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Synthesis and anticancer screening of some novel substituted pyrazole derivatives

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ABSTRACT

Novel derivatives of substituted pyrazole ring incorporated to or fused with other heterocyclic ring systems were synthesized. The key compound 3-chloro-5-(3,4-dimethoxyphenyl)- 1H-pyrazole-4-carbonitrile ***(2)*** *was allowed to react with different substituted hydrazine derivatives to afford the pyrazolo[3,4-c]pyrazol derivatives* ***3a-c*** *respectively, while its condensation with glycine led to the formation of the imidazo[1,2-b] pyrazole derivative* ***4****. The derivatives pyrazolo[1,5-e]tetrazole****5*** *and pyrazolo[3,4-d] pyrimidine****6*** *were obtained upon treatment of* ***2*** *with sodium azide and thiourea, respectively. The tricyclicoxopyrazolo[1,5-b]isoquinoline compound* ***7****was gained by the reaction of* ***2*** *with anthranilic acid. Furthermore, reflux of* ***2*** *with various sulfa drugs afforded the sulfonylphenylpyrazole derivatives* ***8a,b****. Also, the treatment of compound* ***2*** *with morpholine gave the morpholino-pyrazole compound* ***9****. Further reaction of* ***2*** *with different aromatic and heteroaromatic amines led to the formation of substituted aminopyrazole derivatives* ***10a-h****. Moreover, 2-(3,4-dimethoxybenzylidene)malononitrile****(11)*** *was used as an intermediate precursor for the synthesis of various aminopyrazole and aminopyrimidine derivatives* ***12-15a,b****upon its treatment with hydrazine hydrate, phenyl hydrazine, cyanoacetamide and (thio)urea, respectively. Anticancer evaluation represented that the derivatives* ***13****,* ***15b****of potential activity against MCF-7, HCTH-6,HePG-2 carcinoma cell lines using Doxorubicin as a reference drug.*

Key words: 3,4-dimethoxybenzaldehyde, pyrazoles, pyrimidines, arylidenemalononitrile, anticancer screening.

INTRODUCTION

Cancer is still continuing to be a major health problem worldwide. The development of new anticancer therapeutic agents is one of the fundamental goals in medicinal chemistry as cancer causes about 13% of all the death. Surpassing cardiovascular diseases, it is taking the position number one killer due to various factors [1]. Also the treatment of cancer is associated with various side effects which include bone marrow depression, alopecia, drug-induced cancer, hepatotoxicity, and many more. Because of the need and value of anticancer drugs, many laboratories are intensively investigating the chemistry and biology of novel anticancer agents. Also the development of resistance against the existing anticancer drugs and cytotoxicity and genotoxicity of anticancer drugs to the normal cells are other major problems in cancer therapy, keeping research window open in search for newer anticancer molecules [2]. Over the past two decades, pyrazole-containing compounds have received considerable attention owing to their diverse chemotherapeutic potentials including versatile antineoplastic activities through inhibiting different types of enzymes that play important roles in cell division [3-5]. Several reports have found diverse applications for *N*-arylpyrazoles in medicine such as antiviral [6], anti-inflammatory [7] agents, or kinase inhibitors for the treatment of type 2 diabetes, hyperlipidemia, and obesity [8]. Moreover, these compounds have remarkable potential in nanomedicine applications against malignant gliomas [9].

On the other hand, pyrimidine derivativeshave wide applications as antiviral[10], antibacterial[11], antimalarial[12], antihypertensive [13], anti-inflammatory [14] and as anticancer agents [15], beside their uses as precursors in the synthesis of fused ring compounds of different chemotherapeutical activities specially as antitumor agents [16-19].
Based on all these findings,we report here synthesis of new compounds of substituted pyrazole nucleus combined with or fused to various heterocyclic ring systems such as pyrimidine moiety as a trial to get more potent and less toxic anticancer agents.

MATERIALS AND METHODS

Chemistry
All melting points were uncorrected and were taken in open capillary tubes using an Electro thermal IA 9100 digital melting point apparatus. Elemental microanalyses were carried out on Elementar, Vario EL,at Micro analytical laboratory, central services laboratory, National Research center, Dokki, Cairo, Egypt, and were found within ±0.5% of the theoretical values. Infrared spectra were recorded on a FT/IR-6100, Fourier transform infrared spectrometer (Japan) at cm^{-1} scale by using KBr disc technique.^{1}HNMR spectra was determined by using a JEOI EX-270 NMR spectrometer and measured in δ scale using TMS as an internal standard. The mass spectra were measured with a Finnegan MAT SSQ-7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoatedaluminum sheets (Type 60, f 254,Merck,Darmstadt,Germany) and the spots were detected by exposure to UV lamp at λ_{254} nm for few seconds. The chemical names given of the prepared compounds are according to the IUPAC system.

2,3-Dihydro-5-(3,4-dimethoxyphenyl)-3-oxo-1*H*-pyrazole-4-carbonitrile (1):
A solution mixture of 3,4-dimethoxybenzaldehyde (16 gm, 0.1mol) and 2-cyanoacetohydrazide (9.5 gm, 0.1 mol) in absolute ethanol (20 mL) acidified with acetic acid (3mL) was refluxed for 2h. The solid that formed was collected by filtration and recrystallized from ethanol to give compound **1**.Yield 70%;mp166^{0}C; **IR** υ(cm^{-1}): 3431, 3302 (2NH),2024 (CN), 1668 (C=O); **MS**: (m/z) ~$[M+2]^+$247(100%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.83 (6H, s, 2OCH_3); 6.94-7.24 (3H, m , aromatic protons), 8.91, 9.21 (2H, 2s, 2NH); Anal. for $C_{12}H_{11}N_3O_3$ (245.23)Calcd./Found(%): C; 58.77/58.45, H; 4.52/4.02, N; 17.13/16.89.

3-Chloro-5-(3,4-dimethoxyphenyl)- 1*H*-pyrazole-4-carbonitrile (2):
A solution of compound **1** (2.45gm, 0.01mol) in phosphorus oxy chloride (50mL) was heated under reflux for 3h. The reaction mixture was cooled, then poured onto ice/cold water with continues stirring. The formed solid was collected by filtration and recrystallized from methanol to give compound **2**.Yield 65%;mp152^{0}C; **IR** υ(cm^{-1}): 3484 (NH),2221 (CN); **MS**: (m/z)~$[M]^+$ 263, $[M+2]^+$ 265 (10, 3.5 %); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.83 (6H, s, 2OCH_3); 6.94-7.35 (3H,m , aromatic protons) and 9.26 (1H, s, NH); Anal. for $C_{12}H_{10}ClN_4O_2$(263.68) Calcd./Found(%): C; 54.66/54.88, H; 3.82/4.07, N; 15.94/16.44.

General method for preparation of 1,2,3,3a-tetrahydro-4-(3,4-dimethoxyphenyl)-2-(substituted)pyrazolo[3,4-*c*]pyrazol-3-amines3a-c:
A mixture of compound **2**(2.63gm, 0.01mol) andthe appropriatesubstituted hydrazine derivativesnamely: mono hydrazine hydrate, methylhydrazine and phenyl hydrazine (0.01 mol) in absolute ethanol (15mL) was refluxed for 4h. After cooling, the reaction mixture was poured onto ice/cold water, the formed precipitate was filtered off, dried and recrystallized from methanol to give the compounds **3a-c**respectively.

1,2,3,3a-Tetrahydro-4-(3,4-dimethoxyphenyl)pyrazolo[3,4-*c*]pyrazol-3-amine (3a):
Yield 60 %;mp190^{0}C; **IR** υ(cm^{-1}): 3407-3225 (2NH, NH_2); **MS**: (m/z)$[M]^+$~ 261(24%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 1.69 (1H, s, CH of pyrazole ring), 3.61 (1H,s, C*H*-NH_2), 3.83 (6H, s, 2OCH_3); 5.72 (2H, s, NH_2); 6.78-7.33 (3H,m, aromatic protons) and 9.01, 9.58 (2H, 2s, 2NH); Anal. for $C_{12}H_{15}N_5O_2$ (261.12) Calcd./Found(%): C; 55.16/55.45, H; 5.79/5.49, N; 26.80/27.01.

1,2,3,3a-Tetrahydro-4-(3,4-dimethoxyphenyl)-2-methylpyrazolo[3,4-*c*]pyrazol-3-amine (3b):
Yield 65 %; mp165^{0}C; **IR** υ(cm^{-1}): 3432-3245(NH, NH_2); **MS**: (m/z) $[M^+$-2] ~273(5%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 1.57 (1H, s, CH of pyrazole ring), 2.47 (3H, s, CH_3),3.61 (1H, s, C*H*-NH_2), 3.85 (6H, s, 2OCH_3); 5.80 (2H, s, NH_2); 6.94-7.31(3H,m , aromatic protons) and 9.26 (1H, s, NH); Anal. for $C_{13}H_{17}N_5O_2$ (275.31) Calcd./Found(%): C; 56.71/57.11, H; 6.22/6.45, N; 25.44/25.67.

1,2,3,3a-Tetrahydro-4-(3,4-dimethoxyphenyl)-2-phenylpyrazolo[3,4-*c*]pyrazol-3-amine (3c):
Yield 62 %; mp110^{0}C; **IR** υ(cm^{-1}): 3432-3299 (NH, NH_2),; **MS**: (m/z) $[M^+]$ ~ 337 (15%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 1.72 (1H, s, CH of pyrazole ring), 3.61 (1H, s, C*H*-NH_2), 3.85 (6H, s, 2OCH_3); 5.21 (2H, s, NH_2); 6.94-8.01

(8H,m, aromatic protons) and 9.26 (1H, s, NH); Anal. for $C_{18}H_{19}N_5O_2$ (337.38) Calcd./Found(%): C; 64.08/64.37, H; 5.68/5.56, N; 20.76/20.38.

3,5-Dihydro-6-(3,4-dimethoxy phenyl)-3-oxo-2*H*-imidazo[1,2-*b*] pyrazole-7-carbonitrile (4):
A mixture of compound **2**(2.63 gm, 0.01mol) and glycine (0.75 gm, 0.01 mol) in n-butanol (30 mL) was heated under reflux for 8h. The solid separated was refluxed with anhydrous acetic acid (5 mL) for 3 h. The obtained precipitate was filtered off, dried, and recrystallized from methanol to give compound **4**.Yield 79 %; mp180^{0}C; **IR** υ(cm^{-1}): 3345 (NH),2215(CN), 1663 (C=O); **MS**: (m/z) $[M]^+$~ 284(13%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 2.21 (2H, s, CH_2 of pyrazole ring); 3.83 (6H, s, 2OCH_3); 6.94-7.34 (3H,m , aromatic protons) and 9.26 (1H, s, NH); Anal. for $C_{14}H_{12}N_4O_3$ (284.27) Calcd./Found(%): C; 59.15/59.38, H; 4.25/4.61, N; 19.71/20.21.

6-(3,4-Dimethoxyphenyl)-5*H*-pyrazolo[1, 5-*e*]tetrazole-7-carbonitrile (5):
A mixture of compound **2**(2.63 gm, 0.01mol) and sodium azide (0.32 gm, 0.005 mol) in n-butanol(30 mL)was heated under reflux for 9h. The product obtained after cooling was driedand recrystallized from DMF/water to give compound **5**. Yield 83%; mp260^{0}C; **IR**υ(cm^{-1}): 3454 (NH),2215(CN); **MS**: (m/z) $[M]^+$~ 270 (25%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm):3.83 (6H, s, 2OCH_3); 6.94-7.35 (3H,m , aromatic protons) and 9.26 (1H, s, NH); Anal. for $C_{12}H_{10}N_6O_2$ (270.25). Calcd./Found(%) C; 53.33/52.83, H; 3.73/3.47, N; 31.10/30.91.

4-Amino-4,5-dihydro-3-(3,4-dimethoxyphenyl)-3a*H*-pyrazolo[3,4-*d*] pyrimidine-6(7*H*)-thione (6):
A mixture of compound **2**(2.63 gm, 0.01 mol) andthiourea (0.76 gm, 0.01mol)in ethanol (15 mL) was heated under reflux while continuous stirring for 2h. The reaction mixture was cooled and poured onto ice/cold water acidified with hydrochloric acid. Theformed precipitate was filtered off, washed with water and recrystallized from ethanol to give compound **6**.Yield 65 %; mp130^{0}C; **IR** υ(cm^{-1}): 3465-3267 (2NH, NH_2),1165 (C=S); **MS**: (m/z) $[M]^+$~ 305 (10%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 1.63 (1H, s, CH of pyrazole ring), 3.61 (1H, s, C*H*-NH_2), 3.83 (6H, s, 2OCH_3),5.92 (2H, s, NH_2), 6.94-7.21 (3H,m , aromatic protons) and 9.26, 9.36 (2H, 2s,2NH); Anal. for $C_{13}H_{15}N_5O_2S$ (305.36)Calcd./Found(%): C; 51.13/51.25, H; 4.95/4.82, N; 22.94/22.59.

1,9-Dihydro-2-(3,4-dimethoxyphenyl)-9-oxopyrazolo[1,5-*b*]isoquinoline-3-carbonitrile (7):
A mixture of compound **2**(2.63 gm, 0.01 mol) andanthranilic acid (1.4 gm, 0.01 mol)in ethanol (15 mL) was heated under reflux for 2h. The reaction mixture was cooled and poured onto ice/cold water. Theformed precipitate was filtered off, washed with water and recrystallized from ethanol to give compound **7**.Yield 68 %; mp130^{0}C; **IR** υ(cm^{-1}): 3465 (NH),2215 (CN),1678 (C=O); **MS**: (m/z) $[M]^+$~ 345 (13%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.86 (6H, s, 2OCH_3),6.83-7.32(8H,m , aromatic protons) and 9.21 (1H, s, NH); Anal. for $C_{20}H_{15}N_3O_3$ (345.35)Calcd./Found(%): C; 69.56/69.25, H; 4.38/4.72, N; 12.17/12.59.

General method for preparation of 3-(4-(pyridin/(pyrimidin)-2-ylsulfonyl)phenylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitriles (8a,b):
A mixture of compound **2**(2.63gm, 0.01mol) anddifferent appropriateSulpha drugs namely: sulphapyridine and sulphadiazine (0.01 mol) in absolute ethanol (30 mL) was refluxed for 8h. After cooling, the reaction mixture was poured onto ice/cold water,the precipitate formed was filtered off, dried and recrystallized from ethanol to give compounds **8a, 8b** respectively.

3-(4-(Pyridin-2-ylsulfonyl)phenylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitrile (8a):
Yield 76 %; mp146^{0}C; **IR** υ(cm^{-1}): 3484, 3366 (2NH),2215 (CN); **MS**:(m/z) $[M]^+$~ 461 (10%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm):3.75 (6H, s, 2OCH_3); 6.94-7.81 (11H,m , aromatic protons) and 9.26, 9.34 (2H, 2s, 2NH); Anal. for $C_{23}H_{19}N_5O_4S$ (461.49) Calcd./Found(%): C; 59.86/59.62, H; 4.15/3.82, N; 15.18/14.97.

3-(4-(Pyrimidin-2-ylsulfonyl)phenylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitrile (8b):
Yield 70 %; mp189^{0}C; **IR** υ(cm^{-1}): 3463, 3325 (2NH),2215(CN); **MS**: m/z $[M]^+$~ 462 (5%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm):δ 3.83 (6H, s, 2OCH_3); 6.94-7.98(10H,m , aromatic protons) and 9.11, 9.41 (2H, 2s, 2NH); Anal. for $C_{22}H_{18}N_6O_4S$ (462.48) Calcd./Found(%): C; 57.13/56.88, H; 3.92/3.56, N; 18.17/17.87.

5-(3,4-Dimethoxyphenyl)-3-morpholino-1*H*-pyrazole-4-carbonitrile (9):
A mixture of compound **2**(2.63 gm, 0.01 mol) andmorpholine (0.87 mL, 0.01 mol)in dry dioxane (15 mL) containing triethylamine (1 mL) was heated under reflux about for 12h. After cooling, thereaction mixturewas poured onto ice/cold water. Theformed precipitate was filtered off, dried and recrystallized from chloroform to give compound (**9**).Yield 79%; mp120^{0}C; **IR** υ(cm^{-1}): 3484 (NH),2215 (CN); **MS**: m/z $[M-1]^+$~ 313 (20%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 2.79 (4H, m, N-$(CH_2)_2$); 3.61 (4H, m, O-$(CH_2)_2$); 3.83 (6H, s, 2OCH_3); 6.94-7.34 (3H,m ,

aromatic protons) and 9.26 (1H, s,NH); Anal. for $C_{16}H_{18}N_4O_3$ (314.34) Calcd./Found (%): C; 61.13/61.57; H; 5.77/5.95; N; 17.82/18.09.

General method for preparation of 3-(substituted amino)-5-(3,4-dimethoxyphenyl)-1*H*- pyrazole-4-carbonitriles 10a-h:

A mixture of compound **2**(2.63 gm, 0.01mol) and different appropriate amines namely: cyclohexylamine, *p*-bromoaniline, *p*-aminoacetophenone,ethyl 4-aminobenzoate, *o*-aminophenol, *o*-aminothiophenol, 2-aminopyridine and 2-aminothiazole (0.01 mol) in methanol (25 mL) containing few drops of pyridine was heated under reflux for 8h. The formed precipitate was filtered off, dried and thenrecrystallized from methanol to give compounds **10a-h** respectively.

3-(Cyclohexylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitrile (10a):

Yield 73 %; mp186^{0}C; **IR** υ(cm^{-1}): 3475, 3335 (2NH),2215(CN); **MS**: m/z $[M+1]^+$~ 327 (42%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 1.49-1.70 (11H, m,cyclohexyl ring); 3.85 (6H, s, 2OCH_3); 6.94-7.41 (3H,m , aromatic protons) and 8.91, 9.32 (2H, 2s, 2NH); Anal. for $C_{18}H_{22}N_4O_2$ (326.39) Calcd./Found(%): C; 66.24/66.66, H; 6.79/6.34, N; 17.17/16.85.

3-(4-Bromophenylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitrile(10b):

Yield 72 %; mp201^{0}C; **IR** υ(cm^{-1}): 3435, 3360(2NH),2216 (CN); **MS**: m/z $[M]^+$~ 399, $[M+2]^+$~ 401 (8, 7%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.83 (6H, s, 2OCH_3); 6.94-7.58 (7H,m , aromatic protons) and 9.26, 9.42 (2H, 2s, 2NH); Anal. for $C_{18}H_{15}BrN_4O_2$(399.24),Calcd./Found(%): C; 54.15/53.82, H; 3.79/3.48, N; 14.03/13.53.

3-(4-Acetylphenylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitrile (10c):

Yield 75 %; mp192^{0}C; **IR** υ(cm^{-1}): 3464, 3366(2NH),2215(CN); **MS**: m/z $[M]^+$~ 362(15%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 2.35 (3H, s, $COCH_3$); 3.86 (6H, s, 2OCH_3); 6.94-7.58 (7H,m , aromatic protons) and 9.11, 9.36 (2H, 2s, 2NH); Anal. for $C_{20}H_{18}N_4O_3$(362.38)Calcd./Found(%): C; 66.29/66.51, H; 5.01/5.19, N; 15.46/15.83.

Ethyl 4-(4-cyano-5-(3,4-dimethoxyphenyl)-1*H*-pyrazol-3-yl-amino)benzoate (10d):

Yield (79%); mp 186^{0}C; **IR** υ(cm^{-1}): 3470, 3365 (2NH),2217 (CN), 1727 (C=O); **MS**: m/z $[M]^+$~392(35%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 1.40 (3H, t, CH_2CH_3); 3.81 (6H, s, 2OCH_3); 4.12 (2H, q, CH_2CH_3); 6.94-7.57 (7H,m , aromatic protons) and 9.26, 9.31 (2H, 2s, 2NH); Anal. for $C_{21}H_{20}N_4O_4$ (392.41)Calcd./Found(%): C; 64.28/64.00, H; 5.14/5.29, N; 14.28/14.61.

3-(2-Hydroxyphenylamino)-5-(3,4-dimethoxyphenyl)-1*H*- pyrazole-4-carbonitrile (10e):

Yield 72 %; mp205^{0}C; **IR** υ(cm^{-1}): 3484-3356 (OH, 2NH),2218 (CN); **MS**: m/z $[M]^+$~336(15%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.85 (6H, s, 2OCH_3); 6.94-7.35 (7H,m , aromatic protons) and 9.01, 9.35, 10.13 (3H, 3s, 2NH, OH); Anal. for $C_{18}H_{16}N_4O_3$ (336.34) Calcd./Found(%): C; 64.28/64.78, H; 4.79/5.01, N; 16.66/16.99.

3-(2-Mercaptophenylamino)-5-(3,4-dimethoxyphenyl)-1*H*-pyrazole-4-carbonitrile (10f):

Yield 70 %; mp179^{0}C; **IR** υ(cm^{-1}): 3443, 3345 (2NH),2218 (CN); **MS**: m/z $[M]^+$~352 (6%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm):3.83 (6H, s, 2OCH_3); 6.94-7.29 (7H,m , aromatic protons) and 9.26, 9.39 (2H, 2s, 2NH); Anal. for $C_{18}H_{16}N_4O_2S$(352.41) Calcd./Found(%): C; 61.35/61.59, H; 4.58/5.08, N; 15.90/16.40.

5-(3,4-Dimethoxyphenyl)-3-(pyridin-2-ylamino)-1*H*-pyrazole-4-carbonitrile (10g):

Yield 70 %; mp182^{0}C; **IR** υ(cm^{-1}): 3445, 3327 (2NH),2218 (CN); **MS**: m/z $[M]^+$~ 321 (32%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.76 (6H, s, 2OCH_3); 6.94-7.31 (7H,m , aromatic protons) and 9.11, 9.20 (2H, 2s, 2NH); Anal. for $C_{17}H_{15}N_5O_2$(321.33)Calcd./Found(%): C; 63.54/63.33, H; 4.71/5.04, N; 21.79/21.98.

5-(3,4-Dimethoxyphenyl)-3-(thiozol-2-ylamino)-1*H*-pyrazole-4-carbonitrile (10h):

Yield 78 %; mp176^{0}C; **IR** υ(cm^{-1}): 3464, 3360 (2NH),2215 (CN); **MS**: m/z $[M]^+$~ 327 (15%); **^{1}H NMR** (DMSO-d_2) (*δ*ppm): 3.86 (6H, s, 2OCH_3); 6.94-7.30 (5H,m , aromatic protons) and 9.26, 9.31 (2H, 2s, 2NH); Anal. for $C_{15}H_{13}N_5O_2S$(327.36)Calcd./Found(%): C; 55.03/54.73, H; 4.00/3.74, N; 21.39/20.98.

General method for preparation of 4-(3,4-dimethoxybenzylidene)4*H*(4,5-dihydro)pyrazoles 12, 13:

To a solution of compound **11** (2.14gm, 0.01mol) in absolute ethanol (15 mL) containing few drops of piperidine, the appropriate hydrazine derivative namely: mono hydrogen hydrazine hydrate and phenyl hydrazine hydrate (0.01mol) was added. The reaction mixture was heated under reflux for 4h. After cooling, the solution was poured onto ice/cold water, the formed precipitate was filtered off, dried, and recrystallized from ethanol to give compounds **12, 13** respectively.

4-(3,4-Dimethoxybenzylidene)-4*H*-pyrazole-3,5-diamine (12):
Yield 85 %; mp175^{0}C; **IR** υ(cm^{-1}): 3465-3266 (2NH$_2$); **MS**: m/z [M]$^+$~ 246 (35%); **^{1}H NMR** (DMSO-d$_2$) (*δ*ppm): 3.87 (6H, s, 2OCH$_3$); 5.67 (4H, brs, 2NH$_2$); 6.32 (1H, s, -C=C*H*) and6.94-7.36 (3H,m , aromaticprotons); Anal. for $C_{12}H_{14}N_4O_2$ (246.27), Calcd./Found(%): C; 58.53/58.13, H; 5.73/5.60, N; 22.75/22.51.

4-(3,4-Dimethoxybenzylidene)-4,5-dihydro-5-imino-1-phenyl-1*H*-pyrazol-3-amine (13):
Yield 72 %; mp125^{0}C; **IR** υ(cm^{-1}): 34470-3286 (NH, NH$_2$),2215(CN); **MS**: m/z [M-1]$^+$~ 321 (73%); **^{1}H NMR** (DMSO-d$_2$) (*δ*ppm):3.85 (6H, s, 2OCH$_3$); 5.61 (2H, s, NH$_2$); 6.40 (1H, s, -C=C*H*); 6.94-7.32 (8H,m , aromatic protons) and 9.26 (1H, s, NH); Anal. for $C_{18}H_{18}N_4O_2$ (322.36), Calcd./Found(%): C; 67.07/67.50, H; 5.63/5.89, N; 17.38/17.63.

2-(3,4-Dimethoxybenzylidene)-3-amino-2,3,4,5-tetrahydro-5-oxopyridine-4-carbonitrile (14):
To a solution of compound **11**(2.14 gm, 0.01 mol) in sodium ethoxide solution (0.01 mol) [sodium metal (0.23 gm, 0.01 mol) in absolute ethanol (50 mL)], cyanoacetamide (0.84 gm, 0.01 mol) was added and the reaction mixture was refluxed for 4h. The solid that formed upon pouring onto ice-water containing few drops of hydrochloric acid was collected by filtrationand recrystallized from ethanol to give compound **14**.

Yield 80 %; mp288^{0}C; **IR** υ(cm^{-1}): 3456, 3345 (NH$_2$),2220 (CN), 1689 (C=O); **MS**: m/z [M]$^+$~ 285(35%); **^{1}H NMR** (DMSO-d$_2$) (*δ*ppm): 3.60, 3.72 (2H, 2d, 2CHof tetrahydropyridine ring); 3.83(6H, s, 2OCH$_3$); 5.62 (2H, s, NH$_2$); 6.46 (1H, s, -C=C*H*) and 6.94-7.45 (4H,m , aromatic protons) Anal. for $C_{15}H_{15}N_3O_3$ (285.30), Calcd./Found(%): C; 63.15/63.47, H; 5.30/4.98, N; 14.73/14.50.

General method for preparation of 5-(3,4-dimethoxy benzylidene)-4,6-diamino-pyrimidin-2(5*H*)-one (thione) derivatives 15a,b:
To a solution of compound **11** (2.14 gm, 0.01 mol) in sodium ethoxide solution (0.01mol) [sodium metal (0.23gm, 0.01mol) in (50 mL) absolute ethanol], urea or thiourea (0.01 mol) was added.Then the reaction mixture was refluxed for 3h. The solid that formed upon pouring onto ice-water containing few drops of hydrochloric acid, was collected by filtrationand recrystallized from methanol to give compounds **15a,b** respectively.

5-(3,4-Dimethoxybenzylidene)-4,6-diaminopyrimdin-2(5*H*)-one (15a):
Yield (74 %); mp179^{0}C; **IR** υ(cm^{-1}): 3474-3335 (2NH$_2$),1685 (C=O); **MS**: m/z [M]$^+$~ 274(40%); **^{1}H NMR** (DMSO-d$_2$) (*δ*ppm): 3.83 (6H, s, 2OCH$_3$); 5.65 (4H, s, 2NH$_2$); 6.66 (1H, s, -C=C*H*) and 6.94-7.25 (3H,m , aromatic protons); Anal. for $C_{13}H_{14}N_4O_3$ (274.28), Calcd./Found(%): C; 56.93/57.18, H; 5.14/5.64, N; 20.43/20.87.

5-(3,4-Dimethoxybenzylidene)-4,6-diaminopyrimdin-2(5*H*)-thione (15b):
Yield 70 %; mp240^{0}C; **IR** υ(cm^{-1}): 3476-3345 (2NH$_2$), 1163 (C=S); **MS**: m/z [M]$^+$~ 290(5%); **^{1}H NMR** (DMSO-d$_2$) (*δ*ppm):3.73 (6H, s, 2OCH$_3$); 5.65 (4H, s, 2NH$_2$); 6.51 (1H, s, -C=C*H*) and 6.94-7.34 (3H,m , aromatic protons); Anal. for $C_{13}H_{14}N_4O_2S$(290.34) Calcd./Found(%): C; 53.78/53.64, H; 4.86/4.50, N; 19.30/18.80.

Anticancer Evaluation
Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide) to purple formazan[20].

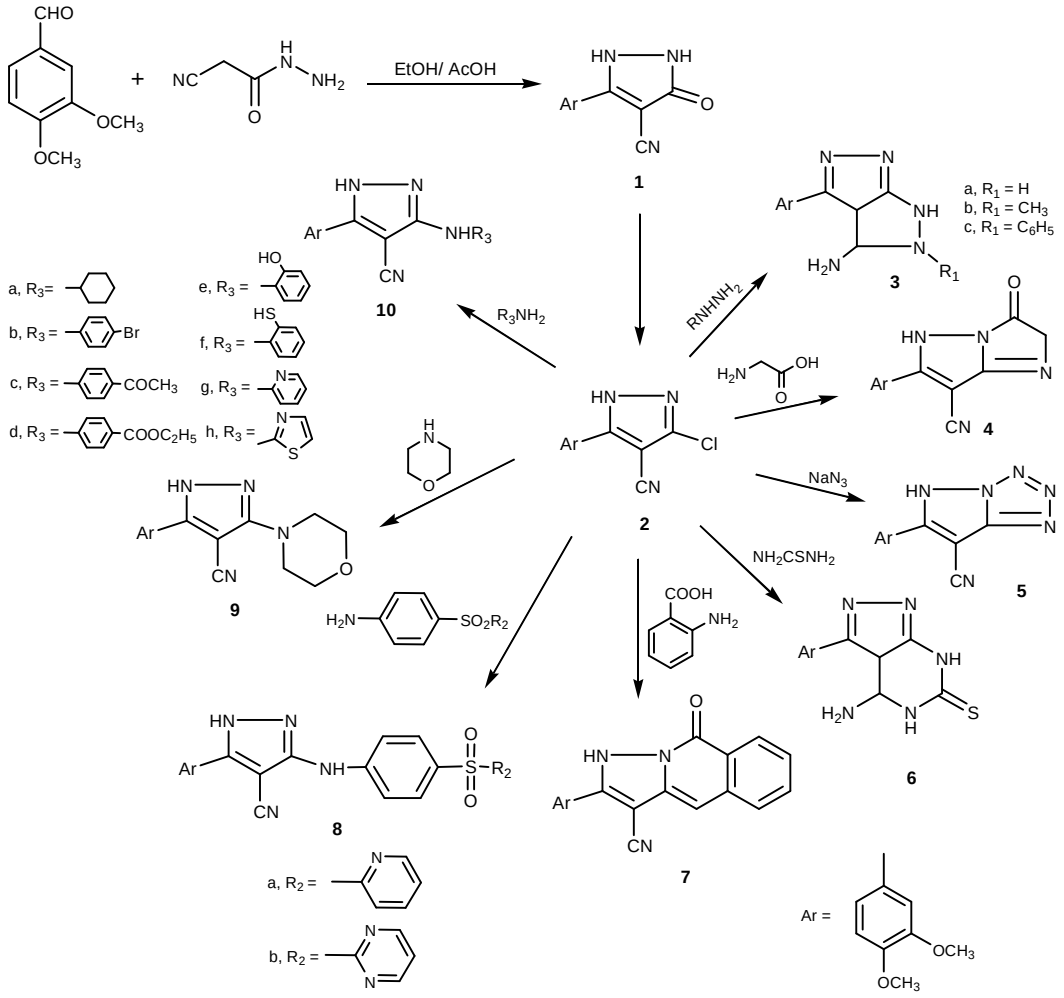
Procedure: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level(Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2- MCF7 and HCT116 – DMEM for A549. The media are supplemented with 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37 ºC under 5% CO2.

Cells were batch cultured for 10 days, then seeded at concentration of 10x10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37 ºC for 24 h under 5% CO2 using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100-50-25-12.5-6.25-3.125-0.78 and 1.56 ug/ml). After 48 h of incubation, medium was aspirated, 40ul MTT salt (2.5µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200µL of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [21,22].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula:

((Reading of extract / Reading of negative control) -1) x 100

A probit analysis was carried for IC50 determination using SPSS 11 program.



Scheme 1

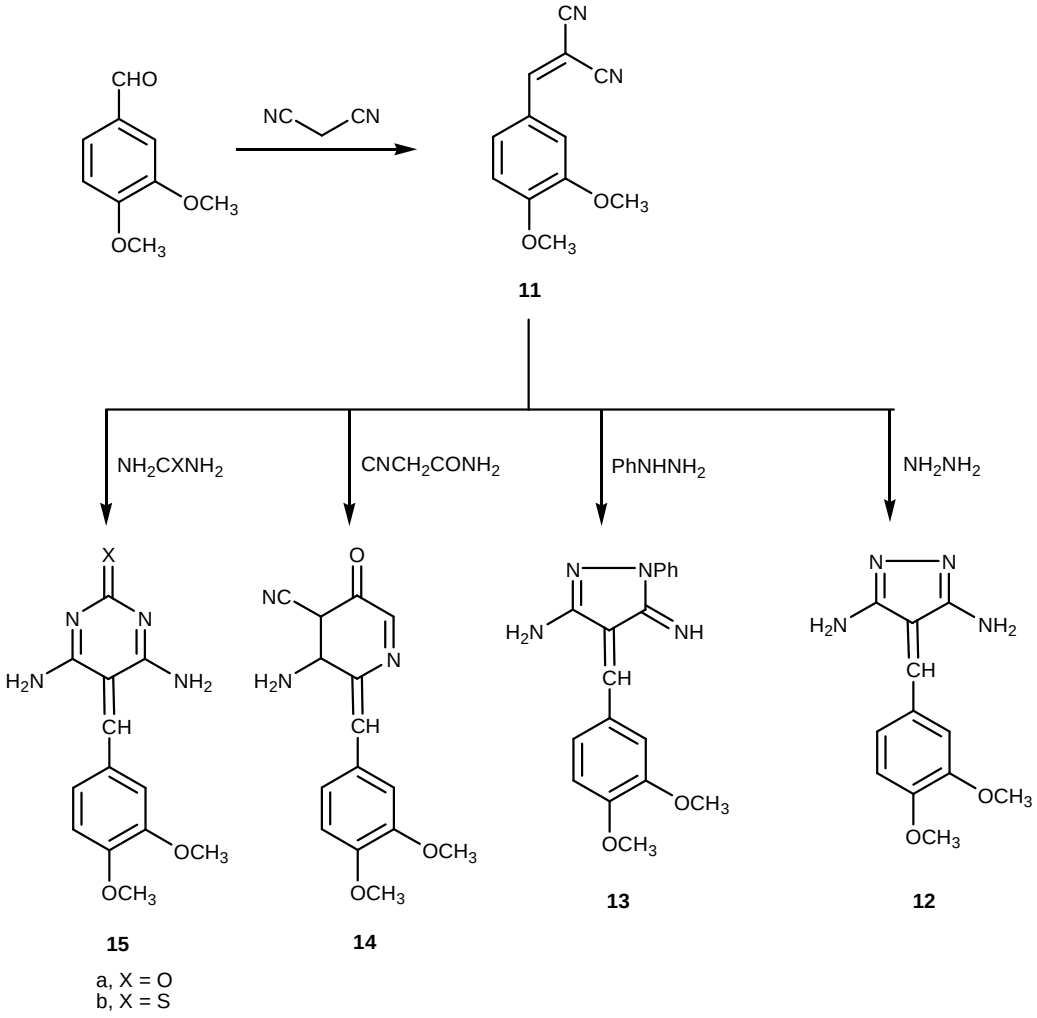
RESULTS AND DISCUSSION

Chemistry

In this study, 3,4-dimethoxybenzaldehyde was condensed with cyanoacetohydrazide in boiling ethanol acidified with acetic acid to affordthe pyrazole derivative **1**, which was heated with phosphorus oxychloride and phosphorus pentachlorideto give 5-chloropyrazole derivative **2**. The chloropyrazole compound **2** was considered a very useful intermediate for building up new derivatives carrying heterocyclic rings combined to or fused with the parent pyrazole moiety. Thus, condensation of **2** with hydrazine hydrate 98%, methyl hydrazine and/or phenyl hydrazine underwent ring closure to give the corresponding pyrazolo[3,4-*c*]pyrazole derivatives **3a-c**. Moreover, cyclo-condensation of compound **2** with glycine in n-butanol produced the imidazo[1,2-*b*]pyrazole derivative **4**, while its cyclization with sodium azide in n-butanol gave the tetrazolo derivative **5**. Furthermore, the reaction of **2** with thiourea in refluxing ethanol gave the pyrazolo-thiopyrimidine derivative **6**. The fused pyrazoloquinazolinone derivative **7** was obtained by thetreatment of compound **2** with anthranilic acid in ethanol. The sulfa derivatives **8a,b** had been synthesized by the reaction of **2** with sulphapyridine and sulphadiazine in absolute ethanol.

Furthermore, the condensation of **2** with morpholine gave the corresponding 3-morpholinopyrazole derivative **9**. On the other hand, upon condensation of compound **2** with different primary amines named: cyclohexyl amine, *p*-bromoaniline, *p*-aminoacetophenone andethyl *p*-aminoethylbenzoate afforded 3-substituted aminopyrazole derivatives **10a-d**, respectively. The reaction of compound **2** with *o*-aminophenol, *o*-aminothiophenol, 4-aminopyridine,2-aminothiazole in methanol containing few drops of pyridine gave the corresponding 3-substituted aminopyrazole derivatives **10e-h**(scheme 1).

Also, this study includes the reaction of 3,4-dimethoxybenzaldehyde with malononitrile in ethanol containing a catalytic amount of triethylamine to afford the arylidenemalononitrile derivative **11** that was cyclized with hydrazine hydrate, phenyl hydrazine to give the substituted pyrazole derivatives **12**, **13**, respectively. While cyclization of **11** with cyanoacetamide, urea and/or thioureaafforded pyrimidinederivatives **14** and **15a,b**, respectively (scheme 2) .



Scheme 2

Anticancer Evaluation

In this work, eleven compounds of the newly synthesized derivatives [1, 3a, 4, 6, 8b, 10a, 10d, 10h, 13, 14, 15b] were chosen as representative examples to evaluate their cytotoxic activity against three different carcinoma cell lines (MCF-7, HCTH-6 , HePG-2). Doxorubicin was used as a reference drug.

According to the obtained results in tables (**1, 2**), the examined carcinoma cell lines appeared to be insensitive to most of the tested compounds. The data showed that moderate cytotoxic activity against both MCF-7 and HCTH-6 carcinoma cells lines was gained by 3,5-diaminopyrazolyl derivative**13**with IC50 : 92.6، 82.6µg/ml respectively.

At the same time, 4,6-diaminopyrimidinyl derivative **15b**exhibited moderate effect against HePG-2 carcinoma cell line of IC50: 92.6µg/ml.

Based on the above mentioned results, the resistance of the tested carcinoma cell lines might be due to the improper fitting of the tested compounds with the protein receptors enzymes that they act on.

Thus, more structural modifications should be carried out to get new effective molecules as cytotoxic agents in cancer therapy field.

Table 1.Anticancer effect of the selected compounds onMCF-7 [Human Caucasian breast adenocarcinoma], HePG-2 [Human hepatocellular carcinoma cell line]andHCT11-6 [Colon cell line]

Sample No	Remarks onMCF-7	Remarks onHePG-2	Remarks on HCT1-6
1	10.4% at 100ppm	38.3% at 100ppm	0% at 100ppm
3a	5.6% at 100ppm	20.4% at 100ppm	13% at 100ppm
4	9.7% at 100ppm	0% at 100ppm	11.8% at 100ppm
6	26.9% at 100ppm	18.7% at 100ppm	36.7% at 100ppm
8b	16.7% at 100ppm	38.1% at 100ppm	7.5% at 100ppm
10a	0% at 100ppm	10.8% at 100ppm	0% at 100ppm
10d	14.6% at 100ppm	38.5% at 100ppm	62.4% at 100ppm
10h	8.2% at 100ppm	13.6% at 100ppm	0% at 100ppm
13	51.7% at 100ppm	30.7% at 100ppm	62.4% at 100ppm
14	21.3% at 100ppm	0% at 100ppm	23.3% at 100ppm
15b	22.5% at 100ppm	52.4% at 100ppm	12.6% at 100ppm
DMSO	3% at 100ppm	1% at 100ppm	1% at 100ppm
Negative control	0 %	0 %	0 %

Table 2.Anticancer effect of the compounds13, 15bonMCF-7, HePG-2 andHCT11-6 cell line

Sample No	LC_{50} (µg/ml)onMCF7	LC_{50} (µg/ml)onHePG 2	LC_{50} (µg/ml) on HCT116
13	92.3		82.8
15b		92.6	
Doxorubicin	26.1	21.6	37.6

***LC_{50}**: Lethal concentration of the sample which causes the death of 50% of cells in 48 h.*

CONCLUSION

This study deals with synthesis of novel derivatives of pyrazole nucleus attached to or fused with various heterocyclic ring systems such as imidazole, tetrazole and (thio)pyrimidine rings. Some of the newly synthesized compounds were examined as anticancer agentsagainst three different carcinoma cell lines (MCF-7, HCTH-6, HePG-2). The derivatives 3,5-diaminopyrazolyl **13** and 4,6-diaminopyrimidinyl **15b** appeared to be of potential cytotoxic activity.

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