World Journal of Chemistry 4 (2): 112-117, 2009
ISSN 1817-3128

New Synthesis of Tetrahydrobenzo [4,5] Thieno [2,3d] Pyrimidine Derivatives and Schiff Bases Derived from 2-aminotetrahydro Benzothiophenes and Hetarylcarboxaldehydes Studies on Their Antitumor and Antimicrobial Activities

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Abstract: 2-Amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide **(1)** reacted with 6-carboxaldehyde chromone derivatives **2a-d** in boiling pyridine to give the corresponding pyrimidine derivatives **3a-d.** On the other hand, compound **l** was reacted with **2a-d** and **5a,b** in boiling ethanol to give the corresponding Schiff bases **4a-d** and **6a,b;** respectively. Also, 2-amino-3-ethyl carboxylate-4,5,6,7-tetrahydrobenzo[b]thiophene **(7)** reacted with **2a,b** and **5a,b** to give **8a,b** and **9a,b,** respectively. Compounds **1,7, 3a-c, 4a,b, 6b** and **8a,b** were tested for their antitumor, antimicrobial and antibacterial activities.

Key word: Chromone · Thieno [2,3-d] pyrimidine · Antitumor · Antimicrobial

INTRODUCTION

It has been reported that chromones and furochromones exhibit diverse biological and physiological activities [1-3]. Since the days of the ancient Egyptians, chromones derivatives have been known as antispasmodics for relief of spasms of the ureter, bile duct, gall bladder and bronchial asthma. Moreover, they are known to be associated with spasmolytic activities [4-6] and antihistaminic activity [7]. Also, Schiff Bases attract much interest both for synthetic and biological point of view [8]. Thus, they can be used in the preparation of pharmaceuticals, plastics, as well as pesticides and can occur as intermediates in much enzymatic reaction [9-14]. Meanwhile, useful pharmacological properties are also associated with presence of 5- and 6-membered heterocyclic rings in drug molecules [15]. This prompted us to design and prepare new pyrimidine and Schiff Bases where in two moieties incorporating heterocycles are linked together through azomethine (-CH=N-) grouping and to study their antitumor, antimicrobial and antibacterial activities.

MATERIALS AND METHODS

All melting points are uncorrected. The IR spectra were recorded on a PyeUnicamSP-1100 Spectrophotometer using KBr discs. The ^{1}H-NMR spectra were recorded on a Varian EM-390-90MHz Spectrometer using DMSO-d_6 as a solvent and TMS as internal standard, chemical shifts are expressed as δ ppm units. Microanalytical data were carried out in Microanalytical Center; Faculty of Science; Cairo University.

Preparationof2-(2-methyl-4-oxo-4H-chromen-6-yl)-3H-benzo [4, 5] Thieno [2, 3] Pyrimidin-4-one Derivatives 3a-d. *General Procedure*: A solution of l (10 mmol) and the appropriate aldehyde **2a-d** (10mmol) in 15 ml pyridine was heated under reflux for 2/3 hrs. After cooling, the reaction mixture was acidified to (pH ~ 6) by addition of 2M HCl. On standing overnight in a refrigerator, the crystalline product was filtered off and recrystallized from appropriate solvent giving **3a-d** (c.f. Table 5).

Preparation of Schiff Bases 4a-d, 6a,b, 8a,b and 9a,b: *General Procedure*: To a suspension of each of 1 and 7 (0.01 mol) and the appropriate hetarylcarboxaldehyde (0.01 mol) in absolute ethanol (40 mL). A few drops of piperidine was added to the reaction mixture then refluxed for 4/5 hrs. Cool the reaction mixture, the solid that separated was filtered off, washed by small amount of cold ethanol and crystallized from appropriate solvent to give the corresponding compounds (c.f. Table 6).

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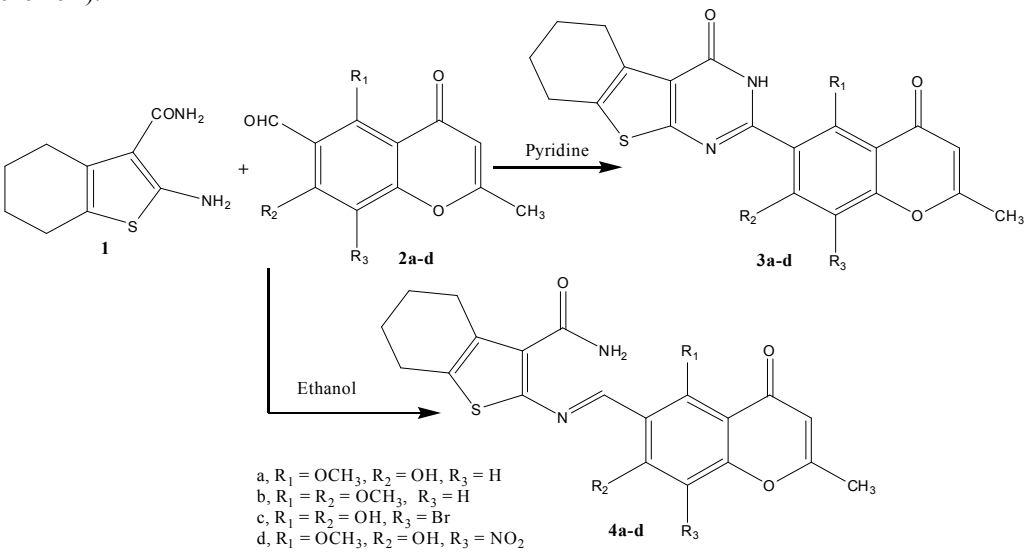
RESULTS AND DISCUSSION

The starting material, 2-amino-4,5,6,7-tetrahydrobenzo [b] thiophene-3-carboxamide **(1)** was prepared from the reaction of cyclohexanone, cyanoacetamide and elemental sulfur in presence of diethylamine following the Gewald reaction condition [16].

The starting 1 was reacted with 6-carboxaldehyde chromone derivatives (**2a-d**) [17] in boiling pyridine, thermal cyclodehydration/dehydrogentation gave the target,2-(2-methyl-4-oxo-4H-chromen-6-yl)-3H-benzo[4,5] thieno [2,3d] pyrimidin-4-one derivatives (**3a-d**) in good yields (Scheme 1).

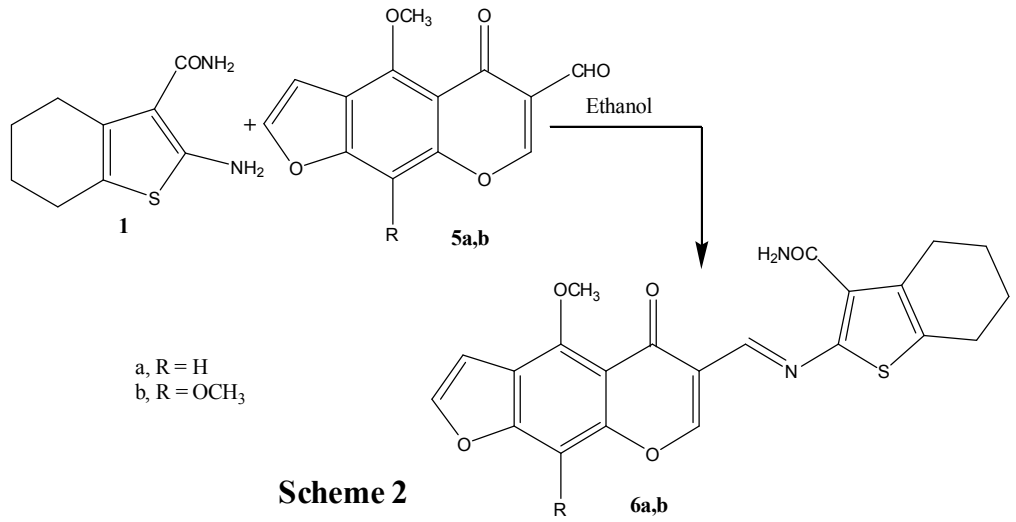
The work was further extended to investigate the behaviour of **(1)** towards (**2a-d**) and furochromones (**5a,b**) [18] in boiling ethanol the corresponding Schiff bases 2-[(2-methyl-4-oxo-4H-chromen-6-yl-methylene)-amino] benzo [b] thiophene-3-carboxamide (**4a-d**) (Scheme 1) and 2-[(4-methoxy-5-oxo-5H-furo[2,3-g]chromen-6-yl-methylene)-amino]benzo[b]thiophene-3-carboxamide (6a,b) were obtained (Scheme 2).

On the other hand, 2-amino-3-ethylcarboxylate-4, 5, 6, **7**-tetrahydrobenzothiophene (7) reacted with (2a,b) and (**5a,b**), the corresponding Schiff bases (**8a,b**) and (**9a,b**), respectively, were obtained (Scheme 3).



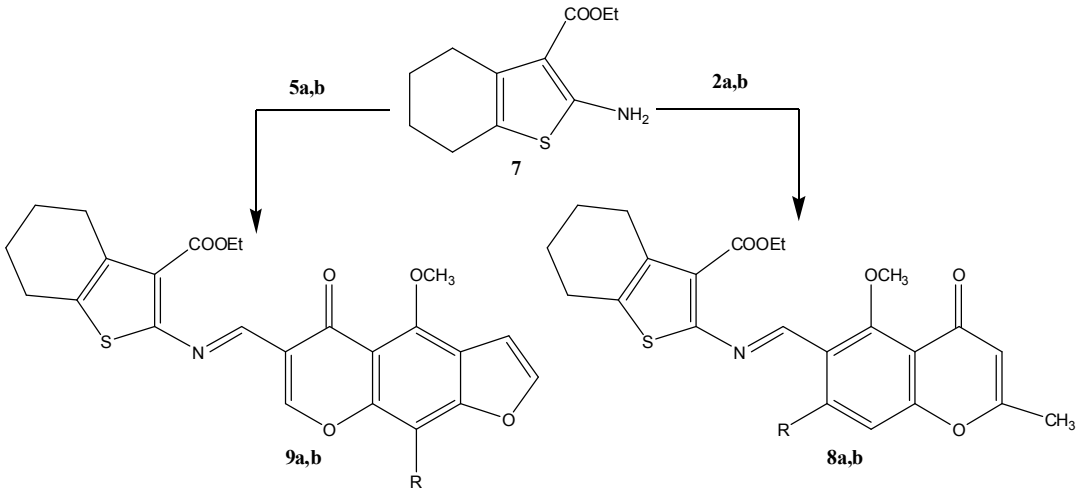
Scheme 1

Scheme 1:



Scheme 2

Scheme 2:



Scheme 3

Scheme 3:

Table 1: Cytotoxicity of some new synthesized compounds

		Decrease in the growth of BG-1		Decrease in the growth of OVCAR3	
Compounds	DoseLevelmol/L	After treatment for 6 days	After treatment for 9 days	After treatment for 6 days	After treatment for 9 days
1	10-9	80±8%	89.8 ± 5.3%	95.2± 12.8%	93.9 ± 17.8%
3a	10-8	70.1±5.7%	70.4± 9.4%	73± 17.7%	88± 7.1%
3b	10-8	54.7±3.7%	40.6± 4.3%	50 ± 5%	62.8± 4.4%
3c	10-8	63.9±4.8%	59.8 ± 5.3%	60.7± 1.7%	65± 7%
4a	10-9	83.9±7.8%	90.8 ± 1.3%	96 ± 7.8%	95± 15%
4b	10-8	42.4±2.4%	39.8 ± 2.3%	42.4 ± 1.7%	55.8± 8.4%
6b	10-9	75±7%	80.6 ± 7.9%	94.3 ± 11.7%	91.2 ± 11.3%
7	10-8	69.7±5.4%	61.5± 5.3%	72.1± 7.7%	68± 8%

Antitumor Activity

Cytotoxicity: All tested compounds Suppresses the Growth of Ovarian Cancer Cells and Transcriptionally Activates the GADD45 Reporter Gene at Concentrations Lower Than 1,25(OH)2D3. Before investigating the in vivo response of ovarian tumor xenografts to EB1089, we first examined the activity of EB1089 in suppressing the growth of ovarian cancer cells in vitro using 1, 25(OH) 2D3 as a control. OVCAR3 and BG-1 cells were treated with vehicle, 1,25(OH)2D3, or the newly synthesized tested compounds at indicated concentrations and the cell growth was determined in MTT assays, as shown in Table 1. 1,25(OH)2D3 at 10-7 mol/L caused a significant decrease in the growth of OVCAR3 and BG-1 cells after treatment for 6 days (43.9 F 7.8% for OVCAR3 and 62.4 F 17.7% for BG-1) and 9 days (59.8 F 5.3% for OVCAR3 and 71.1 F 14.2% for BG-1). The inhibition was not observed for 1, 25(OH) 2D3 at 10-8 or 10-9 mol/L,. In contrast to 1, 25(OH) 2D3, tested compounds lower than at 10-8 mol/L inhibited the growth as shown in Table 1.

Xenografts Evaluation: All the tested compounds Inhibits the Growth of OVCAR3 Tumor Xenografts in Nude Mice without Causing Hypercalcemia. After the growth-suppressing activity of the tested compounds was verified in vitro using OVCAR3 and BG-1 cells, we next investigated its in vivo effect on the growth of OVCAR3 tumor xenografts as described in material and method. Treatment with the tested compounds at 1 μ/d/kg body weight (lower doses give no response) almost suppressed the growth of the tumors (Table 2). In comparison with the placebo controls, the tumor suppression by the tested compounds at both concentrations after 30 days reached statistical significance ($P < 0.01$).

Relative potency of tested compounds to anstrazole was evaluated (c.f. Table 3).

Table 2: Xenografts evaluations of some new synthesized compounds

	% tumor growth suppression at dose level 1 μ/d/kg body weight after time in days					
Compounds	5 days	10 days	15 days	20 days	25 days	30days
1,25(OH)2D3	23±1.2%	55.5±5.4%	77.7±4.4%	80.1±4.2%	98.6±5.4%	100%
1	79±4%	100%	------	------	------	------
3a	65.3±2%	85±3%	85±1%	100%	------	------
3b	39.6±2.1%	61.6±3.8%	83.6±5.3%	97±4.5%	100%	------
3c	40.1±2%	80±3.3%	89±1%	100%	------	------
4a	89.1±3.4%	100%	------	------	------	------
4b	34.5±1.2%	60.16±4.8%	80.6±3.3%	95±4%	100%	------
6b	8.4±3.4%	97.1±3.2%	100%	------	------	------
7	42.4±2.1%	81±1.3%	92±1%	100%	------	------
8a	77.2±2.3%	90.3±3.2%	100%	------	------	------
8b	100%	------	------	------	------	------

Table 3: Relative potency of tested compounds to anstrazole

Compound	Relative potency to anstrozole	Compound	Relative potency to anstrozole
1,25(OH)2D3	1.9	4b	2.34
1	12.5	6b	11.67
3a	8.36	7	5.12
3b	3. 11	8a	10.67
3c	4.56	8b	30.56
4a	19.67	Anstrazole	1.00

Table 4: The antimicrobial activity of compounds 1, 3a, 3b, 3c, 4a, 4b, 6b, 7, 8a and 8b

Comp.No.	*Bacillus subtilis*	*Candid autilis*	*Escherichia coli*	*Staphylococcus aureus*	Pseudo- monas
1	0.40	--	0.30	0.55	0.35
3a	--	--	0.10	0.15	--
3b	--	--	--	0.15	--
3c	0.20	--	0.15	0.30	--
4a	0.25	--	0.25	0.20	--
4b	0.15	--	0.15	0.25	--
6b	--	--	--	--	--
7	0.80	0.40	0.85	0.65	0.50
8a	0.90	0.10	1.25	1.20	0.60
8b	0.15	--	0.15	0.15	--

Antimicrobial Activity: Table shows the effect of compounds **1, 3a, 3b, 3c, 4a, 4b, 6b, 7,8a** and **8b** on the micro-organisms tested. It is on interest to note that the most active compound is followed by the other compounds which are slightly less active.

Antitumor Activity

Cell Culture and Assays for Growth, Apoptosis and Transcriptional Activation of the GADD45 Reporter: OVCAR3 human ovarian cancer cells (HTB-161, obtained from American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 supplemented with15% fetal bovine serum (Life Technologies, Grand Island, NY), 2 mmol/L L-glutamine, 50 units/mL penicillin, 50 Ag/mL streptomycin, 10 mmol/L HEPES, 1mmol/L sodium pyruvate, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate and 10Ag/mL bovine insulin. BG-1 cells were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum. To determine cell numbers, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assays were done as described (15). Absorption at 595 nm (A595nm) was measured on a MRX microplate reader (Dynex Technologies, Chantilly, VA).Cell numbers were calculated based on a standard curve. Apoptosis was determined by flow cytometry analysis after cells were stained with Annexin V-FITC and propidium iodide following manufacturer's instructions (Santa Cruz Biotechnology, Santa Cruz, CA). Transcriptional

Table 5: Physical, analytical and spectroscopic data for **3a-d**

Compound (colour)	m.p.(°C) (solvent)	Yield %	Mol. Formula	Elemental analysis C	H	N	S	Br	IR data (cm^{-1})	MS(M+)m/z
3a*	255-257			61.45	4.42	6.83	7.81	--	3400 (OH); 3270 (NH); 1670,	
(orange)	(acetone)	70	$C_21H_{18}N_2O_5S$	61.12	4.11	6.52	8.13	--	1655 (2C=O); 1630 (C=N)	410
3b	250-252			62.26	4.72	6.60	7.75	--	3310(NH); 1665, 1650 (2C=O);	
(brown)	(acetone)	75	$C_{22}H_{20}N_2O_5S$	62.35	4.48	6.39	7.52	--	1635 (C=N)	424
3c	259-262			51.53	3.48	5.72	6.54	16.34	3390, 3370 (OH); 3325 (NH);	
(orange)	(chloroform)	70	$C_{21}H_{17}N_2O_5SBr$	50.96	4.11	5.93	6.34	15.92	1660,1598(2C=O);1625 (C=N);	
									660 (C-Br)	489
3d**	>280			55.38	3.74	9.23	7.03	--	3390 (OH);3200 (NH); 1675,	
(brown)	(chloroform)	75	$C_{21}H_{17}N_3O_7S$	55.60	3.35	8.95	6.81	--	1665 (2C=O); 1635 (C=N);	
									1530, 1350 (NO2)	455

3a* ^{1}H NMR (DMSO-d_6) (δ ppm): 13.1 (1H,s, OH); 12.2 (1H, br.s, NH); 6.3, 6.8 (2H, 2s, 2CH=C); 3.86 (3H, s, OCH_3); 2.3 (3H, s, CH_3); 2.3-3.1 (8H, m, Saturated protons of tetrahydrobenzothiophene)

3d** ^{1}H NMR (DMSO-d_6) (δ ppm): 13.3(1H,s, OH); 12.4(1H, br.s, NH); 6,5(1H,s, CH=C); 3.76(3H,s, OCH_3); 2.4 (3H,s, CH_3); 2.5-3 (8H, m, Saturated protons of tetrahydrobenzothiophene)

Table 6: Physical, analytical and spectroscopic data for Schiff Bases **4a-d; 6 a,b; 8a,b &9a,b**

Compound (colour)	m.p.(°C) (solvent)	Yield%	Mol. Formula	Elemental analysis C	H	N	S	Br	IR data (cm^{-1})	MS(M+)m/z
4a*	199-200			61.16	4.85	6.79	7.77	--	3350 (OH); 3540, 3380 (NH2);	
(yellow)	(acetone)	75	$C_{21}H_{20}N_2O_5S$	61.50	5.17	6.41	6.59	--	1690, 1660 (2C=O); 1630 (C=N).	412
4b	240-243			61.97	5.16	6.57	7.51	--	3550, 3370 (NH2); 1680, 1655	
(yellow)	(acetone)	70	$C_{22}H_{22}N_2O_5S$	61.62	4.93	7.82	7.32	--	(2C=O); 1635 (C=N)	426
4c	230			50.31	3.56	5.87	6.71	16.75	3380, 3365 (OH); 3540, 3360	
(light brown)	(chloroform)	75	$C_{20}H_{17}N_2O_5SBr$	50.62	3.25	6.15	6.48	17.01	(NH2); 1695, 1665 (2C=O);	
									1630 (C=N); 670 (C-Br)	477
4d**	>280			55.14	4.15	9.19	7.00	--	3390 (OH);3554, 3375 (NH2);	
(brown-red)	(chloroform)	70	$C_{21}H_{19}N_3O_7S$	55.53	4.41	8.85	7.31	--	1690, 1660 (2C=O); 1625 (C=N);	
									1520, 1340 (NO2)	457
6a*	240-243			61.46	4.39	6.83	7.80	--	3500, 3340 (NH2); 1685, 1662	
(brown)	(chloroform)	70	$C_{21}H_{18}N_2O_5S$	61.1	4.71	7.10	7.50	--	(2C=O); 1640 (C=N)	410
8a*	179-180			62.58	5.21	3.17	7.25	--	3385 (OH); 1710, 1680 (2C=O);	
(yellow)	(acetone)	70	$C_{23}H_{23}NO_6S$	62.81	4.79	3.38	7.49	--	1635 (C=N)	441
8b	160-162			63.29	5.49	3.07	7.03	--		
(yellow)	(acetone)	65	$C_{24}H_{25}NO_6S$	63.10	5.21	3.34	6.81	--	1720, 1670 (2C=O); 1640 (C=N)	455
9a*	199-200			63.85	4.56	3.10	7.09	--		
(brown)	(chloroform)	70	$C_{24}H_{21}NO_6S$	64.12	4.23	3.32	7.65	--	1715,1665 (2C=O); 1635 (C=N)	451
9b	203-205			62.37	4.78	2.91	6.65	--		
(orange)	(methanol)	70	$C_{25}H_{23}NO_7S$	62.11	4.52	3.13	6.50	--	1720, 1670 (2C=O); 1640 (C=N)	481

4a* ^{1}H NMR (DMSO-d_6) (δ ppm): 11.5 (1H,s, OH); 8.55 (1H,s, -CH=N-); 5.6 (2H, br, NH_2); 6.2, 6.7 (2H, 2s, 2-CH=C-); 3.55 (3H, s, OCH_3); 2.2 (3H, s, CH_3); 2.8-3.1 (8H, m, Saturated protons of tetrahydrobenzothiophene)

4d**^{1}H NMR (DMSO-d_6) (δ ppm): 11.8(1H,s, OH); 8.4(1H,s, -CH=N-); 5.5(2H, br, NH2); 6.4(1H, s, -CH=C-); 3.6(3H, s, OCH_3); 2.3(3H,s, CH_3); 2.6-3.00(8H, m, Saturated protons of tetrahydrobenzothiophene)

6a* ^{1}H NMR (DMSO-d6) (δ ppm): 8.42 (1H,s, -CH=N-); 8.0-7.1 (2H, H-2 and H-3 furan); 6.3, 6.8 (2H, 2s, 2-CH=C-); 2.8-2.1 (8H, m, Saturated protons of tetrahydrobenzothiophene)

8a* ^{1}H NMR (DMSO-d6) (δ ppm): 12.1 (1H,s, OH); 8.7 (1H,s, -CH=N-); 6.3, 6.8 (2H, 2s, 2-CH=C-); 4.2 (2H,q, CH_2CH_3); 3.6 (3H, s, OCH_3); 2.3 (3H, s, CH_3); 2.8-2.1 (8H, m, Saturated protons of tetrahydrobenzothiophene); 1.6 (3H, t, CH2CH3)

9a* ^{1}H NMR (DMSO-d6) (δ ppm): 8.34 (1H,s, -CH=N-); 8.1, 7.2 (2H, H-2 and H-3 furan); 6.4, 6.9 (2H, 2s, 2-CH=C-); 4.1 (2H,q, CH_2CH_3); 3.5 (3H,s, OCH_3); 2.6-2.3 (8H, m, saturated protons of tetrahydrobenzothiophene); 1.7 (3H, t, CH_2CH_3);. tetrahydrobenzothiophene); 1.7 (3H, t, CH2CH3);.

activation of thevitamin D receptor was done. Briefly, 4 hours posttransfection with GADD45Luc and pCMVgal, OVCAR3 cells were treated with vehicle, tested compound, or 1,25(OH)2D3 in fresh medium for 36 hours. Cells were harvested and luciferase and galactosidase activities were determined.

Growth Suppression of Ovarian Cancer Xenografts in Nude Mice by Nude Mouse Studies of OVCAR3 Tumor Xenografts: All mice were handled according to the Guide for the Care and Use of Laboratory Animals. Mouse studies were carried out following the procedures approved by the Institutional Animal Care and Use Committee at University of South Florida. For inoculation into nude mice, OVCAR3 cells were washed with PBS, digested with trypsin, resuspended in RPMI 1640 containing fetal bovine serum and pooled. After centrifugation, cells were resuspended in Matrigel (BD Biosciences Discovery Labware, Bedford, MA)-RPMI 1640 (1:1) at a concentration of 5 X 106 cells per 100 AL. Cell/Matrigel mixture of 100 AL was injected s.c. into 6-week-old female athymic nu/nu mice (Harlan Sprague-Dawley, Indianapolis, IN) on the dorsal surface. The mice were fed a vitamin D–deficient diet Supplemented with 0.47% calcium (HarlanTeklad, Madison, WI) for the duration of the study. Tumor volumes were monitored by caliper measurement of the length and width and calculated using the formula of length X width X 0.5 the greater of length or width. Treatment was begun when tumors reached volumes of f150 mm3 in average, which took ~4 weeks. Mice were randomized and treated daily by gavage with placebo or tested compound at 0.3 or 1.0 Ag/kg body weight in a volume of 20 AL. Tumor volumes and body weights were monitored every 5 days over the course of treatment. Mice were sacrificed after 30 days of treatments and tumors were removed and fixed in 10% neutral buffered formalin for histologic and immunohistochemical analyses.

Antimicrobial Activity: The microbial strain were used as target organism Bacillus subtilis (gram +ve bacteria), Pseudomonas (gram -ve bacteria), *Escherichia cloi* (gram -ve bacteria), Candida utilis (yeast) and Staphylococcus aureus. The compounds under investigation were insoluble in water, therefore they were dissolved in DMF at concentration of 10 mg\ml and filtered through Bacterial membranes filter (0.45um).

The antimicrobial effects of the compounds were tested by the whole plate method [19]. Spores or cell of tested organisms were mixed with the media before solidification (about 45°C) and the whole mixture was poured into sterile plate for solidification (pH7). 1mg of each compound dissolved in 0.1 ml of DMSO, incubation temperature was 35-37°C for bacteria and 27-30°C for yeast. The toxicity was measured after 24 and 48 hrs for bacteria and 5-7 days for yeast. The above estimation was based on the dia formed. A control experiment with DMSO was also carriedou meter of the inhibition zones formed.

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