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SYNTHESIS OF NOVEL PYRIMIDINE AND FUSED PYRIMIDINE DERIVATIVES AND THEIR *IN VITRO* ANTIMICROBIAL AND CYTOTOXIC EVALUATION

Hanan A. Abdel Fattah[*1], Nermine A. Osman[1], Amany M. AL-Mahmoudy[1], Nermine S. EL-Sayed[2]

[1]Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig-44519 Egypt.

[2]Mansoura University Hospital, Mansoura, Egypt.

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***Correspondence for Author**
Hanan A. Abdel Fattah
Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Zagazig University, Zagazig-44519 Egypt.

ABSTRACT

In the present work some novel pyrimidine and fused pyrimidine derivatives were prepared starting from 1-allyl-2-hydrazinyl-4-oxo-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (**4**) the key intermediate. The structure of new compounds was characterized by IR, ^{1}H, $^{13}CNMR$, mass spectral data and elemental analysis. The *in vitro* antimicrobial activity evaluation for most of new compounds was screened. Most of the tested compounds showed significant antimicrobial activity. Some of the newly synthesized compounds were evaluated for their anticancer activity against breast carcinoma (MCF7) and colon carcinoma (HCT116) cell lines. The anticancer activity showed that compound **4** was the most active one against the two cell lines IC_{50} (3.87, 4.48µg/ml)

KEY WORDS: pyrimidine, fused pyrimidine derivatives, antimicrobial, cytotoxic activities.

INTRODUCTION

Cancer is considered as one of the most health problem.[1] The mortality of patients suffering various type of cancer has become an important issue worldwide. It is realized that neither surgery nor radiation nor the two in combination can adequately control metastatic cancer.[2] Therefore; efforts to care cancer have been focusing on conventional chemotherapy. Unfortunately, this type of treatment usually does not discriminate between dividing normal cells and tumor cells, leading to sever side effects.[3&4] Therefore ,there is an urgent need to

give much attention to update new potent, effective and selective chemotherapeutic agents whether of synthetic or natural origin. Pyrimidine nucleus has been emerged as a promising and attractive scaffold in barbituric acid derivatives and in many chemotherapeutic agents. Also, several pyrimidines have been isolated from the nucleic acid hydrolysis. For example, cytosine is found in both RNA and DNA while uracil is present only in RNA and thymine only in DNA.[5] In addition, 5-Fluorouracile is a pyrimidine analogue which is used in the treatment of various types of cancer such as colon, rectal, breast and ovarian cancer through irreversible inhibition of thymidylate synthase mechanism.[6] Other uracil derivatives possess a variety of biological properties. These properties include antiviral[7-15] and antimicrobial activities.[16-21] Moreover ,several fused pyrimidines were reported to exhibit important biological activities.[22-24] Encouraged by these findings, we thought of preparing several pyrimidine and fused pyrimidine derivatives in an attempt to investigate their possible anticancer as well as antimicrobial activities.

RESULTS AND DISCUSSION

Chemistry

The synthesis strategies of the novel pyrimidine and fused pyrimidine derivatives were depicted in schemes **1**, **2** and **3.**

The starting material 1-allyl-2-mercapto-4-oxo-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile **(1)** was prepared by refluxing an equimolar mixture of ethyl cyanoacetate, allyl thiourea and 3, 4, 5-trimethoxybenzaldehyde in absolute ethanol containing anhydrous potassium carbonate for 27 hrs according to the reported procedure.[25] Alkylation of **1** with either alkyl halides or 4-substituted phenylchloroacetamide derivatives to give the corresponding thioether derivatives **2a-c** and thioacetamide derivatives **2d-g** respectively adopting Ram et al[26] reported procedure.

The 2-substituted amino derivatives **3a-c** were obtained by aminolysis of compound **2a** with different primary and secondary amines according to the procedure used for the synthesis of aminoimidazoline reported by Kadry et al.[27] Similarly, heating **2a** with hydrazine hydrate gave the corresponding 2-hydrazinoderivative **4** the key intermediate (scheme 1). Condensation of the latter with different aromatic aldehydes afforded the corresponding 1-allyl-2-arylidenehydrazino-4-oxo-6- (3,4,5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile **(5a-f)** respectively. Compound **6, 7** and **8** were be synthesized *via* the reaction of the hydrazino compound **4** with maleic anhydride, 2, 5-hexanedione and istatin in glacial

acetic acid respectively at different reaction conditions. Enamine **(9)** was prepared by refluxing the hydrazino **4** with 5, 5-dimethyl-1, 3-cyclohexanedione (dimidone) in toluene. Cyclocondensation of compound **4** with either nitrous acid at room temperature or formic acid under reflux afforded the novel 8-allyl-5-oxo-7-(3,4,5-trimethoxyphenyl)-5, 8 dihydrotetrazolo[1, 5-a]pyrimidine-6-carbonitrile **(10)** and 8-allyl-5-oxo-7-(3, 4, 5-trimethoxyphenyl)-5, 8-dihydro-[1, 2, 4]triazolo[4, 3-a]pyrimidine-6-carbonitrile **(11)** respectively.

Moreover cyclocondensation of compound **4** with acid chloride derivatives unsuccessfully lead to the formation of N-benzoylated products **12a-c.** Upon refluxing the latter compounds with phosphorous oxychloride overnight afforded the target cyclized products 8-allyl-3-(4-substitutedphenyl)-5-oxo-7-(3,4,5-trimethoxyphenyl)-5, 8-dihydro[1,2,4] triazolo[4,3-a]pyrimidine-6-carbonitrile**(13a-c).**Furthermore, compound **14** was prepared by refluxing the hydrazino compound **4** with carbon disulfide in absolute ethanol containing potassium hydroxide.

Biological activity

Preliminary antimicrobial screening

Some of the newly synthesized compounds were screened for their antimicrobial activity using cup plate diffusion method.[28-32] The antimicrobial activity was tested against each of the mentioned strains using Amphotericin B as antifungal, Ampicillin as anti gram postive and Gentamycin as anti gram negative reference (table 1).

The results of *in vitro* antimicrobial activity of the tested compounds summarized in table 1 revealed that Compounds **2d, 2f, 4, 5e** and **13a** were highly active against gram positive bacteria (*Streptococcus pneumonia*) but showed moderate inhibitory activity against (*Bacillis subtilis*) Compounds **2d, 2f, 4, 5a, 5e, 11** and **13a** were highly active against gram negative bacteria (*Escherchia coli*) as well as fungi (*Aspergillus fumigatus* and *Candida albicans*). Compounds **2a, 3c, 5b** and **5d** were devoid of activity against both bacterial and fungal strains.

The minimal inhibitory concentrations (MIC) values were recorded as the lowest concentrations of the substance that had no visible turbidity. Control experiments with DMF and uninoculated media were run parallel to the test compounds under the same conditions.

Compound **5a** had the lowest minimum inhibitory concentration (µg/ml) as shown in (table 2).

Table (1): The *in vitro* antimicrobial activity evaluation of some newly synthesized compounds expressed in diameter (mm) of inhibition zones against the corresponding standard strains of different microorganisms.

Comp. No	Zone inhibition(mm)				
	Bacteria				
	Gram positive bacteria		Gram negative bacteria	Fungi	
	Streptococcus Pneumonie	*Bacillis subtilis*	*Escherichia coli*	*Aspergillus fumigates*	*Candida albicans*
2a	NA	NA	NA	NA	NA
2d	21.3±0.44	23.3±0.67	20.8±0.46	20.7±0.25	21.9±0.44
2f	19.9±0.15	20.8±0.42	19.3±0.53	19.6±0.33	20.8±0.24
3a	17.6±0.44	19.3±0.58	18.2±0.33	18.3±0.34	20.2±0.44
3c	NA	NA	NA	NA	NA
4	21.3±0.20	23.8±0.29	20.8±0.58	20.9±0.63	21.7±0.37
5a	22.7±0.17	25.4±0.32	21.6±0.10	21.3±0.12	22.3±0.58
5b	NA	NA	NA	NA	NA
5d	NA	NA	NA	NA	NA
5e	20.2±0.63	21.4±0.44	19.8±0.37	18.6±0.44	20.3±0.58
7	15.2±0.63	16.2±0.32	14.3±0.46	13.3±0.36	15.2±0.58
11	16.3±0.44	17.2±0.58	19.3±0.58	17.3±0.25	18.6±0.25
12a	16.8±0.25	18.1±0.63	16.2±0.44	15.3±0.25	15.9±0.44
12c	14.3±0.43	16.2±0.53	14.3±0.25	13.4±0.58	15.1±0.58
13a	20.1±0.12	21.3±0.17	19.3±0.33	19.6±0.25	21.9±0.20
13c	17.8±0.17	19.8±0.22	18.2±0.10	18.7±0.11	15.8±0.44
14	17.9±0.17	19.2±0.22	16.9±0.25	14.6±0.39	17.2±0.58
St. Ampicllin	23.8±0.2	32.4±0.3	NT	NT	NT
Gentamicin	NT	NT	25.6±0.3	NT	NT
Amphotericin B	NT	NT	NT	23.7±0.1	25.4±0.1

RCMB:Regional Center for Mycology and Biotechnology Antimicrobial unit test organisms

NA: No activity, data are expressed in the form of mean ± SD

NT: Not tested

Table (2): Antimicrobial activity as MIC (µg/ml) of tested samples against tested microorganisms

Comp. No.	Zone inhibition(mm)				
	Bacteria				
	Gram positive bacteria		Gram negative bacteria	Fungi	
	Streptococcus Pneumoniae	*Bacillis subtilis*	*Escherichia coli*	*Aspergillus fumigates*	*Candida albicans*
5a	0.24	0.06	0.49	0.98	0.49
2d	0.98	0.24	0.98	0.98	0.49
4	0.98	0.12	0.98	0.98	0.49
13a	1.95	0.98	1.95	3.9	0.49
St. Ampicillin	0.12	0.015	NT	NT	NT
Gentamicin	NT	NT	0.03	NT	NT
Amphotericin B	NT	NT	NT	0.12	0.06

***In vitro* antitumor activity**[33-35]

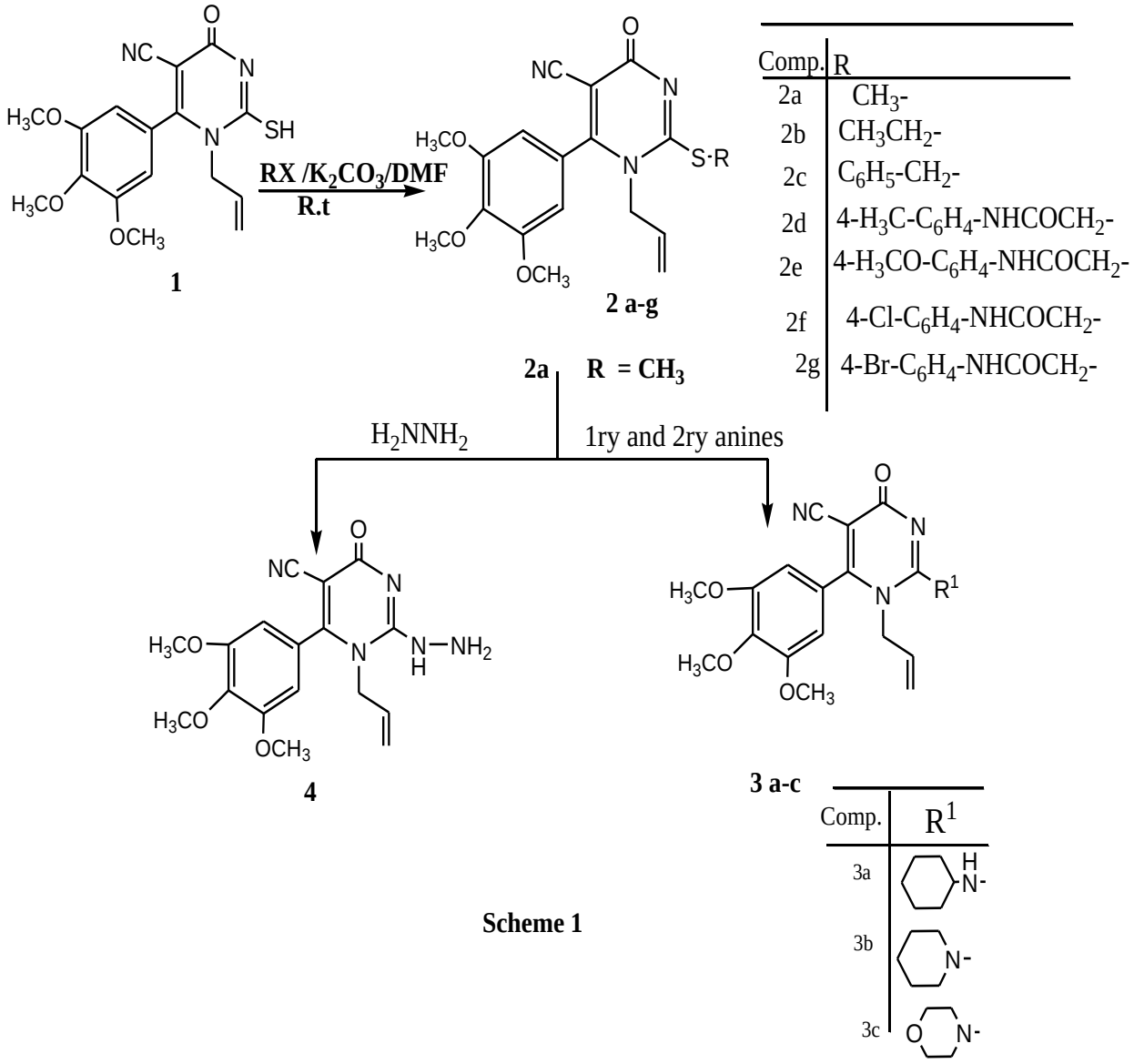
From the newly synthesized compounds, only eight compounds **1, 4, 5a, 8, 10, 11, 13c** and **14** were be selected to be evaluated for their antitumor activity against human breast carcinoma (MCF7) cell line and colon carcinoma (HCT116) cell line . Doxorubicin was used as reference drug. The inhibitory activities were presented as micro molar concentrations of the compound that cause 50% inhibition per unit of enzyme (IC_{50}) under the assay condition. Results of cancer cellular assay are shown in (table3). By investigating the variation in selectivity of tested compounds over the cell lines, it was noticed that most of the compounds under investigation showed significant activity against the breast cancer cell line (MCF7). On the other hand, only compounds **1, 4, 5a, 8, 11** and **14** showed significant activity against the colon cancer cell line (HCT116).

More interestingly compound **4**, showed the highest activity against both cell lines (3.87µg/ml and 4.48 µg/ml respectively).On the contrary, compound **5a** exhibited moderate activity against colon carcinoma cell line (IC_{50}=6.71µg/ml), however, compounds **10** and **13c** have the least activity against the two cell lines. On the other hand hydrazino and hydrazone derivative **4** and **5a** have the high antitumor activity against two cell lines indicating the importance of hydrazine and trimethoxy hydrazone moieties.

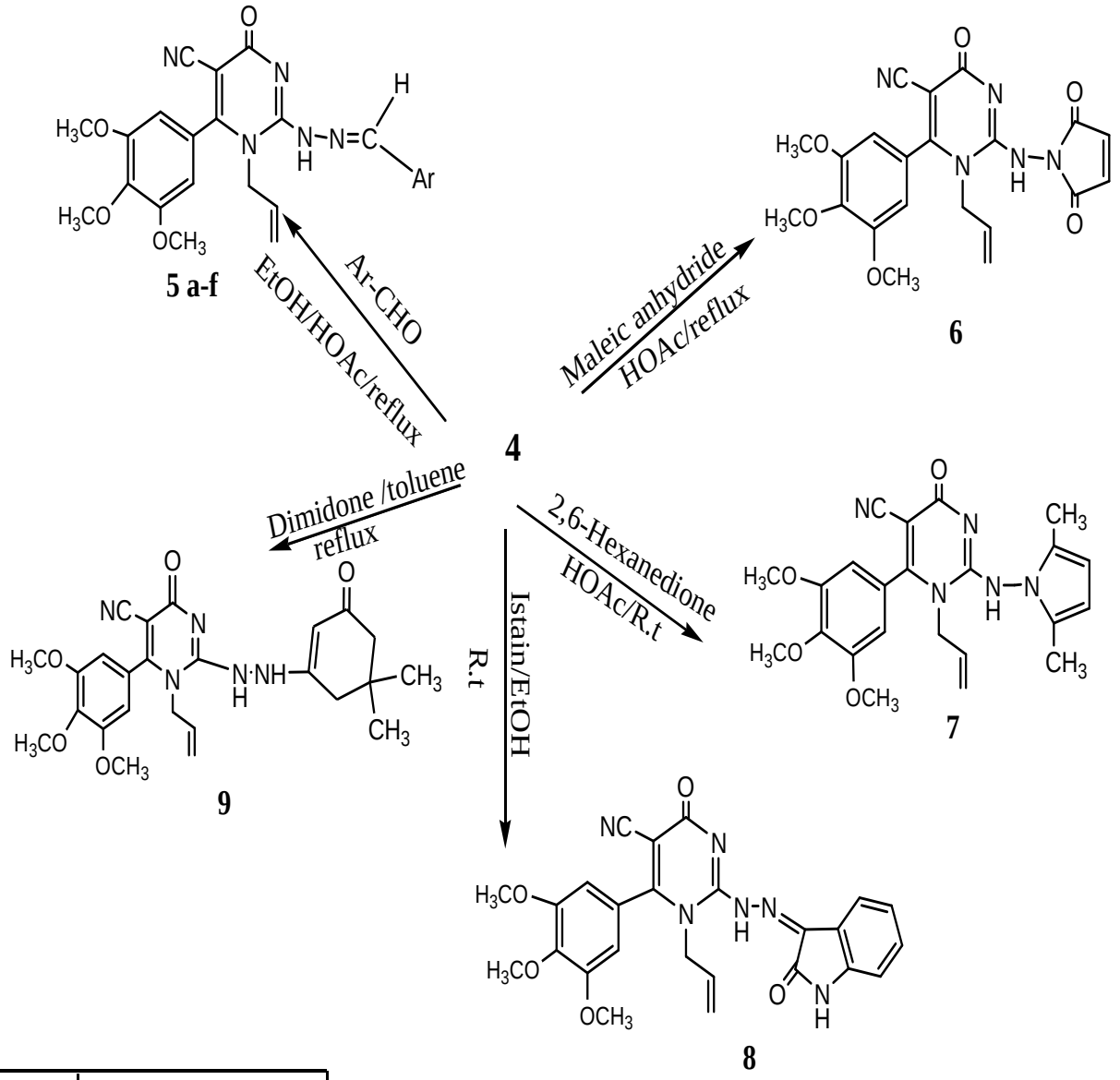
Table (3): The IC_{50} (ug/ml) of some of the new compounds against Breast cancer (MCF7) and colon cancer (HCT-116) cell lines

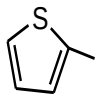
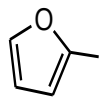
Compound NO.	IC_{50}	
	Breast carcinoma (MCF7) cell line	Colon carcinoma (HCT116) cell line
1	33.3	17.6
4	3.87	4.48
5a	5.36	6.71
8	13.4	11.5
10	32.8	22.6
11	15.4	17.1
13c	21.1	45.8
14	23.1	11.9
Doxorubicin	0.426	0.469

IC_{50} is adose required to inhibit the cell growth by 50%

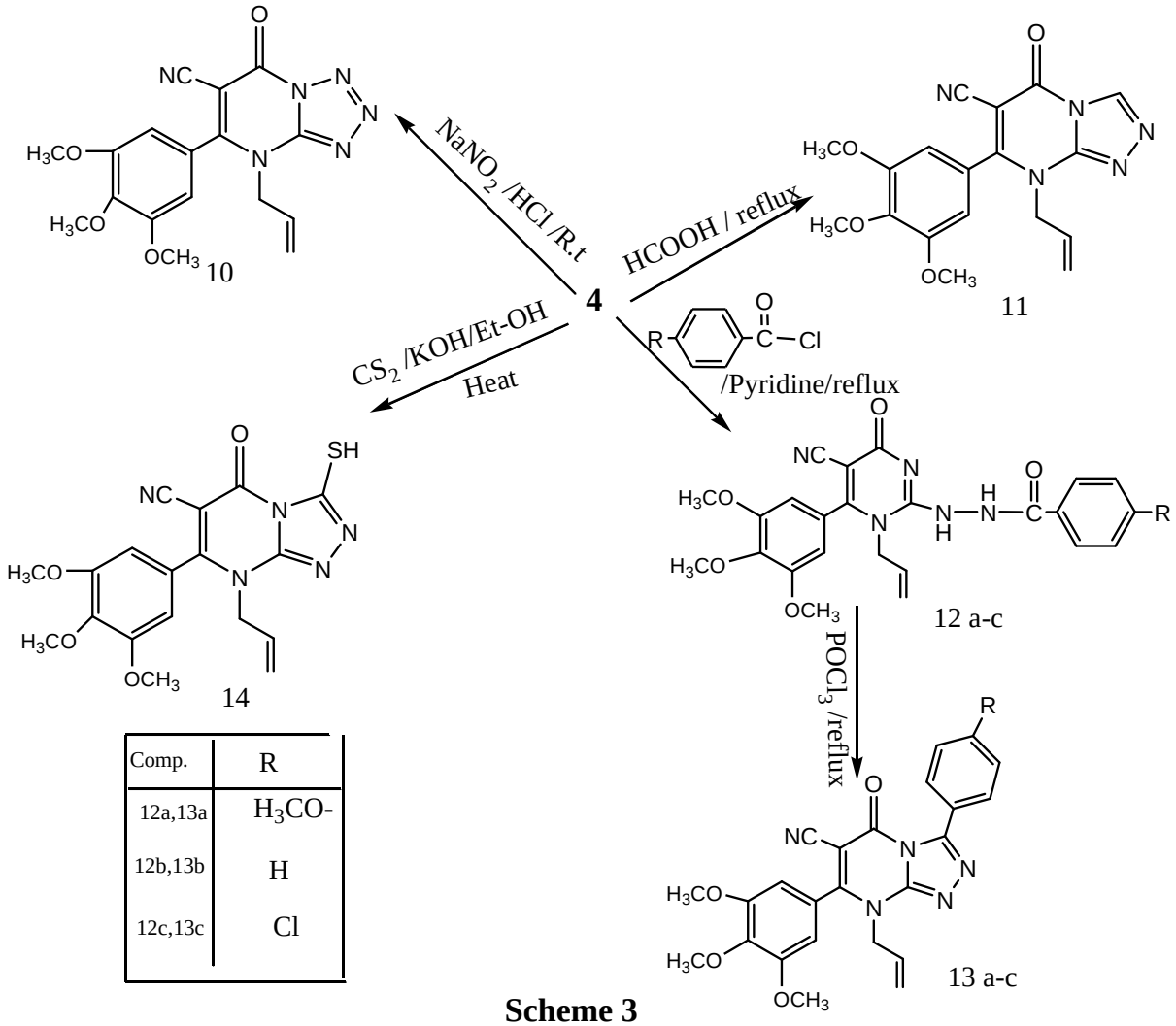


Scheme 1



Comp.	Ar
5a	3,4,5-$(H_3CO)_3C_6H_2$-
5b	4-NO_2-C_6H_4-
5c	4-Cl-C_6H_4-
5d	2,5-$(H_3CO)_2C_6H_3$-
5e	
5f	

Scheme 2



Comp.	R
12a,13a	H_3CO-
12b,13b	H
12c,13c	Cl

Scheme 3

MATERIAL AND METHODS

Melting points were determined with Gallenkamp melting point apparatus and are uncorrected. IR spectra (KBr, cm^{-1}) were recorded on Bruker or Testscan shimadzu FT8000 spectrometer. ^{1}HNMR (300 MHz) spectra were recorded on a Bruker AC 300 MHz spectrometer in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard (chemical shift in ppm).

Mass spectra were determined using a GC/MS Mat 112 S at 70ev spectrometer. Elemental analysis was carried out at the Regional center of Mycology and Biotechnology, Al-Azhar University, Nasr City, Egypt.

TLC was performed on silica gel (Merck 60 F254) and spots were visualized by iodine vapours or irradiation with U V light (254 nm).

Experimental

Chemistry

1-Allyl-2-mercapto-4-oxo-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (1)

Procedure

A mixture of allyl thiourea (1.74g, 15mmol) ethyl cyanoacetate (1.7g, 15mmol), 3, 4, 5 trimethoxy benzaldehyde (2.94g, 15mmol) and anhydrous potassium carbonate (2.5g, 15mmol)in absolute ethanol (100ml) was heated under reflux for 27 hrs. After cooling, the light yellow precipitate obtained was filtered, washed with water and crystallized from DMF/H_2O mixture as yellow crystals. M.p.178-180˚C. yield(60%), Microanalysis for $C_{17}H_{17}N_3O_4S$ (359.40), Calcd : % C, 56.18; H, 4.77; N, 11.69, Found:% C, 56.97; H, 4.79; N, 11.92

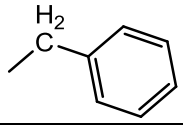
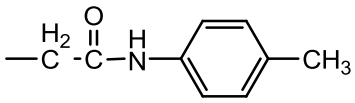
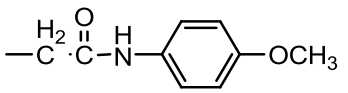
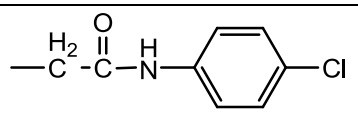
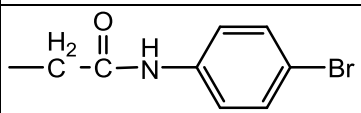
IR (KBr, cm^{-1}): 3196(NH), 3089 (CH, aromatic), 2940 (CH, aliphatic), 2228 ($C\equiv N$), 1692 (C=O), 1588 (C=N), 1533 (C=C), 1323 (C=S). ^{1}HNMR (300MHz, DMSO-d_6) δppm: 3.82 (s, 3H, OCH_3), 3.90 (s, 6H, $2OCH_3$), 4.99-5.01 (m, 2H, CH_2), 5.26-5.32 (t, 2H, $=CH_2$), 5.88-5.94 (m, 1H, =CH), 7.14 (s, 2H, Ar-H), 13.50 (s, 1H, NH, D_2O exchangeable). MS: m/z (%): 361 (13.06) M^++2, 360 (11.85) M^++1, 359 (49.81) M^+, 344 (100), 181 (28.17), 55 (72.57)

1-Allyl-2-(alkylthio)-4-oxo-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile (2a-c) 2-[1-allyl-4-oxo-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile-2-yl]-N-(substitutedphenyl)thioacetamide(2d-g)

General Procedure

To a solution of **1** (0.359g, 1mmol) in DMF (10ml) containing anhydrous potassium carbonate (0.276g, 2mmol) alkyl halide or arylchloroacetamide derivative (1mmol) was added, stirred overnight the reaction mixture was diluted with water and the formed product was filtered and crystallized from ethanol to give the corresponding alkylthio **2 a-c** or thioacetamide derivatives **2 d - g** derivatives respectively see(table4).

Table(4): 1-Allyl-2-(alkylthio)-4-oxo-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile 2(a-c) 2-[1-allyl-4-oxo-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile-2-yl]-N-(substituted phenyl) thioacetamide (2d-g)

Comp No.	R	Yield %	M.P	M.F. (M.W.)	Analysis Calcd/found		
2a	CH_3	93	167-169	$C_{18}H_{19}N_3O_4S$ (373.43)	C H N	57.80 5.10 11.25	57.92 5.08 11.41
2b	CH_3CH_2	71	137-139	$C_{19}H_{21}N_3O_4S$ (387.45)	C H N	58.90 5.46 10.85	59.17 5.54 11.02
2c		84	130-132	$C_{24}H_{23}N_3O_4S$ (449.52)	C H N	64.13 5.16 9.35	64.28 5.22 9.49
2d		40	90-100	$C_{26}H_{26}N_4O_5S$ (506.57)	C H N	61.65 5.17 11.06	61.82 5.22 11.19
2e		50	110-120	$C_{26}H_{26}N_4O_6S$ (522.57)	C H N	59.76 5.01 10.72	59.98 5.13 10.97
2f		54	124-126	$C_{25}H_{23}ClN_4O_5S$ (526.99)	C H N	56.98 4.40 10.63	57.09 4.49 10.81
2g		50	116-118	$C_{25}H_{23}BrN_4O_5S$ (571.44)	C H N	52.55 4.06 9.80	52.68 4.13 9.94

Compound **2a:** IR:(KBr, cm^{-1}): 3086 (CH, aromatic), 2990, 2963 (CH, aliphatic), 2210 ($C\equiv N$), 1661(C=O), 1588 (C=N), 1460 (C=C); ^{1}H-NMR (300MHz, DMSO-d_6) δ ppm: 2.71 (s, 3H, S-CH_3), 3.77 (s, 3H, OCH_3), 3.85 (s, 6H, 2OCH_3), 4.68 (s, 2H, CH_2), 5.19-5.29 (t, 2H, =CH_2), 5.88-5.91 (m, 1H, =CH), 7.40 (s, 2H, ArH); MS: m/z (%) = 375 (11.68) M^++2, 374 (29.91) M^++1, 373 (100) M^+.

Compound **2b:** IR:(KBr, cm^{-1}): 3021 (CH, aromatic), 2969, 2937 (CH, aliphatic), 2219 ($C\equiv N$) 1675 (C=O), 1588 (C=N), 1461 (C=C); ^{1}HNMR(300MHz, DMSO-d_6) δ ppm: 1.38-1.41 (t, 3H, CH_3), 3.29-3.34 (q, 2H, CH_2), 3.78 (s, 3H, OCH_3), 3.84 (s, 6H, 2OCH_3), 4.65 (s, 2H , N-CH_2), 5.19-5.28 (m, 2H, =CH_2), 5.84-5.91(m, 1H, =CH), 7.40 (s, 2H, ArH).

Compound **2c:** IR:(KBr, cm^{-1}): 3063 (CH aromatic), 2999, 2941(CH, aliphatic), 2213 ($C\equiv N$)1672 (C=O), 1586 (C=N), 1495 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δ ppm: 3.76 (s, 3H, OCH_3), 3.77 (s, 6H, 2OCH_3), 4.67 (s, 2H, S-CH_2), 4.69 (s, 2H, N-CH_2), 5.20-5.25 (m, 2H, =CH_2), 5.26-5.28 (m, 1H, =CH), 7.31-7.46 (m, 7H, ArH).

Compound **2d:** IR:(KBr, cm^{-1}): 3310(NH), 3089 (CH, aromatic), 2938 (CH, aliphatic), 2220 (C≡N), 1665 (C=O), 1602 (C=N), 1454 (C=C); ^{1}H NMR(300MHz, DMSO-d_6) δ ppm:2.30 (s, 3H, CH_3), 3.73(s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.40 (s, 2H, S-CH_2), 4.80 (s, 2H, N-CH_2), 5.19-5.25 (m, 2H, =CH_2), 5.80-5.85 (m, 1H, =CH), 7.09-7.47 (m, 6H, ArH), 10.10 (s, 1H, NH, D_2O exchangeable).

Compound **2e:** IR:(KBr, cm^{-1}):3310(NH), 3089(CH, aromatic), 2939, 2838 (CH, aliphatic), 2220 (C≡N), 1678 (C=O), 1590 (C=N), 1503 (C=C); ^{1}H-NMR(300MHz, DMSO-d_6) δppm:3.66 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.31 (s, 2H, CH_2), 4.73-4.81 (m, 2H, CH_2), 5.16-5.25 (t, 2H, =CH_2), 5.88-5.94 (m, 1H, =CH), 7.27-7.50 (s, 6H, ArH), 9.97 (s, 1H, NH, D_2O exchangeable).

Compound **2f:** IR:(KBr, cm^{-1}):3314 (NH), 3089 (CH, aromatic), 2938 (CH, aliphatic), 2221(C≡N) (1674(C=O), 1523(C=N), 1495(C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.75(s, 3H, OCH_3), 3.86(s, 6H, $2OCH_3$), 4.20 (s, 2H, CH2), 4.59-4.65(d, 2H, CH_2), 5.08-5.20(m, 2H, =CH_2), 5.88-5.94 (m, 1H, =CH), 7.23-7.71 (m, 6H, ArH), 10.36 (s, 1H, NH, D_2O exchangeable).

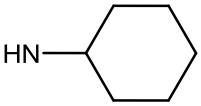
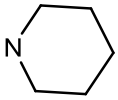
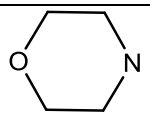
Compound **2g:** IR:(KBr, cm^{-1}): 3305 (NH), 3085 (CH, aromatic), 2938, 2837 (CH, aliphatic), 2220 (C≡N), 1679 (C=O), 1611(C=N), 1497 (C=C); ^{1}HNMR(300MHz, DMSO-d_6) δppm:3.76 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.41(s, 2H, S-CH_2) , 5.01-5.12 (d, 2H, CH_2), 5.17-5.20 (m, 2H, =CH_2), 5.70-5.80 (m, 1H, =CH), 7.10-7.55(m, 6H, ArH), 10.20 (s, 1H, NH, D_2O exchangeable).

1-Allyl-4-oxo-2-(substituted amino)-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile(3a-c)

General Procedure

A mixture of compound **2a** (0.5g, 1mmol) and the appropriate amine (3ml) was heated under reflux for 1h.in oil bath. After cooling and dilution with water; the precipitate was collected and crystallized from ethanol to give yellow crystals of compounds **3 a-c** as shown in (table 5).

Table (5): 1-Allyl-4-oxo-2-(substituted amino)-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (3a-c)

Comp. No.	R	Yield%	M.P˚C	M.F. (M.W.)	Analysis Calcd/Found		
3a		40	113-115	$C_{23}H_{28}N_4O_4$ 424.49	C H N	65.08 6.65 13.20	65.34 6.74 13.47
3b		50	125-127	$C_{22}H_{26}N_4O_4$ 410.47	C H N	64.37 6.38 13.65	64.62 6.49 13.97
3c		95	144-146	$C_{21}H_{24}N_4O_5$ 412.44	C H N	61.15 5.87 13.58	61.37 5.96 13.82

Compound **3a:** IR:(KBr, cm^{-1}):3353(NH), 3088 (CH, aromatic), 2934, 2854 (CH, aliphatic 2211($C\equiv N$), 1669 (C=O), 1512 (C=N), 1460 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:1.20-2.00 (m, 11H, aliphatic H), 3.75 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 4.70 (s, 2H, CH_2), 5.10-5.30 (m, 2H, $=CH_2$), 5.80-5.85 (m, 1H, =CH), 7.47 (s, 2H, ArH).

Compound **3b:** IR:(KBr, cm^{-1}):3081, 3022 (CH, aromatic), 2991, 2933 (CH, aliphatic), 2217 ($C\equiv N$), 1674 (C=O), 1589 (C=N), 1466 (C=C); ^{1}HNMR(300MHz, DMSO-d_6) δppm:1.64 (s, 6H, CH_2), 3.55 (s, 4H, $2CH_2$), 3.77 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 4.50 (s, 2H, CH_2), 5.17-5.23 (m, 2H, $=CH_2$), 5.98-6.05 (m, 1H, =CH), 7.31 (s, 2H, ArH).

Compound **3c:** IR:(KBr, cm^{-1}):3004 (CH, aromatic), 2981, 2958 (CH, aliphatic), 2214 ($C\equiv N$), 1659 (C=O), 1587 (C=N), 1455 (C=C); ^{1}HNMR(300MHz, DMSO-d_6) δppm:3.55 (s, 4H, $2CH_2$), 3.74 (s, 4H, $2CH_2$), 3.75 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 5.40 (s, 2H, CH_2), 5.10-5.12 (d, 2H, $=CH_2$), 5.90-6.00 (m, 1H, =CH), 7.31 (s, 2H, ArH).

1-Allyl-2-hydrazinyl-4-oxo-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile (4)

Procedure

A mixture of **2a** (0.35g, 1mmol) and hydrazine hydrate 99% (5ml) was heated under reflux for 1h.the formed precipitate was filtered off, washed with hot ethanol and crystallized from ethanol as buff crystals, m.p.203-205˚C, Yield(97%) Microanalysis for $C_{17}H_{19}N_5O_4$(357.36); Calcd: % C, 57.14; H, 5.36; N, 19.60; Found:%C, 57.25;H, 5.45;N, 19.40.

IR:(KBr, cm^{-1}) 3313 (NH), 3296, 3257 (NH_2), 3082 (CH, aromatic), 2964, 2938 (CH, aliphatic) , 2205 ($C\equiv N$) 1669 (C=O), 1590 (C=N), 1463 (C=C); ^{1}HNMR(300MHz, DMSO-

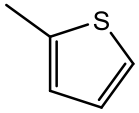
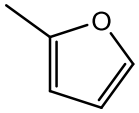
d_6) δppm:3.21 (s, 2H, NH_2, D_2O exchangeable), 3.79(s, 3H, OCH_3), 3.89 (s, 6H, $2OCH_3$), 4.60 (s, 2H, CH_2), 5.19- 5.29 (m, 2H, $=CH_2$), 5.80-6.(m, 1H, =CH), 7.31 (s, 2H, ArH), 9.50 (s, 1H, NH, D_2O exchangeable);^{13}CNMR(75MHz,DMSO-d6)δppm:166,162.02,152.30,147.25,139.45,132.40,119,116.32,107.50,106.80,106.41, 60.14,56.05,41.69. MS: m/z (%) =358 (10.17) M^++1, 357 (10.78) M^+, 317 (93.93), 302 (47.07), 80 (96.48), 64 (72.91).

1-Allyl-2-(arylidenehydrazino)-4-oxo-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidine-5-carbonitrile (5a-f)

General Procedure

A mixture of **4** (0.357g, 1mmol) and the appropriate aromatic aldehyde (1mmol) in glacial acetic acid (5ml) and ethanol (10 ml) was heated under reflux for 6hrs.After cooling and dilution with water, the solid product was collected and crystallized from ethanol to give yellow crystals of **5 a-f** see (table 6).

Table (6): 1-Allyl-2-(arylidenehydrazino)-4-oxo-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (5a-f)

Comp. No.	Ar	Yield%	M.P°C	M.F (M.W.)	Analysis% Calcd/Found		
5a	(3, 4, 5-Trimethoxy)C_6H_2	50	233-235	$C_{27}H_{29}N_5O_7$ 535.55	C H N	60.55 5.46 13.08	60.74 5.53 13.21
5b	4-$NO_2C_6H_4$	55	222-224	$C_{24}H_{22}N_6O_6$ 490.47	C H N	58.77 4.52 17.13	58.89 4.49 17.35
5c	4-ClC_6H_4	60	253-255	$C_{24}H_{22}ClN_5O_4$ 479.92	C H N	60.06 4.62 14.59	60.17 4.68 14.74
5d	(2, 5-Di-methoxy)C_6H_3	65	211-213	$C_{26}H_{27}N_5O_6$ 505.52	C H N	61.77 5.38 13.85	61.94 5.47 14.02
5e		55	218-220	$C_{22}H_{21}N_5O_4S$ 451.50	C H N	58.52 4.69 15.51	58.70 4.74 15.82
5f		60	245-247	$C_{22}H_{21}N_5O_5$ 435.43	C H N	60.68 4.86 16.08	60.87 4.91 16.24

Compound **5a:** IR:(KBr, cm^{-1}):3433 (NH), 3084, 3062 (CH, aromatic), 2952, 2838 (CH, aliphatic), 2228 (C≡N), 1691(C=O), 1580 (C=N), 1457 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm: 3.84 (s, 18H, 6-OCH_3), 4.49 (s, 2H, CH_2), 5.08-5.12 (m, 2H, $=CH_2$), 5.84-

5.85 (m, 1H, =CH), 7.21 (s, 2H, ArH), 7.30 (s, 2H, ArH), 8.65 (s, 1H, N=CH), 9.21(s, 1H, NH, D_2O exchangeable); MS: m/z (%)=535 (6.97) M^+.

Compound **5b:** IR:(KBr, cm^{-1}):3337 (NH), 3082 (CH, aromatic), 2932, 2838 (CH, aliphatic), 2217 (C≡N), 1679(C=O), 1560 (C=N), 1517, 1343 (NO_2), 1476 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.79 (s, 3H, OCH_3), 3.88 (s, 6H, $2OCH_3$), 4.64 (s, 2H, CH_2), 5.18-5.22 (m, 2H, $=CH_2$), 5.84-5.93 (m, 1H, =CH), 7.16-7.20 (d, 2H, ArH), 8.15-8.56 (m, 4H, ArH), 8.86 (s, 1H, N=CH), 11.50 (s, 1H, NH, D_2O exchangeable); MS:m/z (%) =491(7.67) M^++1, 490 (23.89) M^+, 327 (100), 80 (69.32).

Compound **5c:** IR:(KBr, cm^{-1}):3320 (NH), 3082 (CH, aromatic), 2930 (CH, aliphatic), 2222 (C≡N), 1682 (C=O), 1583 (C=N), 1478 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.78 (s, 3H, OCH_3) ,3.87 (s, 6H, $2OCH_3$), 4.65 (s, 2H, CH_2), 5.18-5.21(m, 2H, $=CH_2$), 5.92-6.00 (m, 1H, =CH), 7.52-7.95 (m, 6H, ArH), 8.44 (s, 1H, N=CH), 11.50 (s, 1H, NH, D_2O exchangeable)

Compound **5d:** IR:(KBr, cm^{-1})3322 (NH), 3084 (CH, aromatic), 2938, 2835 (CH, aliphatic), 2215 (C≡N), 1679 (C=O), 1588 (C=N), 1494 (C=C); ^{1}HNMR(300MHz, DMSO-d_6) δppm:3.75 (s, 3H, OCH_3), 3.86 (s, 12H, $4OCH_3$), 4.81(s, 2H, CH_2), 5.18-5.21 (m, 2H, $=CH_2$), 5.95-6.00 (m, 1H, =CH), 7.05-7.49 (m, 5H, Ar-H), 8.65 (s, 1H, N=CH), 11.50 (s, 1H, NH, D_2O exchangeable); MS:m/z(%) = 506 (25.4) M^++1, 505 (31.45) M^+, 327 (100), 79 (20.97), 64 (55.24)

Compound **5e:** IR:(KBr, cm^{-1}):3310 (NH), 3084 (CH, aromatic), 2938, 2836 (CH, aliphatic), 2219 (C≡N), 1676 (C=O), 1502 (C=N), 1477 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) for δppm:3.76 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 4.80 (s, 2H, CH_2), 5.19-5.20 (m, 2H, $=CH_2$) , 5.94-6.00 (m, 1H, =CH), 7.21-8.60 (m, 5H, ArH+3H of thiophene ring), 8.85(s, 1H, N=CH), 11.50 (s, 1H, NH, D_2O exchangeable); MS :m/z(%) =451(18.65)M^+, 327(100), 110(27.53), 96(32.17)

Compound **5f:** IR:(KBr, cm^{-1}): 3322 (NH), 3088 (CH, aromatic), 2937, 2836 (CH, aliphatic), 2222 (C≡N), 1684 (C=O), 1507 (C=N), 1480 (C=C); ^{1}HNMR (300MHz, DMSO-d6) δppm:3.76 (s, 3H, OCH_3), 3.86 (s, 6H, $2OCH_3$), 4.72 (s, 2H, CH_2), 5.17-5.25 (m, 2H, $=CH_2$), 5.94-6.00 (m, 1H, =CH), 6.66-8.11 (m, 5H, ArH+3H of furane ring), 8.31 (s, 1H, N=CH), 11.50 (s, 1H, NH, D_2O exchangeable); MS :m/z(%) =435 (91.07) M^+

1-Allyl-2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-ylamino)-4-oxo-6-(3,4,5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (6)

Procedure

A mixture of **4** (0.357g, 1mmol) and maleic anhydride (0.1g, 1mmol) in glacial acetic acid (5ml) was heated under reflux for 27 hrs .After cooling and dilution with water the solid product was collected and crystallized from ethanol to give buff crystals. m.p. 260-262 ˚C, yield(50%); Microanalysis for $C_{21}H_{19}N_5O_6$ (437.41); Calcd %C, 57.66; H, 4.38; N, 16.01; Found:%C, 57.84;H, 4.45;N, 16.18.

IR:(KBr, cm^{-1}) 3433 (NH), 3081 (CH, aromatic), 2940, 2842 (CH, aliphatic), 2220 ($C\equiv N$), 1736 (C=O), 1677 (C=O), 1590 (C=N), 1462 (C=C); ^{1}HNMR (300MHz, DMSO-d_6)δppm:3.77 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.73 (s, 2H, CH_2), 5.21-5.25 (m, 2H, $=CH_2$), 5.88-5.94 (m, 1H, =CH), 7.07(s, 2H, HC=CH), 7.21(s, 2H, ArH), 10.16 (s, 1H, NH, D_2O exchangeable).

1-Allyl-2-(2,5-dimethyl-1H-pyrrol-1-ylamino)-4-oxo-6-(3,4,5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (7)

Procedure

A mixture of **4** (0.357g, 1mmol) and 2, 5-hexanedione (1mmol) in glacial acetic acid (10ml) was stirred at room temperature overnight. The reaction mixture was diluted with water. The red precipitate obtained was filtered off, washed with water and crystallized from DMF/H_2O mixture as red crystals .m.p.116-119 ˚C, yield (50%); Microanalysis for $C_{23}H_{25}N_5O_4$(435.48); Calcd:% C, 63.44; H, 5.79; N, 16.08; Found:%C, 63.61;H, 5.86;N, 16.23.

IR:(KBr, cm^{-1}) 3260 (NH), 3086 (CH, aromatic), 2934, 2838 (CH, aliphatic), 2216 ($C\equiv N$), 1659 (C=O), 1538 (C=N), 1462 (C=C); ^{1}HNMR (300MHz, DMSO-d_6)δppm:2.02 (s, 6H, $2CH_3$), 3.73 (s, 9H, $3OCH_3$), 4.74 (s, 2H, CH_2), 5.17-5.25 (m, 2H, $=CH_2$), 5.72 (s, 2H, CH, pyrrole), 5.73-6.00 (m, 1H, =CH), 7.22 (s, 2H, ArH), 10.98 (s, 1H, NH, D_2O exchangeable). ^{13}CNMR(75MHz,DMSO-d_6)δppm: 160.86, 153.74, 152.31,1 47.28, 140.43, 139.63, 130.37, 129.98,127.69,124.44,117.14,106.32,103.27,60.15,55.75,42.61,11.13.

1-Allyl-4-oxo-2-(2-(2-oxoindolin-3-ylidene)hydrazinyl)-6-(3,4,5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (8)

Procedure

A mixture of **4** (0.357g, 1mmol) and istain (0.147g, 1mmol) in absolute ethanol(15ml) was stirred at room temperature for 48 hrs .The reaction mixture was diluted with water and the formed product was filtered and crystallized from ethanol as buff crystals.m.p.295-297 ˚C; yield(50%); Microanalysis for $C_{25}H_{22}N_6O_5$(486.48); Calcd:% C, 61.72; H, 4.56; N, 17.28; Found:%C, 61.89;H, 4.63;N, 17.61.

IR:(KBr, cm^{-1}) 3292(NH), 3183 (NH), 3076, 3006 (CH, aromatic), 2943, 2834 (CH, aliphatic), 2220 (C≡N), 1685 (C=O), 1662(C=O), 1538 (C=N), 1460 (C=C); ^{1}HNMR(300MHz, DMSO-d_6)δppm:3.78 (s, 3H, OCH_3), 3.86 (s, 6H, $2OCH_3$), 4.75 (s, 2H, CH_2), 5.24-5.28 (m, 2H, $=CH_2$), 5.92-6.03 (m, 1H, =CH), 6.85-8.17 (m, 6H, ArH), 10.65 (s, 1H, NH, D_2O exchangeable), 11.41(s, 1H, NH, D_2O exchangeable).

1-Allyl-2-(2-(5,5-dimethyl-3-oxocyclohexylidene)hydrazinyl)-4-oxo-6-(3,4,5-trimethoxyphenyl)-1, 4-dihydropyrimidine-5-carbonitrile (9)

Procedure

A mixture of **4** (0.357g, 1mmol) and 5, 5-dimethyl-1, 3-cyclohexanedione (0.14g, 1mmol) in toluene (10ml) was heated under reflux for 48 hrs. The orange precipitate obtained was filtered off washed with petroleum ether and crystallized from DMF/H_2O mixture as orange crystals. m.p.133-135 ˚C, Yield (50%); Microanalysis for $C_{22}H_{29}N_5O_5$ (479.22); Calcd %C, 62.62; H, 6.10; N, 14.60; Found: %C, 62.89;H, 6.15;N, 14.76.

IR:(KBr, cm^{-1}) 3411(NH), 3083 (CH, aromatic), 2953, 2837 (CH, aliphatic), 2218 (C≡N), 1677 (C=O), 1623 (C=O), 1591(C=N), 1416(C=C); ^{1}HNMR (300 MHz, DMSO- d_6) δppm:1.20 (s, 6H ,2 CH_3), 2.20 (s, 2H, CH_2), 2.35 (s, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 4.74 (s, 2H, CH_2), 5.24-5.26 (m, 2H, $=CH_2$), 5.85-5.90 (m, 1H, =CH), 6.60 (s, 1H, =CH). , 7.22 (s, 2H, ArH), 8.91(s, 1H, NH, D_2O exchangeable), 10.21 (s, 1H, NH, D_2O exchangeable).

8-Allyl-5-oxo-7-(3,4,5-trimethoxyphenyl)-5, 8-dihydrotetrazolo[1, 5-a]pyrimidine-6-carbonitrile (10)

Procedure

An aqueous solution of sodium nitrite (5ml, 5%) was added dropwise at 10˚C with stirring over 15 minutes to compound **4** (0.357g, 1mmol) in 2N hydrochloric acid (10 ml).The mixture was allowed to stand at room temperature for 2hrs.The formed precipitate was filtered off, washed with ethanol, crystallized from ethanol as white crystals.m.p.190-192˚C , yield (0.25g, 50%); Microanalysis for $C_{17}H_{16}N_6O_4$(368.35); Calcd% C, 55.43; H, 4.38; N, 22.82; Found :%C, 55.68;H, 4.34;N, 23.01.

IR:(KBr, cm^{-1}) 3089 (CH, aromatic), 2943, 2839 (CH, aliphatic), 2165 ($C\equiv N$), 1673 (C=O), 1565 (C=N), 1416 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.72 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.53 (s, 2H, CH_2), 5.20-5.26 (m, 2H, $=CH_2$), 5.91-6.00 (m, 1H, =CH), 7.22 (s, 1H, ArH), 7.45 (s, 1H, ArH);^{13}CNMR(75MHz,DMSO-d_6)δppm:165.73,159.84,156.74,155.85,152.84,147.67,141.30,130.45,118.60, 107.79,106.81,60.46,59.34,46.48; MS:m/z(%) =369 (22.39) M^++1, 368 (100) M^+, 327 (4.27), 325 (33.95), 297 (37.98), 267 (46.40), 81 (38.20)

8-Allyl-5-oxo-7-(3,4,5-trimethoxyphenyl)-5,8-dihydro-[1,2,4]triazolo[4,3-a]pyrimidine-6-carbonitrile (11)

Procedure

A solution of compound **4** (0.357g, 1mmol) in formic acid (10ml) was refluxed for 12hrs. after cooling and dilution with water. The product was collected and crystallized from ethanol as white crystals.m.p.240-242˚C, Yield (0.4g, 80%); Microanalysis for $C_{18}H_{17}N_5O_4$(367.36); Calcd% C, 58.85; H, 4.66; N, 19.06; Found:%C, 58.98;H, 4.73;N, 19.31

IR:(KBr, cm^{-1}) 3082 (CH, aromatic), 2970, 2939 (CH, aliphatic), 2228 ($C\equiv N$), 1680 (C=O), 1584 (C=N), 1462 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.75 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 4.75-4.77 (d, 2H, CH_2), 5.22-5.28 (m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.17 (s, 2H, ArH), 8.36 (s, 1H, N=CH of triazole ring); ^{13}CNMR(75MHz,DMSO-*d_6)δppm:161,157.02,154.46,152.61,151.08,147.60,140.59,137.57,130.28,118.12,107.8*3,106. 72,60.27,56.25,45.87.

MS: m/z (%) =368 (23.06) M^++1, 367 (100) M^+

N'-(1-allyl-5-cyano-4-oxo-6-(3, 4, 5-trimethoxyphenyl)-1, 4-dihydropyrimidin-2-yl)-4-substituted benzohydrazide (12a-c)

General procedure

Compound **4** (0.5g, 1mmol) was refluxed with acid chloride (10 ml) for 24 hrs.excess acid chloride was distilled off under reduced pressure .The residue washed with hot petroleum ether (60-80˚C).The product was crystallized from ethanol to give compounds **12 a-c** as yellow crystals see (table 7).

Table (7): N'-(1-allyl-5-cyano-4-oxo-6-(3,4,5-trimethoxyphenyl)-1,4-dihydropyrimidin-2-yl)-4-substituted benzohydrazide (11a-c)

Comp. No.	R	Yield%	M.P˚C	M.F. (M.W.)	Analysis Calcd/Found		
12a	H	64	134-136	$C_{24}H_{23}N_5O_5$ 461.47	C H N	62.46 5.02 15.18	62.71 5.09 15.36
12b	Cl	71	144-146	$C_{24}H_{22}ClN_5O_5$ 495.91	C H N	58.13 4.47 14.12	58.37 4.51 14.29
12c	OCH_3	58	128-130	$C_{25}H_{25}N_5O_6$ 491.50	C H N	61.09 5.13 14.25	61.40 5.19 14.47

Compound **12a:** IR:(KBr, cm^{-1}):3437 (NH), 3089 (CH, aromatic), 2941, 2841 (CH, aliphatic) 2206 ($C\equiv N$), 1690 (C=O), 1514 (C=N), 1464 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.70 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.62 (s, 2H, CH_2), 5.16-5.21(m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.22-7.90 (m, 6H, ArH), 10.62 (s, 1H, NH, D_2O exchangeable), 11.80 (s, 1H, NH D_2O exchangeable)

Compound **12b:** IR: (KBr, cm^{-1}):3431(NH), 3089 (CH, aromatic), 2940 (CH, aliphatic), 2217 ($C\equiv N$), 1665 (C=O), 1515 (C=N), 1414 (C=C); ^{1}HNMR (300MHZ, DMSO-d_6) δppm:3.71 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 4.60 (s, 2H, CH_2), 5.14-5.20 (m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.22-7.89 (m, 6H, ArH), 10.62 (s, 1H, NH, D_2O exchangeable), 11.80 (s, 1H, NH, D_2O exchangeable)

Compound **12c:** IR:(KBr, cm^{-1}):3442 (NH), 3089 (CH, aromatic), 2940, 2841(CH, aliphatic) 2216 ($C\equiv N$), 1664 (C=O), 1509 (C=N), 1460 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.61(s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.84 (s, 6H, $2OCH_3$), 4.73 (s, 2H, CH_2), 5.20-

5.24 (m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.04-7.97 (m, 6H, ArH), 10.30 (s, 1H, NH, D_2O exchangeable), 10.64 (s, 1H, NH, D_2O exchangeable).

8-Allyl-3-(4-substituted phenyl)-5-oxo-7-(3,4,5-trimethoxyphenyl)-5, 8-dihydro-[1, 2, 4]triazolo[4, 3-a]pyrimidine-6-carbonitrile(13a-c)

General procedure

Compound **12** (5mmol) was heated under reflux in phosphorous oxychloride (10ml) over night. After cooling and dilution with water, the formed product was filtered off and crystallized from ethanol to give compounds **13 a-c** as buff crystals See (table 8).

Table (8): 8-Allyl-3-(4-substituted phenyl)-5-oxo-7-(3,4,5-trimethoxyphenyl)-5,8-dihydro-[1, 2, 4]triazolo[4, 3-a]pyrimidine-6-carbonitrile

Comp. No.	R	Yield%	M.P°C	M.F. (M.W.)	Analysis Calcd/Found		
13a	H	50	160-162	$C_{24}H_{21}N_5O_4$ 443.45	C H N	65.00 4.77 15.79	65.17 4.74 15.92
13b	Cl	55	172-174	$C_{24}H_{20}ClN_5O_4$ 477.90	C H N	60.32 4.22 14.65	60.58 4.21 14.78
13c	OCH_3	50	208-210	$C_{25}H_{23}N_5O_5$ 473.48	C H N	63.42 4.90 14.79	63.54 4.88 14.95

Compound **13a:** IR:(KBr, cm^{-1}): 3069, 3005 (CH, aromatic), 2963, 2936 (CH, aliphatic), 2229 ($C\equiv N$), 1676 (C=O), 1598 (C=N), 1464 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.60 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 4.43 (s, 2H, CH_2), 5.18-5.22 (m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.18-8.15 (m, 7H, ArH)

Compound **13b:** IR :(KBr, cm^{-1}) :3091 (CH, aromatic), 2927, 2853 (CH, aliphatic), 2225 ($C\equiv N$), 1659 (C=O), 1545 (C=N), 1479 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.76 (s, 3H, OCH_3), 3.83 (s, 6H, $2OCH_3$), 4.44 (s, 2H, CH_2) ,5.18-5.20 (m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.58-8.16 (m, 4H, ArH)

Compound **13c:** IR:(KBr, cm^{-1}):3003(CH, aromatic), 2939, 2841(CH, aliphatic), 2217 ($C\equiv N$), 1674 (C=O), 1545 (C=N), 1463 (C=C); ^{1}HNMR (300MHz, DMSO-d_6) δppm:3.68 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 6H, $2OCH_3$), 4.44 (s, 2H, CH_2), 5.18-5.22 (m, 2H, $=CH_2$), 5.31-5.37 (m, 1H, =CH), 7.04-8.06 (m, 6H, ArH)

8-Allyl-3-mercapto-5-oxo-7-(3,4,5-trimethoxyphenyl)-5, 8-dihydro-[1, 2, 4]triazolo[4, 3-a]pyrimidine-6-carbonitrile (14)

Procedure

A solution of potassium hydroxide (0.07g, 1mmol) and ethanol (5ml)was heated under reflux for 1hr, compound **4** (0.357g, 1mmol), carbon disulfide (1ml) was added and heated under reflux overnight .After cooling and dilution with water and neutralized with glacial acetic acid , the buff precipitate obtained was filtered off, washed with water and crystallized from DMF/H_2O as buff crystals; m.p.105-107˚C; yield(50%); Microanalysis for $C_{18}H_{17}N_5O_4S$(399.42); Calcd%C, 54.13; H, 4.29; N, 17.53; Found:%C, 54.19;H, 4.31;N, 17.68

IR:(KBr, cm^{-1}) 3285(NH), 3089 (CH, aromatic), 2943, 2843 (CH, aliphatic), 2221 ($C\equiv N$), 1672 (C=O), 1592 (C=N), 1484 (C=C), 1341(C=S); ^{1}HNMR (300 MHz, DMSO-d_6) δppm:3.73 (s, 3H, OCH_3), 3.81 (s, 6H, $2OCH_3$), 4.85 (s, 2H, CH_2), 5.18-5.25 (m, 2H, $=CH_2$), 5.94-5.97 (m, 1H, =CH), 7.20 (s, 2H, ArH), 12.01(s, 1H, NH, D_2O exchangeable); MS:m/z (%)=400(36.97) M^++1, 399 (52.94) M^+, 370 (65.55), 356 (93.28), 325 (94.96), 274 (78.99), 209 (100).

Biological activity

Antimicrobial experimental

The antibacterial activities of the samples were determined by the agar well diffusion method.[28-32] Mueller-Hinton agar plates were surface-inoculated with the tested strains suspensions adjusted to match 0.5 McFarland standard and the inocula were spread over the surfaces of plates using sterile cotton swabs. After drying of the plates, cups (10 mm diameter) were punched in the agar and 100 µl of the samples in DMF or the antimicrobial agents were added into the wells. The plates were incubated at 37 ºC for 24 hours. The antibacterial activity was determined by measuring the diameter of the zone of inhibition. The test was repeated three times and the mean inhibition zones were calculated.

Evaluation of the antitumor activity[33-35]

The antitumor activity was evaluated on two carcinoma cell lines, namely MCF-7 and HCT-116 cells. The cell lines were grown as monolayers in growth medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The monolayer's of 10,000 cells adhered at the bottom of the wells in a 96-well micro liter plate (Falcon, NJ, USA) incubated for 24h at 37ºC 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 compound in fresh maintenance medium and incubated at 37ºC. A control of untreated cells was made in the absence of the tested compound. Three 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 followed by cell lysing using 33% glacial acetic acid and read the absorbance at 590nm using ELISA reader after well mixing. The absorbance values from untreated cells were considered as 100% proliferation 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 compounds and ODc is the mean optical density of untreated cells. The 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 50µg/ml gentamycin. The cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two to three times a week.

CONCLUSION

In this study some new pyrimidine and fused pyrimidine derivatives were synthesized and elucidated by spectral data and elemental analysis. Some of the new compounds were investigated for their *in vitro* anticancer activity against breast carcinoma (MCF7) and colon carcinoma (HCT-116) cell lines using Doxorubicin as stander. The results in (table 3) showed that compound **4** was the most active against the two cell lines IC50 (3.87, 5.36 ug / ml). Moreover the *in vitro* antimicrobial activity evaluation for most of new compounds was screened against different strain of Microorganism. The results in (table1) showed that most of the tested compounds have promising antimicrobial activity. The minimal inhibitory activity concentration (MIC) values were recorded where compound **5a** had the lowest (MIC) / ug /ml see (table2). This indicating the importance of both hydrazine and 3,4,5-trimethoxyphenyl moieties.

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