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Synthesis and Anticancer Evaluation of Some New poly functionally Substituted Pyrimidine-2-thione Derivatives

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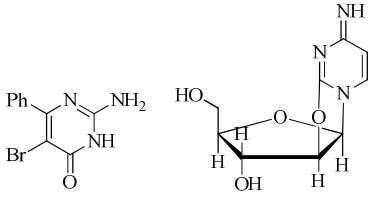
ABSTRACT

*The starting 5-cyano-6-(3,4-dimethoxyphenyl)-2-thiouracil **1** which prepared by reaction of ethyl cyanoacetate with thiourea and 3,4-dimethoxybenzaldehyde, was allowed to react with $POCl_3/PCl_5$ to give the key intermediate 4-chloropyrimidine-2-thione derivative (**2**). Compound **2** underwent several substitution reactions with sulfa drug, p-amino acetophenone and benzocaine to give the corresponding substituted 4-amino derivatives **3**, **4**, **6**, respectively. The hydrazides **5**, α,β-unsaturated ketone **7** and its cyclized products **8**, **9** were also prepared. The imidazopyrimidinthione **10**, its Mannich bases and arylmethylene derivatives **11** and **12** were also prepared. Several other pyridoimidazopyrimidine-thiones and triazolopyrimidines were also synthesized. All newly prepared compounds were biologically evaluated for their anticancer activity against cancer cell lines.*

Keywords: 2-mercaptopyrimidines, sulfa drugs, α,β-unsaturated ketone, Fused pyrimidines, *in vitro* anticancer evaluation.

INTRODUCTION

Pyrimidines are among those molecules that make life possible as being some of the building blocks of nucleic acids DNA and RNA. Various analogues of pyrimidine-2-thiones and thiouracil derivatives possess antimetabolic activity against microbial cells, e.g. *Lactobacillus arabinosus*, *L. leichanii*, and cancer cells, e.g. Sarcoma 180 in mice [1-3]. Several other pyrimidine-2-thione derivatives are reported to possess antimalarial[4], antiprotozol[5], antifungal[6], and anticancer activities[7]. Bropirimine; 2-amino-5-bromo-6-phenyl-4(1H)pyrimidinone8 **I** and anticitabine; 2,2'-anhydro(1β-D-arbinose furanosyl)cyctosine **II[**9] are well-known antiplastic agents of choice.



I **II**

The therapeutic importance of this class of compounds prompted us to synthesized a series of 5-cyano-6-(3,4-dimethoxyphenyl)-pyrimidine-2-thione derivatives and fused pyrimidines to determine their potential chemotherapeutic activity as anticancer agents against cancer cell lines.

MATERIALS AND METHODS

All melting points are uncorrected and were taken on open capillary tubes using electrothermal apparatus 9100. Elemental micro analyses were carried out at microanalytical unit , Central Services Laboratory, National Research Centre, Dokki, Cairo-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 cm^{-1} scale using KBr disc technique at the Central Services Lab. NRC, Dokki, Cairo, Egypt. ^{1}HNMR spectra were determined by using a JEOL EX-270 NMR Spectrometer at Central Services Lab, NRC. Mass spectra were measured with Finnigan M A T SSQ-7000 mass spectrometer at the Central Services, NRC Dokki, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60 F254-Merck, Darmstadt, Germany) and the spots were detected by exposure to UV Lamp at 254 nanometer for few seconds. Nomenclature given for the new compounds are according to the IUPAC System.

6-(3,4-Dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (1)

A mixture of thiourea (7.6g; 0.1mol), ethyl cyanoacetate (11.3g; 1.14mol), veratraldehyde (16.6g; 0.1mol) and Potassium carbonate (13.9g) in 150ml of ethanol was refluxed overnight and cooled. The precipitate thus obtained was filtered off and washed with cold ethanol. The precipitate was dissolved in water at 80^{o}C filtered off, neutralized with glacial acetic acid. The precipitate was filtered off and washed with water to give Product **1** which was separated as yellow crystals (ethanol), yield 80%. mp 205-206 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3354,3406 (NH), 2253 (CN), 1669 (C=O), 1264 (Ar-ether).^{1}H NMR ($CDCl_3$): δ = 3.70 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 6.46-7.20 (m, 3 H, H_{arom}), 12.31 (s, 1H, NH), 12.63 (s, 1H, NH). ppm.MS (EI, 70 eV): *m/z* (%) = 289 (5) (M^+). Anal. for $C_{13}H_{11}N_3O_3S$ (289.31): calcd. C, 53.97; H, 3.83; N, 14.52; S, 11.08; found: C, 54.00; H, 3.56; N, 14.23; S, 11.12.

4-Chloro-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (2)

10g of **1** was heated on water bath with 40ml $POCl_3$ and 5g of PCl_5 for 10h then cooled and poured dropwise on ice/water while stirring. The produced precipitate was filterd off, dried under vacuum and product **2** was separated as brownish yellow crystals (ethanol), yield 77%. mp 136-137 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3406 (NH), 2220 (CN), 1612 (C=N), 1264 (Ar-ether), 1156 (Cl-Ph).^{1}H NMR ($CDCl_3$): δ = 3.71 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 6.46-7.20 (m, 3 H, H_{arom}), 13.03 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 309 (5) (M+2), 307 (16) (M^+). Anal. for $C_{13}H_{10}ClN_3O_2S$ (307.76): calcd. C, 50.73; H, 3.28; Cl, 11.52; N, 13.65; S, 10.42; found: C, 50.45; H, 3.32; Cl, 11.45; N, 13.75; S, 10.40.

General procedures for synthesis of 3a,b

A mixture of compound **2** (0.02mol) and the appropriate sulfa drug, either sulfa pyridine or sulfa diazine (0.02mol) in 50 ml ethanol, then cooled, filtered, dried and crysatllized to give final products **3a,b**.

4-((5-Cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)-*N*-(pyridin-2-yl) benzenesulfonamide (3a)

Product **3a** was separated as yellow crystals (ethanol), yield 65%. mp 173-174 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3402 (NH), 2325 (CN), 1379 (sulphonamide), 1159 (Ar-ether).^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.10-6.60 (m, 4H, H_{arom}), 6.50-7.10 (m, 4 H, pyridine), 7.10-7.40 (m, 3H, H_{arom}), 8.10 (s, 1H, NH), 9.70 (s, 1H, NH, sulphonamide), 13.10 (s, 1H, NH-C=S) ppm. MS (EI, 70 eV): *m/z* (%) = 520 (10) (M^+). Anal. for $C_{24}H_{20}N_6O_4S_2$ (520.58): calcd. C, 55.37; H, 3.87; N, 16.14; S, 12.32; found: C, 55.55; H, 3.77; N, 16.15; S, 12.23.

4-((5-Cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)-*N*-(pyrimidin-2-yl) benzenesulfonamide (3b)

Product **3b** was separated as orange crystals (ethanol), yield 59%. mp 148-149 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3409 (NH), 2320 (CN), 1383 (sulphonamide), 1155 (Ar-ether).^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.50-6.70 (m, 4H, H_{arom}), 7.20-7.70 (m, 3 H, pyrimidine), 7.60-8.10 (m, 3H, H_{arom}), 8.15 (s, 1H, NH), 9.67 (s, 1H,

NH, sulphonamide), 13.09 (s, 1H, NH-C=S) ppm. MS (EI, 70 eV): *m/z* (%) = 521 (7) (M^+). Anal. for $C_{23}H_{19}N_7O_4S_2$ (521.57): calcd. C, 52.96; H, 3.67; N, 18.80; S, 12.30; found: C, 53.00; H, 3.55; N, 18.75; S, 12.35.

General method for synthesis of compounds 4,6

A mixture of compound **2** (0.02mol) and either ethyl-p-aminobenzoate or p-aminoacetophenone (0.01mol) was heated in refluxed ethanol with few drops of con HCl for 5h, then cooled, poured onto ice, cold ammonium hydroxide solution (10%). The resulted precipitate was filtered off and crystallized from the proper solvent.

Ethyl-4-((5-cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino) benzoate (4)

Product **4** was separated as yellow crystals (ethanol), yield 70%. mp 138-139 ^oC. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3412 (NH), 2330 (CN), 1746 (ester C=O), 1170 (Ar-ether). ^1H NMR ($CDCl_3$): δ = 2.30 (t, 3H, CH_3), 3.80 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.30 (q, 2H, CH_2), 6.70-7.50 (m, 7H, H_{arom}), 10.50 (s, 1H, NH), 12.97 (s, 1H, NH). ppm. MS (EI, 70 eV): *m/z* (%) = 434 (12) (M-2). Anal. for $C_{22}H_{20}N_4O_4S$ (436.48): calcd. C, 60.54; H, 4.62; N, 12.84; S, 7.35; found: C, 60.50; H, 4.26; N, 12.89; S, 7.40.

General method of compounds 5a-c

A mixture of compound **4** (0.05mol, 2.2g) and the appropriate hydrazine derivatives, namely, hydrazine hydrate (98%), methyl hydrazine, and/or phenyl hydrazine (0.01mol) was heated under refluxed in 25ml ethanol for 1h, stirred for 24h in room temperature, evaporated under vacuum to half its volume then cooled and poured onto ice/cold water to give the corresponding carbohydrazides **5a-c** respectively.

4-((5-Cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)benzohydrazide (5a)

Product **5a** was separated as brown crystals (chloroform), yield 55%. mp 181-182 ^oC. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3501 (NH_2), 3446 (NH), 2315 (CN), 1690 (amide C=O). ^1H NMR ($CDCl_3$): δ = 3.83 (s, 3H, OCH_3), 3.85(s, 3H, OCH_3), 4.43 (s, 2H, NH_2), 6.50-7.70 (m, 7H, H_{arom}), 9.40 (s, 1H, NH), 10.20 (s, 1H, NH), 12.80 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 422 (5) (M^+). Anal. for $C_{20}H_{18}N_6O_3S$ (422.46): calcd. C, 56.86; H, 4.29; N, 19.89; S, 7.59; found: C, 56.88; H, 4.30; N, 19.90; S, 7.55.

4-((5-Cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)-N'-methylbenzohydrazide (5b)

Product **5b** was separated as yellow crystals (chloroform), yield 53%. mp 111-112 ^oC. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3450 (NH), 2325 (CN), 1679 (amide C=O). ^1H NMR ($CDCl_3$): δ = 2.45 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 4.10 (s, 1H, NH), 6.10-7.50 (m, 7H, H_{arom}), 9.64 (s, 1H, NH), 10.75 (s, 1H, NH), 13.07 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 437 (12) (M+3). Anal. for $C_{21}H_{20}N_6O_3S$ (436.49): calcd. C, 57.79; H, 4.62; N, 19.25; S, 7.35; found: C, 57.80; H, 4.60; N, 19.28; S, 7.36.

4-((5-Cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)-N'-phenylbenzohydrazide (5c)

Product **5c** was separated as orange crystals (chloroform), yield 60%. mp 155-156 ^oC. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3444 (NH), 2330 (CN), 1702 (amide C=O). ^1H NMR ($CDCl_3$): δ = 3.70 (s, 3H, OCH_3), 3.74(s, 3H, OCH_3), 6.10-7.70 (m, 12H, H_{arom}), 9.34 (s, 1H, NH) 10.04 (s, 1H, NH), 10.75 (s, 1H, NH), 13.09 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 495 (7 (M-3). Anal. for $C_{26}H_{22}N_6O_3S$ (498.56): calcd. C, 62.64; H, 4.45; N, 16.86; S, 6.43; found: C, 62.65; H, 4.44; N, 16.89; S, 6.45.

4-((4-acetylphenyl)amino)-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (6)

Product **6** was separated as yellow crystals (ethanol), yield 85%. mp 122-123 ^oC. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3405 (NH), 2260 (CN), 1725 (C=O). ^1H NMR ($CDCl_3$): δ = 2.70 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.10-7.50 (m, 7H, H_{arom}), 10.71 (s, 1H, NH), 13.05 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 406 (15) (M^+). Anal. for $C_{21}H_{18}N_4O_3S$ (406.46): calcd. C, 62.05; H, 4.46; N, 13.78; S, 7.89; found: C, 62.09; H, 4.45; N, 13.80; S, 7.87.

6-(3,4-Dimethoxyphenyl)-4-((4-(3-(4-methoxyphenyl)acryloyl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (7)

A mixture of (0.41g; 0.01mol) of the acetyl derivative **6** and (1.2g; 0.01mol) of *p*-anisaldehyde in 30ml of 10% ethanolic sodium hydroxide solution, was heated for 8h. the reaction mixture was cooled and the precipitated

material was filtered off, air dried to give product **7** which was separated as yellow crystals (ethanol), yield 85%. mp 102-103 °C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3390 (NH), 2550 (CN), 1645 (C=O). ^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.10-7.70 (m, 11H, H_{arom}), 7.50 (d, 1H, =CH), 8.07 (d, 1H, =CH), 10.60 (s, 1H, NH), 12.98 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 525 (25) (M+1). Anal. for $C_{29}H_{24}N_4O_4S$ (524.59): calcd. C, 66.40; H, 4.61; N, 10.68; S, 6.11; found: C, 66.45; H, 4.60; N, 10.70; S, 6.15.

General methods for synthesis of compounds 8a-c

A mixture of the chalcone **7** (5.24g; 0.01mol) and hydrazine namely, 98% hydrazine hydrate, methyl hydrazine and/or phenyl hyrazine (0.01mol) in absolute ethanol (40ml) was heated under reflux for 10h. After cooling, the separated material was filtered, air-dried and crystalized from the proper solvent to give **8a-c**.

6-(3,4-Dimethoxyphenyl)-4-((4-(5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (8a)

Product **8a** was separated as yellow crystals (chloroform), yield 72%. mp 103-104 °C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3480 (NH), 2326 (CN). ^{1}H NMR ($CDCl_3$): δ = 3.59-3.69 (m, 2H, CH_2), 3.73-3.78 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.30-8.10 (m, 11H, H_{arom}), 9.95(s, 1H, NH), 10.65 (s, 1H, NH), 13.08 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 537 (13) (M-1). Anal. for $C_{29}H_{26}N_6O_3S$ (538.62): calcd. C, 64.67; H, 4.87; N, 15.60; S, 5.95; found: C, 64.70; H, 4.90; N, 15.56; S, 5.92.

6-(3,4-dimethoxyphenyl)-4-((4-(5-(4-methoxyphenyl)-1-methyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (8b)

Product **8b** was separated as orange crystals (chloroform), yield 68%. mp 134-135 °C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3500 (NH), 2390 (CN). ^{1}H NMR ($CDCl_3$): δ = 2.60 (s, 3H, N-CH_3), δ = 3.59-3.69 (m, 2H, CH_2), 3.73-3.78 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.54-8.15 (m, 11H, H_{arom}), 10.65 (s, 1H, NH), 13.08 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 552 (10) (M^+). Anal. for $C_{30}H_{28}N_6O_3S$ (552.65): calcd. C, 65.20; H, 5.11; N, 15.21; S, 5.80; found: C, 65.24; H, 5.10; N, 15.22; S, 5.79.

6-(3,4-Dimethoxyphenyl)-4-((4-(5-(4-methoxyphenyl)-1-phenyl-4,5-dihydro-1H-pyrazol-3-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (8c)

Product **8c** was separated as orange crystals (chloroform), yield 75%. mp 122-123 °C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3477 (NH), 2356 (CN). ^{1}H NMR ($CDCl_3$): δ = δ = 3.55-3.68 (m, 2H, CH_2), 3.71-3.75 (m, 1H, CH), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.21-7.80 (m, 16H, H_{arom}), 10.66 (s, 1H, NH), 13.04 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 614 (9) (M^+) Anal. for $C_{35}H_{30}N_6O_3S$ (614.72): calcd. C, 68.39; H, 4.92; N, 13.67; S, 5.22; found: C, 68.40; H, 4.90; N, 13.70; S, 5.20.

6-(3,4-Dimethoxyphenyl)-4-((4-(6-(4-methoxyphenyl)-2-thioxo-1,2,5,6-tetrahydropyrimidin-4-yl)phenyl)amino)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (9)

A mixture of chalcone **7** (5.2g; 0.01mol) and thiourea (0.77g; 0.01mol) in ethanolic solution of sodium hydroxide (30ml, 10%) was heated under reflux for 7h. the reaction mixture was concentrated to half of its volume then poured onto ice cold water. The precipitated material was filterd off to give product **9** was separated as brown crystals (chloroform), yield 65%. mp 132-133 °C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3407 (NH), 2600 (CN), 1190 (C=S). ^{1}H NMR ($CDCl_3$): δ = 2.54-2.70 (m, 2H, CH_2), 3.73-3.80 (m, 1H, CH), 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.30-8.10 (m, 11H, H_{arom}), 9.54(s, 1H, NH), 10.64 (s, 1H, NH), 13.08 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 582 (12) (M^+). Anal. for $C_{30}H_{26}N_6O_3S_2$ (582.70): calcd. C, 61.84; H, 4.50; N, 14.42; S, 11.01; found: C, 61.85; H, 4.49; N, 14.40; S, 11.03.

7-(3,4-Dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c]pyrimidine-8-carbonitrile (10)

A solution of compound **2** (3.1g; 0.01mol) and glycine (0.75g; 0.01mol) in 30ml of n-butanol was heated under reflux for 10h. The separated solid was refluxed with acetic anhydride for 3h, cooled and crystalized to give **10** that was separated as black crystals (ethanol), yield 50%. mp 190-191 °C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3374 (NH), 2357 (CN), 1713 (C=O), 1158 (C=S). ^{1}H NMR ($CDCl_3$): δ = 2.60 (s, 2H, CH_2), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.20-6.70 (m, 3 H, H_{arom}), 13.04 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 328 (35) (M^+). Anal. for $C_{15}H_{12}N_4O_3S$ (328.35): calcd. C, 54.87; H, 3.68; N, 17.06; S, 9.77; found: C, 54.90; H, 3.70; N, 17.05; S, 9.80.

General method for synthesis of 11a-d

A mixture of (1.8g; 0.005mol) of paraformaldehyde and the appropriate amine namely, diethyl amine, diethanol amine, morpholine and/or N-methyl piperazine (0.005mol) in 25ml absolute ethanol was heated till complete solubility of paraformaldehyde followed by addition of (0.632g; 0.002mol) of compound **10** dissloved in 10ml absolute ethanol, then refluxed for 10h, cooled, filtered dried and recrystallized from the proper solvent to give the Mannich bases **11a-d**.

2-((Diethylamino)methyl)-7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c] pyrimidine-8-carbonitrile (11a)

Product **11a** was separated as yellow crystals (ethanol), yield 52%. mp 103-104 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3414 (NH), 2327 (CN), 1707 (C=O), 1200 (C=S). ^{1}H NMR ($CDCl_3$): δ = 1.31 (t, 6H, 2 CH_3), 2.20-2.43 (m, 1H, CH), 2.52-2.81 (m, 2H, CH_2), 2.50 (q, 4H, 2 CH_2), 3.90 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.20-6.80 (m, 3 H, H_{arom}), 12.45 (s, 1H, NH), ppm. MS (EI, 70 eV): *m/z* (%) = 413 (10) (M^+). Anal. for $C_{20}H_{23}N_5O_3S$ (413.49): calcd. C, 58.09; H, 5.61; N, 16.94; S, 7.75; found: C, 58.10; H, 5.60; N, 16.98; S, 7.77.

2-((Bis(2-hydroxyethyl)amino)methyl)-7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo [1,2-c]pyrimidine-8-carbonitrile (11b)

Product **11b** was separated as yellow crystals (ethanol), yield 50%. mp 122-124 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3630 (OH), 3370 (NH), 2400 (CN), 1751 (C=O), 1199 (C=S). ^{1}H NMR ($CDCl_3$): δ = 2.55 (t, 4H, 2 CH_2), 2.20-2.43 (m, 1H, CH), 2.52-2.81 (m, 2H, CH_2), 3.42(t, 4H, 2 CH_2), 3.90 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 4.16 (s, 2H, 2OH), 6.10-6.60 (m, 3 H, H_{arom}), 12.85 (s, 1H, NH), ppm.. MS (EI, 70 eV): *m/z* (%) = 447 (5) (M+2). Anal. for $C_{20}H_{23}N_5O_5S$ (445.49): calcd. C, 53.92; H, 5.20; N, 15.72; S, 7.20; found: C, 53.90; H, 5.25; N, 15.70; S, 7.21.

7-(3,4-Dimethoxyphenyl)-2-(morpholinomethyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo [1,2-c]pyrimidine-8-carbonitrile (11c)

Product **11c** was separated as colorless crystals (ethanol), yield 56%. mp 150-151 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3330 (NH), 2317 (CN), 1727 (C=O), 1116 (C=S). ^{1}H NMR ($CDCl_3$): δ = 2.30 (m, 1H, CH), 2.50-2.68 (m, 2H, CH_2), 3.10 (t, 4H, 2 CH_2), 3.40 (t, 4H, 2 CH_2), 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.30-6.90 (m, 3 H, H_{arom}), 13.05 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 428 (15) (M+1). Anal. for $C_{20}H_{21}N_5O_4S$ (427.48): calcd. C, 56.19; H, 4.95; N, 16.38; S, 7.50; found: C, 56.20; H, 4.93; N, 16.40; S, 7.49.

7-(3,4-Dimethoxyphenyl)-2-((4-methylpiperazin-1-yl)methyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c]pyrimidine-8-carbonitrile (11d)

Product **11d** was separated as yellow crystals (ethanol), yield 58%. mp 176-177 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3339 (NH), 2350 (CN), 1718 (C=O), 1202 (C=S). ^{1}H NMR ($CDCl_3$): δ = 2.10-2.15 (m, 1H, CH), 2.30-2.53 (m, 2H, CH_2), 2.67 (t, 4H, 2 CH_2), 2.75 (t, 4H, 2 CH_2), 3.70 (s, 3H, N-CH_3), 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.10-6.70 (m, 3 H, H_{arom}), 13.07 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 440 (7) (M^+). Anal. for $C_{21}H_{24}N_6O_3S$ (440.52): calcd. C, 57.26; H, 5.49; N, 19.08; S, 7.28; found: C, 57.28; H, 5.50; N, 19.10; S, 7.30.

General method for synthesis of 12a-e

A mixture of compound **10** (0.32g; 0.001mol) and the appropriate aromatic or heteocyclic aldehydes namely, 5-methyl furfurldehyde, p-carboxybenzaldehyde, 2,4-dichlorobenzaldehyde, indole-3-carboxaldehyde, 2-naphthaldehyde in 20ml absolute ethanol was heated under reflux for 10h, then cooled. The product was filtered off, dried under vacuum and was crystalized from the proper solvent to give compounds **12a-e** respectively.

7-(3,4-Dimethoxyphenyl)-2-((5-methoxyfuran-2-yl)methylene)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c]pyrimidine-8-carbonitrile (12a)

Product **12a** was separated as brown crystals (ethanol), yield 62%. mp 206-207 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3407 (NH), 2349 (CN), 1664 (C=O), 1169 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.70 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.30-7.60 (m, 5 H, H_{arom}), 8.50 (s, 1H, =CH), 12.97 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 436 (5) (M^+). Anal. for $C_{21}H_{16}N_4O_4S$ (436.44): calcd. C, 57.79; H, 3.70; N, 12.84; S, 7.35; found: C, 57.80; H, 3.71; N, 12.80; S, 7.33.

4-((8-Cyano-7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-5,6-dihydroimidazo[1,2-c]pyrimidin-2(3H)-ylidene) methyl)benzoic acid (12b)

Product **12b** was separated as brown crystals (ethanol), yield 65%. mp 191-192 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3377 (NH), 2360 (CN), 1705 (C=O), 1690 (C=O), 1159 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 6.60-8.10 (m, 7 H, H_{arom}), 8.80 (s, 1H, =CH), 12.52 (s, 1H, NH), 13.02 (s, 1H, OH) ppm. MS (EI, 70 eV): *m/z* (%) = 460 (5) (M^+). Anal. for $C_{23}H_{16}N_4O_5S$ (460.46): calcd. C, 59.99; H, 3.50; N, 12.17; S, 6.96; found: C, 60.00; H, 3.51; N, 12.12; S, 6.94.

2-(2,4-Dichlorobenzylidene)-7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c] pyrimidine-8-carbonitrile (12c)

Product **12c** was separated as yellow crystals (ethanol), yield 70%. mp 177-178 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3400 (NH), 2370 (CN), 1780 (C=O), 1780 (C=O), 1222 (C=S), 1177 (C-Cl). ^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 6.30-7.80 (m, 6 H, H_{arom}), 8.10 (s, 1H, =CH), 12.40 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 484 (5) (M^+), 486 (20) (M+2), 488 (16) (M+4). Anal. for $C_{22}H_{14}Cl_2N_4O_3S$ (485.34): calcd. C, 54.44; H, 2.91; Cl, 14.61; N, 11.54; S, 6.61; found: C, 54.45; H, 2.90; Cl, 14.60; N, 11.55; S, 6.59.

2-((1H-indol-3-yl)methylene)-7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c] pyrimidine-8-carbonitrile (12d)

Product **12d** was separated as green crystals (ethanol), yield 73%. mp 221-222 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3350 (NH), 2339 (CN), 1746 (C=O), 1160 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.20 (s, 1H, NH), 6.30-7.70 (m, 8 H, H_{arom}), 8.60 (s, 1H, =CH), 6.20 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 455 (12) (M^+) Anal. for $C_{24}H_{17}N_5O_3S$ (455.49): calcd. C, 63.29; H, 3.76; N, 15.38; S, 7.04; found: C, 63.30; H, 3.73; N, 15.41; S, 7.01.

7-(3,4-Dimethoxyphenyl)-2-(naphthalen-2-ylmethylene)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c] pyrimidine-8-carbonitrile (12e)

Product **12e** was separated as green crystals (ethanol), yield 85%. mp 111-112 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3390 (NH), 2330 (CN), 1788 (C=O), 1160 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.20-7.80 (m, 10 H, H_{arom}), 8.60 (s, 1H, =CH), 12.64 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 465 (15) (M^{-1}) Anal. for $C_{26}H_{18}N_4O_3S$ (466.51): calcd. C, 66.94; H, 3.89; N, 12.01; S, 6.87; found: C, 66.95; H, 3.90; N, 12.00; S, 6.85.

General method for preparation of 13a-c (Michael addition)

A mixture of compounds **12a,d,e** (0.01mol) and ethylcyanoacetate (1.5ml; 0.01mol), anhydrous ammonium acetate (0.62g; 0.08mol) in *n*-butanol (10ml) was refluxed for 5h. the reaction mixture was concentrated to half of its volume under reduced pressure. After cooling, the formed precipitate was filtered off, air-dried and recrystallized from the proper solvent to give compounds **13a-c**, respectively.

7-(3,4-Dimethoxyphenyl)-4-(5-methoxyfuran-2-yl)-2-oxo-9-thioxo-1,2,8,9-tetrahydropyrido[3',2':4,5]imidazo [1,2-c]pyrimidine-3,6-dicarbonitrile (13a)

Product **13a** was separated as brown crystals (ethanol), yield 55%. mp 161-162 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3358 (NH), 2394 (CN), 1743 (C=O), 1120 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.75 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.10-6.80 (m, 5 H, H_{arom}), 9.30 (s, 1H, NH), 10.90 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 500 (5) (M^+). Anal. for $C_{24}H_{16}N_6O_5S$ (500.49): calcd. C, 57.60; H, 3.22; N, 16.79; S, 6.41; found: C, 57.64; H, 3.20; N, 16.80; S, 6.44.

7-(3,4-Dimethoxyphenyl)-4-(1H-indol-3-yl)-2-oxo-9-thioxo-1,2,8,9-tetrahydropyrido[3',2':4,5]imidazo[1,2-c]pyrimidine-3,6-dicarbonitrile (13b)

Product **13b** was separated as green crystals (ethanol), yield 55%. mp 181-182 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3393 (NH), 2385 (CN), 1715 (C=O), 1166 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.81 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.10 (s, 1H, NH), 6.15-7.70 (m, 7 H, H_{arom}), 8.54 (s, 1H, =CH), 9.50 (s, 1H, NH), 13.10 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 519 (7) (M^+). Anal. for $C_{27}H_{17}N_7O_3S$ (519.53): calcd. C, 62.42; H, 3.30; N, 18.87; S, 6.17; found: C, 62.39; H, 3.33; N, 18.85; S, 6.20.

7-(3,4-Dimethoxyphenyl)-4-(naphthalen-2-yl)-2-oxo-9-thioxo-1,2,8,9-tetrahydropyrido[3',2':4,5]imidazo[1,2-c]pyrimidine-3,6-dicarbonitrile (13c)

Product **13c** was separated as yellow crystals (ethanol), yield 58%. mp 124-125 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3404 (NH), 2330 (CN), 1720 (C=O), 1205 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.10-7.60 (m, 10 H, H_{arom}), 9.90 (s, 1H, NH), 12.97 (s, 1H, NH), ppm. MS (EI, 70 eV): *m/z* (%) = 530 (11) (M^+). Anal. for $C_{29}H_{18}N_6O_3S$ (530.56): calcd. C, 65.65; H, 3.42; N, 15.84; S, 6.04; found: C, 65.61; H, 3.43; N, 15.89; S, 6.07.

General method for preparation of compounds 14a,b

The foregoing method for preparation of **13** was applied except that malononitrile was used instead of ethyl cyanoacetate. Compounds **12c,e** gave the corresponding **14a,b** respectively.

2-Amino-4-(2,4-dichlorophenyl)-7-(3,4-dimethoxyphenyl)-9-thioxo-8,9-dihydropyrido[3',2':4,5]imidazo[1,2-c]pyrimidine-3,6-dicarbonitrile (14a)

Product **14a** was separated as yellow crystals (ethanol), yield 70%. mp 144-145 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3500 (NH_2), 3388 (NH), 2355 (CN), 1156 (C=S), 1125 (C-Cl). ^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.50 (s, 2H, NH_2), 6.10-7.80 (m, 6 H, H_{arom}), 13.03 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 548 (7) (M^+), 550 (20) (M+2). Anal. for $C_{25}H_{15}Cl_2N_7O_2S$ (548.40): calcd. C, 54.75; H, 2.76; Cl, 12.93; N, 17.88; S, 5.85; found: C, 54.71; H, 2.78; Cl, 12.96; N, 17.90; 5.80.

2-Amino-7-(3,4-dimethoxyphenyl)-4-(naphthalen-2-yl)-9-thioxo-8,9-dihydropyrido[3',2':4,5]imidazo[1,2-c] pyrimidine-3,6-dicarbonitrile (14b)

Product **14b** was separated as yellow crystals (ethanol), yield 79%. mp 199-200 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3489 (NH_2), 3341 (NH), 2315 (CN), 1202 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.80 (s, 2H, NH_2), 6.30-7.10 (m, 10 H, H_{arom}), 13.05 (s, 1H, NH), ppm. MS (EI, 70 eV): *m/z* (%) = 529 (10) (M^+). Anal. for $C_{29}H_{19}N_7O_2S$ (529.57): calcd. C, 65.77; H, 3.62; N, 18.51; S, 6.05; found: C, 65.80; H, 3.59; N, 18.50; S, 6.07.

6-(3,4-Dimethoxyphenyl)-4-hydrazinyl-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (15)

A mixture of compound **2** (3.1g; 0.01mol) and 98% hydrazine hydrate (3ml; 0.01mol) in 20 ml absolute ethanol was refluxed for 5h; after cooling, the separted material was filtered off, washed with cold water, dried to give product **15** that was separated as yellow crystals (ethanol), yield 80%. mp 146-147 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3498 (NH_2), 3340 (NH), 2325 (CN), 1168 (C=S). ^{1}H NMR ($CDCl_3$): δ = 3.80 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 4.30 (s, 2H, NH_2), 4.80 (s, 1H, NH), 6.30-6.70 (m, 3 H, H_{arom}), 13.06 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 301 (15) (M-2). Anal. for $C_{13}H_{13}N_5O_2S$ (303.34): calcd. C, 51.47; H, 4.32; N, 23.09; S, 10.57; found: C, 51.50; H, 4.30; N, 23.10; S, 10.60.

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

A solution of compound **15** (0.01mol) in 20ml of glacial acetic acid was refluxed for 4h, cooled, and poured onto cold water. The precipitated material was filtered off to give product **16** that was separated as yellow crystals (ethanol), yield 75%. mp 180-181 ^{o}C. IR(KBr), $\widetilde{\nu}$ /cm^{-1} : 3373 (NH), 2387 (CN), 1160 (C=S). ^{1}H NMR ($CDCl_3$): δ = 2.50 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 6.50-6.70 (m, 3 H, H_{arom}), 13.10 (s, 1H, NH) ppm. MS (EI, 70 eV): *m/z* (%) = 328 (25) (M^{+1}) Anal. for $C_{15}H_{13}N_5O_2S$ (327.36): calcd. C, 55.03; H, 4.00; N, 21.39; S, 9.79; found: C, 55.00; H, 4.03; N, 21.40; S, 9.80.

BIOLOGICAL ASSAYS

Cell culture

MCF-7 cell lines were obtained from the Karolinska Institute, Stockholm, Sweden. HEPG-2 cells were maintained in RPMI 1640 medium, while MCF-7 Human cancer cells were maintained in DMEM medium (Lonza Biowahittkar, Belgium). All the media were supplemented with 1 % antibiotic-antimycotic mixture (10,000 U ml-1 potassium penicillin, 10,000 µg ml-1streptomycin sulfate, 25 µg ml-1 amphotericin B and 1 % L-glutamine (Biowest, USA).

MTT cytotoxicity assay

Cell viability was investigated using MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (Bio Basic Canada Inc., Canada). This reaction depends on mitochondrial reduction of yellow MTT into purple formazan.

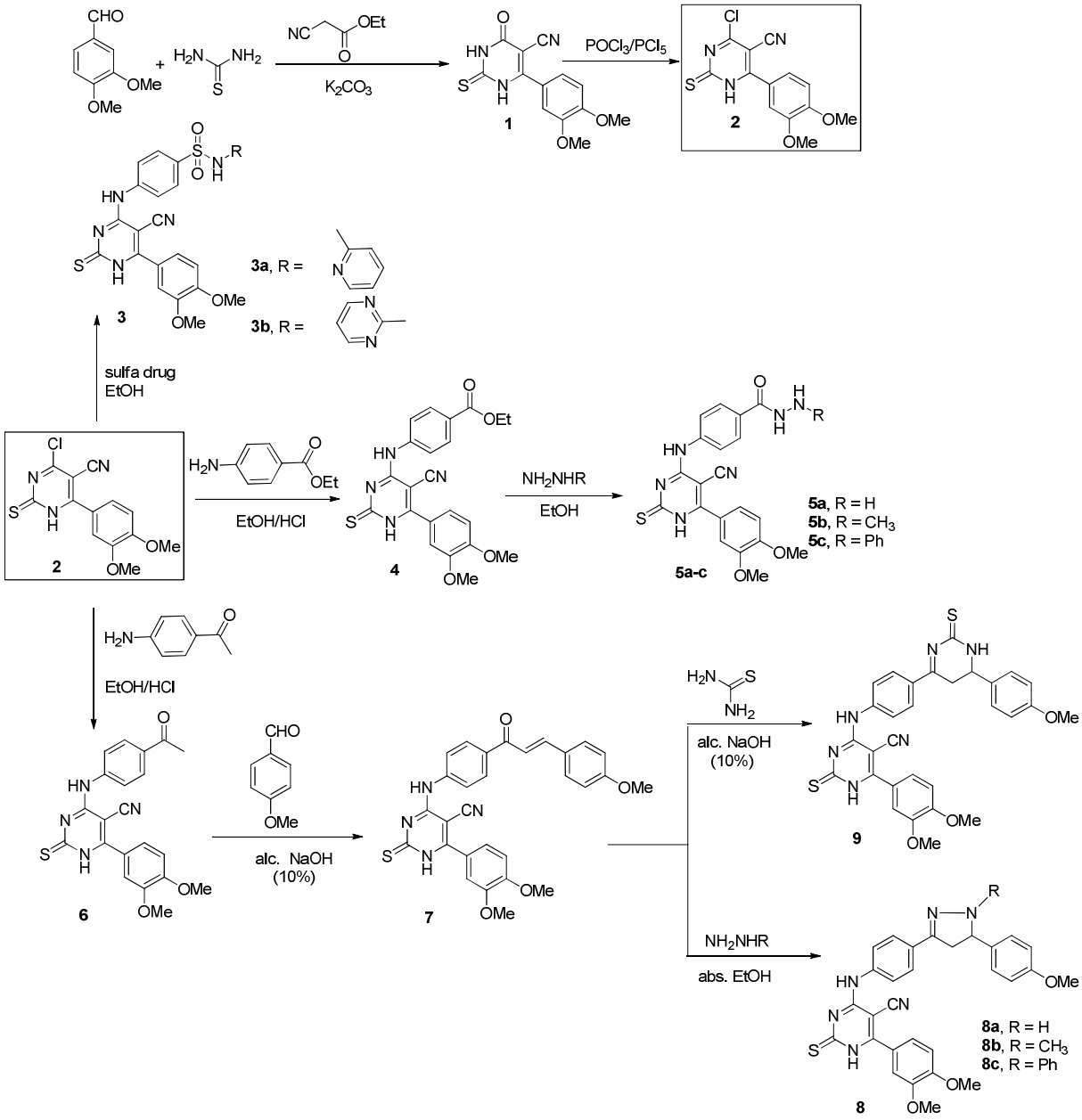
All the preceding steps were carried out in sterile laminar airflow cabinet Biosafety class II level (Baker, SG403INT; Sanford, ME, USA). All incubations were done at 37 ˚C in 5 % CO2 incubator in the humidified atmosphere (Sheldon, TC2323; Cornelius, OR, USA). Cells were seeded into 96-well microtiter plastic plates at the concentration of (104 cells per well) and allowed to adhere for 24 h. Medium was aspirated and fresh medium (without serum) was added to the cells with 100 µg ml-1 in DMSO then the promising compounds which give 100% inhibition will subjected to various concentrations of the promising compounds (100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg ml-1 in DMSO) and incubated for 48 h. The medium was aspirated and 40 µl MTT salts (2.5 µg ml-1) was added to each correctly and incubated for a further 4 hs. To stop the reaction and dissolve any formed formazan crystals, 200 µl of 10 % sodium dodecyl sulfate (SDS) were added to each well and incubated overnight at 37 ˚C. The amount of formazan product was measured at 595 nm with a reference wavelength of 620 nm as a background using a microplate reader (Bio-Rad Laboratories, model 3350, USA). For the untreated cells (negative control), medium was added instead of the test compounds. A positive control Adrinamycin® (doxorubicin) (Mr=579.9) was used as a known cytotoxic natural agent giving 100 % inhibition. Dimethyl sulfoxide (DMSO) was the vehicle used for dissolution of testing compound, and its final concentration on the cells was less than 0.2 %.

RESULTS AND DISCUSSION

CHEMISTRY

For the purpose of this study, the synthetic approach was confined to two general schemes 1 and 2 to obtain the target polyfunctionally substituted pyrimidine-2-thione derivatives and the related fused Pyrimidines. Thus, reaction of thiourea with 3,4-dimethoxybenzaldehyde and ethyl cyanoacetate in alkaline medium according to a reported method[10], afforded the first starting compound 6-(3,4-dimethoxyphenyl)-4-oxo-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**1**) in almost good yield.

Compound **1** was chlorinated with phosphorus oxychloride/phosphorus pentachloride mixture to give the corresponding 4-chloro-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**2**), which in turn was allowed to react with sulfa pyridine and/or sulfadiazine to afford the corresponding 4-(p-substituted sulfamoyl aniline-) derivatives **3a** and **3b** respectively. Reaction of **2** with ethyl *p*-amino-benzoate and/or *p*-aminoacetophenone, afforded the corresponding 4-subsitututed aniline pyrimidine-2-thione derivatives **4** and **6** respectively. Reaction of ethyl 4-((5-cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)benzoate (**4**) with hydrazine hydrate, methyl and/or phenyl hydrazine, gave the corresponding substituted carbohydrazides **5a-c** respectively. Further, reaction of methyl 4-((5-cyano-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)amino)benzoate (**6**) with *p*-anisaldehyde afforded the corresponding α,β-unsaturated ketone **7** which underwent cyclocondensation reaction with either hydrazine hydrate or methyl and phenyl hydrazine in ethanolic sodium hydroxide solution to give the corresponding *N*-substituted-dihydropyrazoline derivatives **8a-c** respectively. While reaction of **7** with thiourea afforded the corresponding polyfunctionally substituted di-(pyridine-2-thione) derivative **9** (Scheme 1).



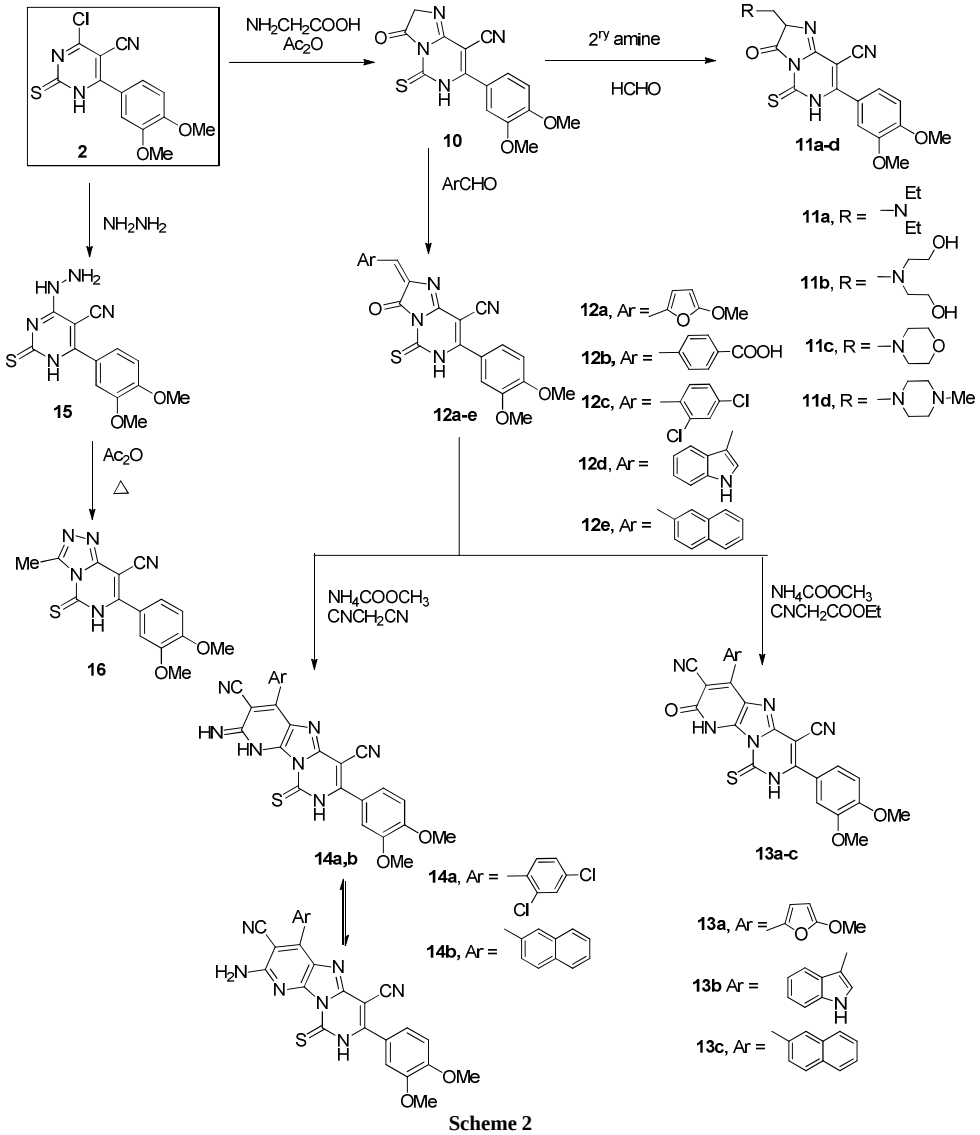
Scheme 1

On the other hand, 4-chloro-6-(3,4-dimethoxyphenyl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile (**2**) was heated under reflux with glycine in butanol followed by cyclization by heating wit acetic anhydride to achieve the corresponding 7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c]pyrimidine-8-carbonitrile (**10**) which was allowed to undergo the Mannich reaction with paraformaldehyde and the appropriate secondary amines, namely, diethylamine, diethanol amine, morpholine, and/or methyl piperazine to give the Mannich bases **11a-d** respectively. Also, compound **10** was allowed to react with the appropriate aromatic aldehyde namely, methyl furfural, p-carboxybenzaldehyde, 2,4-dichlorobenzaldehyde, indole-3-carboxaldehyde and/or naphthalene-2-carboxaldehyde to give the corresponding 2-aryl-7-(3,4-dimethoxyphenyl)-3-oxo-5-thioxo-2,3,5,6-tetrahydroimidazo[1,2-c]pyrimidine-8-carbonitrile derivatives **12a-e** respectively (Scheme 2).

Further, cyclocondensation of compound **12** with ammonium acetate and ethyl cyanoacetate afforded the 2-oxopyridoimidazopyrimidine derivatives **13a-c**, while upon reaction with ammonium acetate and malononitrile, it gave the corresponding pyrido imidazopyrimidine amine derivatives **14a,b** respectively.

Furthermore, compound **2** was allowed to react with hydrazine hydrate in ethanol to give the corresponding 4-hydrazino-pyrimidine-2-thione derivative **15** in good yield. The hydrazine derivative **15** underwent cyclocondensation reaction in acetic anhydride to give the corresponding 1,2,4,-triazinopyrimidine-2-thione derivative **16** (Scheme 2).

All the newly synthesized compounds were structurally investigated by chemical microanalyses and spectral analyses.



Scheme 2

ANTITUMOR ACTIVITY[13-16]

This study exhibited the anti-tumor activity of some of the newly synthesized compounds. Fifteen derivatives were selected as representative examples to examine their *in vitro* anti-tumor activities against Hep-G2 human liver cancer cell line and MCF-7 human breast cancer cell line. The percentage of the intact cells was measured and tabulated in table **1**. The activities of the derivatives against carcinoma cells were compared with the cytotoxicity of

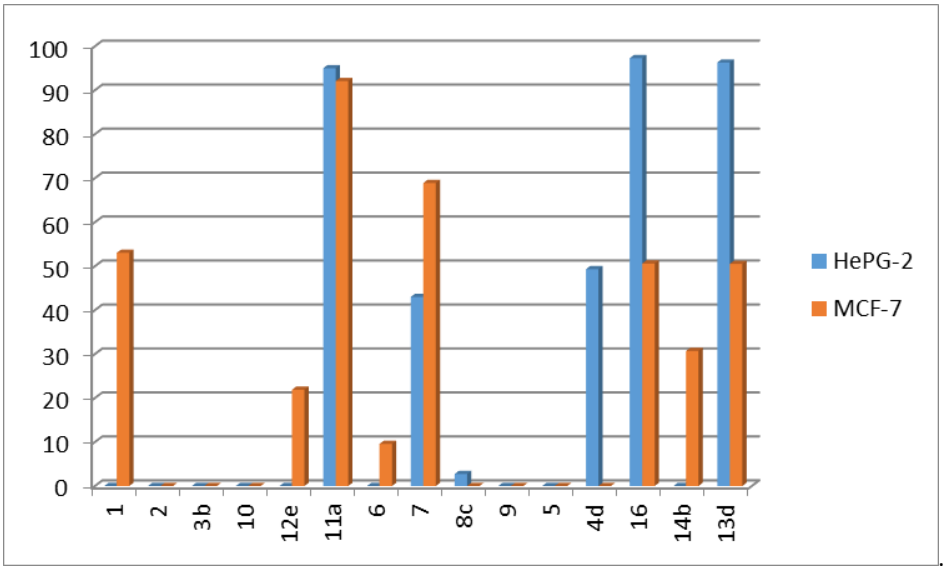
doxorubicin as a reference drug. The results showed that the triazolo[4,3-*c*]pyrimidine derivative **16** and the tri-fused tetrahydropyrido[3',2':4,5]imidazo[1,2-c]pyrimidine derivative **13c** exhibited the highest cytotoxic activity against the liver cancer cells (HepG-2) with inhibition percentage of 97.3, 96.3 %, while they showed moderate potency against the breast carcinoma cell lines MCF-7 (growth inhibition 50.5 %). Fortunately, dual inhibition potency against both HepG-2 and MCF-7 cancer cell lines was gained by the imidazo[1,2-c]pyrimidine derivative **11a**.

Also, the results indicated that the starting thiopyrimidine compound **1** and the acryloyl -phenylamino thiopyrimidine compound **7** showed moderate cytotoxic activity against the MCF-7 cancer cell lines (inhibition growth; 53, 68.9 %) with lack of activity against Hep-G2 cell lines. The rest of the tested derivatives produced no activity.

Further derivatization and optimization of heterocyclic compounds are required to get more active and wide spectrum anti-tumor potency.

Compound	HePG-2*	MCF-7*
1	0	53.04
2	0	0
3b	0	0
4d	49.3	0
5	0	0
6	0	9.59
7	43	68.9
8c	2.73	0
9	0	0
10	0	0
11a	95	92.09
12e	0	21.9
13d	96.3	50.5
14b	0	30.7
16	97.3	50.6

**Results at 100ppm*



	PC3		A549		HCT116		MCF7		HepG2	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
doxorubicin	6.8±1.2	13.8±0.8	0.087±0.9	0.35±0.7	2.2±3.1	5.2±1.9	12.8±1	51.7±0.7	0.6±0.1	1.8±0.2

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