

Synthesis and Anti-Tumor Evaluation of Some New Tranilast Analogous

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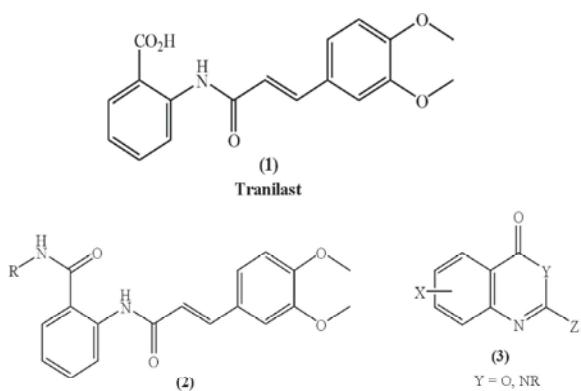
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Abstract: Ring opening reactions of benzoxazinones (6a,b) with oxygen, nitrogen and carbon nucleophiles gave the corresponding benzoates (7), benzamides (8), acrylamides (9 and 11) and the cyanoacetamides (10 a,b). Condensation of compounds (6) with amines under drastic conditions, Yield:ed the quinazolinone derivatives (13). When reacted with thiosemicarbazide and/or phenyl thiourea, compound 6b gave the triazole (14) and / or pyrimidoquinazolinone (15). Some of the new compounds (7a, 7b, 7c, 8b, 8d, 9, 11, 13a, 13b, 14 and 15) were tested for cytotoxic activity using tumor cell line (MCF7).

Key words: Tranilast • Quinazolinones • Antitumor • MCF7 (Breast Carcinoma Cell Lin)

INTRODUCTION

Tranilast (1) is an antiallergic agent that blocks the release of chemical mediators, such as histamine and leukotrienes from mast cells [1-6]. Tranilast (1) possesses antitumor effects (i.e it potentiated the inhibition of the tumor growth induced by several anticancer drugs [7, 8]. It possesses pharmacological approaches to cestenosis prevention [9] and for therapeutic interventions in heart failure [10]. Amides (2) (analogues of Tranilast), showed characteristic activities against smooth muscle cell proliferation [11].



On the other hand, benzoxazinones and quinazolinones (3) (prepared by cyclization of (1) and/or (2)), still have pronounced characteristic biological and pharmacological activities. [12-16].

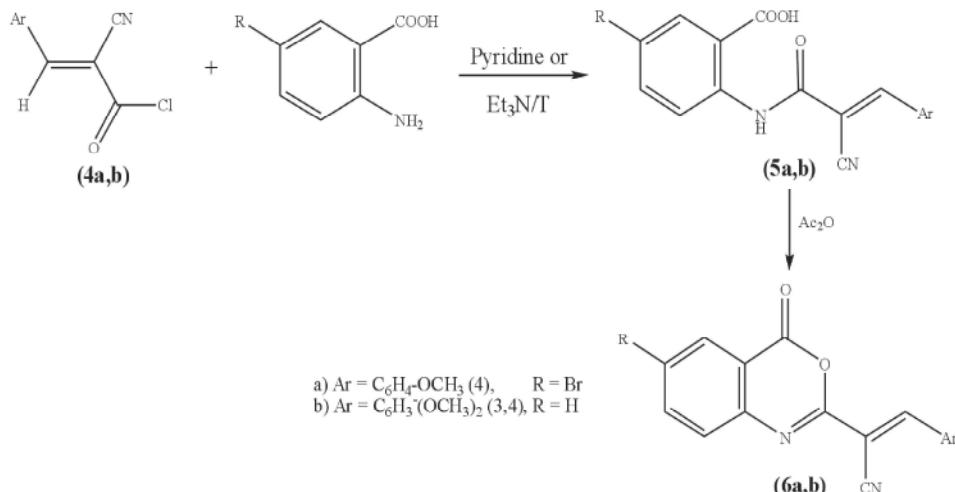
RESULTS AND DISCUSSION

The reported biological and pharmacological activities of benzoxazines initiated our interest in studying the behavior of this ring system towards a variety of nucleophiles. The desired benzoxazinones (6a,b) were prepared by condensation of 2-propenoyl chloride derivatives (4a,b) with anthranilic acid or its 5-bromo derivative in pyridine or triethylamine/toluene *via* the intermediacy of benzoic acid derivatives (5a,b) [17-20]. The structures of 5b and 6b were established by comparison with the authentic samples [20]. Scheme 1.

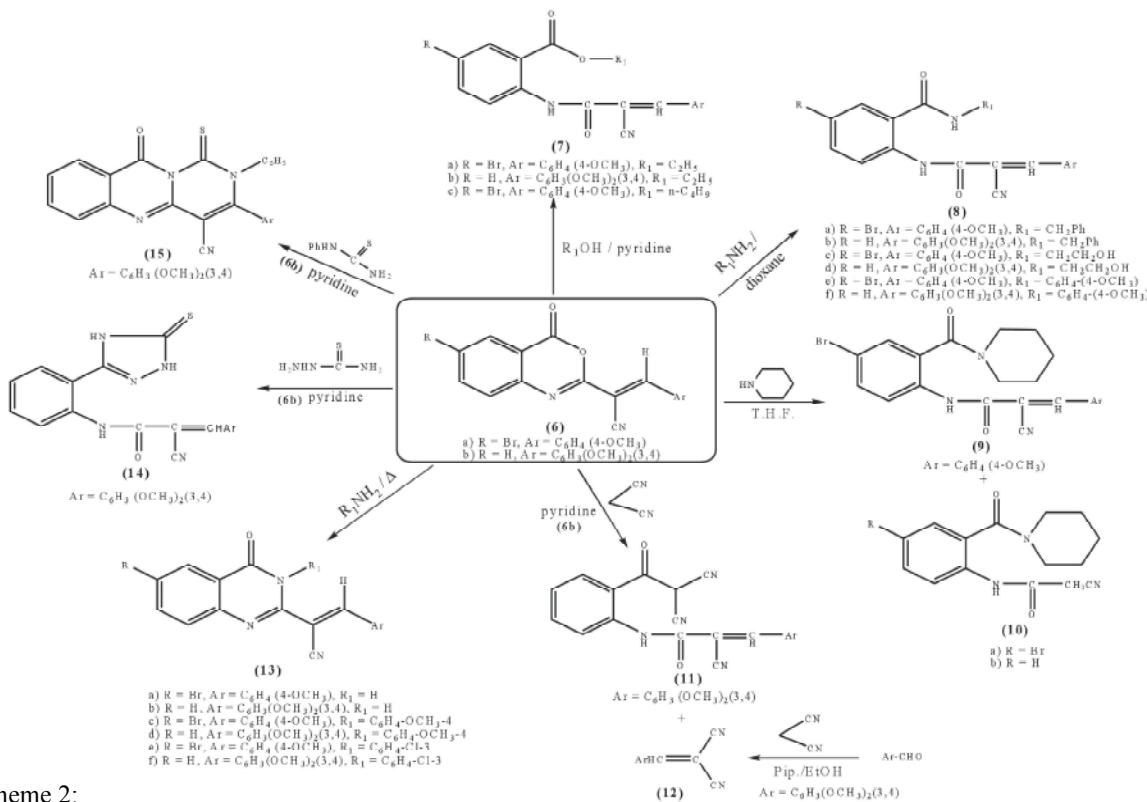
The expected 2-acrylamido benzoates (7a-c) were obtained by treatment of benzoxazinones (6a,b) with ethanol and/or 1-butanol in presence of pyridine (Scheme 2).

Ring opening of 6a,b with primary amines, such as benzylamine, ethanolamine and 4-methoxyaniline, in dioxane gave the corresponding benzamide derivatives (8a-f), while with secondary amines, such as piperidine, (6a) gave a mixture of the acrylamide (9) and cyanoacetamide (10a) while 6b gave 10b only as a sole product (Scheme 2).

Condensation of 6b with malononitrile in presence of pyridine afforded a mixture of the acrylamide (11) and 2-(3,4-dimethoxyphenylmethylene) malononitrile (12) [21], which was identified authentically by condensation of 3,4-dimethoxybenzaldehyde with malononitrile in piperidine (Scheme 2).



Scheme 1:



Scheme 2:

Fusion of 6a,b with ammonium acetate and/or aromatic amines, like, 4-methoxyaniline or 3-chloroaniline gave the corresponding quinazolinones (13a-f) (Scheme 2). Furthermore, fusion of 6b with thiosemicarbazide in neat at 180°C gave the triazole derivative (14) (Scheme 2), via ring opening of benzoxazinone ring, followed by ring closure at the hydrazide moiety.

On the other hand, refluxing 6b with phenyl thiourea in pyridine, Yield:ed the pyrimidoquinazolinone (15)

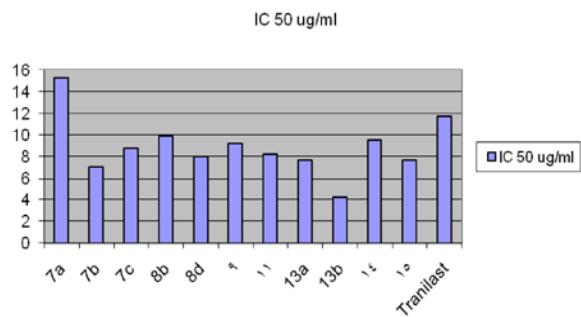
(Scheme 2), through the ring opening-ring closure process, followed by addition on the α,β -unsaturated nitrile with subsequent dehydrogenation of the pyrimidine thione moiety.

Antitumor Activity: The tranilast and its analogous were tested for cytotoxic activity against MCF7 (Breast Carcinoma Cell Lin), Human tumor cell lines were obtained in liquid nitrogen (-180°C) from the American Type

Table 1: The effect of the tranilast and its analogous on MCF7 (Breast Carcinoma Cell Line)

Compound Number	IC 50 ug/ml
7a	15.2
7b	7.08
7c	8.76
8b	9.90
8d	7.95
9	9.22
11	8.17
13a	7.70
13b	4.19
14	9.47
15	7.70
Tranilast	11.7

IC50: the inhibitory concentration of compound which reduce the survival to 50%.



Culture Collection. The tumor cell lines were maintained in the National Cancer Institute, Cairo, Egypt. All tested compounds were proven to have cytotoxic activity against MCF7 (Breast Carcinoma Cell Line).

The IC50 of the tested compounds are listed in table 1.

MATERIAL AND METHOD

- Tumer: Human tumor cell
- MCF7 (Breast Carcinoma Cell Line)

Measurement of Potential Cytotoxicity by Sulfo-Rodamine B (SRB) Assay: Potential cytotoxicity of the compound(s) was tested using the method of Skehan *et al.* [22] and Hiromu *et al.* [23]:

- Cells were plated in 96-multiwell plate (104 cells/well) for 24 hrs before treatment with the compounds to allow attachment of cell to the wall of the plate.
- Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 μ g/ml) were added to the cell monolayer triplicate wells were prepared for each individual dose.

- Monolayer cells were incubated with the compounds for 48 hrs at 37°C and in atmosphere of 5% CO₂.
- After 48 hrs, cells were fixed, washed and stained with Sulfo-Rhodamine-B stain.
- Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA (Ethylene Diamine Tetra Acetic acid) buffer.
- Color intensity was measured in an ELISA (Enzyme Linked Immuno Sorbent Assay) reader.
- The relation between surviving fraction and drug conc. is plotted to get the survival curve of each tumor cell line after the specified compound.

CONCLUSION

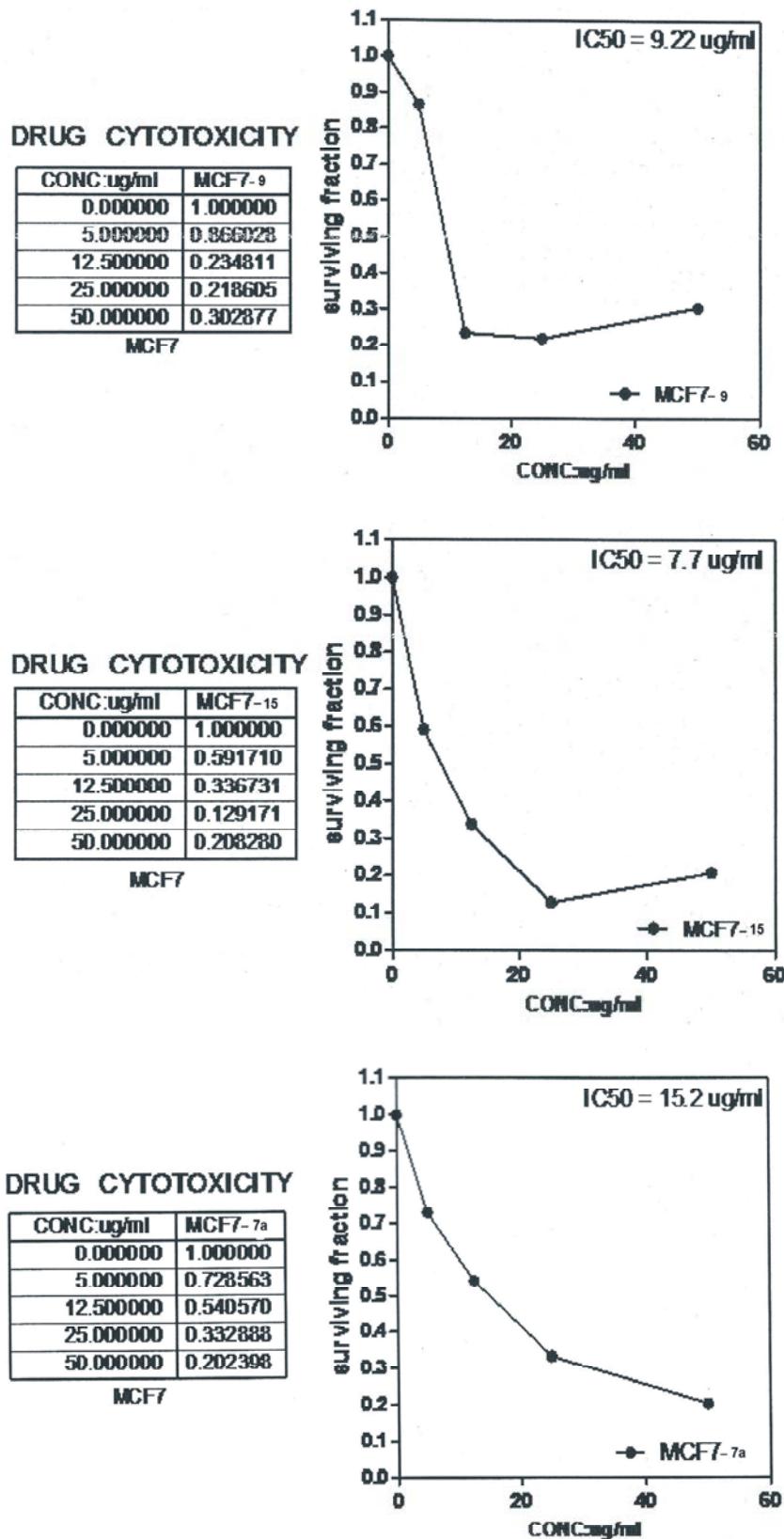
The antitumor activity results indicated that, all the eleven derivatives showed antitumor activity against tested MCF7 (Breast Carcinoma Cell Line) but with varying intensities in comparison to the standard drug: Tranilast. Moreover nine compounds showed higher cytotoxic activity than Tranilast and only two compounds showed lower cytotoxic activity than Tranilast, compounds 7b and 13a showed the highest cytotoxic activity (IC50 equals 7.7 ug/ml).

ACKNOWLEDGEMENT

Our deep regards are to National Cancer Institute, Cairo, Egypt for the fruitful cooperation in the evaluation of anti-tumor activity of the synthesized compounds.

The survival curves of each tumor cell line after the specified compound.

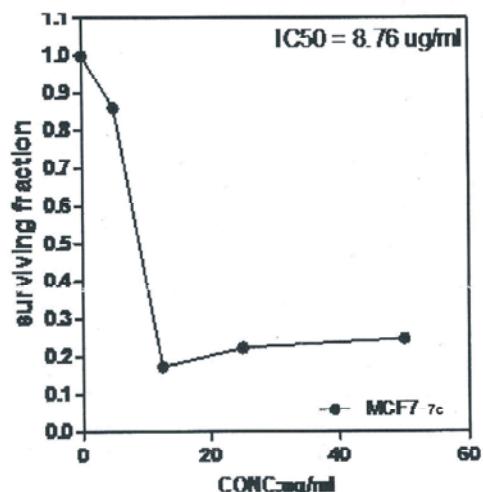
Experimental: Melting points were taken on Griffin and Geory melting point apparatus and are uncorrected. IR spectra were recorded on Pye Unicam SP 1200 spectrophotometer using the KBr wafer technique. ¹H NMR spectra were determined on Varian Gemini 300 MHz using TMS as internal standard. All chemical shifts (δ) are expressed in ppm. And the coupling constants are in Hz. All the NH or OH protons are exchangeable on addition of D₂O. The mass spectra were determined using MP model MS-5988 and Shimadzu single focusing mass spectrometer (70 eV). Elemental analyses were investigated by Elemental analyzer Vario EL III.



DRUG CYTOTOXICITY

CONC:ug/ml	MCF7-7c
0.000000	1.000000
5.000000	0.860986
12.500000	0.172867
25.000000	0.224126
50.000000	0.247776

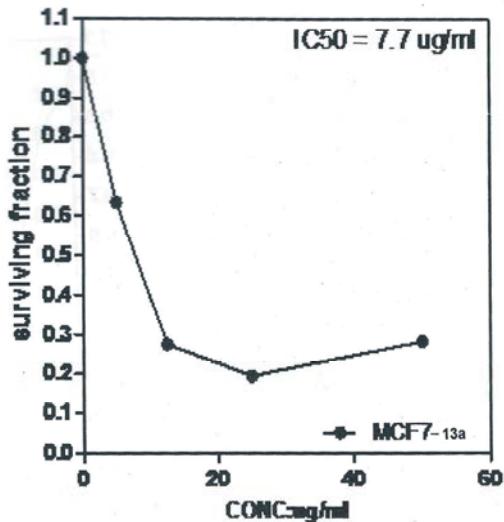
MCF7



DRUG CYTOTOXICITY

CONC:ug/ml	MCF7-13a
0.000000	1.000000
5.000000	0.633846
12.500000	0.274426
25.000000	0.193874
50.000000	0.281388

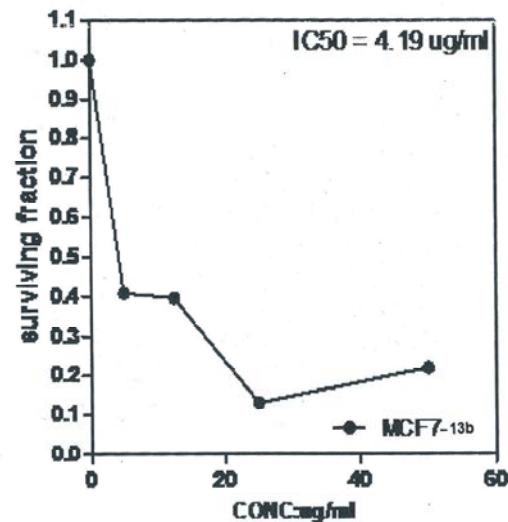
MCF7



DRUG CYTOTOXICITY

CONC:ug/ml	MCF7-13b
0.000000	1.000000
5.000000	0.407678
12.500000	0.396154
25.000000	0.130489
50.000000	0.219927

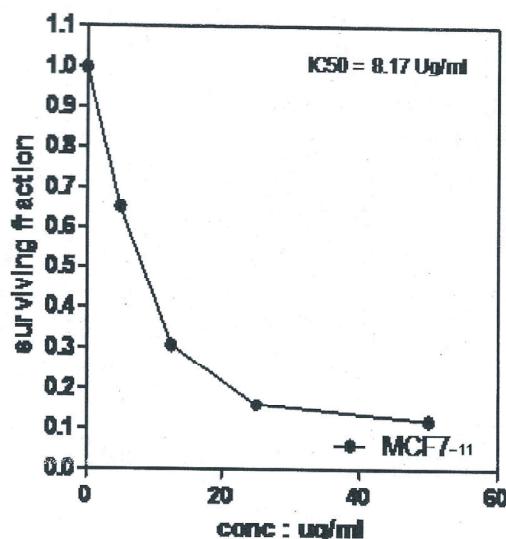
MCF7



DRUG CYTOTOXICITY

Conc ug/ml	MCF7-11
0.000	1.000000
5.000	0.650244
12.500	0.306757
25.000	0.157717
50.000	0.117326

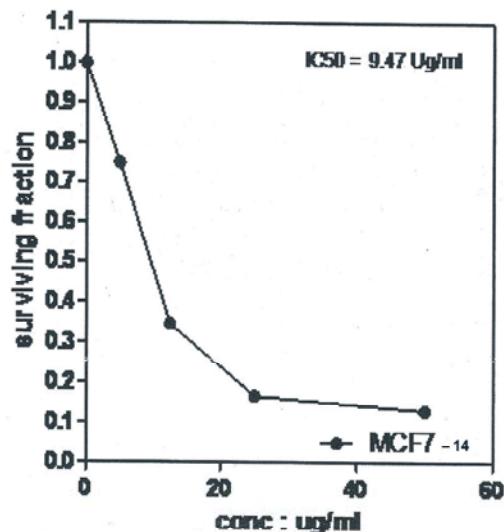
MCF7



DRUG CYTOTOXICITY

Conc ug/ml	MCF7-14
0.000	1.000000
5.000	0.749180
12.500	0.344474
25.000	0.164391
50.000	0.128677

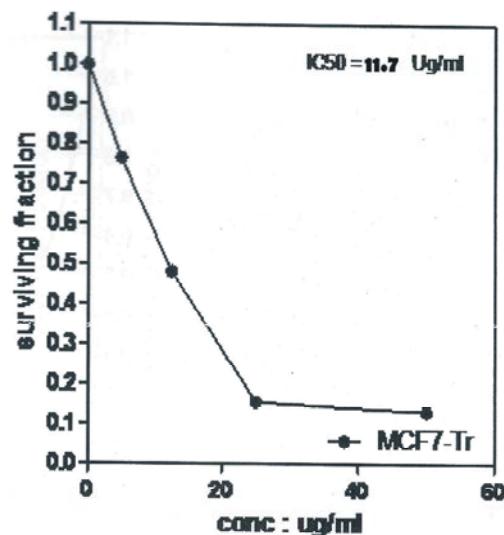
MCF7



DRUG CYTOTOXICITY

Conc ug/ml	MCF7-Tr
0.000	1.000000
5.000	0.763049
12.500	0.479995
25.000	0.153877
50.000	0.129343

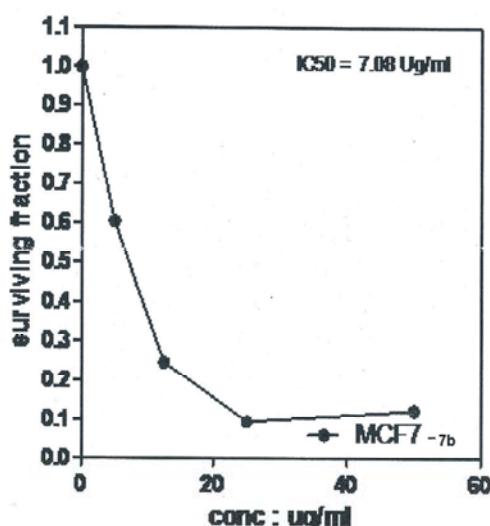
MCF7



DRUG CYTOTOXICITY

Conc ug/ml	MCF7 -7b
0.000	1.000000
5.000	0.603830
12.500	0.245503
25.000	0.093293
50.000	0.122168

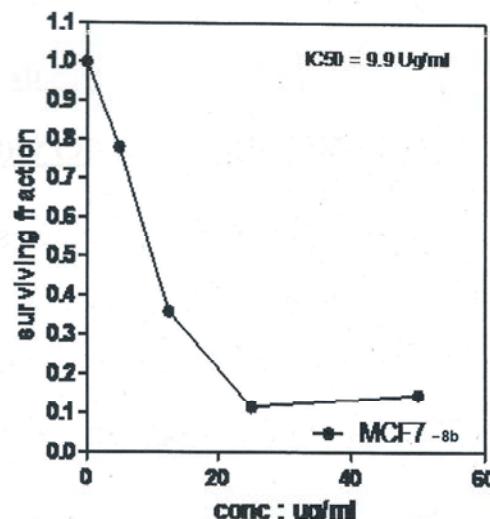
MCF7



DRUG CYTOTOXICITY

Conc ug/ml	MCF7 -8b
0.000	1.000000
5.000	0.780073
12.500	0.358157
25.000	0.114322
50.000	0.146366

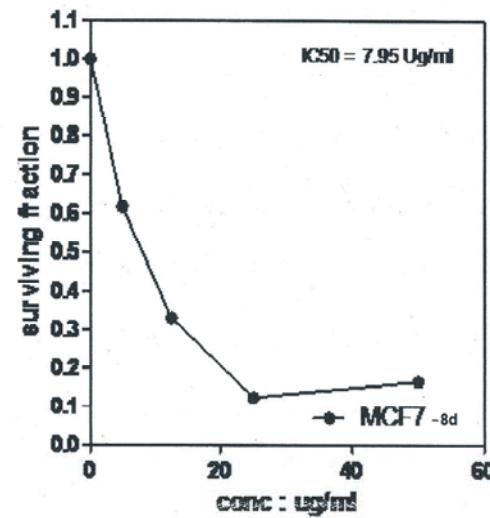
MCF7



DRUG CYTOTOXICITY

Conc ug/ml	MCF7 -8d
0.000	1.000000
5.000	0.616998
12.500	0.330454
25.000	0.123004
50.000	0.164726

MCF7



3-Aryl-2-cyano-2-propenoyl chlorides 4a,b were prepared following the procedure described in the literature [24].

(E)-5-bromo-2-(2-cyano-3-(4-methoxyphenyl)acrylamido)benzoic acid(5a): A mixture of 5-bromoanthranilic acid (0.01 mol, 2.16 g) and 3-(4-methoxyphenyl)-2-cyano-2-propenoyl chloride (0.01 mol, 2.51 g) in dry toluene (50 mL) and pyridine (2-3 drops) was refluxed for 5 hrs. The precipitate formed after concentration of solvent was collected by filtration and recrystallized from ethanol to give 5a. Colour: Green, M.P.: 288-290, Yield: 90 %, FT-IR (KBr, cm⁻¹): 3117 (NH,OH), 2207 (C=N), 1690,1666 (C=O). ¹H NMR (DMSO-d₆) 12.08 (s, 1H, COOH), 8.56 (S, 1H, NH), 8.35 (s, 1H, C=CH), 8.11-7.15 (m7H, Ar-H), 3.87 (s, 3H, OCH₃). Anal. Calced. for C₁₈H₁₃BrN₂O₄: (401.20) C,53.89; H,3.27; N, 6.98 Found: C,53.88; H,3.27; N, 6.97.

(E)-2-(6-Bromo-4-oxo-4H-benzod[*d*][1,3]oxazin-2-yl)-3-(4-methoxyphenyl)-acrylonitrile (6a): The benzoic acid derivative (5a) (0.01mol, 4.01g) was wetted by acetic anhydride (10mL) and the mixture was heated on water bath for 2hrs. The precipitate formed after concentration of solvent was collected and recrystallized form benzene to give 6a. Colour: pale Yellow, M.P.: 278-280, Yield: 80 %, FT-IR (KBr, cm⁻¹): 2222 (C=N), 1772 (C=O), ¹H NMR (DMSO-d₆) 8.56-8.23 (m, 3H, Ar-H), 8.17 (s, 1H, C=CH), 7.63-7.18 (m, 4H, Ar-H), 3.89 (s, 3H, OCH₃). MS: 382 [M-1]⁺ (60), 384 (50), 355 [M+1]⁺ (14.7), 357 (11), 303 (7), 227 (14.4), 225 (19.6), 199 (19.4), 197 (23), 170 (98.5), 168 (100), 158 (33.6), 157 (13.2), 146 (9.6). Anal. Calced. for C₁₈H₁₁BrN₂O₃: (383.20) C, 56.42; H,2.89; N, 7.31 Found: C,56.44; H,2.90;N, 7.30

General method for synthesis of 7a-c: Refluxing of 6a (0.01 mol, 3.83 g) and / or 6b (0.01 mol, 3.34 g), in ethanol and/or n-butanol (20 mL) with few drops of pyridine for 4hrs. After cooling, the precipitate was collected by filtration and recrystallised from a suitable solvent to give 7a-c.

(E)-Ethyl 5-bromo-2-(2-cyano-3-(4-methoxyphenyl)acrylamido)benzoate (7a): Colour: Yellow, M.P.: 260-262, Yield: 45 %, Recrystallized from: ethanol FT-IR (KBr, cm⁻¹): 3212 (NH),2215 (C=N), 1755, 1681 (C=O). ¹H NMR (DMSO-d₆): 11.41 (s, 1H, N H), 8.55-8.34 (m, 3H, Ar-H), 8.32 (s, 1H, =CH), 7.18-7.14 (m, 4H, Ar-H), 4.37 (q, 2H, J= 7.2,OCH₂), 3.87 (s, 3H, OCH₃), 1.35 (t, 3H, J = 6.9, CH₃).

MS: 428 (22), 430 (24), 401 (15.3), 399 (21.2), 186 (100), 158 (20).). Anal. Calced. for C₂₀H₁₇BrN₂O₄: (429.26) C, 55.96; H,3.99; N, 6.53 Found: C,55.99; H,3.97; N, 6.52

(E)-Ethyl 2-(2-cyano-3-(3,4-dimethoxyphenyl)acrylamido)benzoate (7b): Colour: Yellow, M.P.: 140-141, Yield: 50 %, Recrystallized from: toluene FT-IR (KBr, cm⁻¹): 3163 (NH), 2205 (C=N), 1769, 1677 (C=O). ¹H NMR (DMSO-d₆), 11.51 (s, 1H, NH), 8.48-8.35 (m, 3H, J = 8.1, Ar-H), 8.15 (s, 1H, =CH), 7.77-7.66 (m, 4H, Ar-H), 4.37 (q, 2H, J = 7.2, CH₂), 3.87 (s, 6H, 2OCH₃), 1.35 (t, 3H, J = 6.9, CH₃). Anal. Calced. for C₂₁H₂₀N₂O₅: (380.39) C, 66.31; H,5.30; N, 7.36, Found: C,66.35; H,5.30; N, 7.35

(E)-Butyl 5-bromo-2-(2-cyano-3-(4-methoxyphenyl)acrylamido)benzoate (7c): Colour: Yellow, M.P.: 258-260, Yield: 55 %, Recrystallized from: benzene FT-IR (KBr, cm⁻¹): 3230 (NH), 2209 (C=N), 1773, 1680 (C=O). ¹H NMR (DMSO-d₆), 11.4 (s, 1H, NH), 8.45-8.34 (m, 3H, Ar-H), 8.11 (s, 1H, =CH), 8.04-7.14 (m, 4H, Ar-H), 4.34 (t, 2H, J = 6.3, OCH₂), 3.87 (s, 3H, OCH₃), 1.76-1.69 (m, 2H, CH₂), 1.67-1.37 (m, 2H,CH₂), 1.31 (t, 3H, J = 7.2, CH₃). Anal. Calced. for C₂₂H₂₁BrN₂O₄: (457.32) C, 57.78; H,4.63; N, 6.13 Found: C,57.79; H,4.65; N, 6.15

General method for synthesis 8a-f: A mixture of 6a (0.01 mol, 3.83 g) and/or 6b (0.01 mol, 3.34 g) and aromatic amines (0.01mol) namely benzylamine (1.07 g), ethanolamine (0.61 g) or 4-methoxyaniline(1.23 g) in dioxane (50 mL) was refluxed for 5hrs. The precipitate formed after concentration of the solvent under reduced pressure was collected by filtration and crystallized from a suitable solvent to give 8a-f.

(E)-N-Benzyl-5-bromo-2-(2-cyano-3-(4-methoxyphenyl)acrylamido)benzamide (8a): Colour: Yellow, M.P.: 220-221, Yield: 60 %, Recrystallized from: benzene-ethanol mixture (1:1), FT-IR (KBr, cm⁻¹): 3445 (NH), 2206 (C=N), 1676,1632 (C=O). ¹H NMR (DMSO-d₆) 12.74 (s, 2H, NH), 8.46-8.23 (m, 3H, Ar-H), 8.03(s, 1H, =CH), 7.52-7.36 (m, 9H, Ar-H), 4.05 (s, 2H, CH₂), 3.86 (s, 3H, OCH₃). Anal. Calced. for C₂₃H₂₀BrN₃O₃: (490.35) C, 61.24; H,4.11; N, 8.57 Found: C,61.22; H,4.10; N, 8.58

(E)-N-Benzyl-2-(2-cyano-3-(3,4-dimethoxyphenyl)acrylamido)benzamide (8b): Colour: Brown, M.P.: 252-253, Yield: 65 %, Recrystallized from: petroleum ether 80-100 FT-IR (Kbr, cm⁻¹): 3279 (NH), 2202 (C=N), 1679,1634 (C=O). ¹H NMR (DMSO-d₆), 12.31 (s, 1H, NH),

9.38 (s, 1H, NH), 8.31 (s, 1H, =CH), 7.907.70 (m, 3H, J =7.8, Ar-H), 7.27-7.36 (m, 4H, Ar-H), 7.18-7.25 (m, 5H, C_6H_5), 4.52 (s, 2H, CH_2), 3.86 (s, 6H, 2 OCH_3). Anal. Calced. for $C_{26}H_{23}N_3O_4$: (441.48) C, 70.73; H, 5.25; N, 9.25 Found: C, 70.74; H, 5.26; N, 9.51

(E)-5-Bromo-2-(2-cyano-3-(4-methoxyphenyl)acrylamido)-N-(2-hydroxyethyl)-benzamide (8c): Colour: Yellow, M.P.: 278-280, Yield: 60 %, Recrystallized from: ethanol FT-IR (KBr, cm^{-1}): 3522, 3288 (NH), 2212 (C=N), 1688, 1638 (C=O). 1H NMR (DMSO-d₆) 12.283 (s, 1H, NH), 8.932 (s, 1H, NH), 8.43-7.16 (m, 8H, 7Ar-H + 1C=CH), 4.764 (s, 1H, OH), 3.879 (s, 7H, OCH_3+2CH_2). MS: [M+1]⁺ 445 (5.8), [M-1]⁺ 443 (6.2), 385 (17.3), 383 (14.7), 186 (100), 187 (15.4). Anal. Calced. for $C_{20}H_{18}BrN_3O_4$: (444.28) C, 54.07; H, 4.908; N, 9.46 Found: C, 54.12; H, 4.10; N, 9.45

(E)-2-(2-Cyano-3-(3,4-dimethoxyphenyl)-acrylamido)-N-(2-hydroxyethyl)-benzamide (8d): Colour: Brown, M.P.: 256-258, Yield: 70 %, Recrystallized from: ethanol FT-IR (KBr, cm^{-1}): 3285 (NH), 2204 (C=N), 1676, 1636 (C=O). 1H NMR (CDCl₃) 12.147 (s, 2H, NH), 8.69-8.29 (m, 7H, Ar-H), 7.841 (s, 1H, OH), 7.18 (s, 1H, =CH), 3.931 (s, 6H, 2 OCH_3), 3.96-3.88 (m, 4H, CH_2CH_2). MS: 395 (M⁺, 25.3), 364 [M-1]⁺ (3.4), 335 (45.2), 333 (14.6), 307 (15.8), 216 (100), 117 (19). Anal. Calced. for $C_{21}H_{21}BrN_3O_5$: (395.41) C, 63.79; H, 5.35; N, 10.63 Found: C, 63.80; H, 5.34; N, 10.61

(E)-5-Bromo-2-(2-cyano-3-(4-methoxyphenyl)acrylamido)-N-(4-methoxyphenyl)-benzamide (8e): Colour: Yellow, M.P.: 280-281, Yield: 55 %, Recrystallized from: 1,4dioxane, FT-IR (KBr, cm^{-1}): 3290 (NH), 2203 (C=N), 1687, 1638 (C=O). 1H NMR (DMSO-d₆) 11.59 (s, 1H, NH), 10.50 (s, 1H, NH), 8.32 (s, 1H, =CH), 8.31-8.10 (m, 3H, Ar-H), 7.807.62 (m, 8H, Ar-H), 3.77 (s, 6H, 2 OCH_3). Anal. Calced. for $C_{25}H_{20}BrN_3O_4$: (506.35) C, 59.30; H, 3.98; N, 8.30 Found: C, 59.32; H, 3.99; N, 8.31

(E)-2-(2-Cyano-3-(3,4-dimethoxyphenyl)acrylamido)-N-(4-methoxyphenyl)-benzamide (8f): Colour: Yellow, M.P.: 282-283, Yield: 75 %, Recrystallized from: 1,4dioxane FT-IR (KBr, cm^{-1}): 3277 (NH), 2201 (C=N), 1681, 1637 (C=O). 1H NMR (DMSO-d₆) 11.69 (s, 1H, NH), 10.42 (s, 1H, NH), 8.40 (d, 1H, J =8.4, Ar-H), 8.31 (s, 1H, =CH), 7.92 (d, 1H, J =7.8, Ar-H), 7.75 (s, 1H, Ar-H), 7.59-7.69 (m, 5H, Ar-H), 7.31 (t, 1H, J =7.2, Ar-H), 7.18 (d, 1H, J =8.7, Ar-H), 6.95 (d, 1H, J =8.7, Ar-H), 3.90 (s, 9H, 3 OCH_3). MS: M⁺ not observed, 407 (7), 335 (9), 188 (4), 123 (100), 122 (13). Anal. Calced. for $C_{26}H_{21}N_3O_5$: (457.48) C, 68.26; H, 5.07; N, 9.19 Found: C, 68.28; H, 5.08; N, 9.18

Synthesis of 9, 10a and 10b: A mixture of 6a (0.01 mol, 3.83 g) and/or 6b (0.01 mol, 3.34 g) and piperidine (0.01 mol, 0.85 g) in THF (50 mL) was refluxed for 5hrs. The precipitate formed after concentration of the solvent was collected by filtration and crystallized from a suitable solvent to give 9, 10a and 10b respectively.

(E)-N-(4-Bromo-2-(piperidine-1-carbonyl)phenyl)-2-cyano-3-(4-methoxyphenyl)-acrylamide (9): Colour: Yellow, M.P.: 226-227, Yield: 60 %, Recrystallized from: benzene FT-IR (KBr, cm^{-1}): 3448 (NH/OH), 2206 (C=N), 1675 (C=O). 1H NMR (300MHz,DMSO-d₆) 12.6 (s, 1H, N H), 8.47-8.11 (m, 3H, Ar-H), 8.11 (s, 1H, =CH), 8.03-7.83 (m, 4H, Ar-H), 3.86 (s, 3H, OCH_3), 3.00 (t, 4H, $N(CH_2)_2$), 1.65-1.54 (m, 6H, 3C H_2). Anal. Calced. for $C_{23}H_{22}BrN_3O_3$: (468.34) C, 58.98; H, 4.73; N, 8.97 Found: C, 58.99; H, 4.71; N, 8.96

N-(4-Bromo-2-(piperidine-1-carbonyl)phenyl)-2-cyanoacetamide (10a): Colour: Yellow, M.P.: 190-191-280, Yield: 40 %, Recrystallized from: toluene, FT-IR (KBr, cm^{-1}): 3350 (NH), 2200 (C=N), 1686, 1652 (C=O). 1H NMR (300MHz, DMSO-d₆) 12.73 (s, 1H, NH), 8.53-7.44 (m, 3H, Ar-H), 1.55-3.37 (m, 12H, CH_2CN and 10H, Pip. H). Anal. Calced. for $C_{15}H_{16}BrN_3O_2$: (350.21) C, 51.44; H, 4.60; N, 12.00 Found: C, 51.47; H, 4.61; N, 12.02

2-Cyano-N-(2-(piperidine-1-carbonyl)phenyl)acetamide (10b): Colour: reddish Yellow, M.P.: 210-211, Yield: 55 %, Recrystallized from: toluene-ethanol mixture, FT-IR (KBr, cm^{-1}): 3395 (NH), 2205 (C=N), 1638 (C=O). 1H NMR (300MHz,DMSO-d₆) 10 (s, 1H, NH), 7.77-7.30 (m, 4H, Ar-H), 3.31 (s, 2H, CH_2CN), 3.01 (m, 4H, $N(CH_2)_2$), 1.6 (m, 6H, 3 CH_2). MS: 272 (2.7, M⁺+1), 187 (21), 84 (100), 158 (20), 132 (5), 119 (69). Anal. Calced. for $C_{15}H_{17}N_3O_2$: (271.31) C, 66.40; H, 6.32; N, 15.49 Found: C, 66.41; H, 15.47; N, 8.96

(E)-2-Cyano-N-(2-(2,2-dicyanoacetyl)phenyl)-3-(3,4-dimethoxyphenyl)acrylamide (11): A mixture of 6b (0.01 mol, 3.34 g) and malononitrile (0.01 mol, 0.66 g) in pyridine (30 mL) was refluxed for 5hrs. After cooling, the reaction mixture was poured onto ice cold HCl, the precipitate formed was collected, dried and recrystallized from petroleum ether to give 11.

Colour: Brown, M.P.: 280-281, Yield: 55 %, FT-IR (KBr, cm^{-1}): 3269 (NH), 2205 (C=N), 1631 (C=O). 1H NMR (DMSO-d₆- \ddot{a}) 10.33 (s, 1H, NH), 8.90-7.14 (m, 5H, 4H, Ar-H + 1H, =CH), 4.02 (s, 1H, $CH(CN)_2$), 3.82 (s, 6H, 2 OCH_3). Anal. Calced. for $C_{22}H_{16}N_4O_4$: (400.39) C, 66.00; H, 4.03; N, 13.99 Found: C, 66.12; H, 4.11; N, 13.97

General method for synthesis of 13a-f: A mixture of 6a (0.01 mol, 3.83 g) and/or 6b (0.01 mol, 3.34 g) and ammonium acetate (0.04 mol, 3.08 g), 4-methoxyaniline(0.01 mol, 1.23 g) and/or 3-chloroaniline (0.01 mol, 1.27 g) was heated in neat for 3hrs. After cooling, the reaction mixture triturated with hot water or diluted HCl and the precipitate separated was collected, dried and crystallized from a suitable to give 13a-f.

(E)-2-(6bromo-4-oxo-3,4-dimhydroquinazolin-2-yl)-3-(4-methoxyphenyl)acrylonitrile (13a): Colour: Yellow, M.P.: 292-293, Yield: 70 %, Recrystallized from: 1,4dioxane FT-IR (KBr, cm⁻¹): 3445 (NH), 2206 (C=N), 1676 (C=O). ¹H NMR (300MHz,DMSO-d6,) 12.65 (s, 1H, NH), 8.393-8.17 (m, 7H, Ar-H), 7.15 (s, 1H, =CH),, 3.86 (s, 3H, OCH₃). Anal. Calced. for C₁₈H₁₂BrN₃O₂: (382.21) C, 56.56; H, 3.16; N, 10.99 Found: C, 56.58; H, 3.17; N, 10.89

(E)-3-(3,4-dimethoxyphenyl)-2-(4-oxo-3,4-dihydroquinazolin-2-yl)acrylonitrile (13b): Colour: Brown, M.P.: 170-172, Yield: 85 %, Recrystallized from: benzene FT-IR (KBr, cm⁻¹): 3182 (NH), 2204 (C=N), 1671 (C=O). ¹H NMR (300MHz,DMSO-d6,) 12.39 (s, 1H, NH), 8.40 (s, 1H, =CH), 8.11-6.9 (m, 7H, Ar-H), 3.77 (s, 6H, 2OCH₃). Anal. Calced. for C₁₉H₁₅N₃O₃: (333.30) C, 68.46; H, 4.54; N, 12.61 Found: C, 68.48; H, 4.55; N, 12.60

(E)-2-(6-Bromo-3-(4-methoxyphenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)-3-(4-methoxyphenyl)acrylonitrile (13c): Colour: Brown, M.P.: 166-167, Yield: 70 %, Recrystallized from: toluene, FT-IR (KBr, cm⁻¹): 2212 (C=N), 1687 (C=O). ¹H NMR (300MHz,DMSO-d6,) 8.34 (s, 1H, =CH), 8.00-6.76 (m, 11H, Ar-H), 3.82 (s, 6H, 2OCH₃). Anal. Calced. for C₂₅H₁₈BrN₃O₃: (488.33) C, 61.49; H, 3.72; N, 8.60 Found: C, 61.52; H, 3.71; N, 8.58

(E)-3-(3,4-Dimethoxyphenyl)-2-(3-(4-methoxy-phenyl)-4-oxo-3,4-dihydro-quinazolin-2-yl)acrylonitrile (13d): Colour: Brown, M.P.: 180-181,, Yield: 65 %, Recrystallized from: ethanol, FT-IR (KBr, cm⁻¹): 2208 (C=N), 1685 (C=O). ¹H NMR (300MHz, DMSO-d6,) 8.17 (s, 1H, =CH), 7.92-6.87 (m, 11H, Ar-H), 3.85 (s, 9H, 3OCH₃). MS: 439 (35.5, M⁺), 438[M-1] (90), 413 (11.8), 332 (18.3), 302 (28), 251 (37.6), 188 (28), 137 (21), 107 (49.2), 77 (100). Anal. Calced. for C₂₆H₂₁N₃O₄: (439.46) C, 71.06; H, 4.82; N, 9.65 Found: C, 71.09; H, 4.83; N, 9.55

(E)-2-(6-Bromo-3-(3-chlorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)-3-(4-methoxyphenyl)acrylonitrile (13e): Colour: Brown, M.P.: 190-191, Yield:

60 %, Recrystallized from: methanol FT-IR (KBr, cm⁻¹): 2212 (C=N), 1689 (C=O). ¹H NMR (300MHz,DMSO-d6,) 8.244 (s, 1H, =CH), 8.098-7.167 (m, 11H, Ar-H), 3.887 (s, 3H OCH₃). Anal. Calced. for C₂₄H₁₅BrClN₃O₂: (492.75) C, 58.50; H, 3.07; N, 8.53 Found: C, 58.55; H, 3.08; N, 8.55

(E)-2-(3-(3-Chlorophenyl)-4-oxo-3,4-dihydroquinazolin-2-yl)-3-(3,4-dimethoxy-phenyl)acrylonitrile (13f):

Colour: Yellow, M.P.: 158-159, Yield: 80 %, Recrystallized from: benzene FT-IR (KBr, cm⁻¹): 2210 (C=N), 1687 (C=O). ¹H NMR (300MHz,DMSO-d6,) 8.21 (s, 1H, =CH), 7.83-7.11 (m, 11H, Ar-H), 3.88 (s, 6H, 2OCH₃). Anal. Calced. for C₂₅H₁₈ClN₃O₃: (443.88) C, 67.65; H, 4.09; N, 9.47 Found: C, 67.66; H, 4.12; N, 9.45

(E)-2-Cyano-3-(3,4-dimethoxyphenyl)-N-(2-(5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)phenyl)acrylamide (14):

A mixture of 6b (0.01 mol, 3.34 g) and thiosemicarbazide (0.01 mol, 0.91 g) was heated in neat at 160°C for 4hrs. After cooling, the precipitate formed was collected by filtration and recrystallized from toluene to give 14. Colour: Yellow, M.P.: 258-259, Yield: 45 %, FT-IR (KBr, cm⁻¹): 3270 (NH, OH), 2199 (C=N), 1682 (C=O). ¹H NMR (CDCl₃) 11.69 (s, 1H, NH), 8.64-8.29 (m, 5H, 4Ar-H+1H, =CH), 7.94 (s, 1H, NH), 7.67 (s, 1H, NH), 7.64-7.50 (m, 3H, Ar-H), 3.98 (s, 6H, 2OCH₃). Anal. Calced. for C₂₀H₁₇N₅O₃S: (407.45) C, 58.96; H, 4.21; N, 17.19 Found: C, 58.95; H, 4.20; N, 17.18

3-(3,4-Dimethoxyphenyl)-10-oxo-2-phenyl-1-thioxo-2,10-dihydro-1H-pyrimido[6,1-b]quinazoline-4-carbonitrile (15):

A mixture of 6b (0.01 mol, 3.34 g) and phenyl thiourea (0.01 mol, 0.152 g) in pyridine (50 mL) was refluxed for 4hrs. After cooling, the reaction mixture was poured onto ice cold HCl, the solid formed was collected, dried and recrystallized from toluene to give 15. Colour: Brown, M.P.: 230-231, Yield: 40 %, FT-IR (KBr, cm⁻¹): 2203 (C=N), 1682 (C=O). ¹H NMR (CDCl₃) 8.85-8.31 (m, 4H, Ar-H), 7.80-7.56 (m, 5H, C₆H₅), 7.21-6.97 (m, 3H, Ar-H), 3.98 (s, 6H, 2OCH₃). Anal. Calced. for C₂₆H₁₈N₄O₃S: (466.51) C, 66.94; H, 3.89; N, 12.01 Found: C, 66.96; H, 3.87; N, 12.01

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