Egypt. J. Chem. **Vol. 63**, No. 12, pp. 5203 - 5218 (2020)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Design and Synthesis of Novel Pyrazoles, Pyrazolines, and Pyridines from Chalcone Derivatives with Evaluation of Their *In Vitro* Anticancer Activity Against T-47D and UACC-257 Cell Lines

Mangoud M. Mangoud[a*1], Mohamed Z. Hussein[a], Eman A. El-Bordany[b]

[a] *Environmental Research Department, National Centre for Social and Criminological Research, Ibn Khaldoun Square, Mohandesin, Zamalek, Giza 11561, Egypt;*
[b] *Department of Chemistry, Faculty of Science, Ain Shams University, Cairo 32511, Egypt;*

Abstract

Novel series of pyrazoline carbothioamides, acetyl pyrazoles, pyridine-3-carbonitrile, pyridine-2-carbonitriles, and nicotinonitriles were synthesized. The structures of the newly synthesized compounds were established based on their spectral data, elemental analyses and alternative synthetic routes whenever possible. Also, the newly synthesized compounds were screened for their *in vitro* anticancer activity against T-47D (breast cancer human cell line) and UACC-257 (melanoma human cell line) by MTT assay (a colorimetric assay for assessing cell metabolic activity). Experimental results demonstrated that compounds **3a**, **4a**, and **8c** showed excellent *in vitro* activity against against T-47D cell line, while compounds **3d**, **6d**, and **5b** exhibited excellent activity against UACC-257 cell line. Molecular docking studies were performed on compound **4a**, as it showed the most promising activity against T-47D cell line, using Molecular Operation Environment (MOE 2008.10) software. Also, drug-likeness, pharmacokinetics, and toxicity assessment studies were carried out.

Keywords: Anticancer; docking; pharmacokinetics; chalcones; pyrazolines; carbonitriles; cyanopyridines.

1. Introduction

Heterocyclic compounds are considered an exceptionally important class of compounds which play a key role in health care and prescription drug design. Chalcones have been reported to possess various biological activities. In addition they are well known intermediates for the synthesis of various heterocyclic compounds like pyrazoles, pyrazolines, pyridines, etc.

Pyridines are class of organic heterocyclic compounds consisting of six-member ring of five carbons and one nitrogen atom. Pyridines and their derivatives exhibit various pharmacological properties such as antimicrobial [1-3], antiviral [4, 5], antioxidant [2, 6], antidiabetic [7, 8], anticancer [9-11], antimalarial [12], anti-inflammatory [13, 14], analgesic [13, 15], anti-convulsant [13, 15], and antiparkansonian agents [13, 15].

Additionally pyrazoles, which are five-membered two-nitrogen-containing heterocycles, are very important organic compounds. Numerous compounds containing pyrazole moiety are known to exhibit antidiabetic [16], analgesic [17], anti-inflammatory [17, 18], antipyretic [18], antimicrobial [19], antihypertensive [20] and anticancer [21, 22] activities.

Pyrazolines also are well known and important nitrogen-containing 5-membered heterocycles, which were found to have a broad spectrum of biological antitubercular [23], antidepressant [24, 25], anticonvulsant [25, 26], antiproliferative [23, 27, 28], anti-inflammatory [29, 30] and analgesic [31] activities.

This gave us immense confidence to synthesize novel heterocyclic compounds bearing pyrazoline, pyrazole, and pyridine moieties starting from chalcones and screening all the newly synthesized compounds for their *in vitro* inhibition capacity against two human cancer cell lines, melanoma (UACC-257) and breast cancer (T-47D), in

*Corresponding author e-mail: mangoud_online@yahoo.com.; *Tel.: +20-01098476136.*
Receive Date: 27 April 2020, Revise Date: 19 June 2020, Accept Date: 22 June 2020
[1]This paper is abstracted from his PhD.
DOI: 10.21608/EJCHEM.2020.28793.2620

comparison to the well-known anticancer drug cisplatin.

Molecular docking is a very useful and reasonably reliable for prediction of putative binding modes and affinities of ligands for macromolecules. Such methods are gaining popularity because the experimental determination of complex structures is rather difficult and highly expensive. Over the years, the speed and accuracy of computational docking methods have improved, and these methods now play a pivotal role in structure-based drug design. Also, the newly synthesized compounds will be *in silico* assessed for their drug-likeness, pharmacokinetics, and toxicity.

2. Experimental

2.1. *Chemistry*

1-Phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde **(1a)** was readily prepared according to the procedures previously described in the literature [32]. 4-Methylacetophenone was purchased from Riedel-De Haen AG Co., 2-acetyl furan was purchased from loba chemie, and 2-acetylfuran was purchased from Alfa Aesar Co., while *p*-dimethylaminobenzaldhyde was purchased from RESEARCH-LAB FINE CHEM INDUSTRIES. All melting points were determined on an electrothermal apparatus and were uncorrected. IR spectra were recorded (KBr discs) on a Shimadzu FT-IR 8201 PC spectrophotometer. ^{1}H NMR spectra were recorded in $(CD_3)_2SO$ solutions on a Varian Gemini 300 MHz and a Bruker 400 MHz spectrometer and chemical shifts are expressed in δ ppm units using TMS as an internal reference at microanalytical unit, Cairo university. Mass spectra were recorded on a GC-MS QP1000 EX Shimadzu. Elemental analyses were carried out at the Micro-analytical Center of Cairo University. The chemical names given for the synthesized compounds are according to the IUPAC system.

2.1.1. *Synthesis of Chalcone derivatives 3a-d*

General procedure for synthesis:10% NaOH (0.2 g, 5 mmol) was added dropwise to a mixture of the appropriate 2-acetylthiophene (0.63 g, 5 mmol) or 2-acetylfuran (0.54 g, 5 mmol) or 4-methylacetophenone (0.67 ml, 5 mmol) and the appropriate of 1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde (**1a**) (1.27 g, 5 mmol), 4-(dimethylamino)benzaldehyde (0.74 g, 5 mmol) (**1b**) in ethanol (25 ml), at 0-5 ^{0}C while stirring. The precipitate formed was filtered, washed with ethanol, and recrystallized from the appropriate solvent gave **3a-d**, respectively.

(*E*)-3-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-1-(thiophen-2-yl)prop-2-en-1-one (3a)
Yellow solid from glacial acetic acid, yield (1.57g, 87%), mp: 183–184 °C; **IR (KBr, cm^{-1}):** 3065 (=C–H), 1643 (–C=O), 1583 (–C=N); **^{1}H-NMR:** δ 7.24-7.92 (*m*, 11H, Ar-H + thienyl-H + vinyl-H), 8.06 (*d*, *J* = 3.0 Hz, 1H, thienyl-C22-H), 8.14 (*d*, *J* = 2.7 Hz, 1H, thienyl-C24-H), 9.36 (*s*, 1H, pyrazole-H); **MS (m/z, %):** 364 (M+2, 3), 363 (M+1, 6), 362 (M+, 24), 252 (20), 251 (100), 121 (12), 111 (33), 77 (39), 51 (14); **Anal. Calcd. for $C_{20}H_{14}N_2OS_2$** (362.47): C, 66.27; H, 3.89; N, 7.73; S, 17.69; found: C, 66.29; H, 3.88; N, 7.72; S, 17.69.

(*E*)-3-(4-(dimethylamino)phenyl)-1-(thiophen-2-yl)prop-2-en-1-one (3b)
mp: 113-115 ^{0}C, Reported mp: 114-115 ^{0}C. [33]

(*E*)-3-(4-(dimethylamino)phenyl)-1-(furan-2-yl)prop-2-en-1-one (3c)
mp: 105-106 ^{0}C, Reported mp: 95-97 ^{0}C. [34]

(*E*)-3-(4-(dimethylamino)phenyl)-1-(*p*-tolyl)prop-2-en-1-one (3d)
mp :119-121 ^{0}C, Reported value of mp: 124-125 ^{0}C. [35]

2.1.2. *Synthesis of carbothioamide derivatives* 4a-c

To a mixture of the appropriate chalcones **3a-d** (5 mmol) and thiosemicarbazide (0.46 g, 5 mmol) in ethanol (20 ml) 40% NaOH was added then the mixture was refluxed for 3 h. The result solid was collected and recrystallized from the appropriate solvent gave **4a-c**, respectively.

1'-Phenyl-3',5-di(thiophen-2-yl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazole]-2-carbothioamide (4a)
White solid from chloroform, yield (1.32g, 61%), mp: 278-280°C; **IR (KBr, cm^{-1}):** 3437, 3282 (N–H), 3086 (=C–H); **^{1}H-NMR:** δ 3.22 (*dd*, *J* = 0.7 and 3.6 Hz, 1H, pyrazoline-H), 4.06 (*q*, *J* = 11.4 and 3.6 Hz, 1H, pyrazoline-H), 6.18 (*dd*, , *J* = 3.6 and 7.8 Hz, 1H, pyrazoline-H), 7.13 (*dd*, *J* = 3.6 and 1.2 Hz, 1H, thienyl-H), 7.26-7.84 (*m*, 11H, Ar–H + thienyl-H + Pyrazole-H), 8.12 (*s*, 2H, -NH_2); **MS (m/z, %):**438 (M+3, 1), 437 (M+2, 4), 436 (M+1, 8), 435 (M+ , 25), 402 (30), 325 (25), 311 (30), 293 (12), 266 (15), 252 (18), 251 (17), 234 (13), 110 (20), 109 (16), 104 (18), 97 (15), 77 (100), 69 (12), 60 (35), 51 (27); **Anal. Calcd. for $C_{21}H_{17}N_5S_3$** (435.59): C, 57.90; H, 3.93; N, 16.08; S, 22.08; found: C, 57.94; H, 3.91; N, 16.07; S, 22.01.

5-(4-(dimethylamino)phenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (4b)
mp: 218-220 ^{0}C, Reported mp: 220-221 ^{0}C. [36]

5-(4-(Dimethylamino)phenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (4c)
Yellowish white solid from acetonitril, yield (0.9g, 53%), mp: 278-280 ^{0}C; **IR (KBr, cm^{-1}):** 3410 (N–H), 3051 (=C–H), 2989 (-C–H); **^{1}H-NMR:** δ 2.34 (*s*, 3H, *p*-CH_3), 2.83 (*s*, 6H, N-(CH_3)$_2$), 3.04 (*dd*, *J* = 2.4 and 15.3 Hz, 1H, pyrazoline-H), 3.79 (*q*, 1H, pyrazoline-H), 5.80 (*dd*, 1H, pyrazoline-H), 6.62-7.77 (*m*, 8H, Ar–H), 7.93 (*s*, 2H, -NH_2); **MS (m/z, %):**339 (M+1, 2), 338 (M+, 8), 222 (73), 205 (82), 147 (100), 146 (75), 132 (20), 118 (39), 91 (34), 77 (40), 60 (93);

Anal. Calcd. for $C_{19}H_{22}N_4S$ (338.47): C, 67.42; H, 6.55; N, 16.55; S, 9.47; found: C, 67.40; H, 6.54; N, 16.57; S, 9.48.

2.1.3. *Synthesis of Acetyl pyrazolines 5a-c*

A mixture of chalcone (0.01 mol), hydrazine hydrate (0.02 mol) and few drops of glacial acetic acid in 15 ml ethanol was refluxed for 2-3 h, and then the reaction mixture was distilled off to remove the excess solvent, poured into crushed ice. The solid obtained was washed with cold water, dried and recrystllized from an appropriate solvent.

1-(1'-Phenyl-3',5-di(thiophen-2-yl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)ethanone (5a)

Beige solid from glacial acetic acid, yield (1.70g, 81%), mp: 219-220 ^{0}C; **IR (KBr, cm^{-1}):** 3083 (=C–H), 2928 (-C-H), 1651 (-C=O); **^{1}H-NMR:** δ 2.32 (*s*, 3H, -CH_3), 3.21 (*dd*, *J* = 4.7 and 12.6 Hz, 1H, pyrazolinyl-H) , 3.99 (*dd*, *J* = 11.6 and 5.8 Hz, 1H, pyrazolinyl-H), 5.81 (*q*, *J* = 11.6 and 6.8 Hz, 1H, pyrazolinyl-H), 7.12-7.88 (*m*, 11H, Ar-H + thienyl-H), 8.36 (*s*, 1H, pyrazolyl-H); **MS (m/z, %):** 420 (M+2, 12), 419 (M+1, 29), 418 (M+, 100), 377 (23), 376 (76), 375 (56), 344 (17), 343 (66), 266 (14), 251 (38), 237 (18), 226 (24), 110 (20), 77 (87), 51 (23); **Anal. Calcd. for $C_{22}H_{18}N_4OS_2$**(418.53): C, 63.13; H, 4.33; N, 13.39; S, 15.32; found: C, 63.14; H, 4.32; N, 13.40; S, 15.31.

1-(5-(4-(dimethylamino)phenyl)-3-(thiophen-2-yl)-1*H*-pyrazol-1-yl)ethan-1-one (5b)

Pale yellow solid from glacial acetic acid, yield (1.16 g, 74%), mp: 151-152 ^{0}C; **IR (KBr, cm^{-1}):** 3032 (=C–H), 2931 (-C-H), 1662 (–C=O); **^{1}H-NMR:** δ 2.22 (*s*, 3H, -COC$\underline{H}_3$), 2.85 (*s*, 6H, N-($\underline{CH}_3)_2$), 3.12 (*dd*, *J* = 4.1 and 13.6 Hz,1H, pyrazolinyl-H) , 3.80 (*q*, *J* = 11.6 and 6.1 Hz,1H, pyrazolinyl-H), 5.44 (*dd*, *J* = 4.0 and 7.5 Hz,1H, pyrazolinyl-H), 7.65 (*d*, *J* = 0.5 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 6.67 (*d*, *J* = 0.6 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 6.98 (*d*, *J* = 0.6 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.00 (*d*, *J* = 0.6 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.15 (*q*, *J* = 3.7 and 1.2 Hz,1H, thienyl-H), 7.44 (*dd*, *J* = 0.1 and 3.0 Hz, 1H, thienyl-H), 7.72 (*dd*, *J* = 0.8 and 4.2 Hz, 1H, thienyl-H); **MS (m/z, %):** 315 (M+2, 3), 314 (M+1, 10), 313 (M+, 40), 312 (10), 204 (100), 203 (45), 196 (14), 189 (77), 162 (19), 147 (36), 146 (38), 134 (21), 121 (13); **Anal. Calcd. for $C_{17}H_{19}N_3OS$** (313.42): C, 65.15; H, 6.11; N, 13.41; S, 10.23; found: C, 65.17; H, 6.12; N, 13.39; S, 10.22.

1-(5-(4-(Dimethylamino)phenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-1-yl)ethan-1-one (5c)

White solid from glacial acetic acid, yield (0.87g, 54%), mp: 135-137 ^{0}C; **IR (KBr, cm^{-1}):** 3008 (=C–H), 2920 (-C–H), 1666 (-C=O amide); **^{1}H-NMR:** δ 2.27 (*s*, 3H, -COC$\underline{H}_3$), 2.35 (*s*, 3H, *p*-$\underline{CH}_3$), 2.84 (*s*, 6H, N-($\underline{CH}_3)_2$), 3.06 (*dd*, *J* = 4.2 and 13.7 Hz, 1H, pyrazolinyl-H) , 3.75 (*q*, *J* = 11.6 and 6.3 Hz, 1H, pyrazolinyl-H), 5.40 (*dd*, *J* = 4.1 and 7.5 Hz,1H, pyrazolinyl-H), 6.64 (*d*, *J* = 0.6 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 6.67 (*d*, *J* = 0.7 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 6.98 (*d*, *J* = 0.6 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.00 (*d*, *J* = 0.5 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.27 (*d*, *J* = 0.6 Hz,1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.29 (*d*, *J* = 0.6 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.67 (*d*, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.69 (*d*, 1H, *p*-CH_3-C_6H_3-$\underline{H}$); **MS (m/z, %):** 323 (M+2, 3), 322 (M+1, 20), 321 (M+, 87), 320 (14), 205 (19), 204 (74), 203 (39), 190 (18), 189 (100), 162 (28), 147 (60), 146 (70), 121 (18), 120 (17), 115 (23), 91 (37), 77 (31), 65 (20); **Anal. Calcd. for $C_{20}H_{23}N_3O$** (321.42): C, 74.74; H, 7.21; N, 13.07;; found: C, 74.76; H, 7.22; N, 13.04.

2.1.4. *Synthesis of Carbonitriles 6a-d*

First method: Chalcone (10 mmol), ethyl cyanoacetate (1.06 ml, 10 mmol), ammonium acetate (0.77 g, 10 mmol) were refluxed in glacial acetic acid for 3-4 h., then acetic acid was evaporated under reduced pressure, precipitated by crushed ice, filtered, and recrystallized from an appropriate solvent.

Second method: Aldehyde (10 mmol), aryl ketone (10 mmol), ethyl cyanoacetate (1.06 ml, 10 mmol), ammonium acetate (6.00 g, 77 mmol) were refluxed in n-butanol for 3-4 h., then the solvent was evaporated under reduced pressure, precipitated by crushed ice, washed by ethanol and recrystallized from an appropriate solvent.

2-Oxo-4-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile (6a)

Pale yellow solid from glacial acetic acid, yield (1.58 g, 74%), mp: 250-251 ^{0}C; **IR (KBr, cm^{-1}):** 3380 (-N-H), 3149 (=C–H), 2221 (-CN), 1696 (-C=O); **^{1}H-NMR:** δ 7.26 (*s*, 1H, thienyl-H), 7.43-7.90 (*m*, 11H, Ar-H + thienyl-H), 8.19 (*s*, 1H, Pyrazole-$\underline{H}$), 9.12 (*s*, 1H, Pyridine-$\underline{H}$); **MS (m/z, %):** 424 (M-2, 0.7), 321 (19), 320 (81), 277 (23), 276 (92), 129 (10), 104 (11), 95 (18), 85 (18), 84 (17), 83 (26), 81 (17), 77 (100), 69 (35), 57 (54), 55 (48), 51 (37); **Anal. Calcd. for $C_{23}H_{14}N_4OS_2$** (426.51): C, 64.77; H, 3.31; N, 13.14; S, 15.04; found: C, 64.73; H, 3.33; N, 13.13; S, 15.07.

4-(4-(Dimethylamino)phenyl)-2-hydroxy-6-(thiophen-2-yl)nicotinonitrile (6b)

Yellow solid from glacial acetic acid, yield (1g, 63%), mp: 359-361 ^{0}C; **IR (KBr, cm^{-1}):** 3093 (=C–H), 2904 (-C-H), 2214 (-CN), 1635 (-C=O); **^{1}H-NMR:** δ 3.02 (*s*, 6H, ($\underline{CH}_3)_2$-N-), 6.83 (*d*, *J* = 0.5 Hz, 1H, $(CH_3)_2$N-C_6H_3-$\underline{H}$) , 6.85 (*d*, *J* = 0.6 Hz, 1H, $(CH_3)_2$N-C_6H_3-$\underline{H}$), 7.25-7.86 (*m*, 13H, Ar-H + thienyl-H), 7.94 (*s*, 1H, Pyridine-$\underline{H}$), 11.53 (*s*, 1H, -OH); **MS (m/z, %):**323 (M+2, 7), 322 (M+1, 25), 321 (M+, 100), 320 (64), 306 (5), 278 (5), 248 (3), 167 (2), 139 (5), 113 (3), 77 (4), 57 (10), 55 (8); **Anal. Calcd. for $C_{18}H_{15}N_3OS$** (321.40): C, 67.27; H, 4.70; N, 13.07; S, 9.98; found: C, 67.29; H, 4.69; N, 13.07; S, 9.97.

4-(4-(Dimethylamino)phenyl)-6-(furan-2-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (6c)

Yellow solid from glacial acetic acid, yield (1.23g, 61%), mp: 361-363 ^{0}C; **IR (KBr, cm^{-1}):** 3101 (=C–

H), 2897 (-C-H), 2210 (-CN), 1643 (-C=O); **^{1}H-NMR:** δ 3.02 (*s*, 6H, $(\underline{CH_3})_2$-N-), 6.77 (*d*, *J* = 1.5 Hz, 1H, $(CH_3)_2N$-C_6H_3-$\underline{H}$) , 6.78 (*d*, *J* = 3.5 Hz, 1H, $(CH_3)_2N$-C_6H_3-$\underline{H}$), 6.77-7.65 (*m*, 5H, Ar-H + furyl-H), 8.00 (*s*, 1H, Pyridine-H), 12.5 (*s*, 1H, -O$\underline{H}$); **MS (m/z, %):** 307 (M+2, 4), 306 (M+1, 21), 305 (M+, 100), 304 (M-1, 71), 262 (4), 152 (6), 123 (3); **Anal. Calcd. for $C_{18}H_{15}N_3O_2$** (305.33): C, 70.81; H, 4.95; N, 13.76; found: C, 70.83; H, 4.94; N, 13.75.

4-(4-(Dimethylamino)phenyl)-2-hydroxy-6-(*p*-tolyl)nicotinonitrile (6d)

Yellow solid from glacial acetic acid, yield (1.27g, 77%), mp: 327-329 ^{0}C; **IR (KBr, cm^{-1}):** 3429 (-O-H), 3083 (=C–H), 2915 (-C–H), 2208 (-CN), 1703 (-C=O); **^{1}H-NMR:** δ 2.37 (*s*, 3H, *p*-$\underline{CH_3}$-C_6H_4-),3.01 (*s*, 6H, $(\underline{CH_3})_2$-N-), 6.72 (*s*, 1H, Pyridine-H), 6.81 (*d*, *J* = 8.7 Hz, 2H, *C12*,*14*-$\underline{H}$) , 7.31 (*d*, *J* = 8.1 Hz, 2H, *C20*,*C22*-$\underline{H}$), 7.65 (*d*, *J* = 8.4 Hz, 2H, *C11*,*C15*-$\underline{H}$), 6.67 (*d*, *J* = 7.8 Hz, 2H, *C19*,*C23*-H), 12.25 (*s*, 1H, -OH); **MS (m/z, %):** 331 (M+2, 3), 330 (M+1, 24), 329 (M+, 100), 328 (57), 314 (6), 300 (3), 286 (4), 164 (16), 128 (6), 118 (5), 77 (5), 57 (8), 55 (8); **Anal. Calcd. for $C_{21}H_{19}N_3O$**(329.40): C, 76.57; H, 5.81; N, 12.76; found: C, 76.55; H, 5.80; N, 12.79.

2.1.5. *Synthesis of picolinonitriles 8a-d*

First method**:** Chalcone (10 mmol), ω-cyanoacetophenone (1.45 g, 10 mmol), ammonium acetate (0.77 g, 10 mmol) were refluxed in glacial acetic acid for 3-4 h., then acetic acid was evaporated under reduced pressure, precipitated by ice, filtered, washed with ethanol, and recrystallized from an appropriate solvent.

Second method**:** Aldehyde (10 mmol), arylketone (10 mmol), ω-cyanoacetophenone (1.45 g, 10 mmol), ammonium acetate (6.00 g, 77 mmol) were refluxed in n-butanol for 2-3 h., then the solvent was evaporated under reduced pressure, the residue formed was precipitated by crushed ice, washed several times with water and ethanol, and then recrystallized from an appropriate solvent.

3-Phenyl-4-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)picolinonitrile (8a)

Pale yellow solid from glacial acetic acid, yield (1.55 g, 64%), mp: 181-183 ^{0}C; **IR (KBr, cm^{-1}):** 3057 (=C–H), 2217 (-CN); **^{1}H-NMR:** δ 7.21 (*q*, *J* = 4.0 and 0.2 Hz, 1H, thienyl-H), 7.43-7.94 (*m*, 14H, Ar-H + thienyl-H), 8.13 (*s*, 1H, pyrazole-H), 9.26 (*s*, 1H, Pyridine-H); **MS (m/z, %):**486 (M+, 0.22), 328 (84), 276 (45), 105 (69), 77 (100); **Anal. Calcd. for $C_{29}H_{18}N_4S_2$**(486.61): C, 71.58; H, 3.73; N, 11.51; S, 13.18; found: C, 71.57; H, 3.75; N, 11.51; S, 13.17.

4-(4-(Dimethylamino)phenyl)-3-phenyl-6-(thiophen-2-yl)picolinonitrile (8b)

Yellow solid from glacial acetic acid, yield (1.35g, 71%), mp: 232-234 ^{0}C; **IR (KBr, cm^{-1}):** 3037 (=C–H), 2216 (-CN); **^{1}H-NMR:** δ 3.03 (*s*, 6H, $(\underline{CH_3})_2$-N-), 6.88 (*d*, *J* = 0.6 Hz, 1H, -C_6H_3-$\underline{H}$), 6.91 (*d*, *J* = 0.5 Hz, 1H, -C_6H_3-$\underline{H}$), 7.25 (*dd*, *J* = 3.9 and 0.9 Hz, 1H, thienyl-$\underline{H}$), 7.58-7.93 (*m*, 9H, Ar-H + thienyl-H), 8.07 (*s*, 1H, Pyridine-H); **MS (m/z, %):** 383 (M+2, 8), 382 (M+1, 28), 381 (M+, 100), 271 (17), 189 (8), 77 (17), 57 (7); **Anal. Calcd. for $C_{24}H_{19}N_3S$** (381.49): C, 75.56; H, 5.02; N, 11.01; S, 8.41; found: C, 75.54; H, 5.03; N, 11.03; S, 8.40.

4-(4-(Dimethylamino)phenyl)-6-(furan-2-yl)-3-phenylpicolinonitrile (8c)

Yellow solid from glacial acetic acid, yield (1.19g, 65%), mp: 272-274 ^{0}C; **IR (KBr, cm^{-1}):** 3055 (=C–H), 2897 (-C–H), 2214 (-CN); **^{1}H-NMR:** δ 3.02 (*s*, 6H, $(\underline{CH_3})_2$-N-), 6.75 (*q*, *J* = 1.7 and 1.6 Hz, 1H, furyl-H), 6.87 (*d*, *J* = 8.3 Hz, 1H, -C_6H_3-$\underline{H}$), 6.89 (*d*, *J* = 8.8 Hz, 1H, -C_6H_3-$\underline{H}$), 7.41-7.91 (*m*, 9H, Ar-H + furyl-H), 7.98 (*s*, 1H, Pyridine-H); **MS (m/z, %):** 367 (M+2, 4), 366 (M+1, 26), 365 (M+, 100), 348 (6), 321 (10), 146 (4), 105 (6), 77 (12); **Anal. Calcd. for $C_{24}H_{19}N_3O$** (365.43): C, 78.88; H, 5.24; N, 11.50;found: C, 78.90; H, 5.23; N, 11.49.

4-(4-(Dimethylamino)phenyl)-3-phenyl-6-(*p*-tolyl)picolinonitrile (8d)

Pale yellow solid from glacial acetic acid, yield (1.34 g, 69%), mp: 194-195 ^{0}C; **IR (KBr, cm^{-1}):** 3035 (=C–H), 2920 (-C–H), 2214 (-CN); **^{1}H-NMR:** δ 2.39 (*s*, 3H, *p*-CH_3), 3.02 (*s*, 6H, $(\underline{CH_3})_2$-N-), 6.87 (*d*, *J* = 0.6 Hz, 1H, $(CH_3)_2N$-C_6H_3-$\underline{H}$) , 6.9 (*d*, *J* = 0.5 Hz, 1H, $(CH_3)_2N$-C_6H_3-$\underline{H}$), 7.34 (*d*, *J* = 0.6 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.36 (*d*, *J* = 0.8 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 7.58-7.97 (*m*, 7H, Ar-H), 8.03 (*s*, 1H, Pyridine-H), 8.18 (*d*, *J* = 0.6 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$), 8.2 (*d*, *J* = 0.7 Hz, 1H, *p*-CH_3-C_6H_3-$\underline{H}$); **MS (m/z, %):** 391 (M+2, 4), 390 (M+1, 28), 389 (M+, 100), 372 (15), 345 (17), 193 (6), 91 (4), 77 (4); **Anal. Calcd. for $C_{27}H_{23}N_3$** (389.49): C, 83.26; H, 5.95; N, 10.79; found: C, 83.29; H, 5.93; N, 10.78.

2.1.6. *Synthesis of aminonictinonitriles 9a and 9c*

First method: Chalcone (10 mmol), malononitrile (0.66 g, 10 mmol), ammonium acetate (0.77 g, 10 mmol) were refluxed in glacial acetic acid for 3-4 h., then acetic acid was evaporated under reduced pressure, precipitated by ice, filtered, and recrystallized from an appropriate solvent.

Second method: Aldehyde (10 mmol), arylketone (10 mmol), malononitrile (0.66 g, 10 mmol), ammonium acetate (6.00 g, 77 mmol) were refluxed in n-butanol for 2-3 h., then the solvent was evaporated under reduced pressure, precipitated by crushed ice, filtered, washed with ethanol, and recrystallized from an appropriate solvent.

2-Amino-4-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)nicotinonitrile (9a)

Yellow solid from glacial acetic acid, yield (1.57g, 74%), mp: 275-277°C; **IR (KBr, cm^{-1})**: 3397 (N–H), 3187 (=C–H), 2212 (-CN); **^{1}H-NMR:** δ 7.28 (*dd*, *J* = 3.7 and 1.4 Hz, 1H, thienyl-H), 7.45-7.96 (*m*, 12H, Ar–H + thienyl-H), 7.96 (*s*, 1H, pyridine-H), 8.20 (*s*, 1H,

pyrazole-H); **MS(m/z, %):**427 (M+2, 0.3), 426 (M+1, 0.73), 425 (M+, 2), 321 (11), 320 (46), 277 (16), 202 (38), 111 (20), 78 (9), 77 (100), 76 (10), 51 (39); **Anal. Calcd. for $C_{23}H_{15}N_5S_2$** (425.53): C, 64.92; H, 3.55; N, 16.46; S, 15.07; found: C, 64.93; H, 3.53; N, 16.46; S, 15.06.

2-Amino-4-(4-(dimethylamino)phenyl)-6-(furan-2-yl)nicotinonitrile (9c)

Brown solid from glacial acetic acid, yield (1.02g, 67%), mp: 252-254 ^{0}C; **IR (KBr, cm^{-1}):** 3471, 3309 (-NH_2), 3118 (=C–H), 2900 (-C-H), 2202 (-CN); **^{1}H-NMR:** δ 3.00 (*s*, 6H, -CH_3), 6.68-7.55 (*m*, 9H, Ar–H + furyl-H + N-H), 7.88 (*s*, 1H, pyridine-H); **MS (m/z ,%):**306 (M+2, 2), 305 (M+1, 22), 304 (M+, 100), 303 (M-1, 57), 288 (4), 260 (3), 77 (1); **Anal. Calcd. for $C_{18}H_{16}N_4O$** (304.35): C, 71.04; H, 5.30; N, 18.41; found: C, 71.01; H, 5.32; N, 18.42.

2.2. Pharmacology

2.2.1. *Cell culture protocol*

Human T-47D breast cancer cell line and Human UACC-257 skin melanoma were obtained from the American Type Culture Collection (ATCC). Cells were cultured using DMEM (Dulbecco's Modified Eagle Medium) (Invitrogen/Life Technologies) which is the most broadly suitable medium for many adherent cell phenotypes among defined media for cell and tissue culture. DMEM was supplemented with 10% FBS (Hyclone), 10 ug/ml of insulin (Sigma), and 1% penicillin-streptomycin. All of the other chemicals and reagents were purchased from Sigma Aldrich and Invitrogen.

Cells were plated (cells density 1.2 - 1.8 × 10,000 cells/well) in a volume of 100 µl complete growth medium + 100 µl of the tested compound per well in a 96-well plate for 24 hours before the MTT assay.

2.2.2. *Antiproliferative activity*

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) is considered the most widely used for viability assays of eukaryotic cells. In these cases, the nicotinamide-adenine-dinucleotide (NAD(P)H) coenzyme and dehydrogenases from metabolically active cells reduce tetrazolium salts to strongly coloured and lipophilic formazan products, which are then quantified by absorbance (MTT) [37].Each test included a blank containing complete medium without cells. Cultures were removed from the incubator into laminar flow hood and each vial of MTT was reconstituted to be used with 3 ml of the medium. Reconstituted MTT were added in an amount equal to 10% of the culture medium volume. Then, cultures were returned to the incubator for 2-4 hours. After the incubation period, cultures were removed from the incubator and the resulting formazan crystals were dissolved by adding an amount of MTT Solubilisation Solution equal to the original culture medium volume. Absorbance values were measured spectrophotometrically at a wavelength of 450 nm. The background absorbances of the multiwell plates were measured at 690 nm and were subtracted from the 450 nm measurement. The viability of the cells were measured for five different concentrations (100, 25, 6.5, 1.56, and 0.39 µM) of the most promising compounds, then IC_{50} values were calculated.

2.2.3. In silico ADME, pharmacokinetics and drug-likeness screening

The ADME properties which were supposed to be evaluated includes aqueous solubility (QPlogS), blood brain barrier (BBB) permeability (QPlogBB), serum protein binding (QPlogKhsa), apparent MDCK cell permeability (QPPMDCK), gut-blood barrier (QPPCaco), predicted central nervous system activity, skin permeability (QPLogKP) and human oral absorbtion calculations were carried out by an *in silico* ADME screening using QikProp program (Schrödinger software), a QSAR-based model to obtain surrogate chemical property values, which were then integrated to evaluate ADME properties along other various sub-criteria.

Screening for pharmacokinetics and drug-likeness Pharmacokinetics and drug-likeness prediction for the synthesized compounds were also performed by an online tool, SwissADME (http://www.swissadme.ch/index.php), of Swiss Institute of Bioinformatics [38]. These analyses tasks were done to check whether those compounds were inhibitors of isoforms of Cytochrome P450 family such as CYP1A2 and CYP2D6. Additionally, pharmacokinetics (such as gastro intestinal absorption, P-glycoprotein and Blood brain barrier) and drug-likeness prediction such as Lipinski, Ghose, Veber rules and bioavailability score [39-41].

2.2.4. BOILED-Egg model

The brain or intestinal estimated permeation method (BOILED-Egg) is proposed as an accurate predictive model that works by computing lipophilicity and apparent polarity (described by WLOGP and TPSA, respectively). An Egan's egg graph for the synthesized compounds was generated using SwissADME. If plotted molecule falls inside the white ellipse, the probability of a good intestinal absorption is high. If plotted molecule falls inside the yellow ellipse (i.e. the yolk), the probability of a good BBB crossing is high. The white and yolk of the BOILED-Egg are not mutually exclusive and molecules predicted as not absorbed by the GI and BBB non-permeant are located in the grey area or even further outside the range of the plot. [42]

2.2.5. Toxicity risks assessment

The toxicity risk assessment tries to locate substructures within the chemical structure being indicative of a toxicity risk within one of four major toxicity classes. Risk alerts are doesn't mean to be a fully reliable toxicity prediction. Nor should be concluded from the absence of risk alerts that a particular substance would be completely free of any toxic effect. The toxicity risk assessment including mutagenicity, tumorogenicity, irritation and reproductive or developmental toxicity was accomplished along with the overall drug score using OSIRIS Data Warrior version 5.0.0.

2.2.6. Molecular docking

Molecular docking was carried out for compound **4a**, as it showed the most significant anticancer activity against T-47D cancer cell line on, a Core 2 duo 2.20 GHz workstation, 3 GHz memory with windows 7 operating system using molecular operating environment (MOE 2008.10 software) [43]. The crystal structure of the complex of an inhibitor-bound tyrosine kinase was downloaded from protein data bank (PDB ID: 4XG7) [44] which has been retrieved from ***www.rcsb.org*** [45], and was targeted in the docking experiments. The target was prepared for docking by 3D-protonation and partial charges were computed with subsequent energy minimization and using MOE default parameters under CHARMM27 (an all-atom forcefield parameterized for proteins, DNA and RNA). Docking method was validated by re-docking the co-crystallized inhibitor in 4XG7, Ligand conformations were generated with the bond rotation method. These were then placed in the site with the triangle matcher method, and ranked with the london dG scoring function. The retain option specifies the number of poses (100) to pass to the refinement, for energy minimization in the pocket, before rescoring with the london dG scoring function and the retain option specifies the number of poses (20), which gave S value -12.51 kcal/mol and RMSD value of 0.333 A^{o} which is a very good value.

3. RESULTS AND DISCUSSION

3.1. Chemistry

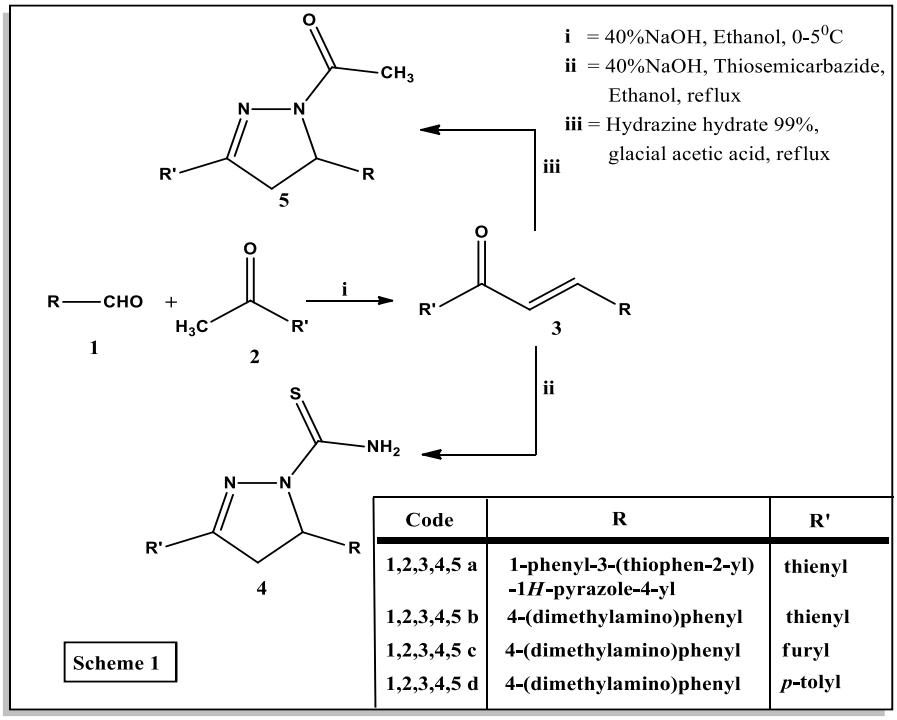
Reaction of **1a** aldhyde with 1-(thiophen-2-yl)ethan-1-one **(2a)** afforded 3-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-1-(thiophen-2-yl)prop-2-en-1-one **(3a)**. The reaction was accomplished using the Claisen-Schmidt [46, 47] condensation by the reaction of equimolar amounts of **1a** and **2a** in ethanol using a 40% NaOH solution as catalyst. Likewise, 3-(4-(dimethylamino)phenyl)-1-(thiophen-2-yl)prop-2-en-1-one **(3b)**,3-(4-(dimethylamino)phenyl)-1-(furan-2-yl)prop-2-en-1-one **(3c)**, and 3-(4-(dimethylamino)phenyl)-1-(*p*-tolyl)prop-2-en-1-one **(3d)** were prepared by the reaction of 4-(dimethylamino)benzaldehyde **(1b)** with **2a**, 1-(furan-2-yl)ethan-1-one **(2b)**, and 1-(*p*-tolyl)ethan-1-one **(2c)**, respectively **(scheme 1)**. The ^{1}H-NMR spectrum of **3a** showed a multiplet at δ 7.24-7.92 due to thienyl hydrogen, vinyl hydrogen and other aromatic hydrogens, a doublet at δ 8.06 due to thienyl-c22 hydrogen, a doublet at δ 8.14 from thienyl-c22 hydrogen, and a singlet at δ 9.36 due to the pyrazole hydrogen while IR spectrum revealed the absence of the peak of the pyrazole aldhyde hydrogen that was expected to appear around 2720 cm^{-1}. The structure of **3a** was also established on the basis of mass spectroscopy and elemental analysis while the structure characterization of compounds **3b**, **3c** and **3d** was previously reported.

The cyclocondensation reaction of chalcones **3a-b** and **3d** with thiosemicarbazide and 40% NaOH in ethyl alcohol under reflux conditions produced 1'-phenyl-3',5-di(thiophen-2-yl)-3,4-dihydro-1'*H*,2*H*-[3,4'-bipyrazole]-2-carbothioamide **(4a)**, 5-(4-(dimethylamino)phenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide **(4b)**, and 5-(4-(dimethylamino)phenyl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide **(4c)**, respectively **(scheme 1)**. The IR spectrum of the compound **4a** afforded pyrazoline thiocarbamoyl group N-H stretching (3437–3282 cm^{-1}). The CH_2 protons of the pyrazoline ring resonated as a pair of doublets of doublets at 3.22 and 4.06 ppm (H_A), 3.86–4.06 ppm (H_B). The N–H protons of the thiocarbamoyl group for compound **4a** were seen at 8.12 ppm as a singlet. The protons belonging to the aromatic ring and the other aliphatic groups are observed with the expected chemical shift and integral values and data from the mass spectrum and elemental analysis were in line with molecular formula. Likewise, The structures **4c** was established on the basis of infrared (IR), ^{1}H NMR and elemental analyses while the structure characterization of compound **4b** was previously reported.

The cyclocondensation reaction of chalcones **3a-b**, and **3d** with hydrazine hydrate in ethanol in the presence of few drops of glacial acetic acid under reflux produced 1-(1'-phenyl-3'-(thiophen-2-yl)-5-(*p*-tolyl)-1'*H*,2*H*-[3,4'-bipyrazol]-2-yl)ethan-1-one **(5a)**, 1-(5-(4-(dimethylamino)phenyl)-3-(thiophen-2-yl)-1*H*-pyrazol-1-yl)ethan-1-one **(5b)**, 1-(5-(4-(dimethylamino)phenyl)-3-(*p*-tolyl)-1*H*-pyrazol-1-yl)ethan-1-one **(5c)**, respectively **(scheme 1)**. Compounds **5a-c** showed bands at 1651, 1662 and 1666 cm^{-1}, respectively, attributed to carbonyl group, also the band at 3083 of compound **5a** may be attributed to the formation of acetyl group. The confirmation of the formation of a new acetyl group was presented by ^{1}H NMR analysis as it revealed a singlet at δ 2.32, 2.22 and 2.27, respectively for compounds **5a-c**. The formation of the pyrazoline ring was confirmed by the three types of pyrazoline hydrogen formation. The CH_2 protons of the

pyrazoline ring appeared as a pair of doublets of doublets at δ 3.21 and 3.99 ppm (H_A), and a quartet at δ 5.81 ppm (H_B) for the compound **5a** .likewise pyrazoline ring formation was confirmed for compounds **5b** and **5c**.

The structures **5a-c** were also established on the basis of mass spectra and elemental analyses.



Code	R	R'
1,2,3,4,5 a	1-phenyl-3-(thiophen-2-yl) -1*H*-pyrazole-4-yl	thienyl
1,2,3,4,5 b	4-(dimethylamino)phenyl	thienyl
1,2,3,4,5 c	4-(dimethylamino)phenyl	furyl
1,2,3,4,5 d	4-(dimethylamino)phenyl	*p*-tolyl

Scheme 1

The four-component one-step modified Hantzch reaction [48] was in the Reactions of **3a** with each of ethyl cyanoacetate, phenacyl cyanide and malononitrile in n-butanol in th presence of ammonium acetate afforded 2-oxo-4-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)-1,2-dihydropyridine-3-carbonitrile **(6a)**, 3-phenyl-4-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)-1,2-dihydropyridine-2-carbonitrile **(8a)**, and 2-amino-4-(1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazol-4-yl)-6-(thiophen-2-yl)nicotinonitrile **(9a)**.

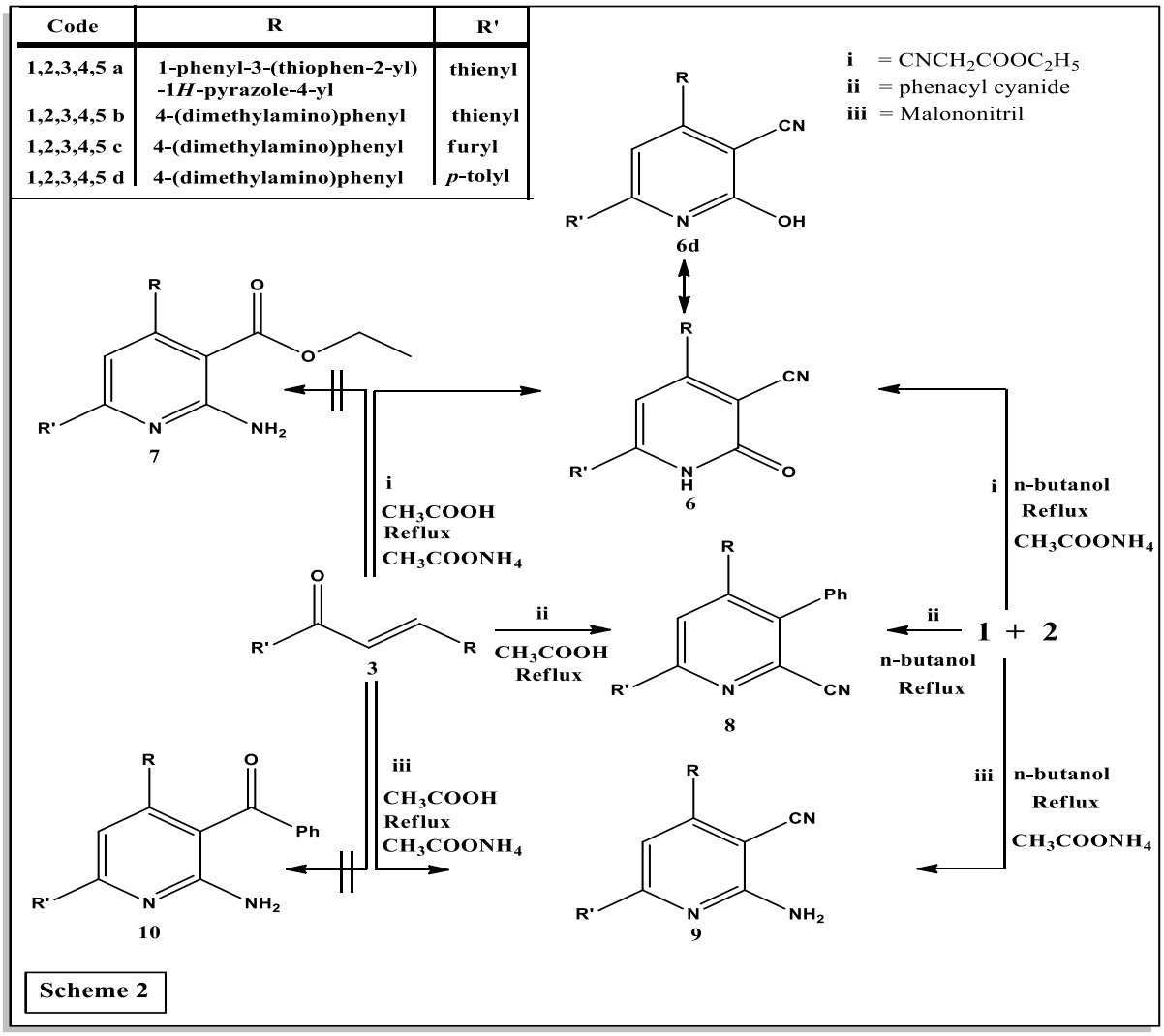
Analogy, refluxing of the appropriate chalcone **3b-d** with the appropriate ethyl cyanoacetate, phenacyl cyanide, and malononitril in glacial acetic acid in presence of ammonium acetate gave pyridine derivatives **6a-d** and **8a-d** (*cf.* **Schemes 2**).

Compounds **6a-d**, **8b-d**, **9a**, and **9d** were synthesized by alternative route by the refluxing of a mixture of **1** and **2** with the appropriate ethyl cyanoacetate, phenacyl cyanide, and malononitril, respectively in n-butanol for 4 hours (**scheme 2**). The products had the same melting point and mixed melting point of those synthesized by the first route.

Structure **7** was excluded as the ester group did not appear in the ^{1}H NMR chart while the structure **6a** was supported by elemental analysis, IR, ^{1}H NMR and mass spectral studies. Its IR spectrum showed absorption bands at 3380, 2221, 1696 cm^{-1} indicating the presence of NH, CN and CO groups, respectively, while pyridine ring formation was confirmed by a singlet at δ 9.12 attributed to pyridinyl hydrogen. Likewise, structures **6b and 6c** have been confirmed. IR spectrum for compound **6d** showed absorption bands at 3429 cm^{-1} suggests the presence of OH groups (i.e. enol form), and the formation of this isomer was confirmed by the absence pyridinyl hydrogen peak that was expected at δ 8 also, ^{1}H NMR chart indicated the presence of a broad peak at δ 12.25 due to phenolic hydrogen (**scheme 2**).

Structure **8a** was elucidated by elemental analysis and spectral data.The ^{1}H-NMR spectrum of **8a** showed the formation of the pyridine ring by a singlet at δ 9.26 due to pyridinyl hydrogen, IR spectrum showed a band at 2217 cm^{-1} attributed to cyanide group. In the same manner, structures **8a-d** were elucidated. (*cf.* Experimental).

Structure **10** was excluded as the ester group as the IR spectrum showed absorption bands at 2212 and 2202 cm^{-1} indicating the presence of CN and CO



groups and this supports structures **9a** and **9c**. Structures of compounds **9a** and **9c** were supported by mass spectra, elemental analyses and the other synthetic route.

3.2. Pharmacology

3.2.1. In vitro anti-proliferative activity

Initially, the anti-proliferative activities of the synthesized compounds were estimated by determining their *in vitro* single-dose growth inhibitory activity. A dose of 100 µg mL^{-1} of each complex was used with the cell lines T-47D and UACC-257 for this purpose. All the synthesized compounds exhibited moderate to high activity against T-47D breast cancer cell line, especially compounds **3b**, **4a**, and **8c** with inhibition values 67.34, 69.64, and 67.95 %, respectively. These values are considered promising as they surpassed that of cisplatin (63.62 %) which is a well-known chemotherapeutic drug and has been used as a first-line therapy for several cancers, including testicular, ovarian, cervical, head, neck and small-cell lung cancers either alone or in combination with other anticancer agents.[49]

Compounds **3d** and **6d** showed potent activity against UACC-257 cell line, with inhibition percentage values (61.24 and 57.41, respectively) just below that of cisplatin (61.76%), while the most potent compound against UACC-257 cell line was **5b** (62.54 %).

Compounds **3a**, **4a**, **5a**, **6a**, **8a**, and **8c** exhibited moderate activity against UACC-257 cell line, while compounds **3b**, **3c**, **4c**, **5c**, and **6c** were the least active compounds against UACC-257 compared to the other synthesized compounds and cisplatin. (See **Table 1** and **fig. 1**)

Also, from the results listed in **table 2** and **figure 2**, compounds **8c**, **9c**, **4a** and **3b** showed *in vitro* cytotoxic activity with IC_{50} values of 1.86, 0.96, 0.55 and 1.46 µM, respectively, against the T-47D cell line which is better than that of cisplatin (3.08 µM). Compounds **5b** showed an *in vitro* cytotoxic activity with IC_{50} value of 2.9 µM against the UACC-257 cell line which was slightly above that of cisplatin 1.76 µM when the cells were subjected to 5 different concentrations of the compounds (**table 3** and **fig. 3**).

Table 1: The anticancer inhibition percentages of the synthesized compounds using MTT assay against T-47D and UACC-257 human cancer cells.

Serial	Comp.	Inhibition %	
		UACC-257	T-47D
1	3a	30.10	56.90
2	3b	12.92	67.34
3	3c	4.88	57.24
4	3d	61.24	38.88
5	4a	31.34	69.64
6	4b	42.68	57.13
7	4c	12.63	60.64
8	5a	39.71	53.54
9	5b	62.54	59.70
10	5c	15.02	37.74
11	6a	35.33	43.91
12	6b	48.53	37.52
13	6c	2.81	59.36
14	6d	57.41	38.50
15	8a	31.92	50.77
16	8b	47.09	42.36
17	8c	36.33	67.95
18	8d	59.35	60.74
19	9a	47.99	47.02
20	9c	21.85	63.97
21	cisplatin	61.76	63.62

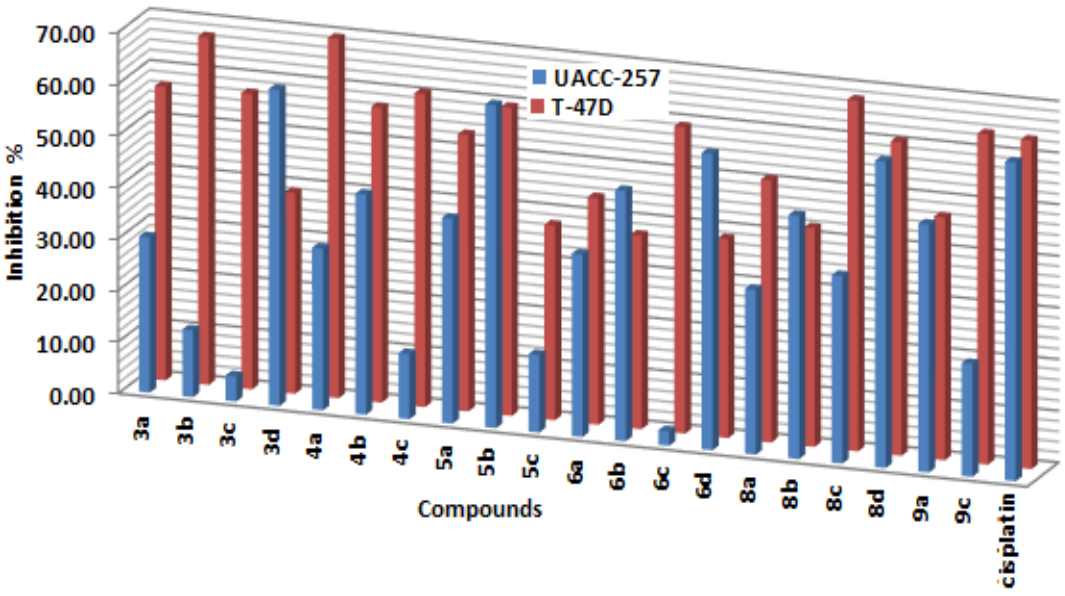


Fig. 1: The anticancer inhibition percentages of a single dose from the synthesized

Also, from the results listed in **table 2** and **figure 2**, compounds **8c**, **9c**, **4a** and **3b** showed *in vitro* cytotoxic activity with IC_{50} values of 1.86, 0.96, 0.55 and 1.46 µM, respectively, against the T-47D cell line which is better than that of cisplatin (3.08 µM). Compounds **5b** showed an *in vitro* cytotoxic activity with IC_{50} value of 2.9 µM against the UACC-257 cell line which was slightly above that of cisplatin 1.76 µM when the cells were subjected to 5 different concentrations of the compounds (**table 3** and **fig. 3**).

Table 2: *In vitro* cytotoxic activity IC_{50} (µM) of compounds **8c**, **9c**, **4a** and **3b** against T-47D cell line.

Ser.	code	Cytotoxicity IC_{50}(uM) against T-47D
1	**8c**	1.86±0.05
2	**9c**	0.96±0.03
3	**4a**	0.55±0.014
4	**3b**	1.46±0.038
5	**Cisplatin**	3.08±0.10

Table 3: *In vitro* cytotoxic activity IC_{50} (µM) of the compound **5b** against UACC-257 cell line.

Ser.	code	Cytotoxicity IC_{50}(uM) against UACC-257
1	**5b**	2.9±0.07
2	**Cisplatin**	1.76±0.046

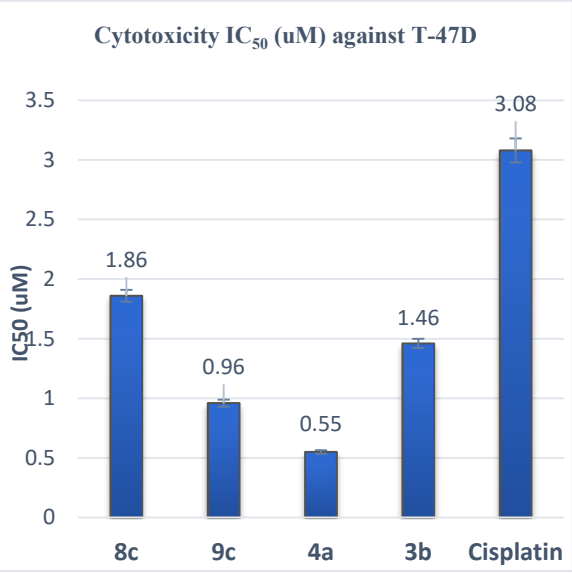


Fig. 2: *In vitro* cytotoxic activity IC_{50} (µM) of compounds **8c**, **9c**, **4a** and **3b** against T-47D cell line.

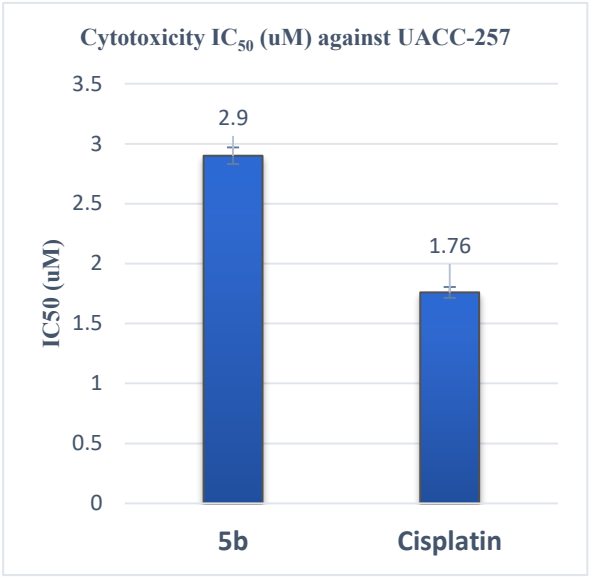


Fig. 3: *In vitro* cytotoxic activity IC_{50} (µM) of the compound **5b** against UACC-257 cell line.

3.2.2. Molecular docking

Docking method validation carried out by MOE program demonstrated that the re-docked compound X7G interacts in the same manner of the co-crystallized one with the reaction site residues. So, this method of is considered a valid one for docking of our compound (**Fig. 4**).

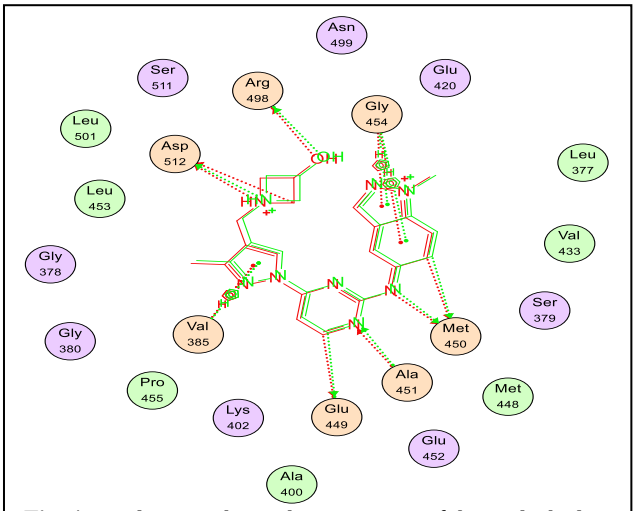


Fig. 4: 2D diagram shows the interaction of the re-docked X7G compound and the co-crystallized one in 4XG7

It gave a docking score value -8.43 kcal/mol which is lower than that of the co-crystallized drug but yet is still considered a very good value. Also, it was relieved, as shown in **fig. 6,** that compound **4a** fits well in the reactive site cavity. Additionally, there is a strong hydrogen bond between nitrogen atom of the amino group of **4a** compound with ASP 512 residue, Also sulfur atom of the carbothioamide group is connected with a hydrogen bond with Asn 499 residue, and there is pi-H between serine 379 and the thienyl

ring directly attached to the pyrazole ring, and the bond lengths were 2.74, 4.09, and 3.68 angstrom, respectively, while the bond energies were -4.9, -1.2, and -0.6 kcal/mol, respectively. (**Fig. 6)**.

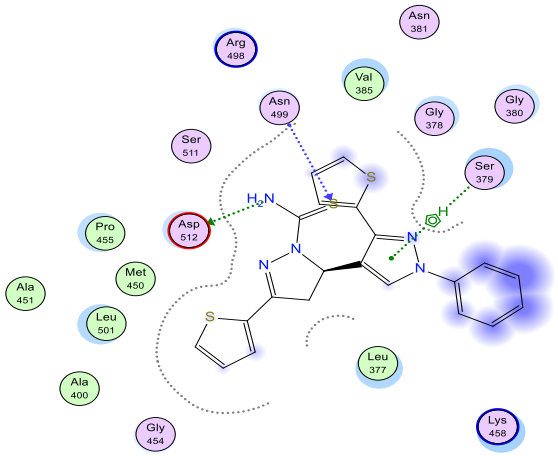


Fig. 6: 2D diagram shows the interaction between compound **4a** with amino acid residues of 4XG7 active

3.2.3. In silico ADME, Pharmacokinetics and drug-likeness evaluation

The ideal oral drug is one that is rapidly and completely absorbed from the GIT, distributed specifically to its site of action in the body, metabolized in a way that does not instantly affect its activity, and eliminated in a perfect manner, without leading to any harmful effects. *In silico* ADME, pharmacokinetics and drug-likeness screening introduces a very important way to recognize or predict these criteria in our synthesized compounds. ADME studies carried out on QikProp program were summarized in **Table 4**. Typically, a low solubility goes along with a bad absorption, and therefore the general aim is to avoid poorly soluble compounds. This property was measured through aqueous solubility (QPlogS). The QPLogS of a compound significantly affect its absorption and distribution characteristics. The calculated QPLogS values of synthesized compounds were within acceptable limits except compounds **3a**, **4a**, **5a**, **6a**, **8a**, **8b**, **8c**, **8d**, and **9a** which were poorly soluble in water. Calculations related to serum protein binding, blood-brain barrier (QlogBB and apparent MDCK cell permeability), gut-blood barrier (QPPCaco), predicted central nervous system activity (CNS), skin permeability (QPLogKP), and human oral absorbtion indicated that these values for the synthesized compounds with the standard ranges generally observed for drugs (**Table 4**).

Predictions performed on SwissADME were summarized in **Table 5**. All compounds showed high GI absorption except **4a**, **6a**, **8a**, and **9a** compounds that had low GI absorption. All compounds have no BBB permeability except **3b**, **3c**, **3d**, **4c**, **5b**, **5c**, **6c**, **6d**, and **8c** however, most of them showed inhibition towards CYP1A2 unlike CYP2D6, which wasn't affected by most of the synthesized compounds. The drug-likeness prediction was also conducted depending on the selected Lipinski, Ghose and Veber rules and bioavailability score. The Lipinski (Pfizer) filter is the pioneer rule-of-five [39]. The Lipinski's Rule of Five states that the absorption or permeation of a molecule is more likely when the molecular weight is under 500 g/mol, the value of log P is lower than 5, and the molecule has utmost 5 H-donor and 10 H-acceptor atoms. Ghose filter (Amgen) [40] defines drug-likeness constraints as follows: calculated log P is between -0.4 and 5.6, MW is between 160 and 480, molar refractivity is between 40 and 130, and the total number of atoms is between 20 and 70. Veber (GSK) [41], rule defines drug-likeness constraints as Rotatable bond count ≤ 10 and polar surface area (PSA) ≤ 140. Screening process with Lipinski Rule of Five showed that all the synthesized compounds meet the criteria of drug-likeness assessment **(Table 5)**. According to the screening process with Ghose rules showed that all compounds matched with the rules except **4a**, **8b**, **8c**, **8d**, and **9a** compounds with one violation, while compound **8a** had 3 violations **(Table 5)**. The screening process with Veber rules demonstrated that all compounds meet the criteria of drug likeness assessment except compound **4a** which had one violation **(Table 5)**.

BOILED-Egg graph (**Fig.7**) showed that all compounds complied with Eagan graph. Compounds **3a**, **3d**, **3c**, **3d**, **5c**, and **6d** were absorbed by brain**.** Also compounds **4c**, **5b**, **6c**, **8c** and **9c** were absorbed by brain in a less manner. The remaining compounds showed gastrointestinal absorption within an acceptable range. Additionally, only **4a**, **6a**, **8b**, and **8d** molecules are predicted to be effluated from the CNS by P-glycoprotein.

3.2.4. Toxicity risks assessment

It is now possible to predict the toxicity risks of compounds through reliable bioinformatics tools. In the present study, we have calculated toxicity risks parameters such as mutagenicity, tumorogenicity, irritation and reproductive or developmental toxicity of all synthesized compounds (**Table 6**). On the one hand, toxicity screening results demonstrated that the synthesized compounds pose no risk of mutagenicity except **3b**, **3c**, and **3d** compounds which were highly mutagenic. On the other hand, most of the compounds were tumorogenic except compounds **3a**, **4a**, **5a**, **6a**, **8a**, and **9a** which posed no risk of tumorogenicity. Additionally, all compounds showed no reproductive risks except **4a**, **4b**, and **4c** compounds which showed high reproductive risks. Also, all the synthesized compounds indicated no risk of irritation. To judge the active compounds' overall potential to qualify for a drug, we calculated overall drug score, which combines drug-likeness, hydrophilicity, aqueous

solubility (QPLogS), MW, and toxicity risk parameters. Predicted active compounds showed their

Table 4: ADME studies results predicted by QikProp program.

Code	Pharmacokinetics					Drug-likeness			
	GI absorp.	**BBB permeant**	**Pgp substrate**	**CYP1A2 inhibitor**	**CYP2D6 inhibitor**	**Lipinski viol.**	**Ghose viol.**	**Veber viol.**	**Bio-availability score**
3a	High	No	No	Yes	No	0	0	0	0.55
3b	High	Yes	No	Yes	No	0	0	0	0.55
3c	High	Yes	No	Yes	No	0	0	0	0.55
3d	High	Yes	No	Yes	Yes	0	0	0	0.55
4a	Low	No	Yes	Yes	No	0	1	1	0.55
4b	High	No	No	No	No	0	0	0	0.55
4c	High	Yes	No	No	No	0	0	0	0.55
5a	High	No	No	Yes	No	0	0	0	0.55
5b	High	Yes	No	Yes	No	0	0	0	0.55
5c	High	Yes	No	Yes	No	0	0	0	0.55
6a	Low	No	Yes	Yes	No	0	0	0	0.55
6b	High	No	No	Yes	No	0	0	0	0.55
6c	High	Yes	No	Yes	Yes	0	0	0	0.55
6d	High	Yes	No	Yes	No	0	0	0	0.55
8a	Low	No	No	No	No	0	3	0	0.55
8b	High	No	Yes	Yes	No	0	1	0	0.55
8c	High	Yes	Yes	Yes	Yes	0	1	0	0.55
8d	High	No	Yes	Yes	No	0	1	0	0.55
9a	Low	No	Yes	Yes	Yes	0	1	0	0.55
9c	High	No	No	Yes	Yes	0	0	0	0.55

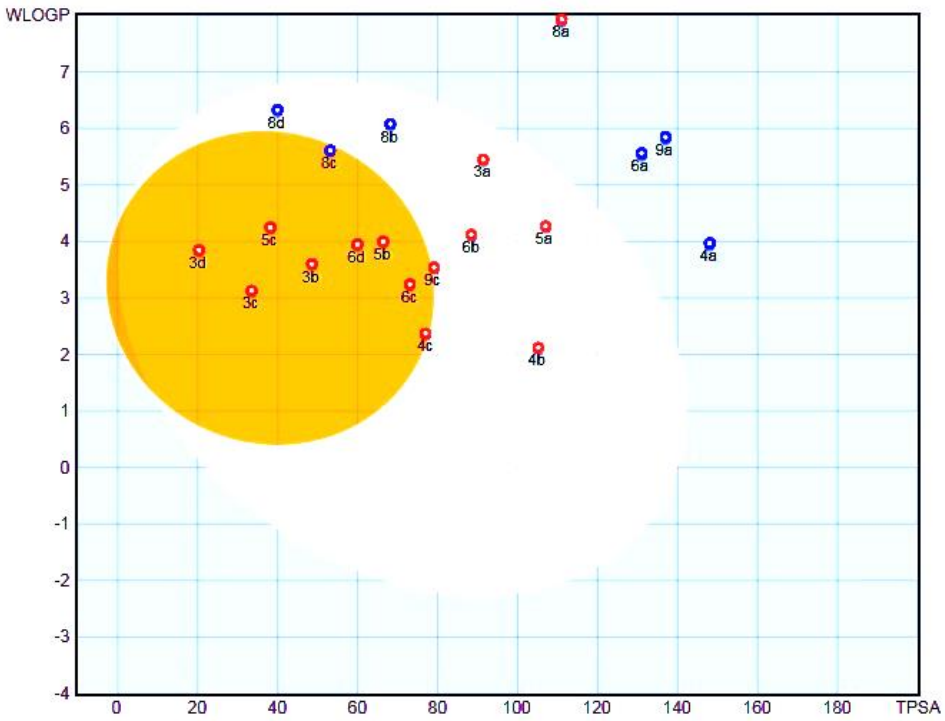


Fig.7: BOILED-Egg graph

Table 5: Pharmacokinetics and drug-likeness prediction for the synthesized compounds by SwissADME.

Code	Pharmacokinetics					Drug-likeness			
	GI absorp.	BBB permeant	Pgp substrate	CYP1A2 inhibitor	CYP2D6 inhibitor	Lipinski viol.	Ghose viol.	Veber viol.	Bio-availability score
3a	High	No	No	Yes	No	0	0	0	0.55
3b	High	Yes	No	Yes	No	0	0	0	0.55
3c	High	Yes	No	Yes	No	0	0	0	0.55
3d	High	Yes	No	Yes	Yes	0	0	0	0.55
4a	Low	No	Yes	Yes	No	0	1	1	0.55
4b	High	No	No	No	No	0	0	0	0.55
4c	High	Yes	No	No	No	0	0	0	0.55
5a	High	No	No	Yes	No	0	0	0	0.55
5b	High	Yes	No	Yes	No	0	0	0	0.55
5c	High	Yes	No	Yes	No	0	0	0	0.55
6a	Low	No	Yes	Yes	No	0	0	0	0.55
6b	High	No	No	Yes	No	0	0	0	0.55
6c	High	Yes	No	Yes	Yes	0	0	0	0.55
6d	High	Yes	No	Yes	No	0	0	0	0.55
8a	Low	No	No	No	No	0	3	0	0.55
8b	High	No	Yes	Yes	No	0	1	0	0.55
8c	High	Yes	Yes	Yes	Yes	0	1	0	0.55
8d	High	No	Yes	Yes	No	0	1	0	0.55
9a	Low	No	Yes	Yes	Yes	0	1	0	0.55
9c	High	No	No	Yes	Yes	0	0	0	0.55

Table 6: The results of toxicity risks assessment screening

Code	Mutagenic	Tumorigenic	Reproductive Effective	Irritant	Drug score
3a	None	none	None	none	0.56
3b	High	high	None	none	0.29
3c	High	high	None	none	0.29
3d	High	high	None	none	0.24
4a	None	none	High	none	0.31
4b	None	high	high	none	0.28
4c	None	high	high	none	0.26
5a	None	none	none	None	0.55
5b	None	high	none	None	0.44
5c	None	high	none	None	0.40
6a	None	none	none	None	0.58
6b	None	high	none	None	0.20
6c	None	high	none	None	0.36
6d	None	high	none	None	0.34
8a	None	none	none	None	0.18
8b	None	high	none	None	0.12
8c	None	high	none	None	0.12
8d	None	high	none	None	0.09
9a	None	none	none	None	0.28
9c	none	high	none	None	0.19

4. Conclusion

In this study, Novel series of pyrazoline carbothioamides, acetyl pyrazoles, pyridine-3-carbonitrile, pyridine-2-carbonitriles, and nicotinonitriles were successfully synthesized from their corresponding chalcones and all of them were evaluated for their *in vitro* anticancer activities against T-47D and UACC-257 human cancer cell lines at a single high dose concentration. Compounds **8c**, **9c**, **4a** and **3b** showed the highest *in vitro* cytotoxic activity against the T-47D cell line compared with cisplatin. Compounds **5b** showed an *in vitro* cytotoxic activity the highest against the UACC-257 cell line which compared with cisplatin. Molecular docking studies performed on compound **4a**, as it showed the most promising activity, with the tyrosine kinase 4XG7 revealed that compound **4a** fits well in the cavity of the active site. Additionally, there are two hydrogen bonds and a pi-H between **4a** compound and ASP 512, Asn 499, and serine 379 residues of the active site, respectively. *In silico* ADME, Pharmacokinetics and drug-likeness evaluation revealed that compound **4a** comply with Lipiniski rule of five but has one Gose violation and one Veber violation. Also, care must be taken as **4a** compound had high predicted reproductive effects while it posed no mutagenicity, tumorogenicity or irritation risks.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgements

We thank the National Centre for Social and Criminological Research for financial Support on this Research.

7. References

1. Neamah N., Khudair A., Al-Jadaan S., Synthesis, characterization, antimicrobial activity and acute toxicity test of a novel 4,4-(4,5,6,7-tetrahydro-[1,2,3-]selenadiazolo[4,5-e] pyridine-4,6-diyl) bis(benzene-1,3-diol). *Egyptian J. Chem.*, **63**(2), 669-681 (2020).

2. Sayed H.H., Flefel E.M., Abd El-Fattah A.M. , El-Sofany W.I. and Hassan A.M., Focus on the synthesis and reactions of some new Pyridine Carbonitrile derivatives as antimicrobial and antioxidant agents. *Egyptian J. Chem.*, **53**(1), 17-35 (2010).

3. Busic V., Pavlovic H., Roca S. and Gaso-Sokac D., Microwave-assisted quaternization of various pyridine derivatives and their antibacterial activity. *Croat. Chem. Acta.*, **90**(3), 425-434 (2017).

4. Amorim R., de Meneses M.D.F., Borges J.C., da Silva Pinheiro L.C., Caldas L.A., Cirne-Santos C.C., de Mello M.V.P., de Souza A.M.T., Castro H.C., de Palmer Paixao I.C.N. and de Mendonça Campos R., Thieno [2,3-b] pyridine derivatives: a new class of antiviral drugs against Mayaro virus. *Arch. Virol.*, **162**(6), 1577-1587 (2017).

5. Stevens M., Pannecouque C., De E.C. and Balzarini J., Inhibition of human immunodeficiency virus by a new class of pyridine oxide derivatives. *Antimicrob. Agents Ch.*, **47**(9), 2951-2957 (2003).

6. Li Q., Zhang C., Tan W., Gu G. and Guo Z., Novel amino-pyridine functionalized chitosan quaternary ammonium derivatives: Design, synthesis, and antioxidant activity. *Molecules*, **22**(1), 156 (2017).

7. Komeili G., Ghasemi F., Rezvani A.R., Ghasemi K., Khadem Sameni F. and Hashemi M., The effects of a new antidiabetic glycinium [(pyridine-2,6-dicarboxylato) oxovanadate (V)] complex in high-fat diet of streptozotocin-induced diabetic rats. *Arch.Physiol. Biochem.*, 1-7 (2019).

8. Duan H., Ning M., Chen X., Zou Q., Zhang L., Feng Y., Zhang L., Leng Y. and Shen J., Design, synthesis, and antidiabetic activity of 4-phenoxynicotinamide and 4-phenoxypyrimidine-5-carboxamide derivatives as potent and orally efficacious TGR5 agonists. *J. Med. Chem.*, **55**(23), 10475-10489 (2012).

9. Androutsopoulos V. P., and Spandidos D. A., Anticancer pyridines induce G2/M arrest and apoptosis *via* p53 and JNK upregulation in liver and breast cancer cells. *Oncol. Rep.*, **39**(2), 519-524 (2018).

10. El-Naggar M., Almahli H., Ibrahim H.S., Eldehna W.M. and Abdel-Aziz H.A., Pyridine-ureas as potential anticancer agents: synthesis and in vitro biological evaluation. *Molecules*, **23**(6), 1459 (2018).

11. Al-Abdullah E.S., Synthesis and anticancer activity of some novel tetralin-6-yl-pyrazoline, 2-thioxopyrimidine, 2-oxopyridine, 2-thioxo-pyridine and 2-iminopyridine derivatives. *Molecules*, **16**(4), 3410-3419 (2011).

12. Bekhit A.A., Hymete A., Damtew A., Mohamed A.M.I. and Bekhit A.E.D.A., Synthesis and biological screening of some pyridine derivatives as anti-malarial agents. *J. Enzyme Inhib. Med. Chem.*, **27**(1), 69-77 (2012).

13. Al-Omar M.A., Amr A.E.G.E. and Al-Salahi R.A., Anti-inflammatory, analgesic, anticonvulsant and antiparkinsonian activities of some pyridine derivatives using 2,6-disubstituted isonicotinic acid hydrazides. *Arch. Pharm.*, **343**(11-12), 648-656 (2010).

14. Hajhashemi V., Mojiri-Froshani H., Saghaei L. and Fassihi A., A study on the anti-inflammatory effects of new derivatives of 3-hydroxy pyridine-4-one. *Adv. Biomed. Res.*, **3(1)**, 134 (2014).

15. Amr A.E.G.E., Sayed H.H. and Abdulla M.M., Synthesis and reactions of some new substituted pyridine and pyrimidine derivatives as analgesic, anticonvulsant and antiparkinsonian agents. *Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry*, **338**(9), 433-440 (2005).

16. Naim M.J., Alam O., Alam M.J., Shaquiquzzaman M., Alam M.M. and Naidu V.G.M., Synthesis, docking, *in vitro* and *in vivo* antidiabetic activity of pyrazole-based 2,4-thiazolidinedione derivatives as PPAR-γ modulators. *Arch. Pharm.*, **351**(3-4), 1700223 (2018).

17. Taher A.T., Sarg M.T.M., Ali N.R.E.S. and Elnagdi N.H., Design, synthesis, modeling studies and biological screening of novel pyrazole derivatives as potential analgesic and anti-inflammatory agents. *Bioorg. chem.*, **89**, 103023 (2019).

18. Do Carmo Malvar D., Ferreira R.T., De Castro R.A., De Castro L.L., Freitas A.C.C., Costa E.A., Florentino I.F., Mafra J.C.M., De Souza G.E.P. and Vanderlinde F.A., Antinociceptive, anti-inflammatory and antipyretic effects of 1,5-diphenyl-1*H*-Pyrazole-3-carbohydrazide, a new heterocyclic pyrazole derivative. *Life Sci.*, **95**(2), 81-88 (2014).

19. Muhammad Z.A., Alshehrei F., Zayed M.E., Farghaly T.A. and Abdallah M.A., Synthesis of novel Bis-pyrazole derivatives as antimicrobial agents. *Mini Rev. Med. Chem.*, **19**(15), 1276-1290 (2019).

20. Trindade N.R., Lopes P.R., Naves L.M., Fajemiroye J.O., Alves P.H., Amaral N.O., Lião L.M., Rebelo A., Castro C.H., Braga V.A. and Menegatti R., The newly synthesized pyrazole derivative 5-(1-(3 Fluorophenyl)-1*H*-pyrazol-4-yl)-2*H*-tetrazole reduces blood pressure of spontaneously hypertensive rats *via* NO/cGMO pathway. *Front. Physiol.*, **9**, 1073 (2018).

21. Sayed A.R., Gomha S.M., Abdelrazek F.M., Farghaly S.M., Hassan A.S., and Metz P., Design, efficient synthesis and molecular docking of some novel thiazolyl-pyrazole derivatives as anticancer agents. *BMC Chem.*, 13, 116 (2019).

22. Nofal Z.M. El-Zahar M.I. and Gouhar R.S., Synthesis, moleculer docking and anticancer screening of some novel Tetrahydronaphthyl Thiazolyl Pyrazoles and other related derivatives. *Egyptian J. Chem.*, **55**(4), 413-435 (2012).

23. Lokesh B.V., Prasad Y.R. and Shaik A.B., Synthesis, biological evaluation and molecular docking studies of new Pyrazolines as an antitubercular and cytotoxic agents. *Curr.* Drug Targets *Infect. Disord.*, **19**(3), 310-321 (2019).

24. Gok S., Demet M.M., Özdemir A. and Turan-Zitouni, G., Evaluation of antidepressant-like effect of 2-pyrazoline derivatives. *Med. Chem. Res.*, **19**(1), 94-101 (2010).

25. Özdemir Z., Kandilci H.B., Gümüşel B., Çalış Ü. and Bilgin A.A., Synthesis and studies on antidepressant and anticonvulsant activities of some 3-(2-furyl)-pyrazoline derivatives. *Eur. J. Med. Chem.*, **42**(3), 373-379 (2007).

26. Beyhan N., Kocyigit-Kaymakcioglu B., Gümrü S. and Aricioglu F., Synthesis and anticonvulsant activity of some 2-pyrazolines derived from chalcones. *Arab. J. Chem.*, **10**, S2073-S2081 (2017).

27. Garazd Y., Garazd M. and Lesyk R., Synthesis and evaluation of anticancer activity of 6-pyrazolinylcoumarin derivatives. *Saudi Pharm. J.*, **25**(2), 214-223 (2017).

28. EL-Torky, A., Keshta, A.T. and Atef, E., Anti-tumor and anti-oxidant activities of some novel synthetic Pyrazoline derivatives. *Biochem. Lett.*, **12**(1), 27-36 (2016).

29. Khalil O.M., Synthesis of some Chalcones and Pyrazolines carrying morpholinophenyl moiety as potential anti-Inflammatory agents. *Arch. Pharm.*, **344**(4), 242-247 (2011).

30. Magda A.A., Abdel-Aziz N.I., Alaa A.M., El-Azab A.S. and ElTahir K.E., Synthesis, biological evaluation and molecular modeling study of pyrazole and pyrazoline derivatives as selective COX-2 inhibitors and anti-inflammatory agents. Part 2. *Bioorg. Med. Chem.*, **20**(10), 3306-3316 (2012).

31. Joshi R.S., Mandhane P.G., Diwakar S.D., Dabhade S.K. and Gill C.H., Synthesis, analgesic and anti-inflammatory activities of some novel pyrazolines derivatives. *Bioorg. Med. Chem. Lett.*, **20**(12), 3721-3725 (2010).

32. Bratenko, M., Chornous, V., Vovk, M., 4-Functionally substituted 3-heterylpyrazoles: XIII. 3-Aryl(heteryl)-4-(4-pyrazolyl)-1,2,3,4-tetrahydropyrimidin-2-ones(thiones). *Russ. J. Org. Chem.*, **41**, 95–97 (2005).

33. Beyhan, N., Kocyigit-Kaymakcioglu, B., Gümrü, S., and Aricioglu, F. Synthesis and anticonvulsant activity of some 2-pyrazolines derived from chalcones. *Arab. J. Chem.*, **10**, S2073-S2081 (2017).

34. Al-Romaizan, A. N., Ahmed, N. S., and Elfeky, S. M. Design, Synthesis, and Biological Evaluation of Triazolyl-and Triazinyl-Quinazolinediones as Potential Antitumor Agents. *J. Chem.*, **2019**, (2019).

35. Syam S., Abdelwahab S.I., Al-Mamary M.A. and Mohan S., Synthesis of chalcones with anticancer activities. *Molecules*, **17**(6), 6179-6195 (2012).

36. Thirunarayanan G. and Sekar K., Solid SiO_2:H_3PO_4 acid catalyzed solvent-free cyclization of aryl 2-propen-1-ones: Synthesis of some 1-thiocarbomyl pyrazolines and spectral correlations of some 3-phenyl-5-(substituted phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamides. *Org. Chem. Ind. J.*, **10**(2), 43–52 (2014).

37. Stockert J.C., Horobin R.W., Colombo L.L. and Blázquez-Castro A., Tetrazolium salts and formazan products in cell biology: Viability assessment, fluorescence imaging, and labeling perspectives. *Acta Histochem.*, **120**(3), 159-167 (2018).

38. Daina A., Michielin O. and Zoete V., SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci.Rep.*, ***7***, 42717 (2017).

39. Lipinski A.C., Lombardo F., Dominy B. W. and Feeney, P. J., Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.*, **46**(1-3), 3-26 (2001).

40. Ghose A.K., Viswanadhan V.N. and Wendoloski J.J., A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J. Comb. Chem.*, **1**(1), 55–68 (1999).

41. Veber D.F., Johnson S.R., Cheng H.Y., Smith B.R., Ward K.W. and Kopple K.D., Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.*, **45**(12), 2615-2623 (2002).

42. Daina A. and Zoete V., A BOILED-Egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem*, **11**, 1117-1121 (2016).

43. Molecular Operating Environment (MOE), 2008.10; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada.

44. Lee S.J., Choi J.S., Han B.G., Kim H.S., Song H.J., Lee J., Nam S., Goh S.H., Kim J.H., Koh J.S. and Lee B.I., Crystal structures of spleen tyrosine kinase in complex with novel inhibitors: structural insights for design of anticancer drugs. *FEBS J.*, **283**(19), 3613-3625 (2016).

45. Berman, H., Henrick, K. and Nakamura, H., Announcing the worldwide protein data bank. *Nat. Struct. Mol. Biol.*, **10**(12), 980-980 (2003).

46. Claisen, L. and Claparède, A., Condensationen von ketonen mit aldehyden. *Ber. Dtsch. Chem. Ges.*, **14**(2), 2460-2468 (1881).

47. Schmidt, J.G., Ueber die Einwirkung von Aceton auf Furfurol und auf Bittermandelöl bei Gegenwart von Alkalilauge. *Ber. Dtsch. Chem. Ges.*, **14**(1), 1459-1461 (1881).

48. Hantzsch, A., Condensationsprodukte aus Aldehydammoniak und ketonartigen Verbindungen. *Ber. Dtsch. Chem. Ges.*, **14**(2), 1637-1638 (1881).

49. Dasari, S. and Tchounwou, P.B., Cisplatin in cancer therapy: molecular mechanisms of action. *Eur. J. Pharmacol.*, **740**, 364-378 (2014).