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SYNTHESIS AND ANTICANCER ACTIVITIES OF THIAZOLES, 1,3-THIAZINES, AND THIAZOLIDINE USING CHITOSAN-GRAFTED-POLY(VINYLPYRIDINE) AS BASIC CATALYST

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Abstract – Three different series of ethylidenehydrazonothiazoles **5a-c**, **6a-c**, ethylidenehydrazono-1,3-thiazines **9a-i** and ethylidenehydrazonothiazolidine **12** have been prepared *via* reactions of ethylidenethiosemicarbazide **3a** or ethylidenethiocarbohydrazide **3b** with α-halocarbonyl compounds **4a-c**, acrylonitrile derivatives **7a-i**, and dimethyl acetylenedicarboxylate **10**, respectively. Different basic catalysts were used in these reactions such as, triethylamine, chitosan, and chitosan-grafted-poly(vinylpyridine) and the latter catalyst has precedence as environmentally friendly basic catalyst. Moreover, the selected newly synthesized products were evaluated for their anti-cancer activity against a colon carcinoma cell line (HCT-116) and liver carcinoma cell line HEPG2 and revealed promising activity especially 1,3-thiazines **9c-i**.

Biocatalysis is one of the most important tools for green chemistry.[1] This technique is based on the use of natural renewable biological materials, such as enzymes[2] and polymers,[3] that provide cleaner methodologies with high selectivity and energy-efficient operation under mild conditions in contrast to the traditional chemical catalysts. Chitosan, the naturally occurring polysaccharides, is a copolymer containing both glucosamine units and acetylglucosamine. It can be extracted from chitin by extensive

deacetylation under alkaline conditions (Figure 1).[4]

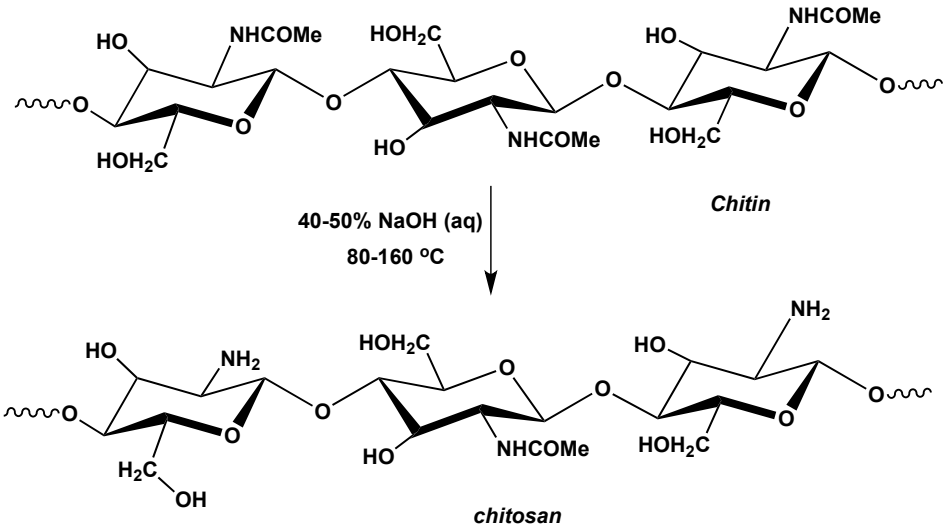


Figure 1. Extraction of chitosan from chitin

Chitosan used as heterogeneous phase transfer basic biocatalyst in heterocyclic synthesis, such as enantioselective syntheses of asymmetric products with chiral center(s)[5] and Michael additions.[6-8] Chitosan was also used to support metal for the preparation of heterogeneous catalysts.[9] One of the main problem of using chitosan that it is highly hygroscopic, leading it to form gels, thereby it could not easily be recycled from the reaction mixture. To overcome this drawback, chitosan-grafted-poly-(vinylpyridine)[10] (Figure 2) has been used as a basic biocatalyst with the following advantages; a) it can be used in the form of beads which increase its catalytic activity,[9] b) easily recycled with the same catalytic efficiency, c) higher basic character due to presence of lone pairs of electrons on nitrogen atoms in pyridine rings.[10]

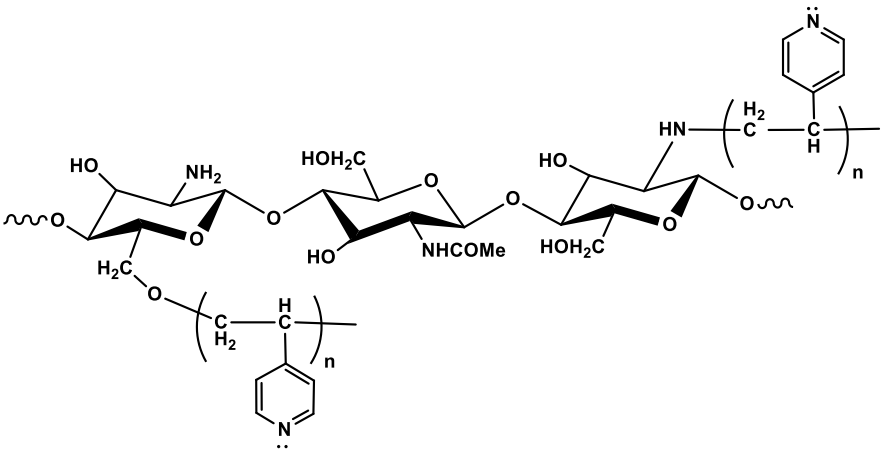
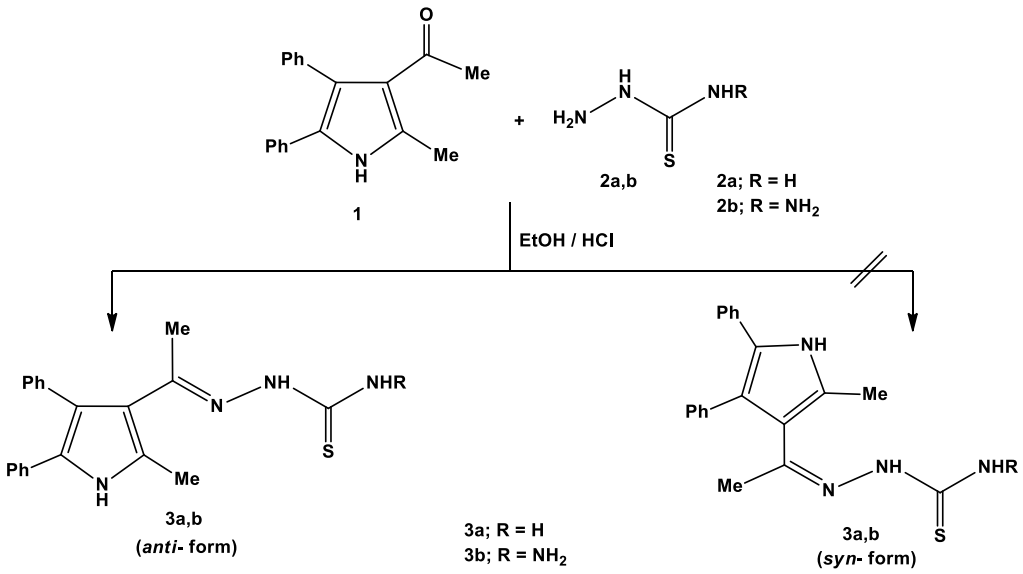


Figure 2. Structure of grafted chitosan

Meanwhile, many heterocyclic compounds containing the S-C-N framework have extensive pharmaceutical applications. Hydrazonothiazoles have demonstrated anticancer activity[11] by inhibition of

histone acetyl transferases (Gcn5 HAT), enzymes are located in the nucleus that are responsible for regulation of gene expression and cooperate with activators to enhance transcription. Also, 2,4-disubstituted thiazoles have been reported as potent anticancer agents for different cell lines.[12-14] Furthermore, 1,3-thiazines are an important group of S,N-containing heterocycles, which revealed selective antitumoral activity against leukemia cells.[15,16] In view of these precedents and as a part of our research interest towards developing new routes for the synthesis of a variety of heterocyclic systems with promising biological and pharmacological activities,[17,18] we present in this report an efficient synthesis of a new series of hydrazonothiazoles and 1,3-thiazines using chitosan-grafted-poly(vinylpyridine) as an eco-friendly biopolymeric basic catalyst. Also, we have examined the anticancer activity of these compounds against different cell lines.

Condensation of 3-acetyl-4,5-diphenyl-2-methyl-1*H*-pyrrole (**1**)[19] with thiosemicarbazide (**2a**) or thiocarbohydrazide (**2b**) in absolute ethanol in the presence of catalytic amount of HCl led to formation of 1-[1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethylidene]thiosemicarbazide (**3a**) or 1-[1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethylidene]thiocarbohydrazide (**3b**), respectively as depicted in Scheme 1.

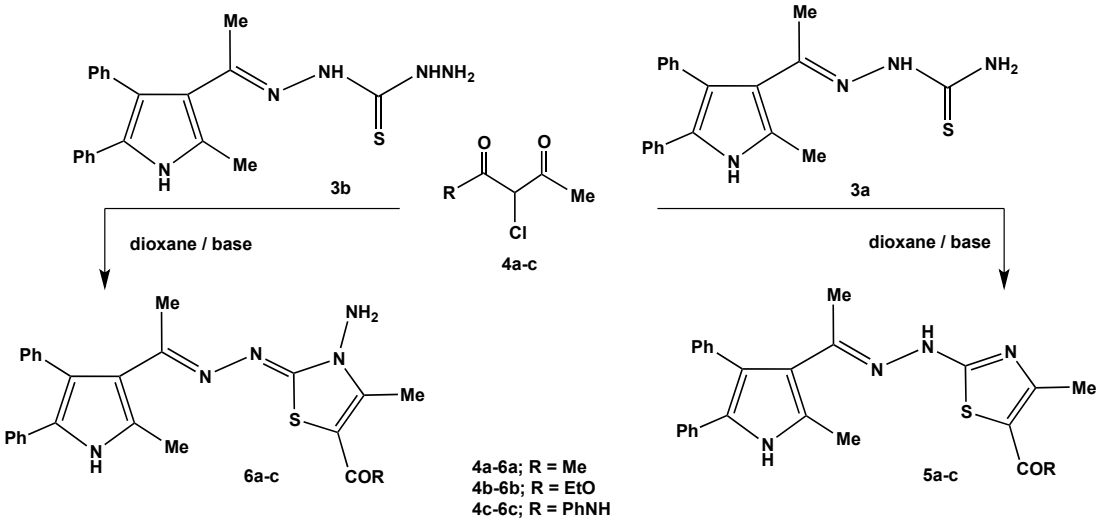


Scheme 1. Synthesis of compounds **3a** and **3b**

An easy way to differentiate between two geometric structures (*syn*, and *anti*) of products **3a,b** could be achieved through NOE difference experiments. ^{1}H NMR spectra of compounds **3a,b** revealed, in each case, two singlet signals at δ = 1.56-1.76 and 2.36-2.37 ppm assignable to methyl group on C2 of pyrrole ring,[19] and another methyl group adjacent to hydrazone (Me-C=N-NH),[20] respectively, in addition to NH signal of hydrazone group (C=N-NH) at δ = 10.82-10.87 ppm (see experimental). Thus irradiating of NH proton at δ = 10.82-10.87 ppm led to enhancement of methyl protons on C2 of pyrrole ring at δ = 1.56-1.76 ppm suggesting (*syn*-) form while in the (*anti*-) form this irradiation did not affect such enhancement. In our case, irradiating of NH proton at δ = 10.82-10.87 ppm did not affect enhancement on

C2-methyl protons on pyrrole ring and led to enhancement of methyl protons adjacent to hydrazone group (Me-C=N-NH) at δ = 2.36-2.37 ppm which confirmed the (*anti*-) form and excluded (*syn*-) form.

Recently, it has been reported that, treatment of ethylidenethiosemicarbazide derivative with appropriate α-haloketones afforded the respective thiazoles.[21-25] However, the reactivity of ethylidenethiocarbohydrazide derivative towards α-haloketones has not been reported. In the present study, we have investigated the reactions of either ethylidenethiosemicarbazide **3a** or ethylidenethiocarbohydrazide **3b** with α-halocarbonyl compounds **4a-c** in dioxane under microwave irradiation (6-10 min) in the presence of different basic catalyst such as triethylamine, chitosan, and chitosan-grafted-poly(vinylpyridine). These reactions afforded ethylidenehydrazonothiazoles **5a-c** and ethylidenehydrazono-aminothiazoles **6a-c** (Scheme 2). At the outset, the performance of different basic catalysts has been explored (Table 1).



Scheme 2. Synthesis of compounds **5a-c** and **6a-c**

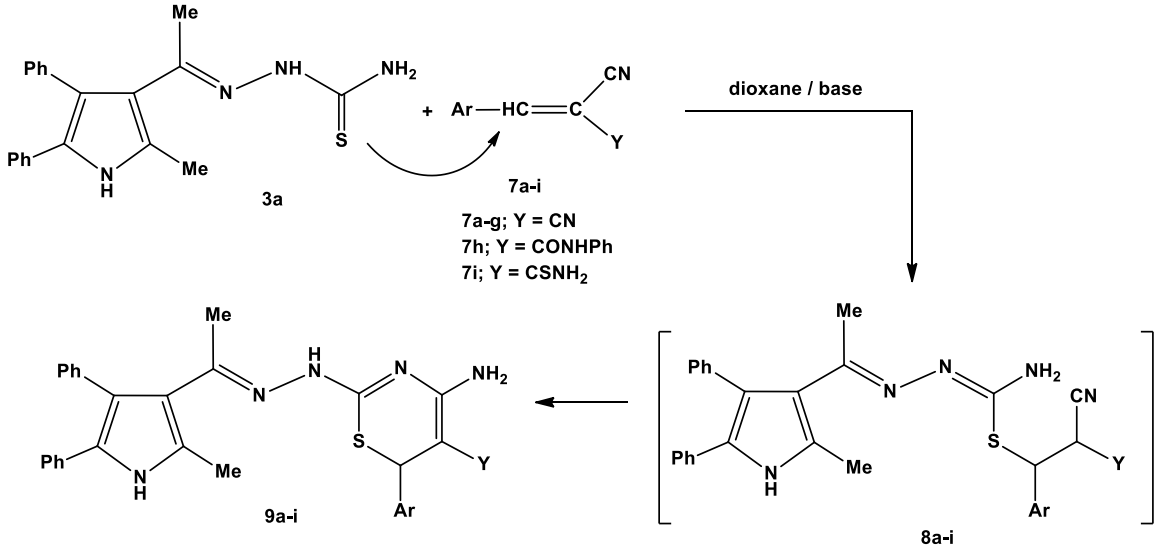
Table 1. Effect of nature of basic catalyst on the product yields of compounds **5a-c** and **6a-c**

Compound no.	R	Time (min)	(%) yield		
			TEA	Chitosan	g-Chitosan
5a	Me	6	62	73	86
5b	OEt	5	61	71	86
5c	PhNH	8	60	72	82
6a	Me	6	64	80	89
6b	OEt	6	54	78	88
6c	PhNH	10	63	77	85

We have observed that under the same reaction conditions the yields of the desired products **5a-c** and **6a-c** increase by changing triethylamine into chitosan. Moreover, using of grafted-chitosan as a basic catalyst has significant increasing effect on the product yields. Elemental analyses and spectral data (^{1}H

NMR, ^{13}C NMR, IR and MS) are compatible with the elucidated structures of the products. For example, ^{1}H NMR spectra of compounds **5a-c** and **6a-c** revealed, in each case, two singlet signals at δ = 2.37-2.42 and 2.58-2.67 ppm assignable to methyl group adjacent to hydrazone group (Me-C=N-NH)[20] and another methyl group on C4 of thiazole ring,[26] respectively (see experimental). In addition, ^{1}H NMR spectra of compounds **6a-c** showed a characteristic broad signal at δ = 3.40-3.64 ppm attributed to amino group which confirmed by absorption bands at ν = 3427-3255 cm^{-1} in IR spectra. The sequence of preparation of target compounds is preceded by displacement of hydrogen chloride followed by dehydrative cyclization to give the isolated products **5a-c** and **6a-c**.

The green protocol for the efficient synthesis of functionalized 1,3-thiazines was extended. Thus, reactions of ethylidenethiosemicarbazide **3a** with different acrylonitrile derivatives such as, arylidenemalononitriles **7a-g,** 2-cyano-*N*,3-diphenylacrylamide (**7h**), and 2-cyano-3-(4-chlorophenyl)-prop-2-enethioamide (**7i**), in dioxane under microwave irradiation (6-8 min) furnished the respective 1,3-thiazines **9a-i** in agreement with literature reports[21,27] concerning the reactions of thiosemicarbazides with substituted acrylonitriles (Scheme 3). In this study we have investigated the effect of nature of basic catalyst such as triethylamine, chitosan, and chitosan-grafted-poly(vinylpyridine) on the percent yields of isolated 1,3-thiazines (Table 2).



Scheme 3. Synthesis of compounds **9a-i**

As shown in Table 2, grafted chitosan has precedence as basic catalyst for synthesis of 1,3-thiazines over chitosan and triethylamine under the same reaction conditions.

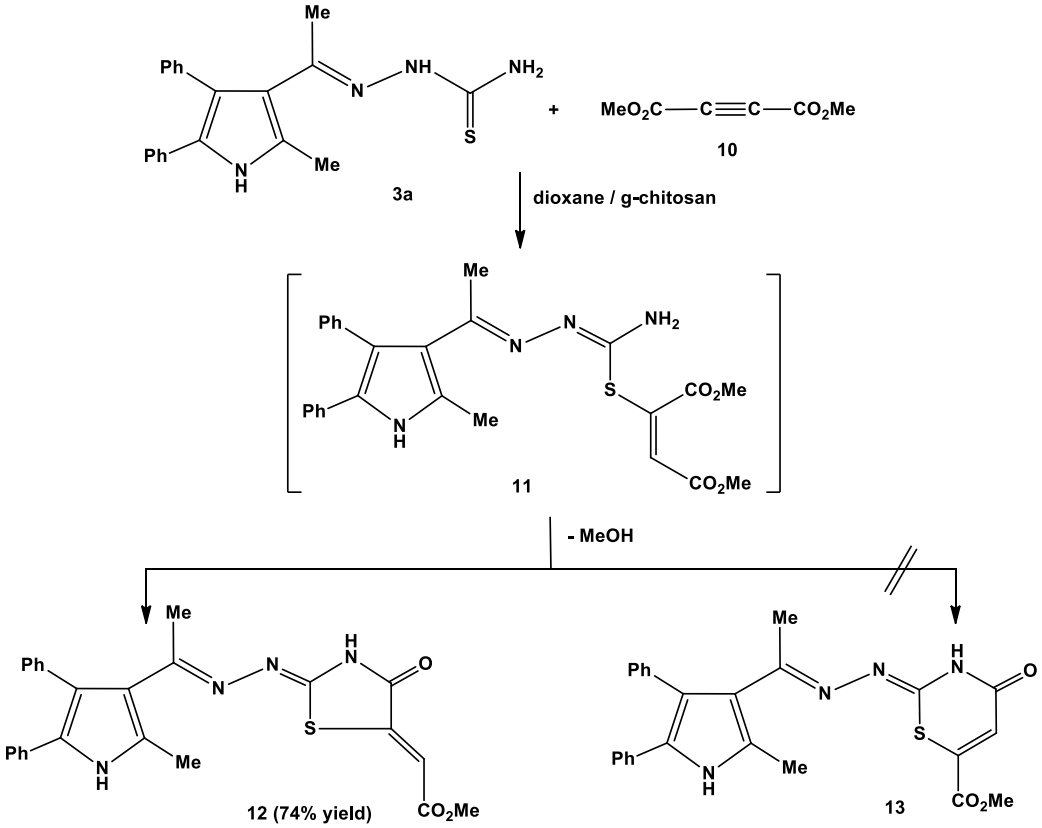
Table 2. Effect of nature of basic catalyst on the product yields **9a-i**

Compound no.	Ar	Y	Time (min)	(%) yield		
				TEA	Chitosan	g-Chitosan
9a	C_6H_5	CN	5	63	75	87
9b	4-ClC_6H_4	CN	5	65	79	89
9c	4-MeC_6H_4	CN	6	60	72	82
9d	4-$MeOC_6H_4$	CN	6	60	73	84
9e	benzo[*b*][1,3]dioxol-2-yl	CN	8	62	76	85
9f	CH=CH-Ph	CN	8	63	77	84
9g	2-furyl	CN	6	65	75	88
9h	C_6H_5	CONHPh	8	60	69	80
9i	4-ClC_6H_4	$CSNH_2$	8	61	66	81

The elucidation for the structures of 1,3-thiazines **9a-i** was based on spectroscopic data (IR, ^{1}H NMR, ^{13}C NMR, MS) and elemental analyses. IR spectra of compounds **9a-g, 9h**, and **9i** revealed characteristic signals at υ = 2185-2220, 1643, and 1300 cm^{-1} assignable to (C≡N), (C=O), and (C=S) groups, respectively. Also, ^{1}H NMR spectra of compounds **9a-i** showed, in each case, two signals at δ = 3.62-3.80 and 4.55-5.35 ppm assignable to CH-thiazine ring[21] and amino group on C4 of thiazine ring.[21,27] The formation of 1,3-thiazines could be suggested by addition of thiol group in thiosemicarbazone moiety into activated double bond to give the non-isolable intermediates **8a-i**. Intramolecluar cyclization of intermediates **8a-i** *via* addition of amino group into nitrile group[28] afforded 6*H*-1,3-thiazine derivatives **9a-i**.

The reactivity of ethylidenethiosemicarbazide **3a** towards activated triple bond has also been investigated. Thus, treatment of ethylidenethiosemicarbazide **3a** with dimethyl acetylenedicarboxylate (DMAD) (**10**) under the employed reaction conditions, using grafted chitosan as a basic catalyst, furnished the corresponding thiazolidin-4-one derivative **12** (Scheme 4). The structure of the isolated product was inferred from its elemental analysis and spectral data [IR and ^{1}H NMR]. Its IR spectrum showed absorption bands at υ = 3366, 3247 (2NH), 1703, 1684 (2C=O), and 1605 (C=N) cm^{-1}, its ^{1}H NMR spectrum revealed a pair of singlet signal at δ 11.19, 12.59 ppm (D_2O-exchangeable) assignable to (2NH) groups and another singlet signal at δ 6.61 ppm due to vinylic-H (C=CH).[21,29,30] Revealing of the latter signal of vinylic-H excluded the isomeric structure of 1,3-thiazin-4-one **13**. To account for the formation of product **12** we assumed that the reaction initially proceeded *via* addition of thiol group in thiosemicarbazone moiety into triple bond to give the non-isolable intermediate **11**. Elimination of

methanol molecule from the latter intermediate afforded the isolated product **12** (cf. Scheme 4).



Scheme 4. Synthesis of compound **12**

Anti-cancer Activity

The anticancer activity of some newly synthesized compounds was determined against a colon carcinoma cell line (HCT-116) and liver carcinoma cell line HEPG2, using doxorubicin as a reference drug. Data generated were used to plot a dose–response curve of which the concentration (μM) of test compounds required to kill 50% of cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments (Table 3). The results showed that 1,3-thiazine derivatives with different aryl moieties at C6 have promising anticancer activity against HCT-116 and HEPG2 cell lines. Structure activity relationship was exemplified by substituting phenyl moiety of **9a** (IC_{50} = 2.67 and 8.85 μM) by electron donating group e.g. (4-Me and 4-OMe groups) affording **9c** and **9d** analogues (IC_{50} = 0.48 & 1.41 and 0.24 & 1.52 μM, respectively), this substitution effectively increased the anticancer activity against both cell lines with IC_{50} close to doxorubicin. On the other hand, substituting phenyl moiety of **9a** by electron withdrawing group e.g. (4-Cl) affording compound **9b**, diminished the activity (IC_{50} = 1.33 & 3.32 μM). Similarly, different aryl or heterocyclic rings at C6 were replaced by phenyl moiety, to afford **9e**-**9i** revealed positive impact on the anticancer activity. SAR of thiazoles **5a-c** and

aminothiazoles **6a-c** was explained by the order of inhibition activity for both cell lines [ethoxycarbonyl (**5b**) > phenylcarboxamide (**5c**) > acetyl (**5a**)] and [phenylcarboxamide (**6c**) > acetyl (**6a**) > ethoxycarbonyl (**6b**)] moieties on C5 of thiazole and aminothiazole ring, respectively (Table 3). Thiazolidin-4-one derivative **12** has weak activities against both cell lines.

Table 3. Cytotoxic activities of selected compounds against tumor cell lines (HCT-116 and HEPG2-1)

Compound No.	IC_{50} (μM)		Compound No.	IC_{50} (μM)	
	HCT-116	HEPG2		HCT-116	HEPG2
Doxorubicin (standard)	0.79	0.72	**Doxorubicin (standard)**	0.79	0.72
5a	11.87	14.51	**9c**	0.48	1.41
5b	1.38	1.57	**9d**	0.24	1.52
5c	6.89	11.70	**9e**	0.53	1.39
6a	4.40	5.91	**9f**	0.45	3.03
6b	46.30	75.69	**9g**	0.30	1.24
6c	2.33	1.17	**9h**	0.32	1.61
9a	2.67	8.85	**9i**	0.61	1.89
9b	1.33	3.32	**12**	18.40	82.10

CONCLUSION

We have developed a green technique for preparation of ethylidenehydrazonothiazoles, ethylidenehydrazono-1,3-thiazines and ethylidenehydrazonothiazolidine by using chitosan-grafted-poly-(vinylpyridine) as environmentally friendly basic catalyst. The synthesized products exhibited high to moderate anti-cancer activities.

EXPERIMENTAL

General

All melting points were determined on an electrothermal Gallenkamp apparatus and are uncorrected. Solvents were generally distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury VXR-300 spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR) and the chemical shifts were related to that of the solvent DMSO-d_6. The mass

spectra were recorded on a GCMS-Q1000-EX Shimadzu and a GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Microwave experiments were carried out using CEM Discover Labmate microwave apparatus (300 W with Chem. Driver Software). Antitumor activity was evaluated by the National Institute of Cancer, Biology Department, Cairo University, Egypt. 3-Acetyl-4,5-diphenyl-2-methyl-1*H*-pyrrole (**1**)[19] was prepared following literature method.

Synthesis of ethylidenethiosemicarbazide 3a and ethylidenethiocarbohydrazide 4a

These compounds were prepared following the procedure described by Gomha *et al*.[21]

1-[1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene]thiosemicarbazide (3a)

White solid (11.83 g, 68%); mp 248-250 °C (EtOH/dioxane); IR (KBr): *v* 3406-3257 (2NH+NH_2), 1590 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.76 (s, 3H, pyrrole-CH_3), 2.36 (s, 3H, $\underline{CH_3}$-C=N-NH), 6.98-7.31 (m, 12H, Ar-H+NH_2), 10.82 (s, 1H, NH), 11.50 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 348 (M^+, 46), 273 (100), 77 (24), 60 (43). *Anal.* Calcd for $C_{20}H_{20}N_4S$ (348.14): C, 68.93; H, 5.79; N, 16.08; S, 9.20. Found: C, 69.11; H, 5.86; N, 16.23; S, 9.41%.

1-[1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene]thiocarbohydrazide (3b)

Yellow solid (10.70 g, 59%); mp 180-182 °C (EtOH); IR (KBr): *v* 3398-3198 (3NH+NH_2), 1596 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.56 (s, 3H, pyrrole-CH_3), 2.37 (s, 3H, $\underline{CH_3}$-C=N-NH), 4.21 (br, 2H, NH_2), 7.07-7.54 (m, 10H, Ar-H), 10.87 (s, 1H, NH), 11.21 (s, 1H, NH), 11.55 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 363 (M^+, 40), 273 (100), 77 (53). *Anal*. Calcd for $C_{20}H_{21}N_5S$ (363.15): C, 66.09; H, 5.82; N, 19.27; S, 8.82. Found: C, 66.18; H, 5.66; N, 19.33; S, 9.01%.

Reactions of 3a and 3b with α-halocarbonyl compounds

Method A

To a solution of **3a** or **3b** (1 mmol) in dioxane (20 mL), containing triethylamine (0.1 g), was added 3-chloro-2,4-pentanedione (**4a**) or ethyl 2-chloro-3-oxobutanoate (**4b**) or *N*1-phenyl-2-chloro-3-oxobutanamide (**4c**) (1 mmol of each). After complete addition, the reaction mixture was irradiated by MW at 400 Watt in a closed Teflon vessel until all the starting material was consumed (6–10 min as monitored by TLC). The mixture was poured into ice/HCl mixture and the solid that precipitated was filtered off, washed with water, dried and finally crystallized from EtOH to give the respective products **5a-c** and **6a-c**.

Method B

To a solution of **3a** or **3b** (1 mmol) in dioxane (20 mL), containing chitosan (0.1 g), was added 3-chloro-2,4-pentanedione (**4a**) or ethyl 2-chloro-3-oxobutanoate (**4b**) or *N*1-phenyl-2-chloro-3-oxobutanamide (**4c**) (1 mmol of each). After complete addition, the reaction mixture was irradiated by MW at 400 Watt in a closed Teflon vessel until all the starting material was consumed (6–10 min as monitored by TLC). The hot solution was filtered off to remove chitosan and the mixture was poured into ice/HCl mixture. The solid that precipitated was filtered off, washed with water, dried and finally crystallized from EtOH to give the respective products **5a-c** and **6a-c**.

Method C

Same procedure in method B using grafted-chitosan (0.1 g) instead of chitosan.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-methyl-5-acetylthiazole (5a).

Yellow solid; mp 210-212 °C; IR (KBr): *v* 3318, 3295 (2NH), 1702 (C=O), 1606 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.74 (s, 3H, pyrrole-CH_3), 2.37 (s, 3H, $\underline{CH_3}$-C=N-NH), 2.44 (s, 3H, $COCH_3$), 2.58 (s, 3H, thiazole-C4-CH_3), 7.09-7.13 (m, 10H, Ar-H), 10.32 (s, 1H, NH-N=), 11.45 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ = 8.22 (thiazole-C4-$\underline{CH_3}$), 13.11 ($\underline{CH_3}$-C=N-NH), 14.42 (pyrrole-$\underline{CH_3}$), 31.54 (CO$\underline{CH_3}$), 116.21, 122.03, 122.68, 123.45, 125.98, 126.53, 127.75, 128.64, 129.22, 130.45, 132.65, 134.78, 137.57, 149.11, 152.53, 170.11 (Ar-Cs + C=N-NH), 186.22 ($\underline{CO}CH_3$) ppm; MS *m/z* (%): 428 (M^+, 65), 273 (100), 258 (54), 77 (18). *Anal*. Calcd for $C_{25}H_{24}N_4OS$ (428.17): C, 70.07; H, 5.64; N, 13.07; S, 7.48. Found: C, 70.11; H, 5.58; N, 13.28; S, 7.39%.

Ethyl 2-[2-{1-(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-methylthiazole-5-carboxylate (5b).

Yellow solid; mp 164-166 °C; IR (KBr): *v* 3308, 3295 (2NH), 1708 (C=O), 1596 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.23 (t, 3H, *J* = 7 *Hz*, $\underline{CH_3}CH_2$), 1.74 (s, 3H, pyrrole-CH_3), 2.39 (s, 3H, $\underline{CH_3}$-C=N-NH), 2.61 (s, 3H, thiazole-C4-CH_3), 4.20 (q, 2H, *J* = 7 *Hz*, $\underline{CH_2}CH_3$), 7.09-7.37 (m, 10H, Ar-H), 10.34 (s, 1H, NH-N=), 11.47 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ = 8.11 (thiazole-C4-$\underline{CH_3}$), 13.08 ($\underline{CH_3}$-C=N-NH), 13.56 (OCH_2-$\underline{CH_3}$), 14.40 (pyrrole-$\underline{CH_3}$), 58.11 (O$\underline{CH_2}CH_3$), 116.28, 121.87, 122.62, 123.40, 125.88, 126.51, 127.54, 128.22, 129.31, 131.05, 132.62, 134.70, 137.61, 149.43, 152.21, 170.09 (Ar-Cs + C=N-NH), 185.92 (C=O) ppm; MS *m/z* (%): 458 (M^+, 25), 273 (70), 113 (89), 84 (100). *Anal*. Calcd for $C_{26}H_{26}N_4O_2S$ (458.18): C, 68.10; H, 5.71; N, 12.22; S, 6.99. Found: C, 68.18; H, 5.59; N, 12.38; S, 7.09%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-methyl-N-phenylthiazole-

5-carboxamide (5c).

Yellow solid; mp 230-232 °C; IR (KBr): *v* 3324-3212 (3NH), 1660 (C=O), 1595 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.76 (s, 3H, pyrrole-CH_3), 2.37 (s, 3H, $\underline{CH_3}$-C=N-NH), 2.63 (s, 3H, thiazole-C4-CH_3), 7.06-7.67 (m, 15H, Ar-H), 9.87 (s, 1H, NH), 10.28 (s, 1H, NH-N=), 11.31 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ = 8.82 (thiazole-C4-$\underline{CH_3}$), 13.75 ($\underline{CH_3}$-C=N-NH), 14.87 (pyrrole-$\underline{CH_3}$), 116.21, 120.43, 122.11, 122.76, 123.07, 124.65, 125.62, 126.53, 126.89, 127.73, 128.64, 129.20, 130.41, 131.77, 132.35, 134.65, 137.32, 148.91, 151.83, 170.31 (Ar-Cs + C=N-NH), 176.65 ($\underline{CO}$NH) ppm; MS *m/z* (%): 505 (M^+, 94), 231 (100), 118 (93), 77 (60). *Anal*. Calcd for $C_{30}H_{27}N_5OS$ (505.19): C, 71.26; H, 5.38; N, 13.85; S, 6.34. Found: C, 71.33; H, 5.19; N, 14.02; S, 6.49%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-3-amino-4-methyl-5-acetyl-2,3-dihydrothiazole (6a).

Yellow solid; mp 224-226 °C; IR (KBr): *v* 3427-3257 (NH+NH_2), 1692 (C=O), 1606 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.65 (s, 3H, pyrrole-CH_3), 2.42 (s, 3H, $\underline{CH_3}$-C=N-NH), 2.67 (s, 3H, thiazole-C4-CH_3), 2.72 (s, 3H, $COCH_3$), 3.40 (s, 2H, NH_2), 7.11-7.34 (m, 10H, Ar-H), 11.46 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ 9.11 (thiazole-C4-$\underline{CH_3}$), 13.34 ($\underline{CH_3}$-C=N-NH), 13.92 (pyrrole-$\underline{CH_3}$), 32.86 (CO$\underline{CH_3}$), 116.20, 121.93, 122.64, 123.03, 125.17, 126.03, 128.05, 128.64, 129.87, 131.45, 132.63, 134.78, 137.57, 149.11, 152.53, 158.11 (Ar-Cs + C=N-NH), 183.20 ($\underline{CO}CH_3$) ppm; MS *m/z* (%): 443 (M^+, 35), 273 (100), 258 (42), 77 (30). *Anal*. Calcd for $C_{25}H_{25}N_5OS$ (443.18): C, 67.69; H, 5.68; N, 15.79; S, 7.23. Found: C, 67.51; H, 5.42; N, 15.67; S, 7.35%.

Ethyl 2-[2-{1-(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-3-amino-4-methyl-2,3-dihydrothiazole-5-carboxylate (6b).

Yellow solid; mp 172-174 °C; IR (KBr): *v* 3423-3255 (NH+NH_2), 1711 (C=O), 1603 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.20 (t, 3H, *J* = 7 *Hz*, $\underline{CH_3}CH_2$), 1.74 (s, 3H, pyrrole-CH_3), 2.39 (s, 3H, $\underline{CH_3}$-C=N-NH), 2.58 (s, 3H, thiazole-C4-CH_3), 3.51 (s, 2H, NH_2), 4.28 (q, 2H, *J* = 7 *Hz*, $\underline{CH_2}CH_3$), 7.11-7.37 (m, 10H, Ar-H), 11.51 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ = 9.23 (thiazole-C4-$\underline{CH_3}$), 13.12 ($\underline{CH_3}$-C=N-NH), 14.11 (pyrrole-$\underline{CH_3}$), 14.74 (OCH_2-$\underline{CH_3}$), 56.11 (O$\underline{CH_2}CH_3$), 116.28, 120.82, 121.92, 123.40, 125.43, 126.90, 127.12, 128.82, 129.11, 130.95, 132.12, 134.62, 137.32, 149.41, 152.21, 158.29 (Ar-Cs + C=N-NH), 186.90 (C=O) ppm; MS *m/z* (%): 473 (M^+, 23), 273 (45), 113 (50), 77 (100). *Anal*. Calcd for $C_{26}H_{27}N_5O_2S$ (473.19): C, 65.94; H, 5.75; N, 14.79; S, 6.77. Found: C, 66.08; H, 5.62; N, 14.54; S, 6.62%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-3-amino-4-methyl-N-phenyl-2,3-

dihydrothiazole-5-carboxamide (6c).

Yellow solid; mp 236-238 °C; IR (KBr): *ν* 3409-3267 (2NH+NH_2), 1660 (C=O), 1600 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.77 (s, 3H, pyrrole-CH_3), 2.37 (s, 3H, $\underline{CH_3}$-C=N-NH), 2.63 (s, 3H, thiazole-C4-CH_3), 3.64 (s, 2H, NH_2), 7.12-7.64 (m, 15H, Ar-H), 9.87 (s, 1H, NH), 11.42 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ = 9.82 (thiazole-C4-$\underline{CH_3}$), 13.15 ($\underline{CH_3}$-C=N-NH), 14.17 (pyrrole-$\underline{CH_3}$), 116.20, 120.43, 121.71, 122.70, 123.14, 124.62, 125.32, 126.58, 126.82, 127.78, 128.20, 129.61, 130.44, 131.12, 132.88, 134.62, 137.30, 148.76, 151.81, 159.01 (Ar-Cs + C=N-NH), 177.05 ($\underline{CO}$NH) ppm; MS *m/z* (%): 520 (M^+, 52), 231 (55), 118 (53), 77 (100). *Anal*. Calcd for $C_{30}H_{28}N_6OS$ (520.20): C, 69.21; H, 5.42; N, 16.14; S, 6.16. Found: C, 69.33; H, 5.29; N, 16.02; S, 6.29%.

Synthesis of 1,3-thiazine derivatives 9a-i

Method A

To a solution of 1-[1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethylidene]thiosemicarbazide (**3a**) (0.348 g, 1 mmol) in dry dioxane (20 mL), containing 0.1 g of triethylamine, was added acrylonitrile derivatives **7a-i** (1 mmol of each). After complete addition, the reaction mixture was irradiated by MW at 400 Watt in a closed Teflon vessel until all the starting material was consumed (6–8 min as monitored by TLC). The mixture was poured into ice/HCl mixture and the precipitate was filtered, washed with MeOH, and crystallized from EtOH to give products **9a-i**.

Method B

To a solution of 1-[1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethylidene]thiosemicarbazide (**3a**) (0.348 g, 1 mmol) in dry dioxane (20 mL), containing 0.1 g of chitosan, was added acrylonitrile derivatives **7a-i** (1 mmol of each). After complete addition, the reaction mixture was irradiated by MW at 400 Watt in a closed Teflon vessel until all the starting material was consumed (6–8 minutes as monitored by TLC). The hot solution was filtered off to remove chitosan then the mixture was poured into ice/HCl mixture and the precipitate was filtered, washed with MeOH, and recrystallized from EtOH to give products **9a-i**.

Method C

Same procedure in method B using grafted-chitosan (0.1 g) instead of chitosan.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-phenyl-6H-1,3-thiazine-5-carbonitrile (9a).

Yellow solid; mp 188-190 °C; IR (KBr): *ν* 3423-3196 (NH_2+2NH), 2185 (C≡N), 1603 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.52 (s, 3H, pyrrole-CH_3), 2.41 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.65 (s, 1H, thiazine-H),

4.55 (s, br, 2H, NH_2), 7.07-7.53 (m, 15H, Ar-H), 10.32 (s, 1H, NH), 11.45 (s, 1H, pyrrole-NH) ppm; ^{13}C-NMR (DMSO-d_6): δ = 13.15 (pyrrole-$\underline{CH_3}$), 16.17 ($\underline{CH_3}$-C=N-NH), 51.87 (C6-thiazine), 110.31 (CN), 116.19, 118.13, 120.43, 120.92, 122.15, 123.91, 124.37, 125.34, 126.18, 126.93, 128.12, 128.88, 129.01, 131.55, 134.22, 139.11, 148.32, 158.44, 161.76, 162.15 (Ar-Cs + C=N-NH) ppm; MS *m/z* (%): 502 (M^+, 45), 371 (72), 80 (100), 77 (47). *Anal*. Calcd for $C_{30}H_{26}N_6S$ (502.19): C, 71.69; H, 5.21; N, 16.72; S, 6.38. Found: C, 71.51; H, 5.38; N, 16.61; S, 6.19%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-(4-chlorophenyl)-6H-1,3-thiazine-5-carbonitrile (9b).

Yellow solid; mp 234-236 °C; IR (KBr): *v* 3419-3192 (NH_2+2NH), 2199 (C≡N), 1603 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.61 (s, 3H, pyrrole-CH_3), 2.42 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.78 (s, 1H, thiazine-H), 4.68 (s, br, 2H, NH_2), 7.09-7.58 (m, 14H, Ar-H), 10.35 (s, 1H, NH), 11.51 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 538 (M^++2, 32), 536 (M^+, 88), 386 (100), 110 (71), 77 (40). *Anal*. Calcd for $C_{30}H_{25}ClN_6S$ (536.15): C, 67.09; H, 4.69; N, 15.65; S, 5.97. Found: C, 66.91; H, 4.48; N, 15.72; S, 6.09%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-(4-methylphenyl)-6H-1,3-thiazine-5-carbonitrile (9c).

Yellow solid; mp 210-212 °C; IR (KBr): *v* 3407-3153 (NH_2+2NH), 2207 (C≡N), 1590 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.77 (s, 3H, pyrrole-CH_3), 2.24 (s, 3H, Ar-CH_3), 2.39 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.64 (s, 1H, thiazine-H), 4.61 (s, br, 2H, NH_2), 7.07-8.73 (m, 14H, Ar-H), 10.00 (s, 1H, NH), 11.50 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 516 (M^+, 44), 386 (60), 91 (25), 77 (100). *Anal*. Calcd for $C_{31}H_{28}N_6S$ (516.21): C, 72.07; H, 5.46; N, 16.27; S, 6.21. Found: C, 71.88; H, 5.35; N, 16.40; S, 6.08%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-(4-methoxyphenyl)-6H-1,3-thiazine-5-carbonitrile (9d).

Yellow solid; mp 182-184 °C; IR (KBr): *v* 3405-3151 (NH_2+2NH), 2220 (C≡N), 1588 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.76 (s, 3H, pyrrole-CH_3), 2.41 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.78 (s, 1H, thiazine-H), 3.91 (s, 3H, OCH_3), 4.58 (s, br, 2H, NH_2), 7.06-8.40 (m, 14H, Ar-H), 10.00 (s, 1H, NH), 11.52 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 532 (M^+, 32), 386 (60), 108 (45), 77 (100). *Anal*. Calcd for $C_{31}H_{28}N_6OS$ (532.20): C, 69.90; H, 5.30; N, 15.78; S, 6.02. Found: C, 69.81; H, 5.45; N, 15.70; S, 6.11%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-(benzo[b][1,3-dioxol-3-yl)-6H-1,3-thiazine-5-carbonitrile (9e).

Yellow solid; mp 154-156 °C; IR (KBr): *v* 3412-3185 (NH_2+2NH), 2197 (C≡N), 1606 (C=N) cm^{-1};

^{1}H-NMR (DMSO-d_6): δ = 1.51 (s, 3H, pyrrole-CH_3), 2.40 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.62 (s, 1H, thiazine-H), 4.61 (s, br, 2H, NH_2), 5.97 (s, 2H, CH_2), 7.05-7.58 (m, 13H, Ar-H), 10.34 (s, 1H, NH), 11.48 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 546 (M^+, 26), 380 (35), 121 (60), 77 (100). *Anal*. Calcd for $C_{31}H_{26}N_6O_2S$ (546.18): C, 68.11; H, 4.79; N, 15.37; S, 5.87. Found: C, 68.24; H, 4.58; N, 15.11; S, 6.09%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-styryl-6H-1,3-thiazine-5-carbonitrile (9f).

Yellow solid; mp 226-228 °C; IR (KBr): *v* 3428-3198 (NH_2+2NH), 2219 (C≡N), 1595 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.75 (s, 3H, pyrrole-CH_3), 2.41 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.80 (s, 1H, thiazine-H), 4.59 (s, br, 2H, NH_2), 6.24 (d, 1H, *J* = 13 *Hz*, CH=), 6.67 (d, 1H, *J* = 13 *Hz*, CH=), 7.07-7.95 (m, 15H, Ar-H), 10.32 (s, 1H, NH), 11.51 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 528 (M^+, 45), 371 (72), 77 (100). *Anal*. Calcd for $C_{32}H_{28}N_6S$ (528.21): C, 72.70; H, 5.34; N, 15.90; S, 6.07. Found: C, 72.53; H, 5.18; N, 16.01; S, 6.22%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-(furan-2-yl)-6H-1,3-thiazine-5-carbonitrile (9g).

Yellow solid; mp 258-260 °C; IR (KBr): *v* 3418-3180 (NH_2+2NH), 2210 (C≡N), 1596 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.75 (s, 3H, pyrrole-CH_3), 2.39 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.68 (s, 1H, thiazine-H), 5.35 (s, br, 2H, NH_2), 7.01-7.38 (m, 13H, Ar-H), 10.34 (s, 1H, NH), 11.48 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 492 (M^+, 26), 380 (35), 77 (100). *Anal*. Calcd for $C_{28}H_{24}N_6OS$ (492.17): C, 68.27; H, 4.91; N, 17.06; S, 6.51. Found: C, 68.12; H, 4.75; N, 17.11; S, 6.39%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-N,6-diphenyl-6H-1,3-thiazine-5-carboxamide (9h).

Yellow solid; mp 244-246 °C; IR (KBr): *v* 3406-3151 (NH_2+3NH), 1643 (CO), 1595 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.76 (s, 3H, pyrrole-CH_3), 2.45 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.72 (s, 1H, thiazine-H), 4.68 (s, br, 2H, NH_2), 7.06-8.28 (m, 20H, Ar-H), 10.41 (s, 1H, NH), 11.51 (s, 1H, pyrrole-NH), 11.63 (s, 1H, NH) ppm; MS *m/z* (%): 596 (M^+, 45), 386 (40), 77 (100). *Anal*. Calcd for $C_{36}H_{32}N_6OS$ (596.24): C, 72.46; H, 5.41; N, 14.08; S, 5.37. Found: C, 72.61; H, 5.28; N, 14.12; S, 5.18%.

2-[2-{1-(2-Methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-amino-6-(4-chlorophenyl)-6H-1,3-thiazine-5-carbothioamide (9i).

Yellow solid; mp 216-218 °C; IR (KBr): *v* 3406-3152 (2NH_2+2NH), 1591 (C=N), 1300 (C=S) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.76 (s, 3H, pyrrole-CH_3), 2.49 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.66 (s, 1H, thiazine-H),

4.56-5.32 (s, br, 4H, $2NH_2$), 7.06-8.22 (m, 14H, Ar-H), 10.00 (s, 1H, NH), 11.52 (s, 1H, pyrrole-NH) ppm; MS *m/z* (%): 572 (M^++2, 14), 570 (M^+, 38), 386 (20), 110 (71), 77 (100). *Anal*. Calcd for $C_{30}H_{27}ClN_6S_2$ (536.15): C, 63.09; H, 4.76; N, 14.71; S, 11.23. Found: C, 62.91; H, 4.58; N, 14.52; S, 11.18%.

Synthesis of methyl 2-[2-{1-(2-methyl-4,5-diphenyl-1H-pyrrol-3-yl)ethylidene}hydrazono]-4-oxothiazolidin-5-ylidene ethanoate (12).

To a solution of 1-[1-(2-methyl-4,5-diphenyl-1*H*-pyrrol-3-yl)ethylidene]thiosemicarbazide (**3a**) (0.348 g, 1 mmol) in dry dioxane (20 mL), contains 0.1 g of grafted chitosan, was added dimethyl acetylenedicarboxylate (0.142 g, 1 mmol). After complete addition reaction mixture was irradiated by MW at 400 Watt in a closed Teflon vessel until all the starting material was consumed (4 minutes as monitored by TLC). The hot solution was filtered to remove grafted chitosan and the precipitate was filtered, washed with MeOH, and recrystallized from EtOH to give product **12**.

Canary yellow solid (0.34 g, 74%); mp 300-302 °C; IR (KBr): *v* 3366, 3247 (2NH), 1703, 1684 (2C=O), 1605 (C=N) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ = 1.74 (s, 3H, pyrrole-CH_3), 2.35 (s, 3H, $\underline{CH_3}$-C=N-NH), 3.78 (s, 3H, $COOCH_3$), 6.61 (s, 1H, C=CH), 7.05-7.33 (m, 10H, Ar-H), 11.49 (s, 1H, pyrrole-NH), 12.59 (s, 1H, NH) ppm; MS *m/z* (%): 458 (M^+, 63), 284 (87), 135 (100), 77 (30). *Anal*. Calcd for $C_{25}H_{22}N_4O_3S$ (458.14): C, 65.48; H, 4.84; N, 12.22; S, 6.99. Found: C, 65.21; H, 4.66; N, 12.12; S, 7.09%.

Evaluation of the antitumor activity using Viability assay:

Human colon carcinoma (HCT-116) and human hepatocellular carcinoma (HEPG2) cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50 µg/mL gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week. Potential cytotoxicity of the compounds was evaluated on tumor cells using the method of Gangadevi and Muthumary.[31] The cells were grown as monolayers in growth RPMI-1640. The monolayers of 10^4 cells adhered at the bottom of the wells in a 96-well microtiter plate incubated for 24 h at 37 °C in a humidified incubator with 5% CO_2. The monolayers were then washed with sterile phosphate buffered saline (0.01 M pH 7.2) and simultaneously the cells were treated with 100 µL from different dilutions of tested sample in fresh maintenance medium and incubated at 37 °C. A control of untreated cells was made in the absence of tested sample. Positive controls containing Doxorubicin drug was also tested as reference drug for comparison. Six wells were used for each concentration of the test sample. Every 24 h the observation under the inverted microscope was made. The number of the surviving cells was determined by staining the cells with crystal violet[32,33] followed by cell lysing using

33% glacial acetic acid and read the absorbance at 590 nm using microplate reader (SunRise, TECAN, Inc, USA) after well mixing. The absorbance values from untreated cells were considered as 100% proliferation. The number of viable cells was determined using microplate reader as previously mentioned before and the percentage of viability was calculated as [1-(ODt/ODc)]x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots.

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