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Synthesis, Docking and Cytotoxicity Evaluation of 3-indolyl heterocycles as Potential Anti-Cancer Agents

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Abstract

5′-cyanomeridianine G analogues produce from 3-cyanoacetyl indole accomplished in to steps by treatment with a mixture of piperidine and triethylorthoformate in DMF and then cyclization of the resulting enaminonitrile with guanidine and thiourea to obtain pyrimidine derivatives and also react with hydrazine and phenyl hydrazine by different mechanisms to obtain pyrazole The biological evaluation against a range of cancer cells lines(i.e. HEPG2,HCT116,MCF7,A549) showed that indolylpyrimidine thione 6 was more cytotoxic against Lung carcinoma cell line (A549) with inhaibition activity of 95.4 % and it has the highst antioxidant activities. While the cyanomeridianine G 5 showed high cytotoxic activity against MCF7, A549 aned HCT116 with 100, 88.6 and 74.6 % inhibition in cell viability then do molecular docking we study effect of compound 6 and 7 on (A549)-Lung carcinoma cell line, (MCF 7)-Human Caucasian breast adenocarcinoma, HCT116 -(Human Colon carcinoma) and HEPG 2 -(Human hepatocellular carcinoma cell line) are examples of host species that can be used to attach ligands (guests). With auto Dock, you can discuss and demonstrate the biological benefits of compounds 6and 7 and 5 as well as those compounds containing active groups and atoms (like S, O, and N) that proms give a good biological activity and can theoretically demonstrate it.

Keywords: Enaminonitrile, Meridianines; indole alkaloids; biological activities,Molecular docking.

1. Introduction

Marine indole alkaloids' biological activity is undoubtedly a result of the unique features and components of marine natural product biosynthesis. Because of the wide range of biological functions that indole alkaloids exhibit, there is inteest in them [1,2]. Eight secondary metabolites (meridianines A–H) registered to far [3], after the initial isolation and characterization of meridianines A–E from the Antarctic tunicate Aplidium meridianum in 1998 [4]. The fundamental structure of meridianine is classified as an indole structure that has been brominated and/or hydroxylated connected by a 2-aminopyrimidine moiety at position C-3 (Structure of meridianins A–H)

A significant increase in the growth inhibitory activation against different kinds of fungi, viruses and bacteria was successfully produced by many indole compounds [5]. Conversely, substituted pyrimidines are very desirable because of their wide range of biological properties, including the suppression of HIV-1 [6], antibacterial [7], anticancer [8], and anti-inflammatory [9] properties. Because the thiopyrimidine bases and their analogues inhibit the synthesis of proteins and poly-nucleic acids and are incorporated into poly-nucleic acids, they have demonstrated therapeutic properties as antiviral,antithyroid, and antitumor activities [10, 11]. As a result, researchers are very interested in these compounds. Given the scarcity of naturally occurring alkaloids, the emphasis is on synthesizing marine indole alkaloids with heterocyclic substituents in the 3-position that have five or six members for biological purposes.

Keeping the previous facts in mind and maintaining our fascination with the production of bioactive compounds derived from heterocycles attached to indole moieties, This work includes the preparation and biological activity of indolyl heterocycle derivatives.

2. Experimental Section

2.1. General Procedures.

The melting points have not been adjusted and were calculated using a Gallenkamp melting point device. FT-IR spectra on a Perkin Elmer FT-IR 1600 by Nujol emulsions between NaCl plates or in KBr with a Bruker Vector 22 Germany spectrophotometer. Varian Mercury 300 MHz spectrometer was used to record the NMR- ^{1}H (300 MHz) and NMR-^{13}C spectra (75.4 MHz) in [D_6] DMSO as solvent and TMS as internal standard. The constants (J) coupling are produce in Hz, and the shifts are scored in δ units (ppm). The overall assignment of NMR spectra for ^{13}C and ^{1}H signals was attempted by double

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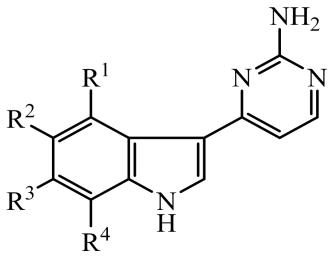
resonance When it was feasible, heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond correlation (HMBC) and double resonance, and investigations were used to fully assign the ^{13}C and ^{1}H signals in the spectra of NMR.

Using a Shimadzu GCMS-QP-1000 EX mass spectrometer, mass spectra were obtained at 70 eV. The Cairo University



Meridianins

A, R^1 = OH, R^2=R^3=R^4= H
B, R^1 = OH, R^2=R^4=H, R^3= Br
C, R^1 = R^3=R^4=H, R^2= Br
D, R^1 = R^2=R^4=H, R^3= Br
E, R^1 = OH, R^2=R^3=H, R^4= Br
F, R^1 = H, R^2= R^3= Br, R^4=H
G, R^1 = R^2 = R^3 = R^4 = H
H R^1= OH, R^2=R^4=Br, R^3=H

Structure of meridianins A–H

Microanalysis Unit used an LECO CHN-932 to conduct microanalyses.

2.2. 3-(*1H*-indol-3-yl)-3-oxopropanenitrile (2) or (3-Cyanoacetylindole).

Yield: 8.39 g (90 %); mp 241 °C (Lit [22]. mp 240 °C).

2.3. 3-Dimethylamino-2-(*1H*-indole-3-carbonyl) acrylonitrile (3).

Crystals, yield (Yellow 78%), mp 169-170 ºC (lit[14]. mp 160-163 ºC);

2.4. 2-(1H-indole-3-carbonyl)-3-(piperidin-1-yl)acrylonitrile (4)

Procedure A: A mixture of 20 mmol of piperidine and (5 mmol)of enaminonitrile and heated by use reflux in (30 mL) of dimethylformamide for 7 hours. Then let it cool to room temperature and emptied into cold water. One molar sodium acetate solution (100 ml) was added to the reaction, which was subsequently filtered, collected, and crystallized from ethanol to produce 4 identical in all respects (TLC, m.p., and NMR) with somewhat loweryields(33%).

Procedure B: 50 millilitres of DMF were added to a mixture containing (0.5 mmoles) of 3-Cyanoacetylindole**2**, (0.6 mmoles) of triethyl orthoformate, and (0.5 mmoles) of piperidine. The mixture was then refluxed for a whole day. The reaction mixture was then poured onto water after cooling. By filtration and crystallized were used to gather to produce compound 4 in 70% yield.

Physical and spectroscopic data: Yellow crystals, yield 70%, mp 160-161ºC; IR (KBr): = (NH) 3218, (CN) 2193, (C=O) 1636 cm^{-1}; (DMSO-*d6*) NMR- ^{1}H: = 1.65 (3CH_2, 6H,m), 3.32 (2CH_2, 4H,m) 7.11-7.21 (H-5,6, 2H,m), 7.45 (2.1 Hz, H-7, , *J* = 7.8, 1H, dd), 7.99 (=CH , 1H s), 8.14 (H-4, 2.1 Hz, *J*= 7.5, 1H, dd), 8.26 (H-2, *J*= 3 Hz, 1H, d), 11.73 (NH, 1H, s). NMR ^{13}C (DMSO-d6): δ = 23.09, 26.49, 57.1, 76.62 (C-CN), 111.70 (C-7), 114.70 (C-3), 120.71 (C-5), 121.75 (CN), 121.98 (C-4), 122.52 (C-6), 126.79 (C-3a), 131.16 (C-2), 135.86 (C-7a), 156.62 (=CH), 181.95 (CO); ms: m/z 279 (M^+). Anal. Calcd for $C_{17}H_{17}N_3O$ (279.34) : C, 73.10; H, 6.13; N, 15.04;. Found: C, C, 73.25; H, 6.23; N, 15.12.

2.5. General procedure to prepare compounds 5 and 6.

For seven hours, K_2CO_3 anhydrous (15.0 mmol, 2.0 g), (10 mmol) enaminonitrile 4, (12.0 mmol) guanidine hydrochloride, or thiourea, and 100% ethanol (30 mL) were heated to reflux temperature. The mixture was cooled before being added to ice water and acidified with a little amount of HCl. After the solidification process, a yellow solid containing 5′-Cyanomeridianin

G **5** and indolylpyrimidine thione **6** was obtained. Upon recrystallization from EtOH, the yields were 69 and 78 percent, respectively.

2.5.1. 2-Amino-4-(1*H*-indol-3-yl)pyrimidine-5-carbonitrile (5), 5′-Cyanomeridianin G.

Physical and spectroscopic data: Solid powder (Off white, 69%), mp 257-258 ºC (lit [14] 258-259 ºC mp). IR (Nujol): ν = 3385 (NH), 3293, 3148 (NH_2),, 2212 (CN), cm^{-1}. (DMSO-*d6*) NMR ^{1}H: m, 2H, H-5′, 6′(= 7.12-7.27,), d, 1H, *J* = 7.5 Hz, H-7′ (=7.50), 2 bs, 2H, NH2 (=7.54 and 7.64), s, 1H, H-6 (= 8.47) , s, 1H, H-2′(=8.570, d, 1H, *J*= 7.2 Hz, H-4′(=8.65), bs, 1H, NH (= 11.95). (DMSO-*d6*) NMR ^{13}C: (C-5)δ= 89.23, (C-3′) =111.56, (C-7′) =112.04, (CN) =119.82, (C-5′) =121.09, (C-6') =122.80, (C-4′) =123.27, (C-3′a) =125.73, (C-2') =130.04, (C-7′a) =136.36, (C-4 or C-2) =163.14, (C-2 or C-4) =163.17, (C-6) =163.35. Anal. Calcd. for $C_{13}H_9N_5$ (235.24): C, 66.37; H, 3.86; N, 29.77. Found: C, 66.35; H, 4.05; N, 29.80.

2.5.2. 4-(*1H*-indol-3-yl)-2-thioxo-1,2-dihydropyrimidine-5-carbonitrile 6.

Physical and spectroscopic data: A pale yellow powder, (78 %); m.p. 220–222 C. (KBr) cm^{-1}IR, (NH) 3330, 2226 (CN), cm^{-1}. (DMSO-d_6) NMR ^{1}H: (m, 2H, H-5′, H-6′) *δ* = 7.21-7.30, (m, 1H, H-7′) *δ* =7.54-7.57, (d, 1H, H-4′) *δ* =7.92, (s, 1H, H-6) *δ* =8.51, (s, 1H, H-2′) *δ* =8.54, (bs, 1H, NH , exchangeable with D_2O) *δ* =12.49, (bs, 1H, NH, exchangeable with D_2O) *δ* =13.24. (DMSO-d_6) NMR ^{13}C: (C-5) *δ* = 93.71, (C -3′) *δ* =109.82, (C-7′) *δ* = 112.87, (CN) *δ* =118.43, (C-4′) *δ* = 118.49, (C-5′) *δ* = 122.02, (C-6′) *δ* =123.52, (C-3′a) *δ* =126.92, (C-2′) *δ* =132.07, (C-7′a) *δ* =136.20, (C-6) *δ* =146.14, (C-2)

δ=162.62, (C=S) δ=164.62. Anal. Calcd. for $C_{13}H_8N_4S$ (252.295): C, 61.89; H, 3.20; N, 22.21; S, 12.71; Found: C, 61.74; H, 3.15; N, 22.26; S, 12.82.

2.6. 3-(*1H*-Indol-3-yl)-1H-pyrazole-4-carbonitrile (7).

After refluxing a combination of 4 (4 mmoles, 1.0 g) and (5 ml) hydrazine hydrate for three hours, cold water was added. A 72% yield of solid product was obtained through filtration and crystallization from ethanol.

Physical and spectroscopic data: mp 218-219 °C; (KBr) cm^{-1} IR, 3349, 3238 (NH), 2227 (CN), cm^{-1}. (DMSO-d_6) NMR ^{1}H: (m, 2H, H-5′, H-6′) δ= 7.11-7.23, (m, 1H, H-7′) δ=7.48-7.50, (s, 1H, pyrazole H-5) δ=7.90, (d, 1H, H-4′) δ=8.05, (s, 1H, H-2′) δ = 8.42, (bs, 1H, NH , exchangeable with D_2O) δ =11.59, (bs, 1H, NH, exchangeable with D_2O) δ =13.22. (DMSO-d_6) NMR ^{13}C: (C-4) δ = 94.30, (C-3′) δ =111.98, (CN) δ =115.85, (C-7′) δ =120.13, (C-4′) δ = 120.56, (C-5′) δ =122.02, (C-6′) δ =122.25, (C-3′a) δ =124.57, (C-2′) δ =128.67, (C-7′a) δ =128.88, (C-3) δ =135.42, (C-5) δ =136.15. ms: m/z 208 (M+). Anal. Calcd. for (208.22) $C_{12}H_8N_4$: C (69.22); H (3.87); N (26.91). Found: C (69.35); H (3.74); N (26.84).

2.7. General procedure to prepare compounds 9a,b

Reflux temperature was used for 7 hours to heat a mixture of enaminonitrile 4 (10 mmol), p-methoxyphenyl hydrazine hydrochloride or p-chlorophenyl hydrazine, K_2CO_3 anhydrous (15.0 mmol ,2.0 g), and 100% ethanol (20 mL). Once the mixture had cooled, it was added to ice water, acidified with hydrochloric acid diluted, and the producing solid was filtered off before being recrystallized in ethanol

2.7.1. (5-Amino-1-(4-methoxyphenyl)-1H-pyrazol-4-yl) (*1H*-indol-3-yl) methanone (9a).

Physical and spectroscopic data: Brown crystal (89%), mp 230-232ºC, IR (Nujol): ν = (NH) 3452, (NH_2) 3290, 3148, (CO) 1650, cm^{-1}; (DMSO-d_6) NMR ^{1}H: s, 3H, OCH_3 (δ = 3.82), s, 2H, NH_2 (δ = 6.81), d, 2H, *J* = 9.0 Hz, H-3′′,5′′ (δ =7.10), m, 2H, H-5′, 6′ (δ =7.14-7.22), m, 1H, H-7′ (δ =7.45-7.47), d, 2H, *J* = 9.0 Hz, H-2′′,6′′ (δ =7.49), s, 1H, H-3 (δ =8.13), m, 1H, H-4′ (δ=8.23-8.25), d, 1H, *J* = 2.7 Hz, H-2′ (δ=8.29), s, 1H, NH (δ=11.86). (DMSO-d_6) NMR ^{13}C: OCH_3 (δ = 55.47), C-4 (δ=104.09), C-7′ (δ=111.88), C-3′′,5′′ (δ=114.58), C-3′ (δ=115.97), C-5′(δ=121.05), C-4′ (δ=121.56), C-6′(δ =122.51), C-2′′,6′′ (δ =125.52), C-3′a (δ =126.40), C-1′′(δ =130.67), C-2′(δ =131.03), C-7′a(δ =136.22), C-3 (δ =139.96), C-5 (δ =150.40), C-4′′ (δ =158.47), CO (δ =182.94). ESI$^+$-MS: 333.33 ([M+1]$^+$). Anal. Calcd. for $C_{19}H_{16}N_4O_2$ (332.36): C, 68.66; H, 4.85; N, 16.86. Found: C, 68.59; H, 5.01; N, 16.89.

2.7.2. (5-Amino-1-(4-chlorophenyl)-1H-pyrazol-4-yl) (1H-indol-3-yl) methanone (9b).

Physical and spectroscopic data: Brown powder (80 %),mp 217.0-219.5ºC; IR (Nujol): ν = 3382 (NH), 3295, 3168 (NH_2), 1653 (CO), cm^{-1}; (DMSO-d_6) NMR ^{1}H: (m, 2H, H-5′, 6′) δ = 6.85 (s, 2H, NH_2, exchangeable with D_2O), δ=7.15 (d, 2H, *J* = 9.0 Hz, (H-3′′,5′′), δ=7.22-7.31, (d, 1H, *J* = 8.4, Hz, H-7′) δ = 7.48, (d, 2H, *J* = 9.0 Hz, (H-2′′,6′′) δ=7.53, (s, 1H, H-3) δ=8.14, (d, 1H, *J* = 2.1 Hz, H-4′) δ = 8.22, (d, 1H, *J* = 2.7 Hz, H-2′) δ=8.29, (s, 1H, NH, exchangeable with D_2O) δ =11.84. Anal. Calcd. for $C_{18}H_{13}ClN_4O$ (336.77): C, 64.20; H, 3.89; N, 16.64; Cl, 10.5. Found: C, 64.30; H, 3.96; N, 16.52; Cl, 10.63.

2.8. Biological evaluation

2.8.1. DPPH·Free radical scavenging assay

By using the procedure which described by Blois [29], the DPPH radical-scavenging capacity of sweet fennel methanolic extract was assessed. One milliliter of 0.1 mM DPPH
was combined with three milliliters of five, ten, twenty, and thirty µg/ml fennel methanolic extracts. The mixture was shaken well and then left at room temperature for thirty minutes. Except for the extracts, all of the reaction reagents were included in the negative control, and butylated 4-hydroxyl toluene (BHT) was the control (positive). After that, the absorbance was calculated at 517 nm and compared to a blank (pure methanol). Stronger ability to scavenge free radicals was indicated by a lower absorbance of the mixture. The DPPH radical's scavenging ability was calculated using the

formula below:

DPPH(1,1-diphenyl-2-picrylhydrazyl): Scavenging action (Inhibition %) =
[(A c –AS/Ac)×100]

Where *As* represented the absorbance while the tested chemicals were present and *Ac* represented the absorbance of the control reaction. IC50: the amounts required to cause 50% inhibition

2.8.2. Ferrous chelating activity

Decker and Welch [30] evaluated the chelating of ferrous ions in sweet fennel. 2 mM FeCl2 (0.1 ml) and 5 mM ferrozine (0.2 ml) solution were added to five milliliters of the 80% tested substances or EDTA solution, which served as a control (positive), at varying doses (5, 10, 20, 30, µg/ml). At room temperature after 10 minutes, the reaction mixture was tested for absorbance at 562 nm. Using the following formula, the ferrous ion chelating capacity percentage was determined:(*Chelating activity* %) = [(*Ac – As/ Ac*) × 100

2.9-Experimental- Molecular docking study

The compounds compound-6(Indolyl Pyrimidine deravatives) and compound-7(IndolyPyrazole deravatives) (designed medicine) atoms exposed to Gasteiger partial charges were employed in both Auto Dock 4.2 and docking calculations. The pattern of ligands-proteins was calculated. making rotatable bonds more understandable and joining nonpolar hydrogen atoms. Following the introduction of fundamental atoms of hydrogen, Kollman standardized atom type charges and solvation parameters were applied by the Auto Dock tools [32-34]. Van der Waals and electrostatic terms were computed by the distance dependent dielectric functions and the Auto Dock parameter set, respectively. To simulate docking, two methods were employed: Solis, Lamarckian genetic algorithm and Wets local search methodology. Additionally, the ligand molecule's initial orientation, torsions and location were determined

2.10. In vitro assay

By using the Skehan et al.[31] approach, the potential cytotoxicity of the investigated substances (100- 0.78µg/ml) against human hepatic cell line (HepG-2), humancoloncancer(HCT116), (MCF7) breast cancer and (A549) lung cancer cell line (A549). In order to allow the cells to adhere to the plate wall, they were plated in 96-multiwell plates (104 cells per well) for 24 hours before to plant treatment. The cell monolayer was treated with various chemical compound concentrations, and triplicate wells were made for each dose. Each chemical was incubated for 48 hours at 37 °C in an environment with 5% CO2 on monolayer cells. Cells were fixed after 48 hourscleaned, and stained with sulforhodamin B dye. Tris EDTA buffer was used to recover the adhered stain after the excess stain was cleaned off with acetic acid. An ELISA reader was used to quantify color intensity. In order to produce the survival curve for each tumor cell, the relationship between the percentage of surviving cells and the component concentrations was displayed.

2.11. Statistical analysis

Data were statistically analyzed using Co-stat computer program statistical package data.

3. Results and discussion

Multifunctional reagents, enaminonitriles have recently been effectively used to synthesize heteroaromatics that would not otherwise be easily accessible [12].

One of the three methods for synthesizing meridianines from indole derivatives that has been reported involves Suzuki cross-coupling with derivatives of indole-3-boronic acid [13]. Bredereck prepare was also reported from α-enaminones [14–16], which was derived from derivatives of 3-acetylindole. Trimethyl silylynone indole compounds have more recently been used to produce meridianines [17].

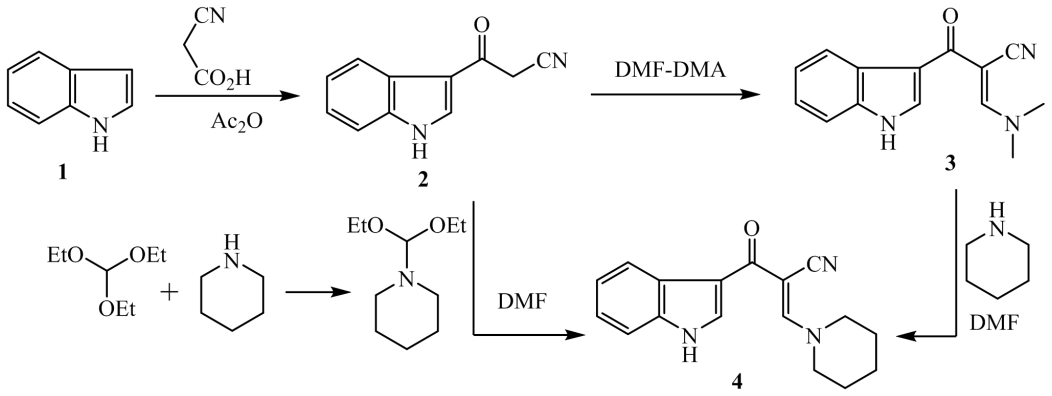
The chemistry of -enaminonitriles [18-21] compounds has received a great interest due to their chemical reactivity, Here, we present the findings from an investigation into the possible applications of 2-(1H-indole-3-carbonyl)-3-(piperidin

-1-yl)acrylonitrile **4**, which is employed as a precursor to 5′-cyanomeridianin G and 3-heteroarylindoles.

Two strategies were studied for the synthesis of enaminonitrile **4**. In the first one, we planned to prepare compound **4** starting from easy procedure for the cyan acetylation of indole [22] leads to cyanoacetylatedindole **2** followed by reactionwithdimethylformamidedimethylacetal (DMF-DMA) to give the corresponding enaminonitrile **3** as we previously reported [23]. Boiling of enaminonitrile **3** with piperidine in DMF furnished the corresponding enaminonitrile **4** in moderate yields.

In view of the low yield of the above synthetic methodology, and to avoid benefit of toxic and expensive (DMF-DMA), compounds **4** could be obtained by alternative procedure requires active methylene to reflux, cyanoacetylatedindole **2,** through the first generation of the created insituamidoacetal,[1(diethoxymethyl)piperidine], which then condenses with an active methylene molecule to yield enaminonitrile 4 (Scheme 1) [12].

Data of spectra (NMR ^{13}C, NMR ^{1}H, MS and IR) and elemental analyses of the reaction products confirmed the assigned structure **4**. The spectra of IR exposed peaks at 3218, 2193 and 1635 cm^{-1}, respectively for:- NH, CN and C=O, functions, ^{1}H NMR spectrum howed two multiplets at 1.65 and 3.32 ppm integrated for 6 and 4 protons, respectively, for the piperidyl-CH_2's, in addition to aromatic indole protons in its expected positions, =CH-N proton at 7.99 ppm and NH indole at 11.73 ppm. Also, ^{13}C NMR data and mass reinforced a proposed structure for **4** (m/z 279, M+).



Scheme 1

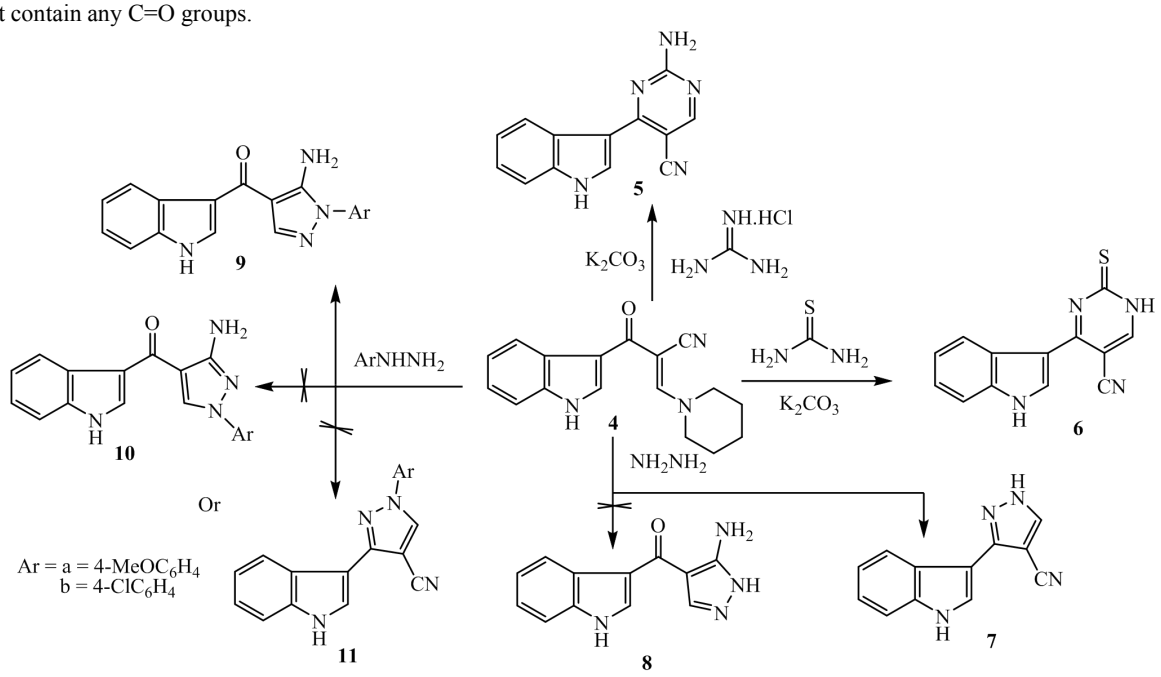
Having now available the new enaminonitrile **4** prompted us to study their synthetic utilities as key intermediates for indole alkaloids with 5-or 6-membered heterocycles.

Thesynthesis of the 2-aminopyrimidine ring was carried out according to the Bredereck technique [15]. Direct conversion of **4** into 5′-cyano meridianine G derivative **5** which involves formation of the 2-aminopyrimidine ring was obtained in 69% of the cases with guanidine hydrochloride treatment in dry ethanol under reflux circumstances with anhydrous potassium carbonate present (Scheme 2).

Furthermore, compounds **6** was conveniently prepared by treatment of the enaminonitrile **4** with thiourea, produced 6 in 78% yields under comparable reaction conditions. Analysis of elemental, IR, mass, and NMR ^{1}H and ^{13}C spectral data were used to determine the structures of compounds 5 and 6 (cf. Experimental Section).

Refluxing 3 molar equivalents from hydrazine hydrate in ethanol with 1 molar equivalent of compound 4 were unsuccessful and the compound produce which was obtained unreacted. The pyrazole derivative was obtained by fusion of compound **4** with 8 molar equivalents of hydrazine hydrate to only be able to purchase 4-cyano-3-(3-indolyl)pyrazole 7.

In addition to a strong band at 2227 cm-1 that was assigned to the CN group, the infrared spectrum of sample 7 revealed two NH stretching bands at 3349 and 3238 cm-1. A singlet signal at 7.90 ppm, which corresponds to pyrazole H-5, was detected in compound 7's ^{1}H NMR spectra. Compound 8's structure was ruled out since the IR and ^{13}C NMR spectra did not contain any C=O groups.



. **Scheme 2**

As anticipated, compound **4** was treated with 4-methoxyphenylhydrazine or 4-chlorophenylhydraune in refluxing ethanol, in a basic medium, produced a single product, and substituted pyrazoles with N-1 or N-2 (**9–11**). Because there was no cyano group in either the ^{13}C NMR or the IR spectra, the structure of the anticipated pyrazole **11** was ruled outAll of the anticipated signals were present in compound **9**'s one-dimensional, one-hertz (^{1}H) NMR analysis, yet this was insufficient to distinguish between structures 9 and 10. Consequently, we acquired the NMR spectra HMQC and HMBC and established a clear assignment in the NMR spectra ^{1}H and ^{13}C (refer to the experimental section). The absence of a correlation peak in the HMBC spectra between the carbon signals at 130.67 (C-1′′) and pyrazole H-3 at 8.13 ppm is specific to structure **9**, not structure **10.**

3.1. Biological activity

According to recent reports, meridianine D, a naturally occurring substance, exhibited mild anticancer effects against several tumor cell lines, but 6-debromomeridianin D, its analogue, showed no antitumor activity at all [24]. However, against the MCF7 breast cancer cell line and the HeLa cervical cell line, the cyano meridianine D derivative demonstrated good cytotoxic activity [14]. Here we report the antitumor and antioxidant activities of 5′-CyanomeridianinG**5**, indolylpyrimidinethione-5-carbonitrile **6** and indolylpyrazole-4-carbonitrile **7**.

3.2. Antioxidant activity

3.2.1. Scavenging activity on DPPH·Radicals

The antioxidant activity of various plant, vegetable, and fruit extracts has been extensively studied using DPPH [25, 26]. In comparison to BHT as a synthetic antioxidant agent, the primary values of radical DPPH scavenging activity, expressed as IC50 against DPPH radical of three chemical structures, 5, 6, and 7, are shown in Fig. (1).

Compounds **5** and **6** demonstrated the highest $\mathbf{IC_{50}}$ (48.46 µg/ml and 62.74 µg/ml respectively), in comparison with **7** (44.65 µg/ml) and standard (42.47 ug/ml). While, butylated hydroxytoluene(BHT), showed the most amount of scavenging activity (10.07 µg/ml). The production of hydroxyl radicals at or close to DNA can eventually cause DNA strand breaks (Floyed, 1981)[27], and they also play a major role in major biological effects such mutagenesis, carcinogenesis, and cytotoxicity (Swartz, 1984)[28]. These

results revealed that **5** and **6** exhibited the lowest DDPH scavenging activitiesas compared either with standard or **7**.

3.2.2. Ferrous ion-chelating activity of chemical compounds

The effect of **5**, **6** and **7** on ferrous ion chelating power is reported in Table (1). The Fe(II) chelating activities of **5**, **6**, **7** and by increasing the concentration of the chemical compounds, EDTA standard were increased (linear relationship and dose- dependent). **5** and **6** display the highest value with Fe(II) chelating activities (52.89 and 47.86 µg/ml, respectively), followed by **7** (40.00 µg/ml) as compared to standard (96.49 µg/ml) at concentration of 30 µg/ml. Table 1 shows that the EDTA standard exhibited the strongest chelating activity across all concentrations tested.

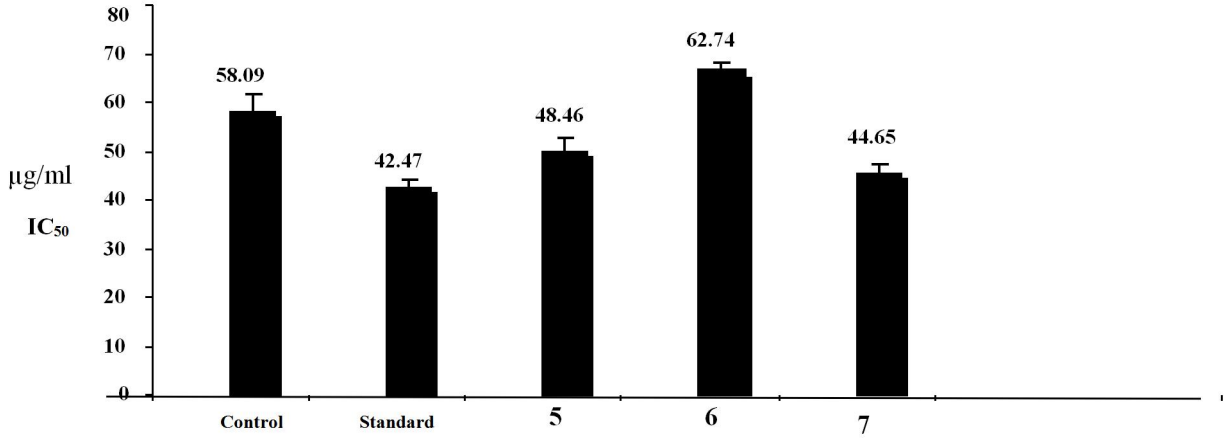


Fig . 1. IC_{50} of DPPH free radical of chemical compounds

All values have a significant difference of p > 0.05 and are the means± SD of three replicates.IC_{50}:50%Inhibition Concentration

Table 1 . Ferrous chelating activity by different synthetic chemical compounds

Compounds	Inhibition %			
	5µg/ml	10 µg/ml	20 µg/ml	30 µg/ml
100% (Control)	9.72 [a] ± 0.12	10.25 [b] ± 0.20	12.57 [a] ± 0.20	13.00 [b] ± 0.12
5	12.64 [b] ± 0.50	30.44 [c] ± 0.42	40.92 [b] ± 0.35	52.89 [c] ± 0.29
6	11.65 [b] ± 0.24	20.04 [c] ± 0.42	33.86 [a] ± 0.42	47.86 [b] ± 0.35
7	9.42 [a] ± 0.10	14.05 [b] ± 0.11	23.87 [a] ± 0.20	40.00 [b] ± 0.12
EDTA standard	19.02 [e] ± 0.06	44.35 [f] ± 0.11	82.32 [e] ± 0.17	96.49 [f] ± 0.06
LSD (P≤ 0.05)	0.72	0.59	0.57	0.58

Each value is the average of three replicates and is deemed statistically significant at p ≥ 0.05 ± standard deviation. The statistical analysis is performed with the SPSS software and the Co-state software (Version 7), with a significance level of P ≤0.05 for unshared letters.

3.3. The cytotoxicity

Table 2 displays the cytotoxicity of the chosen compounds on the survival fractions of HEPG2 and HCT116. Compounds **5**, **6** and **7** recorded inhibition of carcinogenic hepatic cells by 100%, at 100 ppm . However, **5** showed the highest inhibition percent of colon carcinoma cells (74.6 %), followed by **6** (51.5%) . While, **7** recoded the lowest inhibition percent amounting to 12.7% , at the concentration of 100 ppm (Table 3). On the other hand , **5**, and **6** showed the highest inhibition percent against breast adenocarcinoma amounting to 100 and 79.8%, respectively, while **7** demonstrated the lowest activity (Table 4). In addition, all compounds exhibited inhibition activity toward Lung carcinoma cell line (A549) reached to 88.6, 95.4 and 75.6 %, respectively for **5**, **6** and **7** respectively (Table 5).

Table 2 : HEPG 2 (Human hepatocellular carcinoma cell line) inhibition percent
Sample concentration range between (100 to 0.78 µg/ml)

Compunds	**LC_{50}** (µg/ml)	**LC_{90}** (µg/ml)	Remarks
5	16.9	30.5	100% at 100ppm
6	18.8	3408	100% at 100ppm
7	31.6	55.6	100% at 100ppm
DMSO	--------	--------	1% at 100ppm

LC_{50}: Lethal concentration for the sample which causes killing of 50% of cells within 48 hrs
LC_{90}: The concentration which causes killing of 90% of cells within 48 hrs

Table 3 : HCT116 (Human Colon carcinoma) inhibition percent

Compunds	***LC_{50}** (µg/ml)	***LC_{90}** (µg/ml)	Inhibition activity %
5	19.4	33.4	74.6% at 100 ppm
6	17.8	29.8	51.5% at 100 ppm
7	32.4	56.4	12.7% at 100 ppm
Positives control			100% at 100 ppm
DMSO control	3%		
Blank	Zero		

LC_{50}: Lethal concentration for the sample which causes killing of 50% of cells within 48 hrs
LC_{90}: The concentration which causes killing of 90% of cells within 48 hrs

Table 4: Human Caucasian breast adenocarcinoma (MCF 7) inhibition percent

Compunds	**LC_{50}** (µg/ml)	**LC_{90}** (µg/ml)	Remarks
5	30.9	56.9	100% at 100 ppm
6	58.5	103.8	79.8% at 100 ppm
7	----------	----------	22.5% at 100 ppm
Positives control			100% at 100 ppm
DMSO control	3%		
Blank	Zero		

LC_{50}: Lethal concentration for the sample which causes killing of 50% of cells within 48 hrs
LC_{90}: The concentration which causes killing of 90% of cells within 48 hrs

Table 5: Lung carcinoma cell line (A549) inhibition percent

Compunds	***LC_{50}** (µg/ml)	***LC_{90}** (µg/ml)	Remarks
5	52.5	86.9	88.6% at 100 ppm
6	37.9	73.1	95.4 % at 100 ppm
7	70.4	114.4	75.6 % at 100 ppm
Positives control			100% at 100 ppm
DMSO control	2%		
Blank	Zero		

*LC_{50} Lethal concentration for the sample which causes killing of 50% of cells within 48 hrs
*LC_{90}: The concentration which causes killing of 90% of cells within 48 hrs

4-Result and discussion-Molecular Docking

We expect the study of compounds 6(Indolyl Pyrimidine deravatives) and 7(IndolyPyrazole deravatives) on some anticancer proteins according to the biological part as follow:
we study effect of compound 6 and 7 on (A549)-Lung carcinoma cell line, (MCF 7)-Human Caucasian breast adenocarcinoma, HCT116 -(Human Colon carcinoma) and HEPG 2 -(Human hepatocellular carcinoma cell line) are examples of host species that can be used to attach ligands (guests). With auto Dock, you can discuss and demonstrate the biological benefits of compounds 6and 7 and 5 as well as those compounds containing active groups and atoms (like S, O, and N) that

proms give a good biological activity and can theoretically demonstrate it. Figures 2-9 shown that HB plots can produce results that are comparable while displaying a high amount of interaction with each receptor. Based on calculations, inter-hydrogen bonds were clearly observable in all proteins. Three-dimensional or two-dimensional pictures can be used to show docking molecular interactions. The interaction mechanism of the docking molecules can be depicted using both two- and three-dimensional images. (Figures 2-9). H- bonding has emerged as a key intermediate for the interaction between ligand and amino acids in the protein as we examined the impact of compounds 6and 7 on (A549)-Lung carcinoma cell line, (MCF 7)- breast cancer, HCT116 -(Colon cancer) and HEPG 2 -(Liver cancer). Firstly we study compound 6 with choice proteins as follow:

(A549)-Lung cancer (4J1Q), the reaction of bonds result from the interact an amino acid in the protein with compound 6, its made 6 bonds: 4J1Q-1//A/TRP`362 – with length of H bond 3.2 Aº, 4J1Q-1//A/PRO`363 - with length of H bond 2.5 Aº, 4J1Q-1//A/ TRP`365- with length of H bond 3.6 Aº, 4J1Q-1//A/MET`370- with length of H bond 2.8 Aº, 4J1Q-1//A/MET`370- with length of H bond 3.3 Aº, 4J1Q-1//A/GLY` 368- with length of H bond 2.9 Aº, with binding energy = − 7.8 kcal mol^{-1} (Figures 2).

For (MCF 7)-Human Caucasian breast adenocarcinoma (5TWL): the reaction of bonds result from the interact an amino acid in the protein with ligand and occures 8 bonds: 5TWL-h//A/LYS`40– with length of H bond 3.3 Aº, 5TWL-h//A/GLY`20– with length of H bond 2.3 Aº, 5TWL-h//A/ALA`23– with length of H bond 3.1 Aº, 5TWL-h//A/VAL`25– with length of H bond 2.6 Aº, 5TWL-h//A/ILE`149– with length of H bond 3.0 Aº, 5TWL-h//A/ASP`150– with length of H bond 2.7 Aº, 5TWL-h//A/LYS`40– with length of H bond 2.4 Aº, 5TWL-h//A/LYS`40– with length of H bond 2.1 Aº, with binding energy = − 6.7 kcal mol^{-1} (Figures 3).

for HCT116 -5KJU (Human Colon carcinoma) it made 5 bonds: 5KJU –h//A/ALA` 282– with length of H bond 3.2 Aº, 5KJU –h//A/ILE` 302– with length of H bond 2.1 Aº, 5KJU –h//A/YHR` 360– with length of H bond 3.2 Aº, 5KJU –h//A/ILE` 302– with length of H bond 2.1 Aº, 5KJU –h//A/ALA` 282– with length of H bond 3.0 Aº, with binding energy = − 8.4 kcal mol^{-1} (Figures 4).

For last protein, HEPG 2 - 8HYD (Human hepatocellular carcinoma cell line), it mad 6 bonds with compound 6 as follow: 8HYD –h//A/ASN` 210– with length of H bond 3.4 Aº, 8HYD –h//A/CYS` 198– with length of H bond 3.1 Aº, 8HYD –h//A/AS9` 118– with length of H bond 2.1 Aº, 8HYD –h//A/PHE` 62– with length of H bond 3.6 Aº, 8HYD –h//A/AS9` 118– with length of H bond 2.5 Ao, 8HYD –h//A/AS9` 118– with length of H bond 2.7 Aº

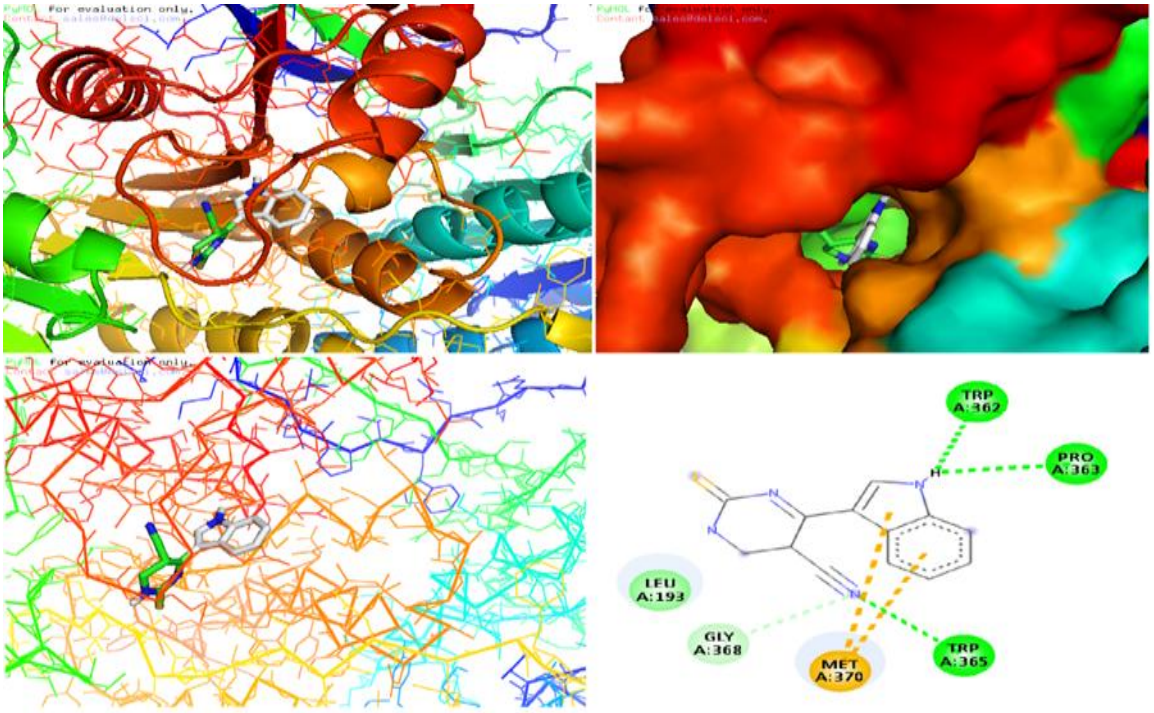


Figure 2. 3D plot interaction of compound 6 with *4J1Q-(A549)-Lung carcinoma*

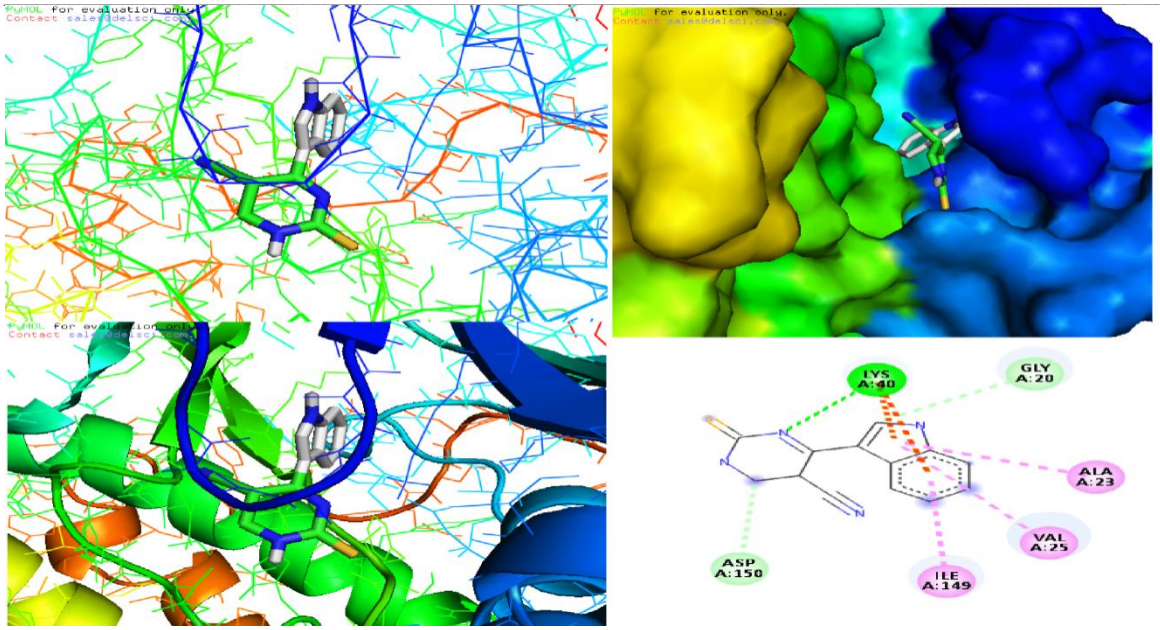


Figure 3. 3D plot interaction of compound 6 with All-6-5TWL-(MCF 7)-Human breast

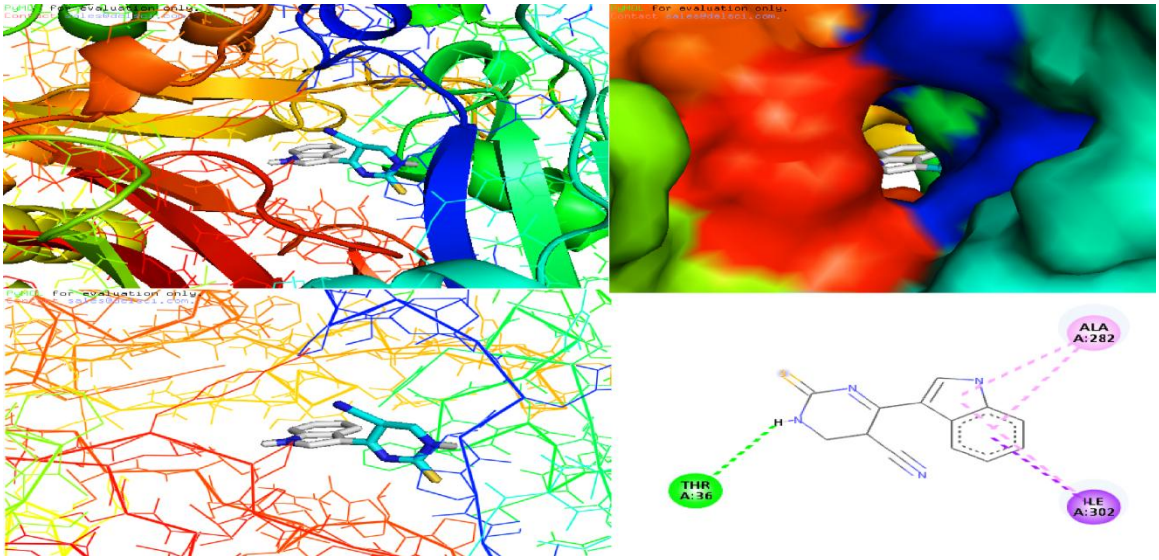


Figure 4. 3D plot interaction of compound 6 with All-5KJU-HCT116 -Human Colon.

However, when we examined the effect of compound 7 on amino acids of anticancer-Receptor proteins as follow:

(A549)-Lung carcinoma cell line(4J1Q), the reaction of bonds result from the interact an amino acid in the protein with compound 6, its made 5 bonds: 4J1Q -1//A/SER`317– with length of H bond 3.2 Aº, 4J1Q -1//A/LEU`193– with length of H bond 2.5 Aº, 4J1Q -1//A/MET`370– with length of H bond 2.4 Aº, 4J1Q -1//A/TRP`365– with length of H bond 3.1 Aº, 4J1Q -1//A/SER`316– with length of H bond 2.7 Aº, with binding energy = − 8.0 kcal mol^{-1} (Figures 6).

For (MCF 7)-Human Caucasian breast adenocarcinoma (5TWL): the interaction which occures between amino acid in protein with ligand creates 13 bonds: 5TWL -h//A/ILE`17– with length of H bond 3.2 Aº, 5TWL -h//A/LEU`139– with length of H bond 2.4 Aº, 5TWL -h//A/ LEU`139– with length of H bond 2.7 Aº, 5TWL -h//A/ LEU `86– with length of H bond 3.1 Aº, 5TWL -h//A/GLU`87– with length of H bond 2.5 Aº, 5TWL -h//A/CYS`89– with length of H bond 3.4 Aº, 5TWL -h//A/ALA`38– with length of H bond 2.9 Aº, 5TWL -h//A/VAL`17– with length of H bond 3.7 Aº, 5TWL -h//A/ILE`149– with length of H bond 2.7 Aº, 5TWL -h//A/CYS`70– with length of H bond 2.8 Aº, 5TWL -h//A/ALA`38– with length of H bond 2.6 Aº, 5TWL -h//A/VAL`17– with length of H bond 3.0 Aº, 5TWL -h//A/CYS`70– with length of H bond 2.1 Aº, with binding energy = − 6.9 kcal mol^{-1} (Figures 7).

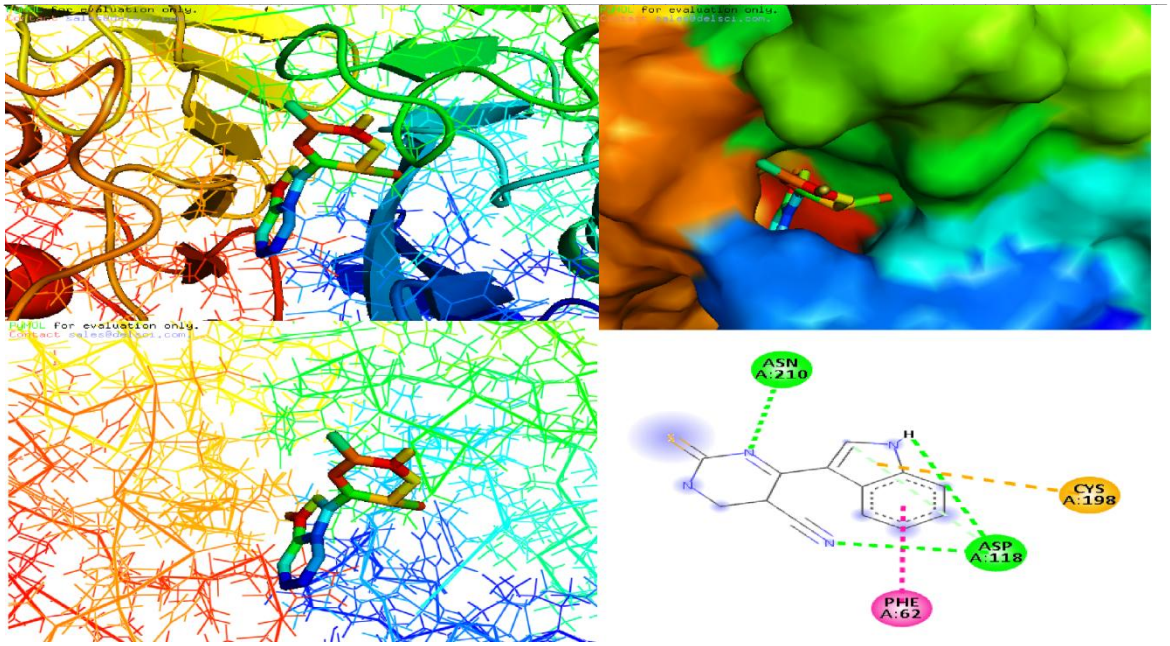


Figure 5. 3D plot interaction of compound 6 with All-8HYD-HEPG 2 -Human hepato.

For HCT116 -5KJU (Human Colon carcinoma) it made 8 bonds: 5KJU –h//A/THR` 36– with length of H bond 3.1 Aº, 5KJU –h//A/LEU` 162– with length of H bond 2.5 Aº, 5KJU –h//A/ASP` 157– with length of H bond 3.1 Aº, 5KJU –h//A/GLY` 158– with length of H bond 2.7 Aº, 5KJU –h//A/ALA` 156– with length of H bond 2.7 Aº, 5KJU –h//A/MET` 151– with length of H bond 3.5 Aº, 5KJU –h//A/ASP` 157– with length of H bond 2.8 Aº, 5KJU –h//A/MET` 151– with length of H bond 2.9 Aº , with binding energy = − 7.7 kcal mol^{-1} (Figures 8).

In the end, protein, HEPG 2 - 8HYD (Human hepatocellular carcinoma cell line), it mad 6 bonds with compound 6 as follow: 8HYD –h//A/ASN` 210– with length of H bond 3.1 Aº, 8HYD –h//A/TYR` 67– with length of H bond 3.4 Aº, 8HYD –h//A/HIS` 240– with length of H bond 2.5 Aº, 8HYD –h//A/TRP` 87– with length of H bond 3.6 Aº, 8HYD –h//A/HIS` 240– with length of H bond 2.5 Aº, 8HYD –h//A/AS9` 118– with length of H bond 2.7 Aº , with binding energy = − 8.1 kcal mol^{-1} (Figures 9).

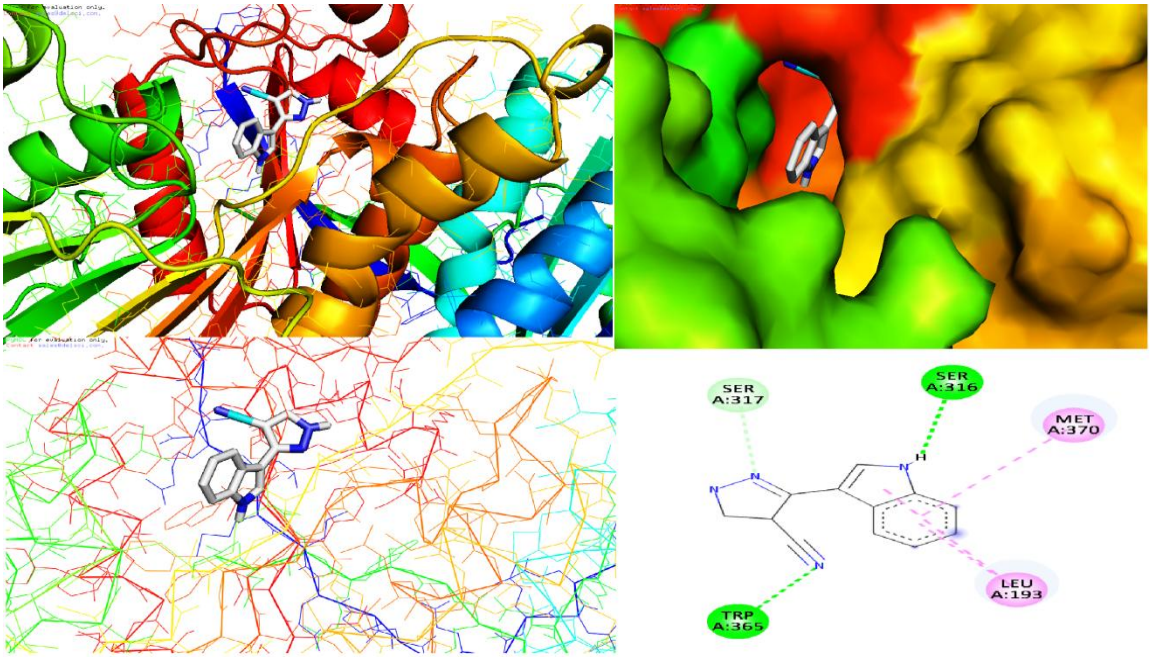


Figure 6. 3D plot interaction of compound 7 with *All-4J1Q-(A549)-Human Lung.*

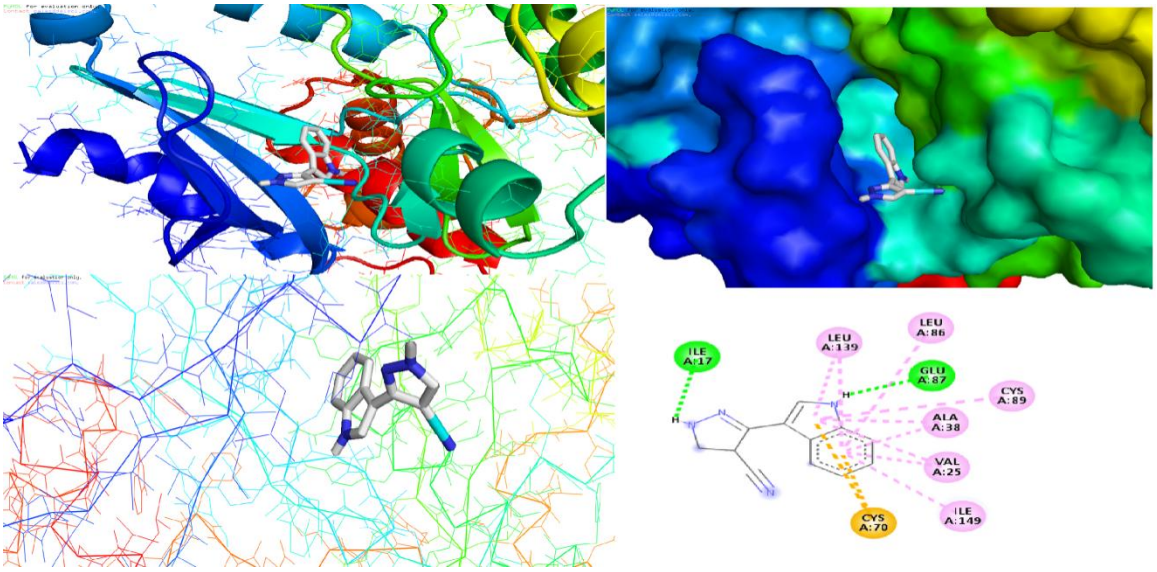


Figure 7. 3D plot interaction of compound 7 with All-5TWL-(MCF 7)-Human breast.

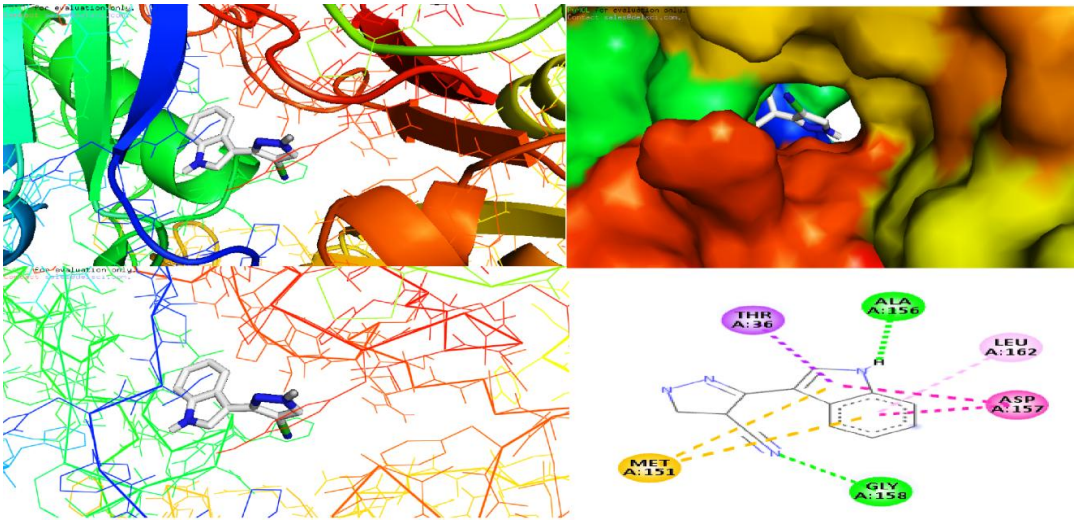


Figure 8. 3D plot interaction of compound 7 with All-5KJU-HCT116 -Human Colon.

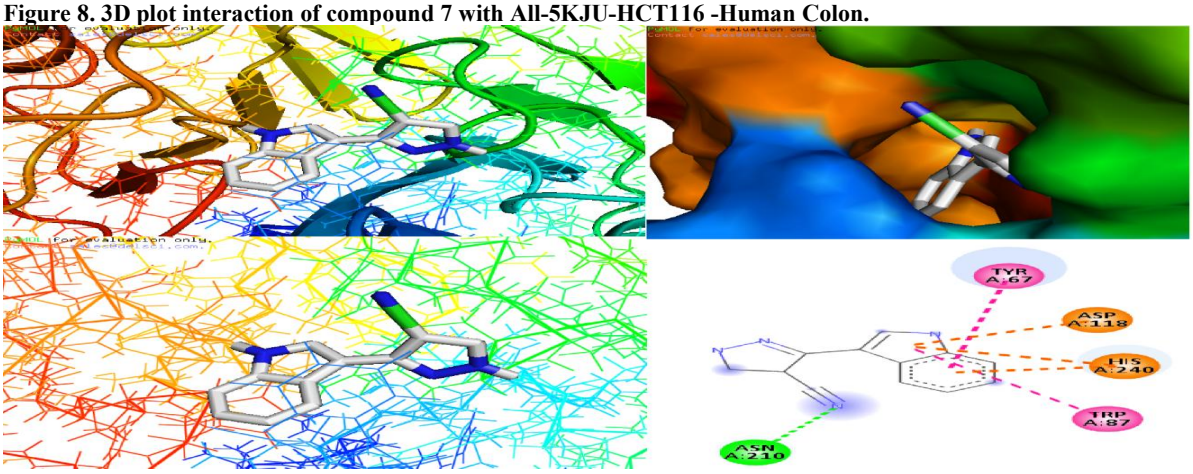


Figure 9. 3D plot interaction of compound 7 with All-8HYD-HEPG 2 -Human hepatocellular.

5. Conclusion

The easily prepared enaminonitrile **4** has been connverted into the coressponding 5′-cyanomeridianin G and indolylpyrimidine thione analogues by treatment with amino guanidine and thiourea. The indolylpyrazole derivatives were also prepared.Anticancer screening of cyano compounds **5-7** showed that indolylpyrimidine thione **6** was more cytotoxic against Lung carcinoma cell line (A549) with ihaibition activity of 95.4 % and it has the highst antioxidant activities. While the cyanomeridianin G **5** showed high cytotoxic activity against MCF7, A549 and HCT116 with 100, 88.6 and 74.6 % inhibition in cell viability, respectively. the aforementioned discussion demonstrates that compound 6 and compound 7's activity, which results from their heteroatoms (O, N, and S) being in good positions, is what gives them good energy, a high number of hydrogen bonds, an RMSD for the compound with respect to the selected proteins, and a good connection with amino acids, which provides a nice compatibility with the experimental part.

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