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ORIGINAL PAPER

Synthesis, reactions, and biological activity of 1,4-benzothiazine derivatives

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Abstract 6,7-Dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-one reacts with dimethylformamide dimethylacetal (DMF-DMA) to give the novel enaminone 2-(dimethylaminomethylene)-6,7-dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-one. The reaction of the latter with various active methylene compounds afforded pyrido[3,2-*b*][1,4]benzothiazines. Also, coupling of the enaminone with diazotized aniline derivatives gave 2-(arylhydrazono)-6,7-dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-ones. Spectral data indicated that the latter compounds exist predominantly in the hydrazone tautomeric form. In addition, coupling of the enaminone with diazotized heterocyclic amines afforded tetra- and pentaheterocyclic ring systems. The antitumor and antimicrobial activity of some of the synthesized compounds was screened.

Keywords Enaminone · 2*H*-1,4-Benzothiazin-3(4*H*)-one · Cytotoxic activity · Antimicrobial activity

Introduction

Enaminones are versatile reagents, and their utility in heterocyclic synthesis has recently earned considerable attention [1–4]. In our previous papers [5–14], we were interested in the azo-hydrazone tautomerism of the arylazo heterocycles, as many of them are useful in the field of material sciences and theoretical chemistry [15, 16]. In addition to these applications, azo compounds are used as photosensitive species in photographic or electrophotographic systems and are the dominant organic photoconductive materials in commercial copiers [17]. Benzothiazines are biologically interesting molecules with established utility in the pharmaceutical and agrochemical industries. Compounds with these ring systems have diverse pharmacological activity such as anticancer [18–20], antiproliferative [18–20], antimicrobial [21, 22], and antifungal activity [23, 24]. Herein we report synthesis of a new enaminone as a precursor for synthesis of several fused benzothiazines and hydrazones which are expected to be biologically active.

Results and discussion

Concurrent reduction and dehydrative cyclization of compound **1** with tin in hydrochloric acid afforded 6,7-dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**2**). Conversion of the latter into the required enaminone **3** was carried out by a procedure differing from that reported for synthesis of other 2-(dimethylaminomethylene)-1,4-benzothiazines [25]. Thus, treatment of **2** with dimethylformamide dimethylacetal (DMF-DMA) under solvent-free conditions furnished a single product [examined by thin-layer chromatography (TLC)] that was identified as 2-(dimethylaminomethylene)-6,7-dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**3**) (Scheme 1). Elemental analysis and spectral data were in complete accordance with the assigned structure **3**; for example, the ^{1}H nuclear magnetic resonance (NMR) spectrum of compound **3** revealed two singlet signals at $\delta =$ 3.09 and 7.26 ppm, characteristic for *N*,*N*-dimethylamino and

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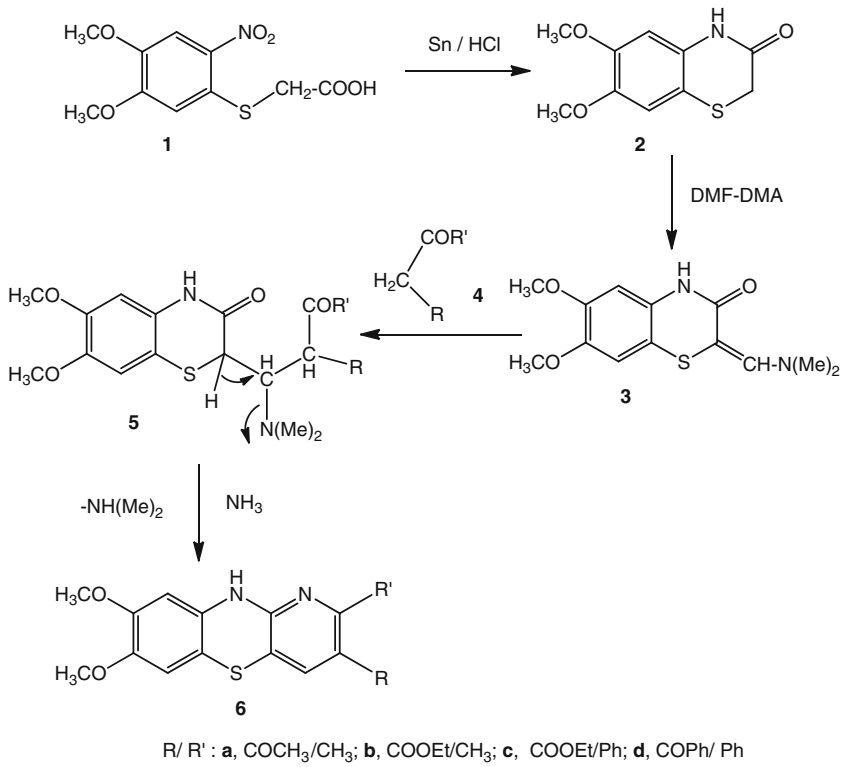
Scheme 1

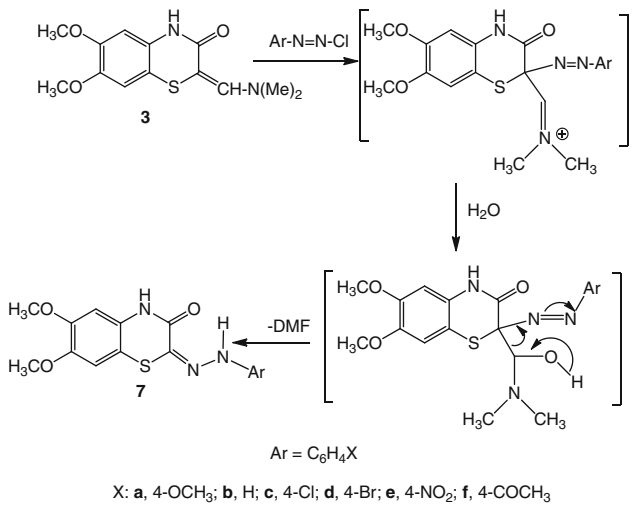


the (*E*)-configuration of exocyclic C=CH protons, respectively [26, 27].

Reaction of enaminone **3** with active methylene compounds acetylacetone (**4a**), ethyl acetoacetate (**4b**), ethyl benzoylacetate (**4c**), and dibenzoylmethane (**4d**) in glacial acetic acid in presence of ammonium acetate gave the corresponding pyrido[3,2-*b*][1,4]benzothiazine derivatives **6a**–**6d** (Scheme 1). The structures of these products were assigned based on their ^{1}H NMR spectra, which showed characteristic singlet signals at $\delta = 7.98$–8.14 ppm characteristic for the pyridine-4H [28].

In conjunction with our interest in the azo-hydrazone tautomerism [5–14], we report here on the coupling reaction of the enaminone **3** with diazotized anilines, giving the respective arylazo derivatives **7a**–**7f** (Scheme 2). On the basis of elemental analyses, infrared (IR), ^{1}H NMR, and ultraviolet (UV) spectra (see Experimental), the isolated products were assigned structure **7**.

Compounds **7** can exist in one or more of five tautomeric forms: the hydroxy-azo form **7A**, the keto-hydrazone form **7B**, hydroxy-hydrazone **7C**, CH-keto-azo form **7D**, and CH-hydroxy-azo tautomeric form **7E** (Fig. 1). Of these five forms, the tautomeric form **7B** seems to be the form of choice for the studied compounds, as it is consistent with their electronic absorption spectra and ^{1}H NMR spectra. This conclusion is consistent with other literature reports on the tautomerism of analogous arylazo derivatives of benzothiazine [29, 30]. For example, like typical hydrazones



Scheme 2

[5, 6, 9], the electronic absorption spectra of **7** in dioxane revealed in each case two characteristic absorption bands in the regions 395–371 and 316–299 nm (Table 1), and the spectra of compound **7b** (taken as a typical example of the series prepared) in different solvents exhibit little, if any, solvent dependence (Table 1). Also, their IR revealed in each case two NH and carbonyl carbon absorption bands in the regions 3,437–3,310, 3,270–3,182, and 1,671–1,652 cm^{-1}, respectively. The ^{1}H NMR spectra are characterized by two singlet signals assignable to two NH protons at

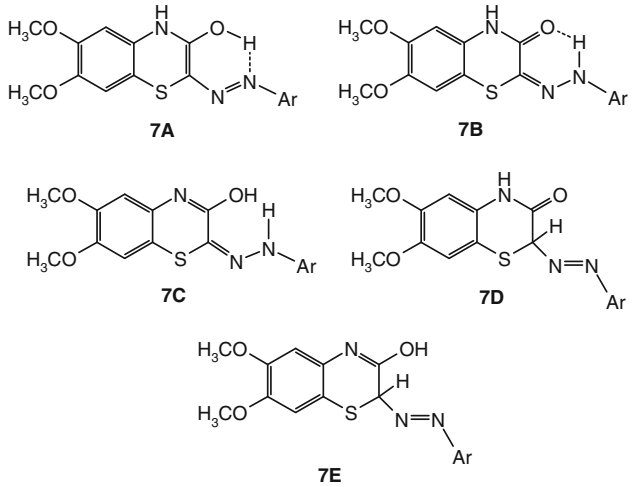


Fig. 1 Possible tautomeric forms of compounds **7**

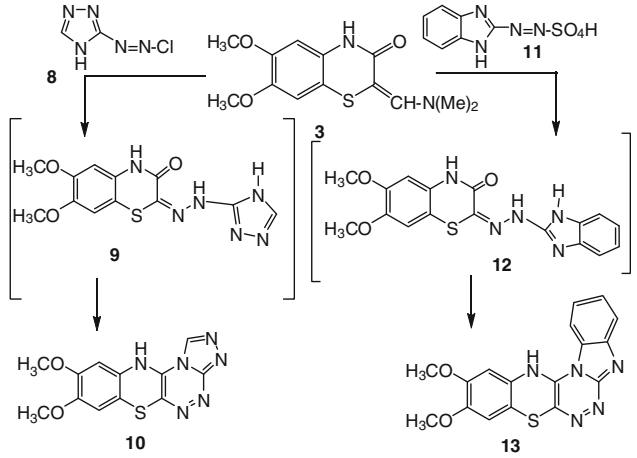
Table 1 UV spectral data of compounds **7a**–**7f** in dioxane

Compound	λ_{max} (nm) (log ε)
7a	389 (4.47), 300 (4.91)
7b[a]	377 (4.20), 299 (4.44)
7c	371 (4.13), 300 (4.59)
7d	379 (4.48), 306 (4.28)
7e	382 (4.57), 304 (4.54)
7f	395 (4.73), 316 (4.21)

[a] Solvent λ_{max} (log ε): acetic acid 376 (4.21), 300 (4.35); ethanol 375 (4.51), 301 (4.02); acetone 374 (4.00), 299 (4.27); DMF 375 (3.99), 301 (4.12)

$\delta = 10.90$–10.69 and 10.20–9.74 ppm. The formation of the products **7a**–**7f** is assumed to take place via Japp–Klingemann-type cleavage of dimethylformamide to afford the product **7**, as illustrated in Scheme 2. This finding indicated that the isolated product was found in tautomeric form **7B**.

The foregoing results prompted us to investigate the behavior of enaminone **3** towards some diazotized heterocyclic amines. Thus, coupling reaction of enaminone **3** with the diazonium salt of 3-amino-1,2,4-triazole (**8**) in pyridine afforded the corresponding hydrazone **9**, which undergoes in situ intramolecular cyclization to afford 8,9-dimethoxy-11*H*-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino [6,5-*b*][1,4]benzothiazine (**10**) (Scheme 3). The structure of the isolated product **10** was established by its spectroscopic [IR, ^{1}H NMR, and mass spectrometry (MS)] data and elemental analyses (see Experimental). Its mass spectrum revealed the molecular ion peak at $m/z = 302$, and its ^{1}H NMR spectrum showed a characteristic signal at $\delta = 8.13$ ppm assignable to the triazole CH proton.



Scheme 3

Table 2 Cytotoxic activity of tested compounds

Compound	IC_{50} (μg/cm^3)		
	HCT-116	HEPG2	MCF7
6a	4.19	4.97	–
7c	4.79	6.69	–
7e	3.74	5.79	10

IC_{50}, 50% inhibition concentration

In a similar manner, enaminone **3** coupled readily with the diazonium salt of 2-aminobenzimidazole (**11**) under the same experimental conditions to afford a single product **13** according to TLC (Scheme 3). The structure of the isolated product is based on the elemental analysis and spectral data (see "Experimental").

Antitumor screening test

The cytotoxic effects of three products **6a**, **7c**, and **7e** were tested against colon cancer cell line HCT-116, liver carcinoma cell line HEPG2-1, and human breast cell line MCF-7. They were evaluated in the National Institute of Cancer, Cairo, Egypt. As shown in Table 2, the analysis of the data obtained indicated that all tested compounds showed reactivity against HCT-116 cell line more than HEPG2 and MCF.

Antimicrobial activity

The products **3**, **6a**, **6d**, **7a**, **7c**–**7e**, **9**, and **11** were screened for their antibacterial activity (in nutrient agar broth) and antifungal activity (in Dox's medium and Sabouraud's agar) by agar diffusion method [31, 32] at concentration of 20 mg/cm^3 using dimethyl sulfoxide (DMSO) as solvent and blank. Compounds were tested for activity against

Table 3 Antimicrobial activity of the tested compounds

Compound	Inhibition zone diameter (mm/mg sample)			
	E. coli	*S. aureus*	*A. flavus*	*C. albicans*
3	16	17	15	14
6a	12	13	–	–
6d	12	13	–	–
7a	13	13	–	13
7c	15	15	–	12
7d	14	14	–	12
7e	14	13	–	12
9	15	13	–	13
11	13	13	–	13
Tetracycline	30	30	–	–
Amphotericin	–	–	18	21

Solution concentration of 20.0 mg/cm^3 was tested

E. coli: *Escherichia coli*, Gram-negative bacteria

S. aureus: *Staphylococcus aureus*, Gram-positive bacteria

A. flavus: *Aspergillus flavus*, fungus

C. albicans: *Candida albicans*, fungus

Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*), in addition to the pathogenic fungi *Aspergillus flavus* and *Candida albicans*. The antimicrobial screening results were measured by the average diameter of the inhibition zones, expressed in mm, and are depicted in Table 3. The results showed that all tested compounds displayed significant activities against *E. coli* and *S. aureus*. Compounds **3**, **7a**, **7c**–**7e**, **9**, and **11** showed moderate activity against *C. albicans*, but only one compound, enaminone **3**, showed a high degree of activity against *A. flavus*.

Conclusion

The novel enaminone **3** was proved to be a valuable synthon for synthesis of fused 1,4-thiazines, such as pyrido[3,2-*b*][1,4]benzothiazine derivatives, 2-(arylhydrazono)-6,7-dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-ones, 8,9-dimethoxy-11*H*-[1,2,4]triazolo[3′,4′:3,4][1,2,4]triazino[6,5-*b*][1,4]benzothiazine (**10**), and 10,11-dimethoxy-13*H*-benzimidazo[2′,1′:3,4][1,2,4]triazino[6,5-*b*][1,4]benzothiazine (**13**). The structure of the newly synthesized compounds was established on the basis of mass, IR, ^{1}H NMR, and elemental analyses. Also, the tautomeric structure of the hydrazone compounds was discussed. The antitumor activity of selected products showed that these compounds are reactive against HCT-116 cell line more than HEPG2 and MCF. In addition, the antimicrobial activity of some selected products showed significant activities against *E. coli*, *S. aureus*, and *A. flavus*.

Experimental

All melting points were determined by using an electrothermal Gallenkamp apparatus. Solvents were generally distilled and dried by standard literature procedures prior to use. IR spectra were measured using a Pye-Unicam SP300 instrument in potassium bromide discs. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury VXR-300 spectrometer (300 or 400 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR), and chemical shifts were related to that of the solvent DMSO-d_6. Mass spectra were recorded using GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, with ionizing voltage of 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. 2-(4,5-Dimethoxy-2-nitrophenylthio)acetic acid (**1**) and 6,7-dimethoxy-2*H*-1,4-benzothiazin-3(4*H*)-one (**2**) were prepared as previously described [33, 34].

2-(Dimethylaminomethylene)-6,7-dimethoxy-2H-1,4-benzothiazin-3(4H)-one (**3**, $C_{13}H_{16}N_2O_3S$)

A mixture of 2.25 g compound **2** (10 mmol) and 2 cm^3 DMF-DMA was heated under reflux for 10 h. The reaction mixture was triturated with ethanol to give a solid product that was collected by filtration and crystallized from ethanol to give compound **3** as orange crystals. Yield 2.52 g (90%); m.p.: 218–220 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.09 (s, 6H, 2CH$_3$), 3.65 (s, 6H, 2 OCH$_3$), 6.57 (s, 1H, ArH), 6.71 (s, 1H, ArH), 7.26 (s, 1H, = CH), 9.49 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 43.08, 56.12, 56.53, 81.63, 101.74, 108.96, 110.02, 132.01, 144.68, 145.39, 146.72, 148.37, 167.30 ppm; IR (KBr): $\bar{\nu}$ = 3,159 (NH), 1,652 (C=O) cm^{-1}; MS: *m/z* (%) = 281 (M$^+$ + 1, 33), 280 (M$^+$, 83), 266 (15), 265 (58), 100 (61), 85 (30), 77 (40), 68 (100).

*Reaction of enaminone **3** with active methylene compounds **4a–4d***

To a solution of 1.40 g **3** (5 mmol) in 15 cm^3 glacial acetic acid in the presence of 0.5 g ammonium acetate was added acetylacetone, ethyl acetoacetate, ethyl benzoylacetate, or dibenzoylmethane (5 mmol). The reaction mixture was heated under reflux for several hours. The reaction was followed by TLC. The solvent was evaporated under reduced pressure, and the solid precipitate was collected by filtration and washed with ethanol to give the respective product **6a**–**6d**.

3-Acetyl-7,8-dimethoxy-2-methyl-10H-pyrido-[3,2-b][1,4]benzothiazine (**6a**, $C_{16}H_{16}N_2O_3S$)

Yellow solid, yield 0.95 g (60%); m.p.: 298–300 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.32 (s, 3H, CH$_3$), 2.67

(s, 3H, $COCH_3$), 3.08 (s, 3H, OCH_3), 3.59 (s, 3H, OCH_3), 6.60 (s, 1H, ArH), 6.75 (s, 1H, ArH), 8.14 (s, 1H, pyridine-H), 10.30 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 14.26$, 21.61, 56.27, 56.69, 102.90, 109.21, 111.30, 127.55, 128.34, 133.26, 140.69, 144.64, 148.32, 160.37, 164.80, 199.0 ppm; IR (KBr): $\bar{\nu} = 3{,}162$ (NH), 1,710 (C=O) cm^{-1}; MS: *m/z* (%) = 317 ($M^+ + 1$, 1), 316 (M^+, 2), 192 (9), 110 (12), 83 (5).

Ethyl 7,8-dimethoxy-2-methyl-10H-pyrido[3,2-b][1,4]-benzothiazine-3-carboxylate (**6b**, $C_{17}H_{18}N_2O_4S$)
Pale yellow solid, yield 1.07 g (62%); m.p.: 170–172 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.28$ (t, $J = 7$ Hz, 3H, CH_3), 2.70 (s, 3H, CH_3), 3.66 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 4.30 (q, $J = 7$ Hz, 2H, CH_2), 6.61 (s, 1H, ArH), 6.83 (s, 1H, ArH), 8.48 (s, 1H, pyridine-H), 10.19 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 14.56$, 25.00, 56.14, 56.50, 61.76, 102.82, 109.59, 111.53, 123.20, 125.90, 131.56, 140.42, 145.12, 148.57, 161.80, 165.40, 165.69 ppm; IR (KBr): $\bar{\nu} = 3{,}202$ (NH), 1,720 (C=O) cm^{-1}; MS: *m/z* (%) = 347 ($M^+ + 1$, 7), 346 (M^+, 24), 137 (25), 75 (100).

Ethyl 7,8-dimethoxy-2-phenyl-10H-pyrido[3,2-b][1,4]-benzothiazine-3-carboxylate (**6c**, $C_{22}H_{20}N_2O_4S$)
Yellow solid, yield 1.16 g (57%); m.p.: 154–156 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 1.20$ (t, $J = 7$ Hz, 3H, CH_3), 3.70 (s, 6H, $2OCH_3$), 4.39 (q, $J = 7$ Hz, 2H, CH_2), 6.72 (s, 1H, ArH), 6.92 (s, 1H, ArH), 7.20–7.78 (m, 5H, ArH), 8.06 (s, 1H, pyridine-H), 10.82 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 14.54$, 56.11, 56.70, 62.05, 103.66, 110.34, 112.46, 119.60, 124.65, 127.91, 129.34, 129.56, 130.71, 133.15, 137.21, 139.47, 145.20, 160.97, 164.28, 165.23 ppm; IR (KBr): $\bar{\nu} = 1{,}728$ (C=O) cm^{-1}; MS: *m/z* (%) = 409 ($M^+ + 1$, 6), 408 (M^+, 25), 331 (61), 91 (83), 77 (100).

3-Benzoyl-7,8-dimethoxy-2-phenyl-10H-pyrido-[3,2-b][1,4]benzothiazine (**6d**, $C_{26}H_{20}N_2O_3S$)
Pale yellow solid, yield 62%; m.p.: 222–224 °C; ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.69$ (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 6.68 (s, 1H, ArH), 6.86 (s, 1H, ArH), 7.10–7.74 (m, 10H, ArH), 7.98 (s, 1H, pyridine-H), 10.11 (s, 1H, NH) ppm; IR (KBr): $\bar{\nu} = 3{,}223$ (NH), 1,689 (C=O) cm^{-1}; MS: *m/z* (%) = 441 ($M^+ + 1$, 10), 440 (M^+, 25), 103 (43), 91 (100), 77 (62).

Preparation of 2-(arylhydrazono)-6,7-dimethoxy-2H-1,4-benzothiazin-3(4H)-ones ***7a–7f***

To a stirred solution of 1.40 g enaminone **3** (5 mmol) in 20 cm^3 ethanol was added 0.7 g sodium acetate trihydrate (0.005 mol), and the mixture was cooled in an ice bath to 0–5 °C. To the resulting solution, while being stirred, was added dropwise over a period of 20 min a solution of the appropriate arenediazonium chloride, prepared as usual by diazotizing the respective aniline (5 mmol) in 3 cm^3 hydrochloric acid (6 M). The whole mixture was then left in a refrigerator overnight. The precipitated solid was collected, washed with water, and finally crystallized from the appropriate solvent to give the respective hydrazone **7a–7f**.

6,7-Dimethoxy-2-(4-methoxyphenylhydrazono)-2H-1,4-benzothiazin-3(4H)-one (**7a**, $C_{17}H_{17}N_3O_4S$)
Pale yellow solid, yield 75%; m.p.: 352 °C (ethanol/dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.50$ (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 6.69 (s, 1H, ArH), 6.89 (s, 1H, ArH), 7.42 (d, $J = 8$ Hz, 2H, ArH), 7.90 (d, $J = 8$ Hz, 2H, ArH), 10.20 (s, 1H, NH), 10.90 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 56.30$, 56.64, 57.47, 102.20, 109.22, 115.85, 120.11, 123.53, 129.27, 129.89, 142.10, 145.18, 147.34, 156.21, 163.10 ppm; IR (KBr): $\bar{\nu} = 3{,}426$, 3,200 (2NH), 1,652 (C=O) cm^{-1}; MS: *m/z* (%) = 360 ($M^+ + 1$, 28), 359 (M^+, 28), 265 (88), 122 (96), 108 (64), 77 (92), 57 (100).

6,7-Dimethoxy-2-phenylhydrazono-2H-1,4-benzothiazin-3(4H)-one (**7b**, $C_{16}H_{15}N_3O_3S$)
Yellow solid, yield 80%; m.p. > 320 °C (ethanol/dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.72$ (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.84 (s, 1H, ArH), 6.88 (s, 1H, ArH), 7.22–7.36 (m, 5H, ArH), 9.74 (s, 1H, NH), 10.81 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 56.36$, 56.58, 103.94, 109.0, 118.13, 119.24, 119.78, 132.24, 142.57, 144.25, 145.61, 147.27, 154.01, 162.24 ppm; IR (KBr): $\bar{\nu} = 3{,}310$, 3,212 (2NH), 1,662 (C=O) cm^{-1}; MS: *m/z* (%) = 329 (M^+, 31), 224 (52), 196 (32), 152 (23), 105 (45), 95 (34), 93 (87), 77 (100).

2-(4-Chlorophenylhydrazono)-6,7-dimethoxy-2H-1,4-benzothiazin-3(4H)-one (**7c**, $C_{16}H_{14}ClN_3O_3S$)
Orange solid, yield 90%; m.p.: 300 °C (ethanol); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.62$ (s, 3H, OCH_3), 3.74 (s, 3H, OCH_3), 6.62 (s, 1H, ArH), 6.82 (s, 1H, ArH), 7.20 (d, $J = 8$ Hz, 2H, ArH), 8.01 (d, $J = 8$ Hz, 2H, ArH), 9.86 (s, 1H, NH), 10.54 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 56.35$, 56.59, 104.28, 110.05, 116.31, 118.0, 121.08, 129.14, 132.35, 143.11, 145.08, 148.34, 154.07, 165.24 ppm; IR (KBr): $\bar{\nu} = 3{,}327$, 3,185 (2NH), 1,671 (C=O) cm^{-1}; MS: *m/z* (%) = 366 ($M^+ + 2$, 6), 365 ($M^+ + 1$, 25), 364 (M^+, 45), 363 (63), 224 (100), 196 (17), 127 (45), 111 (36), 99 (27), 75 (28).

2-(4-Bromophenylhydrazono)-6,7-dimethoxy-2H-1,4-benzothiazin-3(4H)-one (**7d**, $C_{16}H_{14}BrN_3O_3S$)
Orange solid, yield 89%; m.p.: 324–326 °C (ethanol/dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.60$ (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 6.65 (s, 1H, ArH), 6.78 (s, 1H, ArH), 7.80 (d, $J = 9$ Hz, 2H, ArH), 8.21 (d, $J = 9$ Hz, 2H, ArH), 9.89

(s, 1H, NH), 10.69 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 56.10$, 56.42, 102.51, 108.66, 116.18, 119.28, 120.36, 132.08, 136.47, 142.12, 145.70, 146.94, 156.21, 164.97 ppm; IR (KBr): $\bar{\nu} = 3{,}429$, 3,182 (2NH), 1,669 (C=O) cm^{-1}; MS: *m/z* (%) = 410 ($M^+ + 2$, 2), 409 ($M^+ + 1$, 38), 408 (M^+, 11), 407 (28), 224 (100), 211 (12), 171 (20), 108 (13), 91 (46), 85 (16), 64 (30).

6,7-Dimethoxy-2-(4-nitrophenylhydrazono)-2H-1,4-benzothiazin-3(4H)-one (**7e**, $C_{16}H_{14}N_4O_5S$)
Red solid, yield 89%; m.p.: 306–308 °C (dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.59$ (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.81 (s, 1H, ArH), 6.93 (s, 1H, ArH), 7.92 (d, $J = 9$ Hz, 2H, ArH), 8.10 (d, $J = 9$ Hz, 2H, ArH), 10.02 (s, 1H, NH), 10.80 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 56.30$, 56.62, 102.18, 112.24, 116.64, 117.08, 125.18, 130.29, 132.40, 145.27, 145.11, 150.43, 154.25, 165.61 ppm; IR (KBr): $\bar{\nu} = 3{,}430$, 3,270 (2NH), 1,668 (C=O) cm^{-1}; MS: *m/z* (%) = 375 ($M^+ + 1$, 15), 374 (M^+, 100), 225 (20), 224 (98), 210 (16), 196 (25), 150 (19), 108 (27), 91 (17), 85 (21), 76 (36), 64 (67).

2-(4-Acetylphenylhydrazono)-6,7-dimethoxy-2H-1,4-benzothiazin-3(4H)-one (**7f**, $C_{18}H_{17}N_3O_4S$)
Orange crystals, yield 89%; m.p.: 270 °C (ethanol/dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 2.60$ (s, 3H, $COCH_3$), 3.69 (s, 6H, $2OCH_3$), 6.75 (s, 1H, ArH), 6.84 (s, 1H, ArH), 7.34 (d, $J = 9$ Hz, 2H, ArH), 7.86 (d, $J = 9$ Hz, 2H, ArH), 10.16 (s, 1H, NH), 10.90 (s, 1H, NH) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): $\delta = 24.16$, 56.21, 56.60, 102.24, 111.56, 115.27, 118.64, 128.02, 130.28, 131.84, 144.38, 146.0, 153.29, 155.34, 164.05, 198.28 ppm; IR (KBr): $\bar{\nu} = 3{,}437$, 3,220 (2NH), 1,710, 1,656 (2C=O) cm^{-1}; MS: *m/z* (%) = 372 ($M^+ + 1$, 7), 371 (M^+, 32), 311 (10), 224 (54), 211 (12), 120 (61), 105 (26), 91 (33), 84 (28), 77 (64), 55 (100).

*Preparation of compounds **10** and **13***

A stirred solution of 1.40 g enaminone **3** (5 mmol) in 20 cm^3 pyridine was cooled in an ice bath to 0–5 °C. To the resulting solution, while being stirred, was added dropwise over a period of 20 min a solution of the appropriate heterocyclic diazonium salt, prepared as usual by diazotizing the respective heterocyclic amine (5 mmol) in 3 cm^3 nitric acid. The whole mixture was then left in a refrigerator overnight. The precipitated solid was collected, washed with water, and finally crystallized from the appropriate solvent to give the respective compounds **10** or **13**.

8,9-Dimethoxy-11H-[1,2,4]triazolo[3′,4′:3,4][1,2,4]-triazino[6,5-b][1,4]benzothiazine (**10**, $C_{12}H_{10}N_6O_2S$)
Yellow solid, yield 82%; m.p.: 260–262 °C (dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.72$ (s, 6H, $2OCH_3$), 2.82 (s, 3H, $COCH_3$), 6.90 (s, 2H, ArH), 8.13 (s, 1H, triazole-H), 11.01 (s, 1H, NH) ppm; ^{13}C NMR (75 MHz, DMSO-d_6): $\delta = 56.48$, 56.50, 107.05, 112.38, 120.59, 122.87, 128.94, 130.22, 132.35, 145.55, 149.24, 156.00 ppm; IR (KBr): $\bar{\nu} = 3{,}325$ (NH) cm^{-1}; MS: *m/z* (%) = 302 (M^+, 16), 301 (26), 196 (45), 164 (50), 108 (58), 84 (45), 81 (34), 77 (37), 52 (100).

10,11-Dimethoxy-13H-benzimidazo[2′,1′:3,4][1,2,4]-triazino[6,5-b][1,4]benzothiazine (**13**, $C_{17}H_{13}N_5O_2S$)
Yellow solid, yield 92%; m.p. 246–248 °C (dioxane); ^{1}H NMR (300 MHz, DMSO-d_6): $\delta = 3.46$ (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 6.92 (s, 1H, ArH), 7.02 (s, 1H, ArH), 10.55 (s, 1H, NH) ppm; IR (KBr): $\bar{\nu} = 3{,}210$ (NH) cm^{-1}; MS: *m/z* (%) = 351 (M^+, 100), 91 (43), 77 (97).

Pharmacology

Cytotoxic activity against human breast cancer (MCF-7) in vitro
The method applied is similar to that reported by Skehan and Storeng [35] using Sulfo-Rhodamine-B stain (SRB). Cells were plated in a 96-multiwell plate (10^4 cells/well) for 24 h before treatment with the test compound to allow attachment of cells to the wall of the plate, then different concentrations of the compound under test (0, 1.0, 2.5, 5, and 10 μg/cm^3) were added to the cell monolayer in triplicate wells per individual dose. The monolayer cells were incubated with the compounds for 48 h at 37 °C in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with SRB stain. Excess stain was washed with acetic acid, attached stain was recovered with Tris–ethylenediamine tetraacetic acid (EDTA) buffer, and color intensity was measured in an enzyme-linked immunosorbent assay (ELISA) reader. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of tumor cell line, and the IC_{50} was calculated. The results are summarized in Table 2.

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