.1 (m, 7H, ArH), 5.6 (s, 1H, CH), 5.45 (d, 2H, NH_2), 3.65 (s, 3H, OCH_3), MS: m/z, 319(M^{+}, 84.9%), 320(M+1, 94.2%), 321(M+2, 81.4%), 171(100%). Anal.Calcd. for $C_{15}H_{17}N_3O_3S$ (319.38): C, 56.41; H, 5.37; N, 13.16; S, 10.04. Found: C, 56.22; H, 5.38; N, 13.04.

3.1.6 1-[(2,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]thiourea ***(5)***

Reddish brown powder; yield (57%), mp: > 300°C; I.R: 1222-3449 (NH, OHs, NH_2), MS: m/z, 304(M^{+}, 67.07%), 305(M+1, 75.61%), 306(M+2, 63.41%), 174(100%). Anal.Calcd. for $C_{15}H_{16}N_2O_3S$ (304.36): C, 59.19; H, 5.30; N, 9.20; S, 10.54. Found: C, 59.03; H, 5.20; N, 9.41.

3.1.7 N-[(3,4-Dihydroxyphenyl)(4-(dimethylamino)phenyl)methyl]acetamide ***(6)***

Grey powder; yield (53%), mp: >300°C; I.R: 1609(CO), 1243-3368(NH, OHs), MS: m/z, 300(M^{+}, 60.0%), 301(M+1, 66.3%), 302(M+2, 63.2%), 52(100%). Anal.Calcd. for $C_{17}H_{20}N_2O_3$ (300.35): C, 67.98; H, 6.71; N, 9.33. Found: C, 67.62; H, 6.70; N, 9.46.

3.1.8 1-[(3,4-Dihydroxyphenyl)(4-(dimethylamino)phenyl)methyl]urea ***(7)***

Grey powder; yield (63%), mp: 120°C; I.R: 1647(CO), 1223-3349 (NH, OHs, NH_2), MS: m/z, 301(M^{+}, 9.9%), 302(M+1, 5.8%), 303(M+2, 6.1%), 121(100%). Anal.Calcd. for $C_{16}H_{19}N_3O_3$ (301.34): C, 63.77; H, 6.36; N, 13.94. Found: C, 63.52; H, 6.28; N, 14.13.

3.1.9 1-[(3,4-Dihydroxyphenyl)(4-(dimethylamino)phenyl)methyl]thiourea ***(8)***

Grey powder; yield (70%), mp: 188°C; I.R: 1203-3335 (NH, OHs, NH_2), MS: m/z, 317(M^{+}, 33.3%), 318(M+1, 35.8%), 63(100%). Anal.Calcd. for $C_{16}H_{19}N_3O_2S$ (317.41): C, 60.54; H, 6.03; N, 13.24; S, 10.10. Found: C, 60.42; H, 5.94; N, 13.40.

3.1.10 1-[2-(3,4-Dihydroxyphenyl)-1,3-dioxo-2,3-dihydro-1H-inden-2-yl]urea ***(9)***

Brown powder; yield (65%), mp: >300°C; I.R: 1640, 1666 (COs), 1211-3337(NH, OHs, NH_2), MS: m/z, 312(M^{+}, 28.5%), 313(M+1, 25.1%), 57(100%). Anal.Calcd. for $C_{16}H_{12}N_2O_5$ (312.28): C, 61.54; H, 3.87; N, 8.97. Found: C, 61.33; H, 3.82; N, 9.13.

3.2 Synthesis of 4-(4-Methoxyphenyl)-2-methyl-4H-benzo[e][1,3]oxazin-7-ol ***(2)***

Resorcinol (1.1g, 0.01mol), anisaldehyde (1.56g, 0.01mol) and acetamide (0.6g, 0.01mol) and $POCl_3$ (10 ml) were mixed. The reaction mixture was heated on water bath for 2 h. The solution was poured onto [crushed ice/HCl] with stirring. The precipitate was filtered and washed with water. The product was recrystallized from ethanol to give red powder.

Authentic sample **(2)**

A mixture of **1a** (2.9 g, 0.01 mol) and $POCl_3$ (10 ml) was heated on water bath for 2 h. The solution was poured onto [crushed ice/HCl] with stirring. The precipitate was filtered and washed with water. The product was recrystallized from ethanol to give red powder.

Yield (55%), mp: >300°C; I.R: 3403 (OH), MS: m/z, 269(M^{+}, 16.7%), 270(M+1, 13.9%), 271(M+2, 14.4%), 94(100%). Anal.Calcd. for $C_{16}H_{15}NO_3$ (269.3): C, 71.36; H, 5.61; N, 5.20. Found: C, 71.21; H, 5.60; N, 5.74.

3.3 Synthesis of 4-[(3,4-Dihydroxyphenyl)(4-methoxyphenyl)methyl]thiosemicarbazide ***(4b)***

Catechol (1.1g, 0.01mol), anisaldehyde (1.56g, 0.01mol), thiosemicarbazide (0.91, 0.01mol), and $AlCl_3$/ethanol (0.5gm/50ml) were mixed. The reaction mixture was heated on water bath for 2 h. The solution was poured onto [crushed ice/HCl] with stirring. The precipitated product was filtered and washed with water. The product (**4b**) was recrystallized from dioxane to give dark violet powder; yield (72%), mp: 155-157°C; I.R: 3211-3449(NH, OHs, NH_2), MS: m/z, 319(M^{+}, 60.6%), 320(M+1, 19.2%), 134(100%). Anal. Calcd. for $C_{15}H_{17}N_3O_3S$ (319.38): C, 56.41; H, 5.37; N, 13.16; S, 10.04. Found: C, 56.23; H, 5.40; N, 12.98.

4. Antitumor activity studies

4.1 Sulforhodamine-B(SRB)assay of cytotoxic activity

MCF-7 (breast carcinoma cell line), HepG-2 (hepatocellular carcinoma cell line) were obtained frozen in liquid nitrogen (-180°C) from the American type culture collection. The tumor cell lines were maintained in the National cancer Institute, Cairo, Egypt, by serial sub-culturing. Potential cytotoxicity of **1a** and **1b** were tested using the method of Skehan *et al*.

4.2 Principle

The sensitivity of the human tumor cell lines to thymoquinone was determined by the SRB assay. SRB is a brought pink aminoxanthrene dye with two sulfonic groups. It a protein stain that binds to the amino group of intracellular proteins under mildy acidic conditions to proceed a sensitive index of cellular protein content.

4.3 Procedure

1-Cells were used when 90% confluence was reached in T25 flasks. Adherent cell lines were harvested with 0.025% trypsin. Vealrlity was determined by trypan blue exclusion using the invested microscope (Olympus 1x70, Tokyo, Japan).

2-Cells were seeded in 96- well microliter plates at a concentration of $5x10^4 – 10^5$ cell / well in a fresh medium and left to attach to the plates for 24h.

3-After 24h, cells were incubated with the appropriate concentration ranges of drugs, completed to total of 200µ volume / well using fresh medium and incubation was continued for 24, 48 and 72h. Cells were treated with vehicle alone. For each drug concentration, 4 wells were used.

4-Following 24, 48 and 72h.Treatment, the cells were fixed with 50µl cold 50% trichloroacetic acid for 1h at 4°C.

5-Wells were washed 5 times with distilled water and stained for 30 min at room temperature with 50µl 0.4% SRB dissolved in 1% acetic acid.

6-The wells were then washed 4 times with 1% acetic acid.

7-The plates were air-dried and the dye was solublized with 100µl / well or mMtris base (Ph 10.5) for 5 mm on a shaken (orblal shaken 0520, Boeco, Germany) at 1600 rpm.

8-The optical density (O.D.) of each well was measured spectrophotometrically at 564 nm with an ELIZA microplate reader (Meler tech Σ960, U.S.A.).The mean background absorbance was automatically substracted and mean values of each drug concentration were calculated. The relation between survival fraction and compound concentration was plotted to get the survival curve of each tumor all lines (Fig.2), the IC50 values (the concentrations of thymoquinone required to produce 50% inhibition of cell growth (Fig.3)

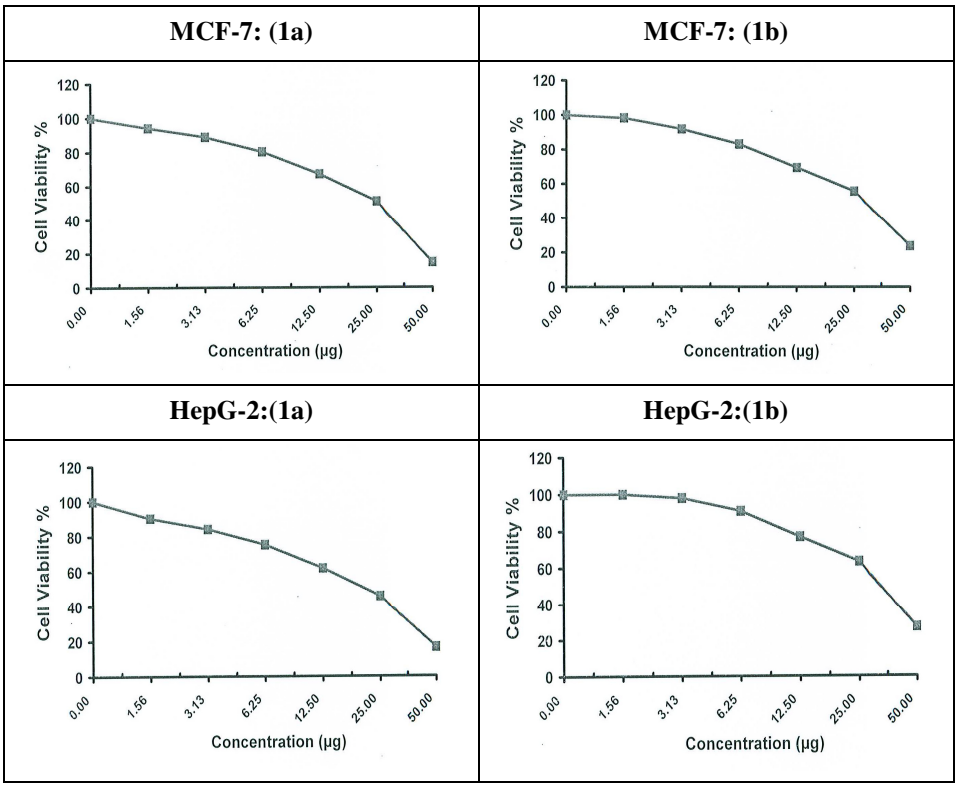
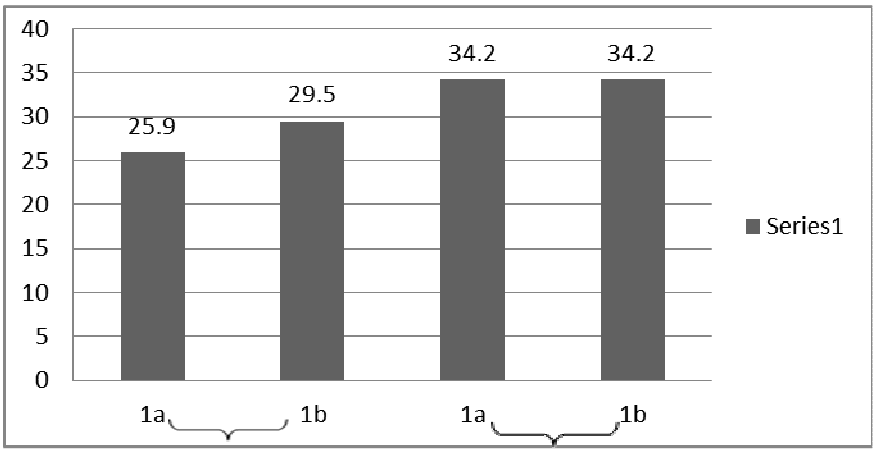


Fig. (2): Anti-tumor cytotoxicity of different concentrations (µg/ml) of compounds 1a and 1b. Against different human cancer cell lines *in vitro*.

4.4 Cytotoxicity against different human cancer cell lines in vitro

For evaluation of anti-tumor cytotoxicity of compounds **1a**, **1b** three different human cancer cell lines were used: **MCF-7** (breast carcinoma cell line), **HepG-2** (hepatocellular carcinoma cell line). Cytotoxicity and **IC50** values of the tested compounds are shown in Fig.**2** and Fig.**3**. The survival fractions were gradually decreased as the concentration of the tested compounds was increased (table**1**). From figure **3**, it has been shown that **1a**, **1b** are the compounds of high **IC50** which means that they are effective cytotoxic drugs; accordingly these compounds can be used as cytotoxic drug for breast carcinoma cell and for liver carcinoma cell.



MCF-7 **HepG-2**

Fig. (3): 0-10 very potent cytotoxic drug, 10-20 moderate cytotoxic drug, >20 very weak cytotoxic drug**.**

Table (1): effect of some new prepared compounds on different types of tumor cells as cytotosxic drug

Con.µg/ml	MCF-7		HepG-2	
	1a	1b	1a	1b
0	100	100	100	100
1.56	94.21	98.28	90.34	100
3.125	89.02	91.87	84.14	97.92
6.25	80.45	83.12	75.36	90.87
12.5	67.37	69.48	61.93	76.84
25	51.24	55.76	45.82	63.29
50	14.78	23.82	16.46	27.31

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