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ARTICLE

Synthesis and anticancer evaluation of some novel *N,O,S* heterocyclic compounds pendant to 3-methyl-5-cyano-6-(3,4-dimethoxyphenyl)pyrimidine and other related fused pyrimidines

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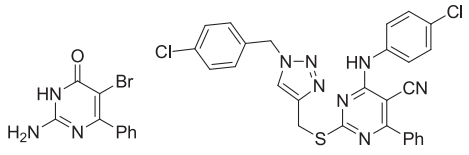
Abstract

The key starting compound 5-cyano-6-(3,4-dimethoxyphenyl)-2-thiouracil (**1**) was synthesized and allowed to undergo electrophilic substitution with methyl iodide to give the corresponding 6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile (**2**). Nucleophilic substitution on compound **2** with hydrazine hydrate led to the corresponding 2-hyrazinopyrimidone intermediate **3**. Compound **3** underwent several substitution and cyclization reactions with β-ketoester, β-ketone, alkyl halides, arylisothiocyanate, or aromatic aldehydes followed by cyclization reactions to give the corresponding *N,O,S* heterocyclic compounds incorporated into pyrimidine moiety and/or the related *S*-triazino[3,4-*b*]pyrimidine derivatives **4**-**18**. Anticancer evaluation of some representative examples of newly synthesized compounds was carried out against MCF-7, HCT116 cell lines. Some of the newly synthesized compounds showed significant activity.

1 | INTRODUCTION

Pyrimidines are among those molecules that make life possible as being building blocks of nucleic acids DNA and RNA. DNA damaged by exogenous physical and chemical agents is the most common cause of cancer. Conversely, some of the commonly used anticancer drugs kill malignant cell by damaging their DNA. Several substituted pyrimidine derivatives are reported to possess anticancer activity. Broprimine **I**,[1] 2-amino-5-bromo-6-phenylpyrimidin-4(3*H*)-one, is a well-known antineoplastic agent. Also, several 2-(((1-(4-chlorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)thio)-4-((4-chlorophenyl)amino)-6-phenylpyrimidine-5-carbonitrile (**II**) are recently reported[2] as potential anticancer agents against MGC-803, EC-109, MCF-7, and B16-F10 cell lines.



I II

Several other pyrimidine derivatives have been reported to possess diverse chemotherapeutic activities such as antiviral, antimalarial, antiprotozoal, antifungal, and antimycotic agents.[3–6] The therapeutic importance of this class of compounds prompted as to synthesize a new series of 3-methyl-4-oxo-5-cyano-6-(3,4-dimethoxyphenyl) pyrimidines and other related *S*-triazino[3,4-*b*]pyrimidines as a continuation of previous work[7–16] in our drug research program for anticancer evaluation against cancer cell lines.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

For the purpose of this study, the synthetic approach was confined to three general schemes (1–3) to obtain the target polyfunctionally substituted pyrimidinones and the related fused pyrimidines. The first starting compound, 6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) in almost good yield was previously prepared by our research group,[10] via the reaction of thiourea with 3,4-dimethoxybenazldehyde and ethylcyanoacetate in alkaline medium. Compound **1** was allowed to undergo electrophilic substitution with methyl iodide in sodium carbonate to give the corresponding 4-(3,4-dimethoxyphenyl)-1-methyl-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (**2**). Nucleophilic substitution on compound **2** with hydrazine hydrate led to the corresponding strategic intermediate 4-(3,4-dimethoxyphenyl)-2-hydrazinyl-1-methyl-6-oxo-1,6-dihydro- pyrimidine-5-carbonitrile (**3**) (Scheme 1). Compound **3** was investigated by spectral and elemental analyses. Thus, the IR spectrum of the hydrazino derivative **3** showed the appearance of NH_2 and NH bands at 3417, 3324, 3198 cm^{-1}. Further support for the structure of compound **3** was gained from its ^{1}H-NMR spectrum, which showed a broad singlet signals corresponding to NH_2 and NH groups at 9.90 and 10.76 ppm, respectively, (cf. Section 3).

Compound **3** underwent several cyclization reactions to give the corresponding *N*,*O*, and/or *S* heterocyclic compounds pendant to pyrimidine moiety. Thus, reaction of **3** with acetoacetic ester afforded the corresponding 2-(3-methylpyrazolone-1-yl-pyrimidine derivative **4**, while cyclization on of **3** using acetyl acetone afforded the corresponding 2-(3,5-dimethylpyrazole-1-yl)pyrimidine derivative **5**. Reaction of **3** with acid anhydrides, newly maleic anhydride, or phthalic anhydride in glacial acetic acid for 6 hours, afforded the corresponding pyrrolidindione and/or the phthalimido derivatives **6a**,**b**, respectively. Also, reaction of **3** with chloroacetylchloride and/or chloroacetone gave the corresponding alkyl hydrazine derivatives **7a**,**b** respectively. Compounds **7a**,**b** upon cyclization with *o*-aminophenol and/or 4-chloro-*o*-aminophenol afforded the corresponding benzo(1,4) oxazine derivatives **8a**,**b**, respectively.

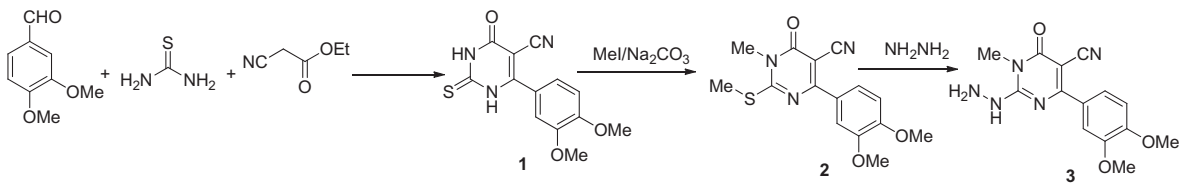
On the other hand, reaction of **3** with substituted arylisothiocyanate, namely, phenyl and/or benzyl isothiocyanate, gave the corresponding 2-substituted thiosemicarbazide derivatives **9a**,**b**, respectively (Scheme 2). Further, reactions of compounds **9a**,**b** with chloroacetic acid afforded the corresponding thiazolidinone derivatives **10a**,**b** respectively, while upon reaction with malonic acid, it gave the thiobarbituric acid derivatives **11a**,**b** respectively. The structure of compound **11** was strongly confirmed by its IR spectrum, which showed the vibrational coupling of the carbonyl groups (cf. Section 3). Also, reaction of **9** with phenacyl bromide gave the corresponding phenyl thiazolidone derivatives **12a**,**b**, respectively (Scheme 3).

Furthermore, reactions of compounds **3** with appropriate aldehydes, namely, indole-3-carboxaldehyde and/or naphthalene-2-carboxaldehyde, gave the corresponding Schiff bases **13a**,**b**, which upon cyclization with thiosalicylic acid, it gave the corresponding benzothiazinone derivatives **14a**,**b**, respectively. On the other hand, cyclization of the Schiff bases **13a**,**b** using lead acetate afforded the corresponding *S*-triazolo[4,3-*a*]pyrimidine-6-carbonitrile **15a**,**b** respectively (Scheme 4).

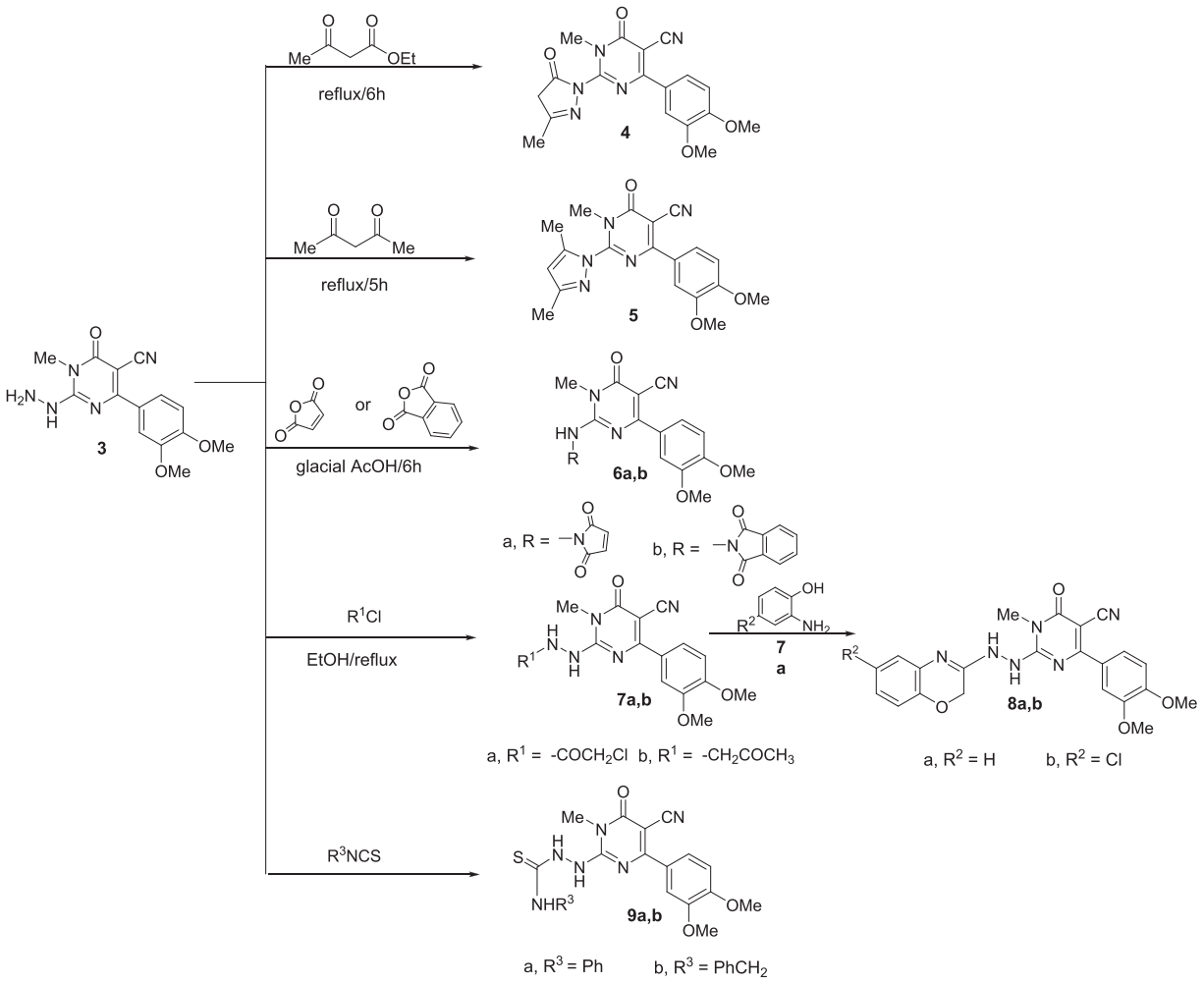
Finally, upon cyclization of compound **3** with either trimethyl orthoformate or formic acid, it gave the corresponding *S*-triazolo[3,4-*b*]pyrimidine derivative **16** in almost good yield, while upon cyclization using ethyl choroformate, it gave the corresponding 5-(3,4-dimethoxyphenyl)-8-methyl-3,7-dioxo-2,3,7,8-tetrahydro-[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile (**17**). Also, upon cyclization of compound **3** with acetic anhydride, it gave the 5-(3,4-dimethoxyphenyl)-3,8-dimethyl-7-oxo-7,8-dihydro-[1,2,4]triazolo[4,3-*a*]pyrimidine-6-carbonitrile (**18**), while upon cyclization with diethyl oxalate, it gave the corresponding triazinopyrimidinetrione derivative **19** (Scheme 5). All newly prepared compounds were investigated by elemental and spectroscopic analyses.

2.2 | Evaluation of cytotoxic activity

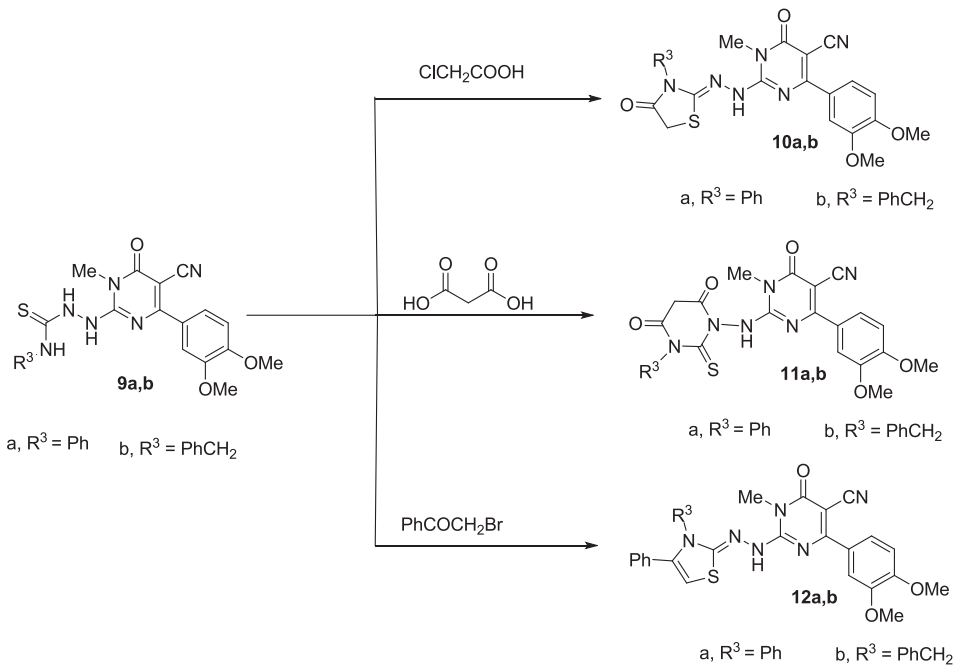
The new pyrimidinone and the related fused pyrimidine derivatives were evaluated as cytotoxic agents against



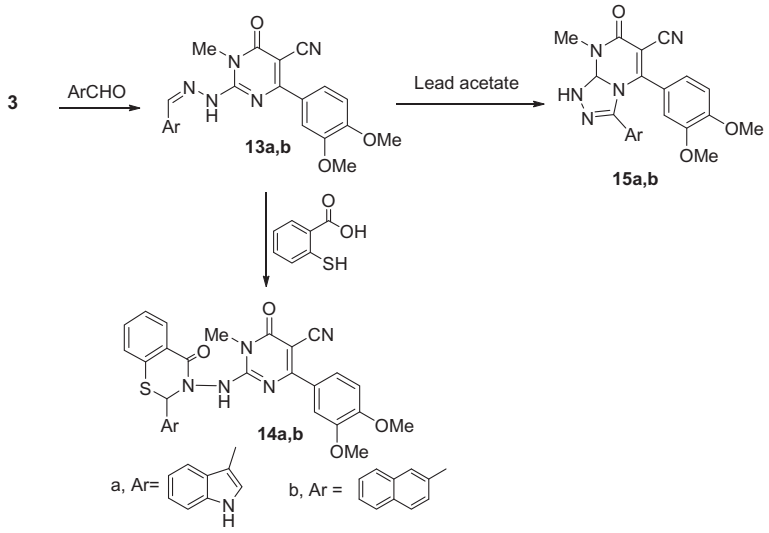
SCHEME 1 Synthesis of compounds **1** to **3**



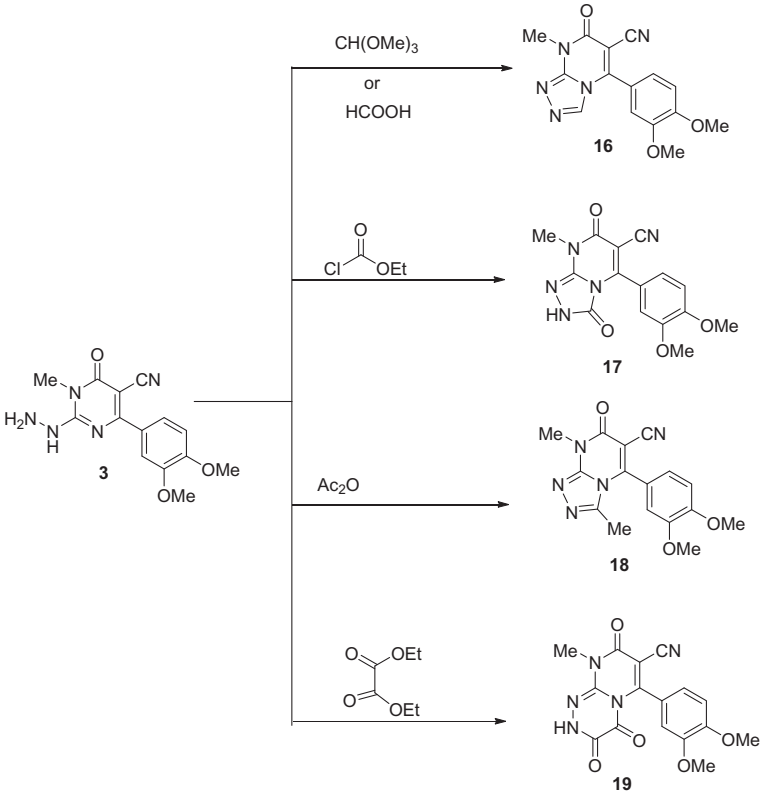
SCHEME 2 Conversion of compound **3** into compounds **4**-**9**



SCHEME 3 Formation of compounds **10**-**12**



SCHEME 4 Formation of compounds **13-15**



SCHEME 5 Conversion of compound **3** into compounds **16-19**

two types of cancer cell lines; human breast (MCF-7) and human colon cancer cell (HCT116) using MTT assay. The resultant data were expressed as IC_{50} (μg/mL) in Tables 1 and 2. To determine the safety of the new compounds, the cytotoxic activity of the active derivatives was examined against the normal skin fibroblast BJ1. It has been noticed that the parent hydrazino-pyrimidinone derivative **3** produced moderate cytotoxic activity against MCF-7 cancer cells of IC_{50}; 66.6 μg/mL. Equivalent potency was obtained upon conjugation of the parent pyrimidinone nucleus with thiazolidinone ring as compound **10a** of IC_{50}; 66.2 μg/mL. On the other hand, the inhibitory potency against MCF-7 cancer cells was increased by the fused *S*-triazolo[4,3-*a*]pyrimidine derivative (compound **15**) producing IC_{50}; 50 μg/mL. The combination of the basic pyrimidinone scaffold with benzothiazinone ring (compound **14a**) was favorable for the activity. It boosted the anticancer potency approximately by 1.7 folds (IC_{50}; 36 μg/mL) comparing with the starting pyrimidinone **3**. Furthermore, the parent

TABLE 1 Sample concentration range between 100 and 0.78 μg/mL using MTT assay

Sample Code	LC_{50}, μg/mL	LC_{90}, μg/mL	Remarks
12	--------	--------	60.1% at 100 ppm
13[a]	54.2	81.3	96.5% at 100 ppm
14a[a]	56.4	89.2	93.2% at 100 ppm
15[a]	25.3	35.9	100% at 100 ppm
16	--------	--------	39.5% at 100 ppm
17[a]	49.0	78.8	97.2% at 100 ppm
18	--------	--------	34.2% at 100 ppm
19	--------	--------	61.3% at 100 ppm
3[a]	49.5	89.8	84.2% at 100 ppm
6	--------	--------	49.5% at 100 ppm
7	--------	--------	63.1% at 100 ppm
8	--------	--------	29.1% at 100 ppm
9	--------	--------	67.2% at 100 ppm
10a[a]	28.7	48.1	100% at 100 ppm
11	--------	--------	31.2% at 100 ppm
DMSO	--------	--------	1% at 100 ppm
Negative control	--------	--------	0%

Note. LC_{50}: lethal concentration of the sample, which causes the death of 50% of cells in 48 hours. LC_{90}: lethal concentration of the sample, which causes the death of 90% of cells in 48 hours.
[a]Compounds that show positive response.

TABLE 2 The sample was tested against the human tumor cell line(s)

Sample Code	LC_{50}, μg/mL	LC_{90}, μg/mL	Remarks
13	--------	--------	34.1% at 100 ppm
14a[a]	36.6	64.8	94.2% at 100 ppm
15[a]	50.6	79	98.2% at 100 ppm
16	--------	--------	40.1% at 100 ppm
17	--------	--------	51.2% at 100 ppm
18	--------	--------	11.4% at 100 ppm
19	--------	--------	29.5% at 100 ppm
6	--------	--------	20.1% at 100 ppm
8	--------	--------	24.3% at 100 ppm
7	--------	--------	54.2% at 100 ppm
9	--------	--------	17.1% at 100 ppm
10a[a]	66.2	109.8	72.3% at 100 ppm
11	--------	--------	54.2% at 100 ppm
12	--------	--------	51.3% at 100 ppm
3[a]	66.6	111	72.3% at 100 ppm
DMSO	--------	--------	3% at 100 ppm
Negative control	--------	--------	0%

Note. LC_{50}: lethal concentration of the sample, which causes the death of 50% of cells in 48 hours. LC_{90}: lethal concentration of the sample, which causes the death of 90% of cells in 48 hours.
[a]Compounds that show positive response.

hydrazino-pyrimidinone derivative **3** produced moderate growth inhibitory activity against colon cancer cell of IC_{50}; 49.5 μg/mL, while its thiazolidinone **10a** and *S*-triazolo[4,3-*a*]pyrimidine analogues **15** remarkably increased the activity by 1.7 and 2 folds, of IC_{50}; 28.7 and 25.3 μg/mL, respectively. Alternatively, an observable reduction in growth inhibition activity was detected by the pyrimidine-Schiff base **13** and pyrimidine-benzothiazinone conjugate **14** producing IC_{50}; 54.2 and 56.4 μg/mL, respectively comparing with the parent compound **3**. Fortunately, no active derivatives produced any cytotoxic activity against the normal fibroblast cells confirming its complete safety profile against the human normal cells. It could be concluded that the tested derivatives are more effective against human colon cancer cell line than the human breast cancer cell lines. The fused *S*-triazolo[4,3-*a*]pyrimidine derivative **15** produced dual cytotoxic activity against both types of human breast and colon cancer cell lines. Despite the benzothiazinone ring is favorable for the anticancer potency against MCF-7 cells, the thiazolidinone one is more active against HCT116 cancer cells. Further structural modification is required to improve the anticancer activity of the previous newly synthesized pyrimidine compounds.

2.2.1 | Cytotoxic activity test

The sample was tested against the human tumor cell line(s):

1. HCT116 (colon cell line)
2. MCF-7 (human Caucasian breast adenocarcinoma)

3 | EXPERIMENTAL

3.1 | Chemistry

All melting points are uncorrected and were taken on open capillary tubes using electrothermal apparatus 9100. Elemental microanalyses were carried out at microanalytical unit, Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt, using Vario Elementar and were found within + or −0.5% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform infrared spectrometer at per centimeter scale using KBr disc technique at the Central Services Lab. NRC, Dokki, Giza, Egypt. ^{1}HNMR and ^{13}CNMR spectra were determined by using a JEOL

EX-NMR Spectrometer—300 MHz and 75 MHz—at Central Services Lab, (Faculty of Science, Cairo University, Cairo, Egypt). Mass spectra were measured with Finnigan M A T SSQ-7000 mass spectrometer at the Central Services, NRC Dokki, Giza, Egypt. Nomenclature given for the new compounds are according to the IUPAC System.

3.1.1 | Synthesis of 4-(3,4-dimethoxyphenyl)-1-methyl-2-(methylthio)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (2)

To a solution of compound **1** (2.9 g, 0.01 mol) in DMF (30 mL), potassium carbonate (4 g) and 0.03 mol of methyl iodide were added and stirred for 3 hours at room temperature. The reaction mixture was diluted with cold water and the precipitate was filtered off. The product **2** was separated as brownish yellow crystals (ethanol), yield 85%. mp 146°C to 137°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 2222 (CN), 1719 (C═O).^{1}H NMR ($CDCl_3$): δ (ppm) 2.90 (s, 3H, S-CH_3), 3.10 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 6.60 to 7.10 (m, 3H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 317 (20) (M^+) Anal. for $C_{15}H_{15}N_3O_3S$ (317.36): calcd. C, 56.77; H, 4.76; N, 13.24; S, 10.10; found: C, 57.00; H, 5.09; N, 12.74; S, 10.16.

3.1.2 | Synthesis of 4-(3,4-dimethoxyphenyl)-2-hydrazinyl-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (3)

A suspension of compound **2** (3.2 g, 0.01 mol) in ethanol (25 mL) was added with 15 mL of hydrazine hydrate, and the reaction mixture was refluxed for 5 hours then cooled with cold water, and the precipitate was filtered off and washed several times with cold water to give product **3**, which was recrystallized from ethanol as brown crystals, yield 90%. mp 152°C to 154°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3417, 3324, 3198 (NH_2, NH), 2279 (CN), 1699 (C═O).^{1}H NMR ($CDCl_3$): δ (ppm) 2.70 (s, 3H, N-CH_3), 3.10 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.50 to 7.20 (m, 3H, H_{arom}), 9.90 (s, 2H, NH_2, exchangeable), 10.76 (s, 1H, NH, exchangeable) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6) δ/ppm: 28.7 (CH_3), 57.4, 58.2 (2 O-CH_3), 98.2, 112.3, 112.8, 119.5, 126.9, 131.8, 146.4, 149.2, 154.1, 162.5 (Ar-C), 171.3 (C═O). MS (EI, 70 eV): *m/z* (%) = 301 (80) (M^+) Anal. for $C_{14}H_{15}N_5O_3$ (301.30): calcd. C, 55.81; H, 5.02; N, 23.24; found: C, 56.02; H, 4.88; N, 22.83.

3.1.3 | Synthesis of 4-(3,4-dimethoxyphenyl)-1-methyl-2-(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (4)

A mixture of compound **3** (1.35 g, 0.05 mol) and ethyl acetoacetate (0.65 mL, 0.005 mol) in ethanol (40 mL) was refluxed for 6 hours. The solvent was concentrated under reduced pressure and the formed precipitate, which obtained on cooling was filtered off and dried to give product **4** that separated as yellow crystals (chloroform), yield 68%. mp 170°C to 172°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 2219 (CN), 1720 (C═O), 1650 (C═N).^{1}H NMR (DMSO):δ (ppm) 2.3 (s,3H,CH_3), 2.80 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 4.40 (s, 2H, CH_2), 6.51 to 6.90 (m, 3H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 368 (30) (M^+) Anal. for $C_{18}H_{17}N_5O_4$ (367.36): calcd. C, 58.85; H, 4.66; N, 19.06; found: C, 58.96; H, 4.41; N, 19.52.

3.1.4 | Synthesis of 4-(3,4-dimethoxyphenyl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (5)

The foregoing procedures for preparation of compound **4** were applied except that acetyl acetone was used instead of ethyl acetoacetate to give product **5** as yellowish brown crystals (chloroform), yield 55%. mp 196°C to 197°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 2228 (CN), 1648 (C═N), 1667 (C═O). ^{1}H NMR (DMSO): δ (ppm) 2.54 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 2.80 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.30 to 6.80 (m, 4H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 365 (15) (M^+) Anal. for $C_{19}H_{19}N_5O_3$ (365.39): calcd. C, 62.46; H, 5.24; N, 19.17; found: C, 62.68; H, 5.41; N, 19.36.

3.1.5 | General procedures for synthesis of 6a,b

A mixture of compound **3** (0.27 g, 0.001 mol) and the appropriate acid anhydride, namely, maleic anhydride and phthalic anhydride (0.001 mol) in glacial acetic acid (10 mL), was heated under refluxed for 6 hours; the reaction mixture was cooled and poured onto ice/cold water; the formed precipitate was filtered off, dried, and recrystallized from the proper solvent to give compounds **6a**,**b**.

4-(3,4-Dimethoxyphenyl)-2-((2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)amino)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6a)

Product **6a** was separated as yellow crystals (chloroform), yield 53%. mp 176°C to 178°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3340 (NH), 2234 (CN), 1735, 1709, 1698 (C═O), 1647 (C═N). ^1H NMR (DMSO): δ (ppm) 2.80 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.20 (s, 1H, NH, exchangeable), 6.50 to 7.37 (m, 5H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 381 (15) (M^+) Anal. for $C_{18}H_{15}N_5O_5$ (381.34): calcd. C, 56.69; H, 3.96; N, 18.37; found: C, 56.33; H, 4.40; N, 18.74.

4-(3,4-Dimethoxyphenyl)-2-((1,3-dioxoisoindolin-2-yl)amino)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (6b)

Product **6b** was separated as yellow crystals (ethanol), yield 63%. mp 103–105°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3344 (NH), 2213 (CN), 1742, 1719, 1708 (C═O), 1647 (C═N). ^1H NMR (DMSO): δ (ppm) 2.65 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.50 (s, 1H, NH, exchangeable), 6.60 to 7.50 (m, 7H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 431 (25) (M^+) Anal. for $C_{22}H_{17}N_5O_5$ (431.12): calcd. C, 61.25; H, 3.97; N, 16.23; found: C, 61.09; H, 4.16; N, 15.81.

3.1.6 | General procedures for synthesis of 7a,b

A mixture of compound **3** (0.27 g, 0.001 mol) and the appropriate alkyl halides, namely, 2-chloroacetyl chloride and/or 2-chloroacetone (0.001 mol) in absolute ethanol (30 mL), was refluxed for 6 hours; the reaction mixture was cooled and poured onto ice/cold water; the formed precipitate was filtered off, dried, and recrystallized from the proper solvent to give compounds **7a**,**b**, respectively.

2-Chloro-*N*′-(5-cyano-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl) acetohydrazide (7a)

Product **7a** was separated as brown crystals (ethanol), yield 56%. mp 270°C to 271°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3365 (NH), 2221 (CN), 1672, 1656 (C═O), 1648 (C═N), 773 (C-Cl). ^1H NMR (DMSO):δ (ppm) 2.70 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 6.10 (s, 1H, NH), 6.60 to 7.50 (m, 3H, H_{arom}), 10.30 (s, 1H, NH, exchangeable), ppm. MS (EI, 70 eV): *m/z* (%) = 379(M + 2, 6), 377 (20) (M^+) Anal. for $C_{16}H_{16}ClN_5O_4$ (377.09): calcd. C, 50.87; H, 4.27; N, 18.54; found: C, 51.09; H, 4.60; N, 18.99.

***N*′-(5-Cyano-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-3-oxobutanehydrazide (7b)**

Product **7b** was separated as brown crystals (ethyl acetate), yield 79%. mp 182°C to 184°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3343 (NH), 2227 (CN), 1720, 1680 (C═O), 1647 (C═N). ^1H NMR (DMSO): δ (ppm) 2.67 (s, 3H, CH_3CO), 2.80 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 4.50 (s, 2H, CH_2), 5.80 (s, 1H, NH, exchangeable), 6.30 (s, 1H, NH, exchangeable), 6.20 to 6.90 (m, 3H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 357 (35) (M^+) Anal. for $C_{17}H_{19}N_5O_4$ (357.14): calcd. C, 57.14; H, 5.36; N, 19.60; found: C, 57.26; H, 5.01; N, 20.10.

3.1.7 | General procedures for synthesis of 8a,b

A mixture of compound **7a** (0.30, 0.001 mol) and the appropriate phenol, namely, 2-amino phenol and/or 2-amino-4-chlorophenol (0.001 mol) in absolute ethanol (10 mL), with few drops of dil.HCl. The reaction mixture was refluxed for 10 hours; the reaction mixture was cooled and poured onto ice/cold water; the formed precipitate was filtered off, dried, and recrystallized from ethanol to give compounds **8a**,**b**.

2-(2-(2*H*-Benzo[*b*][1,4]oxazin-3-yl)hydrazinyl)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (8a)

Product **8a** was separated as yellow crystals (ethanol), yield 53%. mp 153°C to 154°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3390 (NH), 2218 (CN), 1695 (C═O), 1644 (C═N). ^1H NMR (DMSO): δ (ppm): 2.80 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.10 (s, 2H, CH_2-O), 6.50 (s, 1H, NH, exchangeable), 6.92 (s, 1H, NH, exchangeable), 7.20 to 8.11 (m, 7H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 432 (13) (M^+) Anal. for $C_{22}H_{20}N_6O_4$ (432.44): calcd. C, 61.10; H, 4.66; N, 19.43; found: C, 61.31; H, 4.83; N, 19.61.

2-(2-(7-Chloro-2*H*-benzo[*b*][1,4]oxazin-3-yl) hydrazinyl)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (8b)

Product **8b** was separated as yellow crystals (ethanol), yield 50%. mp 169°C to 170°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3383 (NH), 2218 (CN), 1678 (C═O), 1638 (C═N), 1173 (C-Cl). ^1H NMR (DMSO): δ (ppm) 2.70 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 4.60 (s, 2H, CH_2), 5.60 (s, 1H, NH, exchangeable), 6.72 (s, 1H, NH, exchangeable), 6.20 to 7.50 (m, 6H, H_{arom}) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6) δ/ppm: 28.2 (CH_3), 57.6, 57.9 (2 O-CH_3), 71.8(O-CH_2), 95.7, 111.9, 112.3, 115.8, 116.5, 124.9, 125.1,

126.3, 128.9, 130.0, 131.8, 132.0, 136.8, 149.2, 149.7, 154.6 155.5 (Ar-C), 170.2 (C═O). MS (EI, 70 eV): *m/z* (%) = 468(M + 2, 5), 466 (13) (M^+) Anal. for $C_{22}H_{19}ClN_6O_4$ (466.88): calcd. C, 56.60; H, 4.10; N, 18.00; found: C, 56.84; H, 3.91; N, 18.12.

3.1.8 | General procedures for synthesis of 9a,b

A mixture of compound **3** (0.27 g, 0.001 mol) and the appropriate isothiocyanate, namely, phenyl and benzyl isothiocyanate (0.001 mol) in dry benzene (30 mL), was heated under refluxed for 6 hours; the formed precipitate on cooling was filtered off, dried, and recrystallized from the proper solvent to give compounds **9a**,**b**.

2-(5-Cyano-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl)-*N*-phenylhydrazinecarbothioamide (9a)

Product **9a** was separated as yellow crystals (ethanol), yield 58%. mp 146°C to 147°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3426 (NH), 2230 (CN), 1689 (C═O), 1648 (C═N), 1136 (C═S). ^{1}H NMR (DMSO): δ (ppm) 2.50 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.20 (s, 1H, NH, exchangeable), 6.90 to 7.50 (m, 8H, H_{arom}), 10.80 (s, 1H, NH, exchangeable), 11.60 (s, 1H, NH, exchangeable) ppm. MS (EI, 70 eV): *m/z* (%) = 437 (15) (M^{+1}) Anal. for $C_{21}H_{20}N_6O_3S$ (436.13): calcd. C, 57.79; H, 4.26; N, 19.25; found: C, 58.15; H, 4.36; N, 19.36.

N-Benzyl-2-(5-cyano-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidin-2-yl) hydrazinecarbothioamide (9b)

Product **9b** was separated as yellow crystals (ethanol), yield 65%. mp 110°C to 111°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3419 (NH), 2288 (CN), 1686 (C═O), 1644 (C═N), 1137 (C═S). ^{1}H NMR (DMSO): δ (ppm) 2.90 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.70 (s, 2H, CH_2), 6.30 (s, 1H, NH), 10.50 (s, 1H, NH, exchangeable), 11.80 (s, 1H, NH, exchangeable), 6.60 to 7.80 (m, 8H, H_{arom}) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6) δ/ppm: 26.7 (CH_3), 50.3 (CH_2-Ph), 57.2, 57.5 (2 O-CH_3), 96.1, 112.0, 112.3, 115.5, 119.8, 126.5, 126.9, 128.1, 130.1, 138.2, 149.5, 149.8, 153.6 155.8 (Ar-C), 170.5 (C═O), 185.0 (C═S). MS (EI, 70 eV): *m/z* (%) = 450 (18) (M^+) Anal. for $C_{22}H_{22}N_6O_3S$ (450.52): calcd. C, 58.65; H, 4.92; N, 18.65; found: C, 58.92; H, 5.36; N, 18.36.

3.1.9 | General procedures for synthesis of 10a,b

A mixture of compounds **9a**,**b** (0.001 mol) and chloroacetic acid (0.4 g, 0.01 mol) and sodium acetate (0.1 g) in absolute ethanol was heated under refluxed for 10 hours; the formed precipitate was filtered off, dried, and recrystallized from the proper solvent to give compounds **10a**,**b**.

4-(3,4-Dimethoxyphenyl)-1-methyl-6-oxo-2-(2-(4-oxo-3-phenylthiazolidin-2-ylidene) hydrazinyl)-1,6-dihydropyrimidine-5-carbonitrile (10a)

Product **10a** was separated as yellowish brown crystals (ethanol), yield 70%. mp 182°C to 183°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3449 (NH), 2318 (CN), 1725, 1677 (C═O), 1647 (C═N). ^{1}H NMR (DMSO): δ (ppm) 2.64 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2CO), 6.50 (s, 1H, NH, exchangeable), 6.50 to 7.80 (m, 8H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 476 (25) (M^+) Anal. for $C_{23}H_{20}N_6O_4S$ (476.13): calcd. C, 57.97; H, 4.23; N, 17.64; found: C, 58.16; H, 4.07; N, 17.97.

2-(2-(3-Benzyl-4-oxothiazolidin-2-ylidene) hydrazinyl)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (10b)

Product **10b** was separated as yellowish brown crystals (ethanol), yield 63%. mp 211°C to 212°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3438 (NH), 2303 (CN), 1689 (C═O), 1644 (C═N). ^{1}H NMR (DMSO): δ (ppm) 2.78 (s, 3H, N-CH_3), 3.10 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 2H, N-CH_2), 4.40 (s, 2H, CH_2CO), 6.30 (s, 1H, NH, exchangeable), 6.20 to 7.90 (m, 8H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 490 (10) (M^+) Anal. for $C_{24}H_{22}N_6O_4S$ (490.14): calcd. C, 58.76; H, 4.52; N, 17.13; found: C, 58.88; H, 4.35; N, 17.81.

3.1.10 | General procedures for synthesis of 11a,b

A mixture of compounds **9a**,**b** (0.001 mol) and malonic acid (1.0 g, 0.01 mol) in absolute ethanol (20 mL) was heated under refluxed for 15 hours; the reaction mixture was cooled and poured onto ice/cold water contains few drops of HCl; the formed precipitate was filtered off, dried, and recrystallized from the proper solvent to give compounds **11a**,**b**.

4-(3,4-Dimethoxyphenyl)-2-((4,6-dioxo-3-phenyl-2-thioxotetrahydropyrimidin-1(2*H*)-yl)amino)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (11a)

Product **11a** was separated as yellow crystals (ethanol), yield 58%. mp 191-192°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3432 (NH), 2257 (CN), 1725, 1701, 1667 (C═O), 1642 (C═N), 1161 (C═S). ^{1}H NMR (DMSO): δ (ppm) 2.59 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.20 (s, 2H, CH_2),

5.60 (s, 1H, NH, exchangeable), 6.50 to 7.80 (m, 8H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) 505 (22) (M^{+1}) Anal. for $C_{24}H_{20}N_6O_4S$ (504.12): calcd. C, 57.14; H, 4.00; N, 16.66; found: C, 57.48; H, 4.18; N, 16.42.

2-((3-Benzyl-4,6-dioxo-2-thioxotetrahydropyrimidin-1(2*H*)-yl)amino)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (11b)

Product **11b** was separated as yellow crystals (ethanol), yield 62%. mp 177°C to 178°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3330 (NH), 2249 (CN), 1730, 1705, 1685 (C═O), 1645 (C═N), 1204 (C═S). ^{1}H NMR (DMSO): δ (ppm) 2.80 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2CO), 4.50 (s, 2H, N-CH_2), 5.80 (s, 1H, NH, exchangeable), 6.63 to 7.80 (m, 8H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 517 (19) (M^{-1}) Anal. for $C_{25}H_{22}N_6O_4S$ (518.14): calcd. C, 57.91; H, 4.28; N, 16.21; found: C, 58.02; H, 3.92; N, 16.43.

3.1.11 | General procedures for synthesis of 12a,b

A mixture of compounds **9a**,**b** (0.001 mol) and phenacyl bromide (1.9 g, 0.01 mol) in absolute ethanol (20 mL) was heated under refluxed for 8 hours; the solvent evaporated under reduced pressure; the formed precipitate was collected, washed with K_2CO_3 solution, dried, and recrystallized from the proper solvent to give compounds **12a**,**b**.

4-(3,4-Dimethoxyphenyl)-2-(2-(3,4-diphenylthiazol-2(3*H*)-ylidene) hydrazinyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (12a)

Product **12a** was separated as yellow crystals (ethanol), yield 56%. mp 113°C to 114°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3327 (NH), 2236 (CN), 1690 (C═O), 1619 (C═N). ^{1}H NMR (DMSO): δ (ppm) 2.75 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 5.80 (s, 1H, NH, exchangeable), 6.30 to 7.90 (m, 14H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 536 (13) (M^+) Anal. for $C_{29}H_{24}N_6O_3S$ (536.16): calcd. C, 64.91; H, 4.51; N, 15.66; found: C, 65.10; H, 4.27; N, 15.39.

2-(2-(3-Benzyl-4-phenylthiazol-2(3*H*)-ylidene) hydrazinyl)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (12b)

Product **12b** was separated as yellow crystals (ethanol), yield 71%. mp 135°C to 136°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3421 (NH), 2305 (CN), 1702 (C═O), 1648 (C═N). ^{1}H NMR (DMSO): δ (ppm) 2.70 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.60 (s, 3H, OCH_3), 4.20 (s, 2H, N-CH_2), 5.67 (s, 1H, NH, exchangeable), 6.50 to 8.10 (m, 14H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 549 (28) (M-1) Anal. for $C_{30}H_{26}N_6O_3S$ (550.18): calcd. C, 65.44; H, 4.76; N, 15.26; found: C, 65.78; H, 5.04; N, 15.08.

3.1.12 | General procedures for synthesis of 13a,b

A mixture of compound **3** (2.7 g, 0.01 mol) and indole-3-carboxaldehyde and/or naphthalene-2-carboxaldehyde (0.01 mol) in absolute ethanol (30 mL) was heated under refluxed for 6 hours; the reaction mixture was cooled, and the formed precipitate was filtered off, dried, and recrystallized from the proper solvent to give Schiff bases **13a**,**b**.

2-(2-((1*H*-Indol-3-yl)methylene)hydrazinyl)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (13a)

Product **13a** was separated as brownish yellow crystals (ethanol), yield 80%. mp 232°C to 233°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3423 (NH), 2230 (CN), 1692 (C═O), 1648 (C═N). ^{1}H NMR (DMSO): δ (ppm) 2.80 (s, 3H, N-CH_3), 3.30 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.09 (s, 1H, NH, exchangeable), 6.80 to 7.80 (m, 8H, H_{arom}), 8.50 (s, 1H, NH, exchangeable), 9.81(s, 1H, N═CH) ppm. ^{13}C-NMR (75 MHz, DMSO-d_6) δ/ppm: 27.9 (CH_3), 56.2, 56.7 (2 O-CH_3), 83.4, 94.6, 111.0, 111.5, 111.8, 112.2, 115.8, 119.4, 121.9, 122.5, 123.1, 126.3, 127.4, 138.1, 138.6, 148.5, 149.8, 151.2 159.8 (Ar-C), 170.5 (C═O). MS (EI, 70 eV): *m/z* (%) = 428 (35) (M^+) Anal. for $C_{23}H_{20}N_6O_4$ (428.16): calcd. C, 64.48; H, 4.71; N, 19.62; found: C, 64.09; H, 4.61; N, 19.25.

4-(3,4-Dimethoxyphenyl)-1-methyl-2-(2-(naphthalen-2-ylmethylene)hydrazinyl)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (13b)

Product **13b** was separated as brownish yellow crystals (ethanol), yield 75%. mp 101°C to 102°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3438 (NH), 2283 (CN), 1689 (C═O), 1644 (C═N). ^{1}H NMR (DMSO): δ (ppm) 2.74 (s, 3H, N-CH_3), 3.20 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.14 (s, 1H, NH, exchangeable), 6.50 to 7.90 (m, 10H, H_{arom}), 9.40 (s, 1H, N═CH) ppm. MS (EI, 70 eV): *m/z* (%) = 439 (25) (M^+) Anal. for $C_{25}H_{21}N_5O_3$ (439.16): calcd. C, 68.33; H, 4.82; N, 15.94; found: C, 68.08; H, 4.49; N, 16.16.

3.1.13 | General procedures for synthesis of 14a,b

A mixture of Schiff bases **13a**,**b** (0.001 mol) and thiosalicylic acid (1.54 g, 0.01 mol) in dry benzene

(30 mL) was heated under refluxed for 15 hours; the solvent evaporated under reduced pressure; the obtained residue was treated with petroleum ether. The solid precipitate was filtered off, washed with petroleum ether, dried, and recrystallized from the proper solvent to give compounds **14a**,**b**.

2-((2-(1*H*-Indol-3-yl)-4-oxo-2*H*-benzo[e][1,3]thiazin-3(4*H*)-yl)amino)-4-(3,4-dimethoxyphenyl)-1-methyl-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (14a)

Product **14a** was separated as yellow crystals (ethanol), yield 78%. mp 174°C to 175°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3429 (NH), 2210 (CN), 1698, 1686 (C═O), 1648 (C═N). ^1H NMR (DMSO): δ (ppm) 2.64 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.10 (s, 1H, CH), 5.40 (s, 1H, NH, exchangeable), 6.60 to 8.10 (m, 12H, H_{arom}), 8.30 (s, 1H, NH, exchangeable) ppm. MS (EI, 70 eV): *m/z* (%) = 565 (22) (M^{+1}) Anal. for $C_{30}H_{24}N_6O_4S$ (564.16): calcd. C, 63.82; H, 4.28; N, 14.88; found: C, 64.32; H, 4.11; N, 14.07.

4-(3,4-Dimethoxyphenyl)-1-methyl-2-((2-(naphthalen-2-yl)-4-oxo-2*H*-benzo[e][1,3]thiazin-3(4*H*)-yl)amino)-6-oxo-1,6-dihydropyrimidine-5-carbonitrile (14b)

Product **14b** was separated as yellow crystals (methanol), yield 69%. mp 197°C to 198°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3430 (NH), 2220 (CN), 1690, 1675 (C═O), 1648 (C═N). ^1H NMR (DMSO): δ (ppm) 2.65 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.20 (s, 1H, CH), 5.50 (s, 1H, NH, exchangeable), 6.50 to 7.60 (m, 14H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 575 (20) (M^+) Anal. for $C_{32}H_{25}N_5O_4S$ (575.16): calcd. C, 66.77; H, 4.38; N, 12.17; found: C, 66.06; H, 4.65; N, 12.31.

3.1.14 | General procedures for synthesis of compounds 15a,b

Compounds **13a** and/or **13b** (0.001 mol) was dissolved in 15 mL of acetic acid with stirring at 45°C; lead tetraacetate (1.7 g) was added portion wise at interval of 3 hours. The excess solvent evaporated; the reaction mixture was poured onto ice/cold water; the formed precipitate was filtered off, washed with water, dried, and recrystallized from the proper solvent to give compounds **15a**,**b**.

5-(3,4-Dimethoxyphenyl)-3-(1*H*-indol-3-yl)-8-methyl-7-oxo-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (15a)

Product **15a** was separated as yellow crystals (ethanol), yield 77%. mp 280°C to 281°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3441 (NH), 2267 (CN), 1688 (C═O), 1646 (C═N). ^1H NMR ($CDCl_3$): δ (ppm) 2.85 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.60 (s, 1H, CH), 6.10 (s, 1H, NH, exchangeable), 6.30 to 7.90 (m, 8H, H_{arom}), 10.80 (s, 1H, NH, exchangeable) ppm. MS (EI, 70 eV): *m/z* (%) = 429 (30) (M + 1) Anal. for $C_{23}H_{20}N_6O_3$ (428.16): calcd. C, 64.48; H, 4.71; N, 19.62; found: C, 64.67; H, 4.48; N, 19.94.

5-(3,4-Dimethoxyphenyl)-8-methyl-3-(naphthalen-2-yl)-7-oxo-1,7,8,8a-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (15b)

Product **15b** was separated as yellow crystals (ethanol), yield 64%. mp 220-221°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3424 (NH), 2272 (CN), 1683 (C═O), 1637 (C═N). ^1H NMR (DMSO): δ (ppm) 2.90 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.30 (s, 1H, CH), 6.70 to 8.20 (m, 10H, H_{arom}), 10.80 (s, 1H, NH, exchangeable) ppm. MS (EI, 70 eV): *m/z* (%) = 439 (25) (M^+) Anal. for $C_{25}H_{21}N_5O_3$ (439.16): calcd. C, 68.33; H, 4.82; N, 15.94; found: C, 67.98; H, 4.93; N, 16.43.

3.1.15 | General procedures for synthesis of compounds 16-19

A solution of compound **3** (1.35 g, 0.05 mol) and formic acid, ethylchloroacetate, acetic anhydride, and/or diethyl malonate (25 mL) was heated under refluxed for 4 hours; the reaction mixture was cooled and poured onto ice/cold water; the formed precipitate was filtered off, dried, and recrystallized from the proper solvent to give compounds **16**-**19**.

5-(3,4-Dimethoxyphenyl)-8-methyl-7-oxo-7,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (16)

Product **16** was separated as yellow crystals (methanol), yield 75%. mp 188°C to 189°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 2250 (CN), 1673 (C═O), 1645 (C═N). ^1H NMR (DMSO): δ (ppm) 2.70 (s, 3H, N-CH_3), 3.40 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.50 to 7.34 (m, 4H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 311 (30) (M^+) Anal. for $C_{15}H_{13}N_5O_3$ (311.10): calcd. C, 57.87; H, 4.21; N, 22.50; found: C, 57.71; H, 4.43; N, 22.00.

5-(3,4-Dimethoxyphenyl)-8-methyl-3,7-dioxo-2,3,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (17)

Product **17** was separated as yellow crystals (methanol), yield 95%. mp 232°C to 233°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3470 (NH), 2276 (CN), 1687, 1675 (C═O), 1635 (C═N). ^1H NMR (DMSO): δ (ppm) 2.70 (s, 3H, N-CH_3), 3.50 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 6.70 to 6.90 (m, 3H, H_{arom}),

10.66 (s, 1H, NH, exchangeable), ppm. MS (EI, 70 eV): *m/z* (%) = 327 (98) (M^+) Anal. for $C_{15}H_{13}N_5O_4$ (327.10): calcd. C, 55.05; H, 4.00; N, 21.40; found: C, 55.49; H, 4.23; N, 21.16.

5-(3,4-Dimethoxyphenyl)-3,8-dimethyl-7-oxo-7,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (18)

Product **18** was separated as brownish yellow crystals (ethanol), yield 73%. mp 165°C to 166°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 2304 (CN), 1694 (C═O), 1653 (C═N). ^1H NMR (DMSO): δ (ppm) 2.50 (s, 3H, CH_3), 2.90 (s, 3H, N-CH_3), 3.40 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 6.50–6.80 (m, 3H, H_{arom}) ppm. MS (EI, 70 eV): *m/z* (%) = 325 (25) (M^+) Anal. for $C_{16}H_{15}N_5O_3$ (325.12): calcd. C, 59.07; H, 4.65; N, 21.53; found: C, 58.60; H, 4.77; N, 21.03.

6-(3,4-Dimethoxyphenyl)-9-methyl-3,4,8-trioxo-3,4,8,9-tetrahydro-2*H*-pyrimido[2,1-c][1,2,4]triazine-7-carbonitrile (19)

Product **19** was separated as brownish yellow crystals (ethanol), yield 66%. mp 219°C to 220°C. IR (KBr), $\tilde{\nu}/cm^{-1}$: 3375 (NH), 2305 (CN), 1704, 1687 (C═O), 1644 (C═N). ^1H NMR (DMSO): δ (ppm) 2.85 (s, 3H, N-CH_3), 3.60 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.60 to 6.90 (m, 3H, H_{arom}), 10.30 (s, 1H, NH, exchangeable), ppm. ^{13}C-NMR (75 MHz, DMSO-d_6) δ/ppm: 26.9 (CH_3), 57.1, 57.4 (2 O-CH_3), 81.7, 111.1, 111.6, 115.6, 122.5, 127.5, 145.6, 149.4, 149.9, 159.6 (Ar-C), 160.1, 162.8, 164.2 (3C═O). MS (EI, 70 eV): *m/z* (%) = 355 (18) (M^+) Anal. for $C_{16}H_{13}N_5O_5$ (355.09): calcd. C, 54.09; H, 3.69; N, 19.71; found: C, 54.42; H, 3.86; N, 19.55.

4 | CYTOTOXIC ACTIVITY

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to purple *formazan*.[17] All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for MCF-7, HCT116, and BJ1. The media were supplemented with 1% antibiotic-antimycotic mixture (a potassium penicillin of 10 000 U/mL, a streptomycin sulfate of 10 000 μg/mL, and an amphotericin B of 25 μg/mL), 1% L-glutamine and 10% fetal bovine serum and kept at 37°C under 5% CO_2.

Cells were batch cultured for 10 days then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well micro titer plastic plates at 37°C for 24 hours under 5% CO2 using a water-jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media were aspirated; fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of samples to give a final concentration of (100-50-25-12.5-6.25-3.125 -0.78 and 1.56 ug/mL). After 48 hours of incubation, medium was aspirated; 40 uL of MTT salts (2.5 μg/mL) were added to each well and incubated for further 4 hours at 37°C under 5% CO2. To stop the reaction and dissolving the formed crystals, 200 μL of 10% sodium dodecyl sulfate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control, which composed of 100 μg/mL, was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions.[18,19]

The absorbance was then measured using a micro plate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula: ([reading of extract/reading of negative control] − 1) × 100. A probity analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

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