ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

SYNTHESIS, DOCKING AND PHARMACOLOGICAL EVALUATION OF SOME NEW QUINOLINE DERIVATIVES

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Received on 20-08-2016 *Accepted on 25-09-2016*

Abstract

A general route to the synthesis of quinolone derivatives was developed. Pyrano quinoline-3-carbimidic acid derivative (2) was obtained from 8-hydroxy quinoline and cyanoacrylamide derivative(1) and then was reacted with choloro acetyl chloride, hydrazine hydrate, thiourea, p f.benzaldehyde, triethyl orthoformate,acetic anhydride, , ethyl cyano acetate to give a number of quinolone derivatives (3-10). A number of quinoline-3-carbimidic acid derivatives (11-13) yielded when compound (10) reacted with Thioglycolic acid, phenyl isothiocyanate, salicyldehyde,DMF-DMA. Pyrano [3, 2-h] quinoline-3-carbimidic acid derivative (14) yielded when compound (13) reacted with hydrazine hydrate 98%. The compounds were used to evaluate their antitumor potency on three human tumor cell lines namelyprostatic adenocarcinoma (PC3), human colorectal carcinoma (HCT116) and human liver hepatocellular carcinoma (HepG2).Docking simulation was performed to show the position of compounds 3, 10, and 13 into the Methionine Synthase structure active site to determine the probable binding model. Compounds 3, 10 and 13 may be potential anticancer agents with potent inhibitory activity in tumor growth inhibition.

Keywords: Quinoline, Pyrano quinoline, Cyanoacrylamide derivative, Molecular dockin, Pharmcological studies.

Introduction

Quinoline derivatives constitute an important class of nitrogen-containing heterocyclic, Quinoline is the key structural element for many drugs having broad spectrum of action, a new class of 4-quinolinecarboxylic acid analogues were designed as hDHODH inhibitors and anticancer agents[1] ·4Hpyranoquinoline -3-carbonitrile derivatives was synthesized in 2015 using p-methoxy or p-fluoro benzylidene malonitrile with 8-hydroxyquinoline [2] .The various pharmacological properties of quinoline derivatives attracted great attention in the last few decades as their vast occurrence in natural products and drugs [3]. Quinoline derivatives were synthesized and explored for

their analgesic activity[4] as antiallergenic agents[5] in treating Alzheimer's disease (AD) [6] as anticancer[7, 8] antitinephritic[9] antitumor[10] and anti-inflammatory activities. Various derivatives of quinoline have been employed in the synthesis of antihypertensive, antifungal, and antibacterial drugs. Therefore, the synthesis of such compounds has attracted strong interest. Our thesis provides some lights as small contribution in depth view of work done so far on quinoline and its biological activities [11].

Experimental Section

Materials

All melting points are uncorrected and were taken on an electro-thermal capillary melting point apparatus using .Melting Point, Digital, Advanced, SMP30. The elemental analyses for C, H, and N were done on Vario EL III. Infrared spectra were recorded on a Jasco FT/IR-6100; Fourier transforms, Infrared spectrometer at cm-1 scale using the KBr disc technique. ^{1}H- NMR spectra were determined by using a JEOl EX-270 NMR spectrometer. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer. Follow-up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminum sheets (Type 60, F 254) and the spots were detected by exposure to a UV analysis lamp at λ 254/366 nm for a few seconds.

Synthesis of 2-amino-4-(3,4-dimethoxyphenyl)-4H-pyrano[3,2-h]quinoline-3-carbimidic acid (2).

A solution of (1) (0.01 mol) and 8-hydroxy quinoline (0.01 mol) in ethanol (20 ml) containing piperidine (0.5 ml) was heated for 30 min[2].The solid that formed was recrystallized from ethanol forming the compound (2) with yield 90 % (m.p.186-190℃).Elemental *Anal*. Calc. for $C_{21}H_{19}N_3O_4$ (377.39): *C, 66.83; H, 5.07; N, 11.13*. Found C, 66.76; H, 5.21; N, 11.01.IR: 3414 cm^{-1} (OH), 3389- 3314 cm^{-1} (NH_2), 3049(NH), 1626 cm^{-1} (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.48 (s,1H, OH exchanged by D_2O),3.75,3.76(s,6H,2OCH_3), 4.16 (s,1H,CH of pyran), 4.42(s, H, NH exchanged by D_2O), 6.95-7.09 (m,8H, pyridine and aromatic rings),8.85(s, 2H, NH_2 exchanged by D_2O).

Synthesis of 2-(chlorocarbonylamino)-4-(3,4-dimethoxyphenyl)-4H-pyrano[3,2-h]quinoline-3-carbimidic acid(3).

In an ice bath, a solution of compound (2) (0,001mol) and triethylamine (0.5 mL) in dry DMF (10 mL) was stirred for 15 min. Chloroacetyl chloride (0,001 mol) was then added in portions, and the reaction mixture was stirred for 2 h. After completion of the reaction, the mixture was poured, with continuous stirring, on to crushed ice. The solid formed was collected by vacuum filtration, washed with ethyl acetate, and recrystallized from ethanol forming the compound (3)[13]with yield 60 % (m.p.209-211℃).

Elemental *Anal*. Calc. for $C_{22}H_{18}ClN_3O_5$ (439.85): *C, 60.07; H, 4.12; Cl, 8.06; N, 9.55*. Found C, 59.87; H, 4.43; Cl, 8.21; N, 9.21.IR: 3407-3369 cm^{-1} (2OH) 3149(NH), 1597 cm^{-1} (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.75,3.79(s,6H,2OCH_3), 4.41(s, H, NH exchanged by D_2O),4.16 (s,1H,CH of pyran), 6.93-7.09 (m,8H, pyridine and aromatic rings), 10.46,10.34 (s,2H,2OH exchanged by D_2O),.

Synthesis of 7-(3,4-dimethoxyphenyl)-7H-1,6-dihydropyrano[2,3-c]pyrazol-3-amine[3,2-h]quinolin(4).

A mixture of (2) (0.005 mol) and hydrazine hydrate (1 ml ,80%) in ethanol (20 ml) was refluxed for 5 h and then cooled , the precipitated product was collected by filtration, washed with ethanol and dried, recrystallization from forming the compound (4)[14] with yield 85 % (m.p.224-225℃).Elemental *Anal*. Calc. for $C_{21}H_{18}N_4O_3$ (374.39): *C, 67.37; H, 4.85; N, 14.96*. Found C, 67.51; H, 4.64; N, 15.01.IR: 3420cm^{-1}(NH_2), 3052(NH), 1625cm^{-1} (C=N). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.71,3.70(s,6H,2OCH_3), 4.46 (s,1H,CH of pyran), 6.92-7.55 (m,8H, pyridine and aromatic rings), 9.81(s, 2H, NH_2 exchanged by D_2O),10.16 (s, H,NH exchanged by D_2O).

Synthesis of 7-(3,4-dimethoxyphenyl)-10-mercapto-7H-pyrano[2,3-d]pyrimidine[3,2-h]quinolin-8-ol(5).

A mixture of (2) (0.01 mol) and thiourea (0.01 mol) was fused in an oil bath at 180 C for 1-3h after cooling and dilution eith ethanol (30 ml) the solid product formed was filtered off and recrystallized from ethanol[15] forming the compound (5) with yield 90 % (m.p.130-131℃).Elemental *Anal*. Calc. for $C_{22}H_{17}N_3O_4S$ (419.45): *C, 63.00; H, 4.09; N, 10.02; S, 7.64*. Found C, 63.21; H, 4.24; N, 10.13; S, 7.78.IR: 3371cm^{-1}(OH), 3060(NH), 1593(C=N), 1261(C=S). ^{1}H-NMR (DMSO-d_6, δ, ppm): 3.74,3.73(s,6H,2OCH_3), 4.66 (s,1H,CH of pyran), 6.86-7.09 (m,8H, pyridine and aromatic rings), 9,63 (s, H, OH exchanged by D_2O),10.21(s, H, NH exchanged by D_2O).

Synthesis of 7-(3,4-dimethoxyphenyl)-10-phenyl-7H-pyrano[2,3-d]pyrimidine[3,2-h]quinolin-8-ol(6).

A mixture of compound (2) (0.01mol) and p- f- benzaldehyde (0.01mol) in ethanol (20mL) with fewdrops of TEA was refluxed in oil bath with stirring for 10–12h. The reaction mixture was cooled, and poured into ice/water and the solid obtained was recrystallized from ethanol to give (6)[16]with yield 82 % (m.p.100-105℃).Elemental *Anal*. Calc. for $C_{28}H_{21}N_3O_4$ (463.48): *C, 72.56; H, 4.57; N, 9.07*. Found C, 72.43; H, 4.68; N, 9.21.IR: 3406cm^{-1}(OH),3031(NH),1606(C=N).^{1}H-NMR (DMSO-d_6, δ, ppm): 3.79,3.69(s,6H,2OCH_3), 4.66 (s,1H,CH of pyran), 6.81-7.22 (m,12H, pyridine and aromatic rings), 10.21(s, H, OH exchanged by D_2O).

Synthesis of 7-(3,4-dimethoxyphenyl)- 7H-pyrano[2,3-d]pyrimidine[3,2-h]quinolin-8-ol(7).

A mixture of (2) (0.005 mol) and triethyl orthoformate (5 ml) in AcOH (10 ml) was refluxed for 2 h. The solid that formed was recrystallized from ethanol forming the compound (7)[16] with yield 85 % (m.p.113-116℃).Elemental

Anal. Calc. for $C_{22}H_{17}N_3O_4$ (387.39): *C, 68.21; H, 4.42; N, 10.85*. Found C, 68.43; H, 4.60; N, 10.67.IR: 3362, 3057cm^{-1}(NH), 1590(C=N), 1678(C=O).^{1}H-NMR (DMSO-d_6, δ, ppm): 3.72,3.67(s,6H,2OCH3), 4.64 (s,1H,CH of pyran), 6.82-7.48 (m,9H, pyridine pyrazol and aromatic rings), 7.87(s, H, NH exchanged by D_2O).

Synthesis of 7-(3,4-dimethoxyphenyl)-10-methyl-7H-pyrano[2,3-d]pyrimidine[3,2-h]quinolin-8-ol(8).

A mixture of (2) (0.005 mol) and Ac2O (20 ml) was heated under refluxed for 6 h then after cooling, the excess Ac2O was removed under pressure to give viscous material which was washed several times with EtOH. The solid that formed was recrystallized from ethanol forming the compound (8)[16] with yield 90% (m.p.93-95℃).Elemental *Anal*. Calc. for $C_{23}H_{19}N_3O_4$ (401.41): *C, 68.82; H, 4.77; N, 10.47*. Found C, 68.67; H, 4.91; N, 10.29.IR: 3386, 3057cm^{-1}(NH), 1595(C=N), 1764(C=O).^{1}H-NMR (DMSO-d_6, δ, ppm): 2.08 (s,3H,CH_3),3.70,3.69(s,6H,2OCH_3), 4.66 (s,1H,CH of pyran), 6.83-7.54 (m,8H, pyridine and aromatic rings), 7.94(s, H, NH exchanged by D_2O).

Synthesis of (Z)-4-(3,4-dimethoxyphenyl)-2-((dimethylamino)methyleneamino)-4H-pyrano[3,2-h]quinoline-3-carbimidic acid(9).

To a solution of compound (2) (0.004 mol) in dioxane (20 ml) the DMF-DMA (0,004 mol) was added. The reaction mixture was heated under reflux for 4 h [14]. then filtered and recrystallized from ethanol forming the compound (9) with yield 70 % (m.p.100-103℃).Elemental *Anal*. Calc. for $C_{24}H_{24}N_4O_4$ (432.47): *C, 66.65; H, 5.59; N, 12.96*. Found C, 66.51; H, 5.72; N, 12.67.IR: 3414 cm^{-1} (OH), 3049(NH),1626 cm^{-1}(C=N),^{1}H-NMR (DMSO-d_6, δ, ppm):3.17,3,24(s,6H, 2OCH_3), 3.77,3.81(s,6H,2OCH_3), 4.41(s, H, NH exchanged by D_2O),4.16 (s,1H,CH of pyran), 6.86-7.09 (m,8H, pyridine and aromatic rings), 2.48 (s,H,OH exchanged by D_2O).

Synthesis of 2-(2-cyanoacetamido)-4-(3,4-dimethoxyphenyl)-4H-pyrano[3,2-h]quinoline-3-carbimidic acid(10).

A mixture of compound (2) (0.005 mol) and ethyl cyano acetate (0.005 mol) in glacial AcOH (25 ml) and a catalytic amount of concentrated acid (H2SO4) (6-8 drops) was refluxed for 3 h the reaction mixture was then cooled diluted with H_2O (10 ml) and neutralized with NH_3 solution. The crude product was collected by filtration, washed with H2O and recrystallized from ethanol forming the compound (10)[17] with yield 85 % (m.p.165-169℃).Elemental *Anal*. Calc. for $C_{24}H_{20}N_4O_5$ (444.44): *C, 64.86; H, 4.54; N, 12.61*. Found C, 64.66; H, 4.76; N, 12.49.IR: 3418cm^{-1}(OH), 3039(2NH), 2208(C≡N), 1634(C=N), 1746(C=O).

^{1}H-NMR (DMSO-d_6, δ, ppm): 2,43 (s, H, OH exchanged by D_2O),3.24 (s,2H,CH_2), 3.70,3.65(s,6H,2OCH_3), 4.40 (s,1H,CH of pyran), 6.83-7.09 (m,8H, pyridine and aromatic rings),8.22,7.53(s, 2H,2NH exchanged by D_2O).

Synthesis of 4-(3,4-dimethoxy phenyl)-2-(3-(4-oxo-4,5-dihydro thiazol-2-yl) propanamido)-4H-pyrano [3,2-h] quinoline-3-carbimidic acid(11).

Thioglycolic acid (0,004 mol) was added to a solution of compound (10)(0,004 mol) in dioxane(20cm) the reaction mixture was heated under reflux for 10 h and then left to cool to room temperature, the separated crystalline product was filtered off, dried and recrystallized from ethanol forming the compound (11)[18] with yield 80 % (m.p.118-120℃).Elemental *Anal*. Calc. for $C_{27}H_{24}N_4O_6S$ (532.57): *C, 60.89; H, 4.54; N, 10.52; S, 6.02*. Found C, 60.73; H, 4.87; N, 10.65; S, 6.34.IR: 3418cm^{-1}(OH), 3039, 3020(2NH), 1573(C=N), 1658(C=O). ^{1}H-NMR (DMSO-d_6, δ, ppm):1.74-1.89 (m,4H,2CH_2), 2,48 (s, H, OH exchanged by D_2O), 3.70,3.65(s,6H,2OCH_3), 4.05(s,2H,CH2 of thiazone),4.40 (s,1H,CH of pyran), 6.83-7.09 (m,8H, pyridine and aromatic rings),8.22,7.53(s, 2H,2NH exchanged by D_2O).

Synthesis of 4-(3,4-dimethoxyphenyl)-2-(2-imino-2H-chromene-3-carboxamido)-4H-pyrano[3,2-h]quinoline-3-carbimidic acid(12).

To a mixture of (10) (0.01 mol), and salicylaldehyde (0.01 mol) in ethanol (15mL) were added few drops of TEA. The reaction was heated on water bath for 4h and poured into ice water. The obtained product was filtered, washed with water, and recrystallized from the ethanol to give (12)[19] with yield 80% (m.p.200-204℃).Elemental *Anal*. Calc. for $C_{31}H_{24}N_4O_6$ (548.55): *C, 67.88; H, 4.41; N, 10.21*. Found C, 67.65; H, 4.32; N, 10.44. IR: 3429cm^{-1}(OH), 3039(2NH), 1623(C=N), 1796(C=O).^{1}H-NMR (DMSO-d_6, δ, ppm): 2,48 (s, H, OH exchanged by D_2O), 3.60,3.75(s,6H,2OCH_3), 4.40 (s,1H,CH of pyran), 6.73-7.67 (m,13H, pyridine,pyran and aromatic rings),8.22-7.53(s, 3H,3NH exchanged by D_2O).

Synthesis of (E)-2-(2-cyano-3-(dimethylamino) acrylamido)-4-(3,4-dimethoxy phenyl)-4H-pyrano [3,2-h] quinoline-3-carbimidic acid(13).

To a solution of compound (10) (0,004 mol) in dioxane (20 ml) the DMF-DMA (0,004 mol) was added. The reaction mixture was heated under reflux for 4 h, then filtered and recrystallized from ethanol forming the compound (13)[14]with yield 75 % (m.p.169-171℃).Elemental *Anal*. Calc. for $C_{27}H_{25}N_5O_5$ (499.52): *C, 64.92; H, 5.04; N, 14.02*. Found C, 64.78; H, 4.84; N, 14.30.IR:3418cm^{-1}(OH), 3039(2NH), 2208(C≡N),1634(C=N), 1746(C=O).

^{1}H-NMR (DMSO-d_6, δ, ppm):2,48 (s, H, OH exchanged by D_2O), 3.17,3,24(s,6H, 2OCH_3), 3.60,3.75(s,6H,2OCH_3), 4.40 (s,1H,CH of pyran), 6.73-7.67 (m,8H, pyridine and aromatic rings),8.22,7.53(s, 2H,2NH exchanged by D_2O).

Synthesis of 2-(5-amino-1H-pyrazole-4-carboxamido)-4-(3,4-dimethoxy phenyl)-4H-pyrano[3,2-h] quinoline-3-carbimidic acid(14).

A solution of compound (13) (0,004 mol) in DMF (30 ml) and hydrazine hydrate 98% (0,004 mol) was refluxed for 4h [14] and then separated solid was filtered and recrystallized from a mixture of benzene ethanol forming the compound (14) with yield 70 % (m.p.186-1189℃).Elemental *Anal*. Calc. for $C_{25}H_{22}N_6O_5$ (486.48): *C, 61.72; H, 4.56; N, 17.28*. Found C, 61.56; H, 4.43; N, 17.46. IR: 3402cm^{-1}(OH), 3314, 3201(NH_2), 3129-3039(4NH), 1635(C=O). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2,48 (s, H, OH exchanged by D_2O), 3.60,3.75(s,6H,2OCH_3), 4.40 (s,1H,CH of pyran), 6.73-7.67 (m,9H, pyridine,pyrazol and aromatic rings),7.48(s,2H,NH_2),8.22-7.53(s, 3H,3NH exchanged by D_2O).

Antitumor activity

Material: All cell lines were brought from ATCC via Vacsera tissue culture laboratories. All media were purchased from Lonza, Belgium, serum from Gibco, trypsin and MTT from Biobasic Canada.

In vitro antitumor bioassay on human tumor cell lines

Cell culture—HepG2 cell line was maintained in RPMI-1640, PC3 and HCT116 cell lines were maintained in DMEM high glucose with l-glutamine, 10% foetal bovine serum at 37°C in 5% CO_2 and 95% humidity. Cells were sub-cultured using trypsin versene 0.15%.

Viability test—After about 24h of seeding 10000 cells per well (in 96-well plates), when cells have reached 60-70% confluence, the medium was changed to serum-free medium containing a final concentration of the test samples of 100 µM in triplicates. The cells were treated for 72h. 100µM of Doxorubicin was used as a positive control and serum free medium was used as a negative control.

In vitro antitumor bioassay on human normal cell line

Cell culture Human retinal pigmented epithelial cell line RPE1 cell line was maintained in DMEM F12 medium, 10% foetal bovine serum at 37°C in 5% CO_2 and 95% humidity. Cells were sub-cultured using trypsin versene 0.15%.

Viability test After about 24h of seeding 40000 cells per well (in 96-well plates), when cells have reached 60-70% confluence, the medium was changed to serum-free medium containing a final concentration of the test samples of 100 µM in triplicates. The cells were treated for 72h. Serum free medium was used as a negative control.

Cell viability in both cases was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay as described by [20-25].

The equation used for calculation of percentage cytotoxicity: (1-(av(x)/(av(NC)))*100. Where: Av: average, X: absorbance of sample well measured at 595nm with reference 690nm, NC: absorbance of negative control measured at 595nm with reference 690nm. The calculation of the IC_{50} is done by performing 4 concentrations of the compounds on the cells in triplicates and the results are used in the SPSS program using linear regression probit analysis. The equation used for calculation of selectivity index: IC_{50} of normal cells / IC_{50} of cancer cell line

Molecular docking Analysis

Methionine synthase (MetS) is one of the folic acid cycle enzymes that catalyses the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine to produce methionine and tetrahydrofolate. The catalysis occurred via the coenzyme cobalamin cofactor (CH_3-Cb)[26]. This enzyme has been linked to the pathogenesis of anemias, neurodegenerative disorders and cancer. [27]

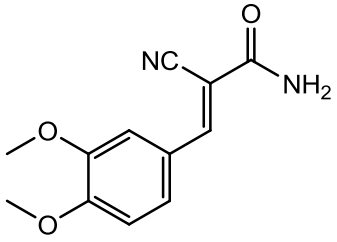
The docking studies are carried out using Molecular Operating Environment (MOE) 2008.10 (Moe source: Chemical Computing Group Inc., Quebec, Canada, 2008). Firstly, a Gaussian Contact surface around the binding site is drawn. The surface surrounds the van der Waals surface of a molecule (filling in solvent inaccessible gaps). After that the docking studies are implemented to evaluate the binding free energy of the inhibitors within the macromolecules. The Dock scoring in MOE software is carried out using London dG scoring function and has been enhanced by using two different refinement methods, the Force-field and Grid-Min pose to confirm that refined poses satisfy the specified conformations. We allowed rotatable bonds; the best 10 poses are retained and analyzed for the binding poses best score.

The Molecule Builder tool in MOE was used to build the 3D structures. Energy minimization is carried out through Force-field MMFF94x Optimization using gradient of 0.0001 for determining the lower energy conformations with the most favorable geometry. Hydrogen atoms and partial charges were added to the protein with the protonation 3D application in MOE.

Results and Discussion

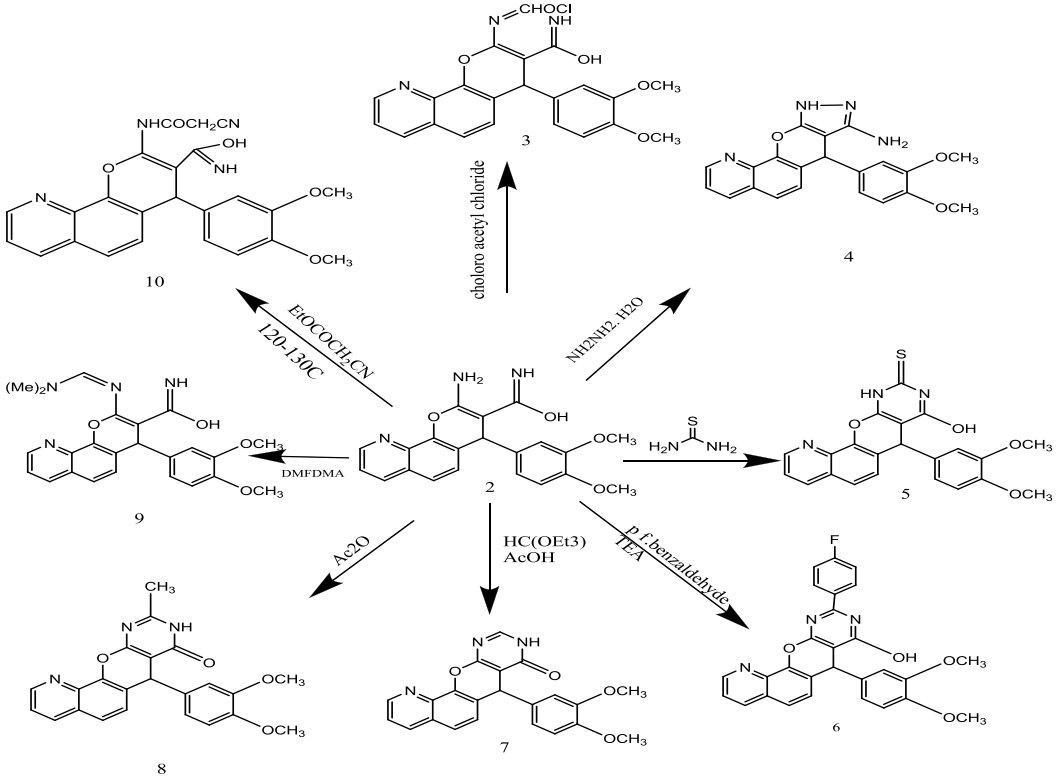
Chemistry

Starting compound(2) was synthesized by Michael addition of 8-hydroxy quinoline on cyanoacrylamide (1) derivative(which prepared by Knoevenagel condensation of veratraldehyde with cyanoacetamide[12] in presence of sodium hydroxide via fusion).



(1)

The 4H-pyrano [3, 2-h] quinoline-3-carbimidic acid derivative (2) by reaction with different reagents such choloro acetyl chloride undergoes cyclization to afford (3), or using hydrazine hydrate to give (4), or thiourea forming (5), p f.benzaldehyde to give(6), triethyl orthoformate to give (7), acetic anhydride to give(8), dimethyl formamide dimethyl acetal to give (9)Furthermore, when compound (2) reacted with ethyl cyano acetate gave compound (10)(Scheme 1).



(Scheme 1)

^{1}H-NMR (DMSO-d_6) spectra of compound (2) revealed signals at2.48 (s,1H, OH exchanged by D_2O),3.75,3.76(s,6H,2OCH_3), 4.16 (s,1H,CH of pyran), 4.42(s, H, NH exchanged by D_2O), 6.95-7.09 (m,8H, pyridine and aromatic rings),8.85(s, 2H, NH_2 exchanged by D_2O).

IR spectra of compound (3) exhibited absorption bands at 3407-3369 cm^{-1} (2OH) 3149(NH), 1597 cm^{-1} (C=N).

^{1}H-NMR (DMSO-d_6) spectra of compound (4) revealed signals at3.71,3.70(s,6H,2OCH3), 4.46 (s,1H,CH of pyran), 6.92-7.55 (m,8H, pyridine and aromatic rings), 9.81(s, 2H, NH_2 exchanged by D_2O),10.16 (s, H,NH exchanged by D_2O).IR spectra of compound (5) exhibited absorption bands at 3371cm^{-1}(OH), 3060(NH), 1593(C=N), 1261(C=S).

^{1}H-NMR (DMSO-d_6) spectra of compound (6) revealed signals at3.79,3.69(s,6H,2OCH3), 4.66 (s,1H,CH of pyran), 6.81-7.22 (m,12H, pyridine and aromatic rings), 10.21(s, H, OH exchanged by D_2O).IR spectra of compound (7) exhibited absorption bands at 3362, 3057cm^{-1}(NH), 1590(C=N), 1678(C=O).

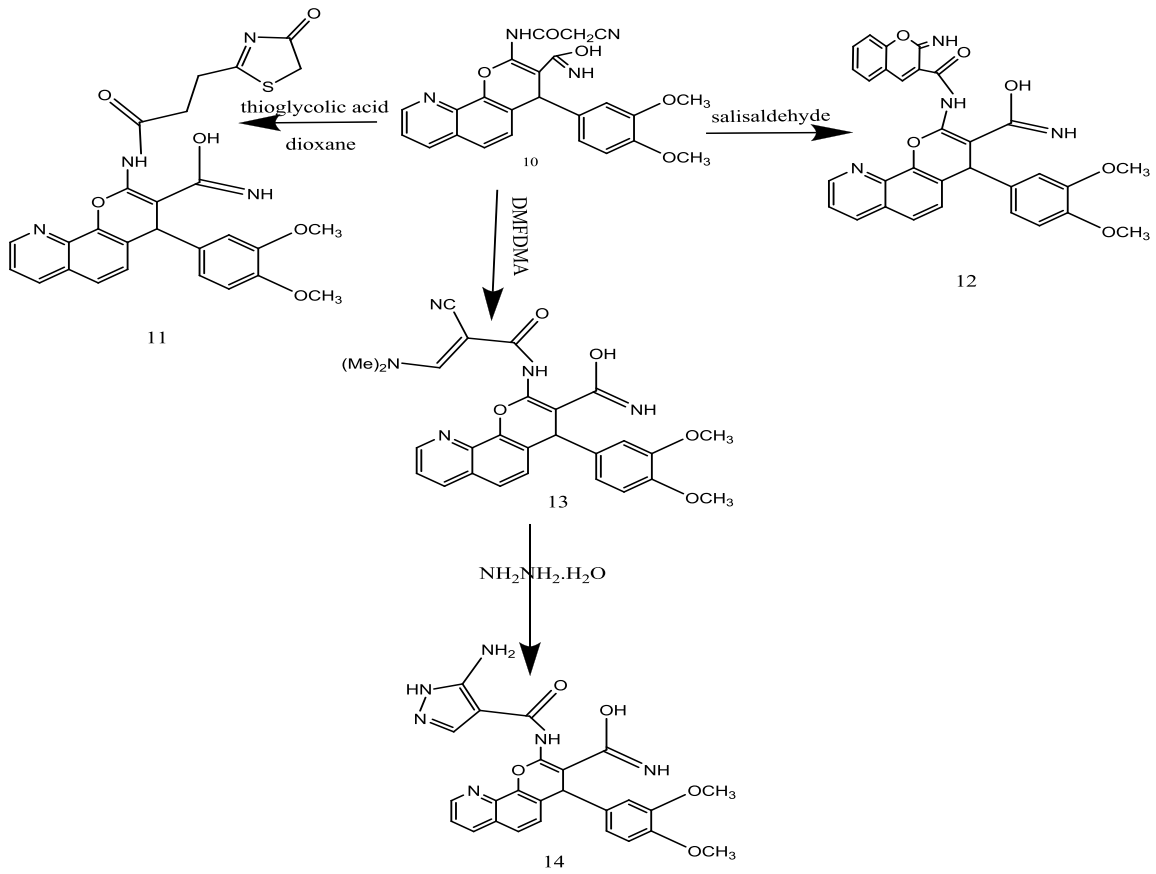
^{1}H-NMR (DMSO-d_6) spectra of compound (8) revealed signals at2.08 (s,3H,CH_3),3.70,3.69(s,6H,2OCH3), 4.66 (s,1H,CH of pyran), 6.83-7.54 (m,8H, pyridine and aromatic rings), 7.94(s, H, NH exchanged by D_2O).

IR spectra of compound (9) exhibited absorption bands at 3414 cm^{-1} (OH), 3049(NH), 1626 cm^{-1}(C=N).

IR spectra of compound (10) exhibited absorption bands at 3418cm^{-1}(OH), 3039(2NH), 2208(C≡N), 1634(C=N), 1746(C=O).

^{1}H-NMR (DMSO-d_6) spectra of compound (10) revealed signals at2,43 (s, H, OH exchanged by D_2O),3.24 (s,2H,CH_2), 3.70,3.65(s,6H,2OCH3), 4.40 (s,1H,CH of pyran), 6.83-7.09 (m,8H, pyridine and aromatic rings), 8.22,7.53(s, 2H,2NH exchanged by D_2O).

Compound (10) when reacted with Thioglycolic acid, phenyl isothiocyanate, salicyldehyde,DMF-DMA gave 2-(substituted)-4H-pyrano [3, 2-h] quinoline-3-carbimidic acid derivatives(11-13)While when compound (13) reacted with hydrazine hydrate gave 2- (substituted)pyrazole-4H-pyrano [3, 2-h] quinoline-3-carbimidic acid derivatives (14) (Scheme 2).



(Scheme 2)

^{1}H-NMR (DMSO-d_6) spectra of compound (11) revealed signals at1.74-1.89 (m,4H,2CH_2), 2,48 (s, H, OH exchanged by D_2O), 3.70,3.65(s,6H,2OCH_3), 4.05(s,2H,CH2 of thiazone),4.40 (s,1H,CH of pyran), 6.83-7.09 (m,8H, pyridine and aromatic rings),8.22,7.53(s, 2H,2NH exchanged by D_2O).

IR spectra of compound (12) exhibited characteristic absorption bands at 3429cm^{-1}(OH), 3039(2NH), 1623(C=N), 1796(C=O). ^{1}H-NMR (DMSO-d_6) spectra of compound (13) revealed signals at2,48 (s, H, OH exchanged by D_2O), 3.17,3,24(s,6H, 2OCH_3), 3.60,3.75(s,6H,2OCH_3), 4.40 (s,1H,CH of pyran), 6.73-7.67 (m,8H, pyridine and aromatic rings),8.22,7.53(s, 2H,2NH exchanged by D_2O).

IR spectra of compound (14) exhibited characteristic absorption bands at 3402cm^{-1}(OH), 3314, 3201(NH_2), 3129-3039(4NH), 1635(C=O).

Antitumor activity

The results of effect of the compounds on prostatic adenocarcinoma (PC3), human colorectal carcinoma (HCT116) and human liver hepatocellular carcinoma (HepG2) cell lines showing in following (Table 1).

Table-1. The cytotoxic effect of the samples at 100 µM on human prostatic adenocarcinoma (PC3), human colorectal carcinoma (HCT116) and human liver hepatocellular carcinoma (HepG2) cell lines.

	PC3	HCT116	HepG2
1	14%	27%	31%
2	66%	67%	70%
3	95%	96%	94%
4	66%	72%	73%
5	60%	62%	70%
6	65%	70%	64%
7	65%	61%	65%
8	63%	63%	65%
9	60%	67%	65%
10	75%	81%	69%
11	0%	18%	43%
12	48%	71%	51%
13	73%	75%	44%
14	18%	18%	20%

Dox	100%	100%	100%

Table-2. IC_{50} of the active compound (which gave more than 90% activity at 100µM) on human tumor cell line.

Compd.	IC_{50}, μM		
	HCT116	HepG2	PC3
3	35	14	13
Doxorubicin	2.2	0.6	6.8

Table 3. IC_{50} of the active compound (which gave more than 90% activity at 100µM) on.

	IC_{50}, μM
	RPE1
3	18

Table 4. The selectivity index of the active compound on human retinal pigmented epithelial cell line RPE1 cell line.

	RPE1/HCT116	RPE1/HepG2	RPE1/PC3
3	0.5	1.3	1.4

Discussion

Among the compounds which were screened at 100 µM on three human tumor cell lines, only compound 3 gave an activity over 90% on all the cell lines. After calculation of its IC_{50} on cancer cells, it was also tested on normal RPE1 cell line.

On calculating its selectivity index, it appeared not to be selective in case of colon (0.5), whereas it was of certain selectivity in cases of liver (1.3) and prostate (1.4) cell lines.

Molecular docking Analysis

Binding model of compounds 3, 10, 13 into Methionine Synthase structure

In an effort to elucidate the possible mechanism by which the title compounds can induce anticancer activity and guide further SAR studies, molecular docking of the potent inhibitors 3 ,10 and 13 into the binding site of Methionine Synthase were performed on the binding model based on the Methionine Synthase complex structure (pdb: 1Q8J) .

Binding models of compounds 3, 10 and 13 and Methionine Synthase were depicted in Figs. 2, 3 and 4 In the binding model, compound 3 is nicely bound to the Methionine Synthase with two hydrogen bond donor with ALA 325 and GLY 326 at distance 2.53 A˚ and 2.10 A˚ respectively, in addition one hydrogen bond acceptor with ASN 538 at distance 2.81 A˚ and it give score -13.7008 kcal/mol.

Compound 10 is nicely bound to the Methionine Synthase with one hydrogen bond donor with GLU320 at distance 3.04 A˚ , besides ,one hydrogen bond acceptor with ARG 516 at distance 2.38 A˚ and it give score -9.4910 kcal/mol.

Compound 13 is nicely bound to the Methionine Synthase with two hydrogen bond acceptor with ASN 411 and ASN508 at distance 3.29 A˚, 3.08 A˚ respectively and it give score -7.4168 kcal/mol.

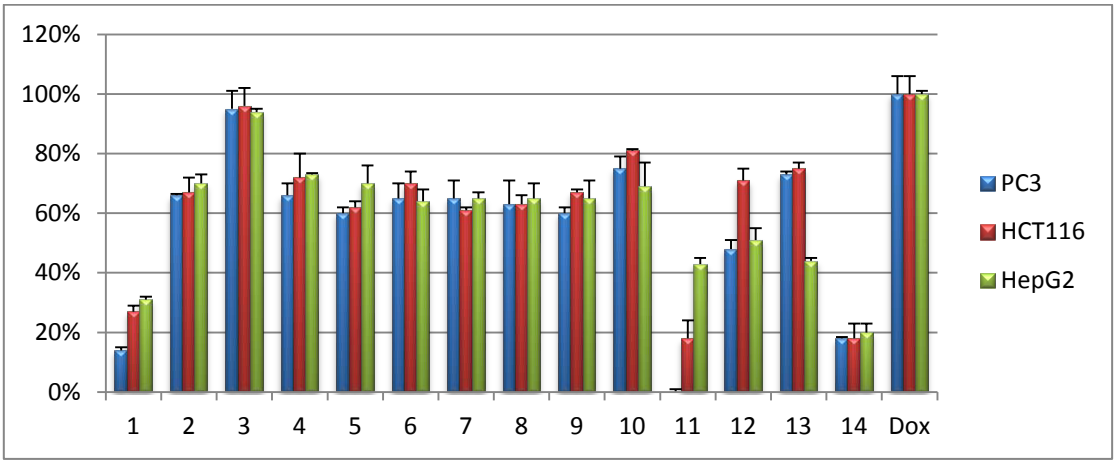


Fig. 1. The cytotoxic effect of the samples at 100 micro mole on human prostatic adenocarcinoma (PC3), human colorectal carcinoma (HCT116) and human liver hepatocellular carcinoma (HepG2) cell lines.

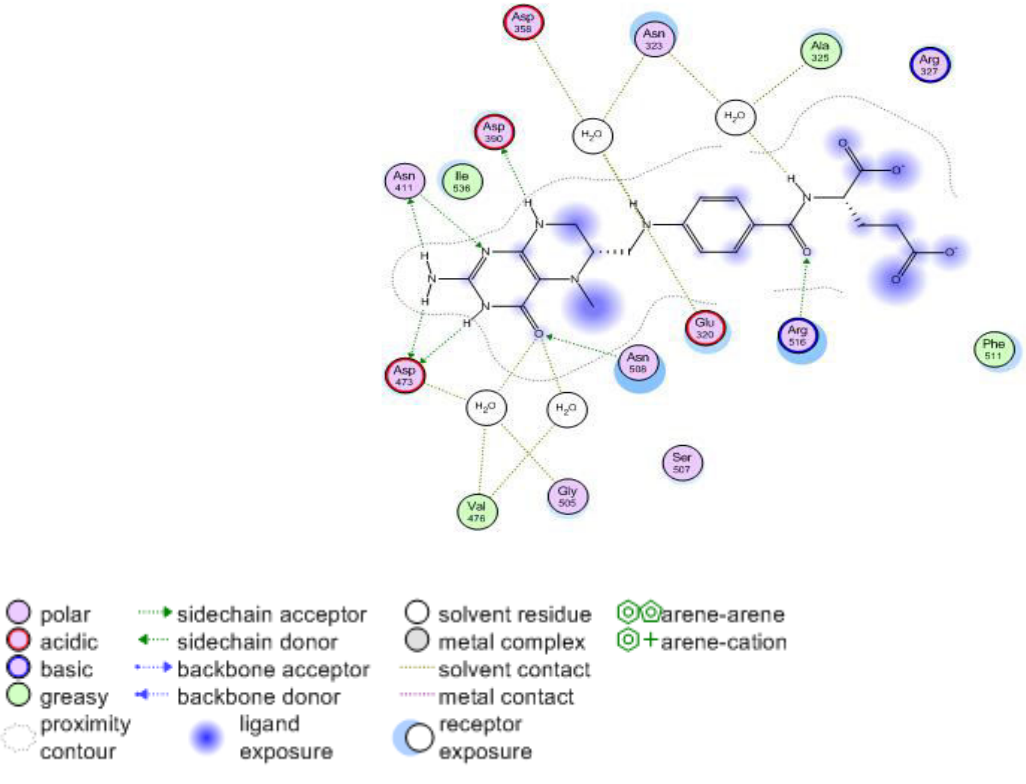


Fig 2: Molecular docking modeling of the native ligand C2F (5-methyl -5,6,7,8-tetrahydrofolic acid) with Methionine Synthase it is exhibited four H-bond donor with ASP 390,ASP411,ASP473 and ASP473 at distance 1.93 A˚ ,2.02 A˚ ,2.03 A˚ and 1.78 A˚ respectively and four H-bond acceptor with ASN 411,ASN508,ARG516 and ARG516 at distance 2.96 A˚ ,2.98 A˚ ,2.88 A˚ and 2.99 A˚ respectively and it is gave score - 15.481 kcal/mol.

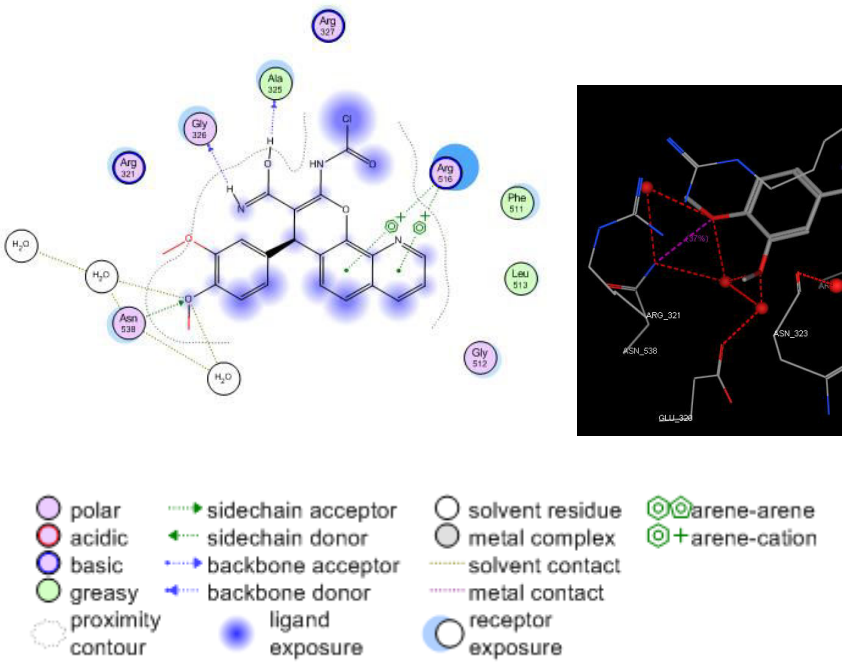


Fig. 3. Molecular docking modeling of compound **3** with Methionine Synthase: for lucidity, only interacting residues are displayed. Left: 2D model of the interaction between compound **3** and the Methionine Synthase binding site. Right: 3D model of the interaction between compound 3 and the Methionine Synthase binding site.

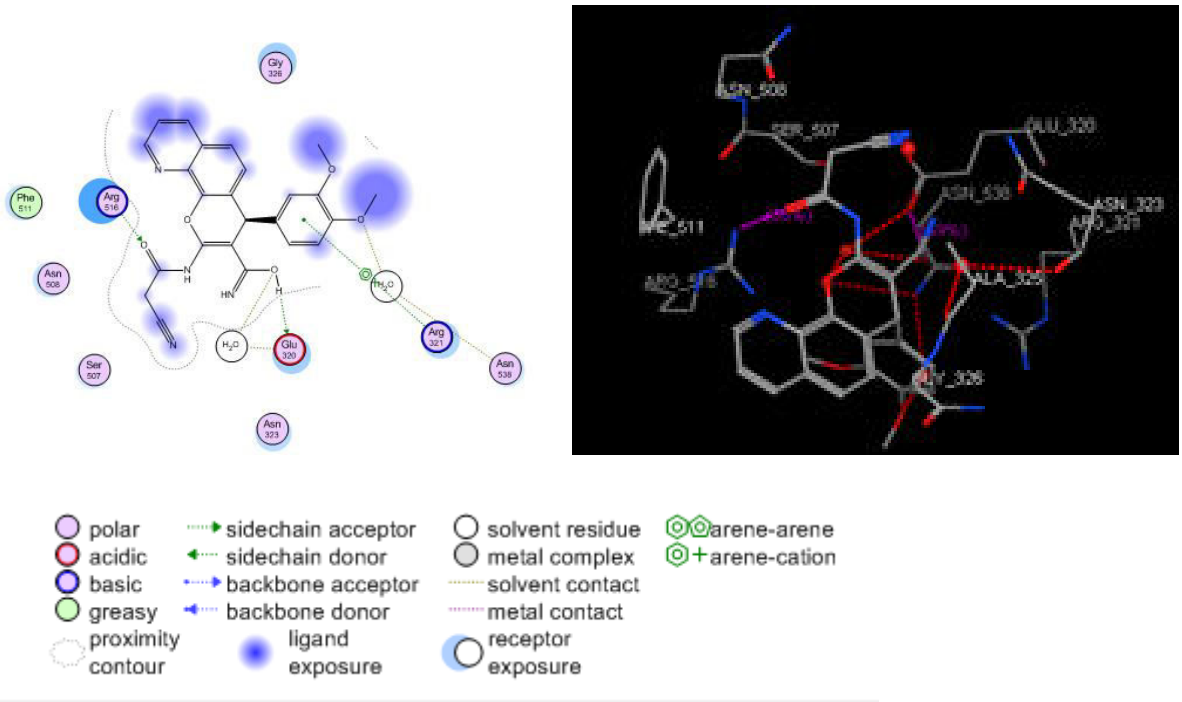


Fig. 4. Molecular docking modeling of compound 10 with Methionine Synthase : for lucidity, only interacting residues are displayed. Left: 2D model of the interaction between compound 10 and the Methionine Synthase binding site. Right: 3D model of the interaction between compound 10 and the Methionine Synthase binding site.

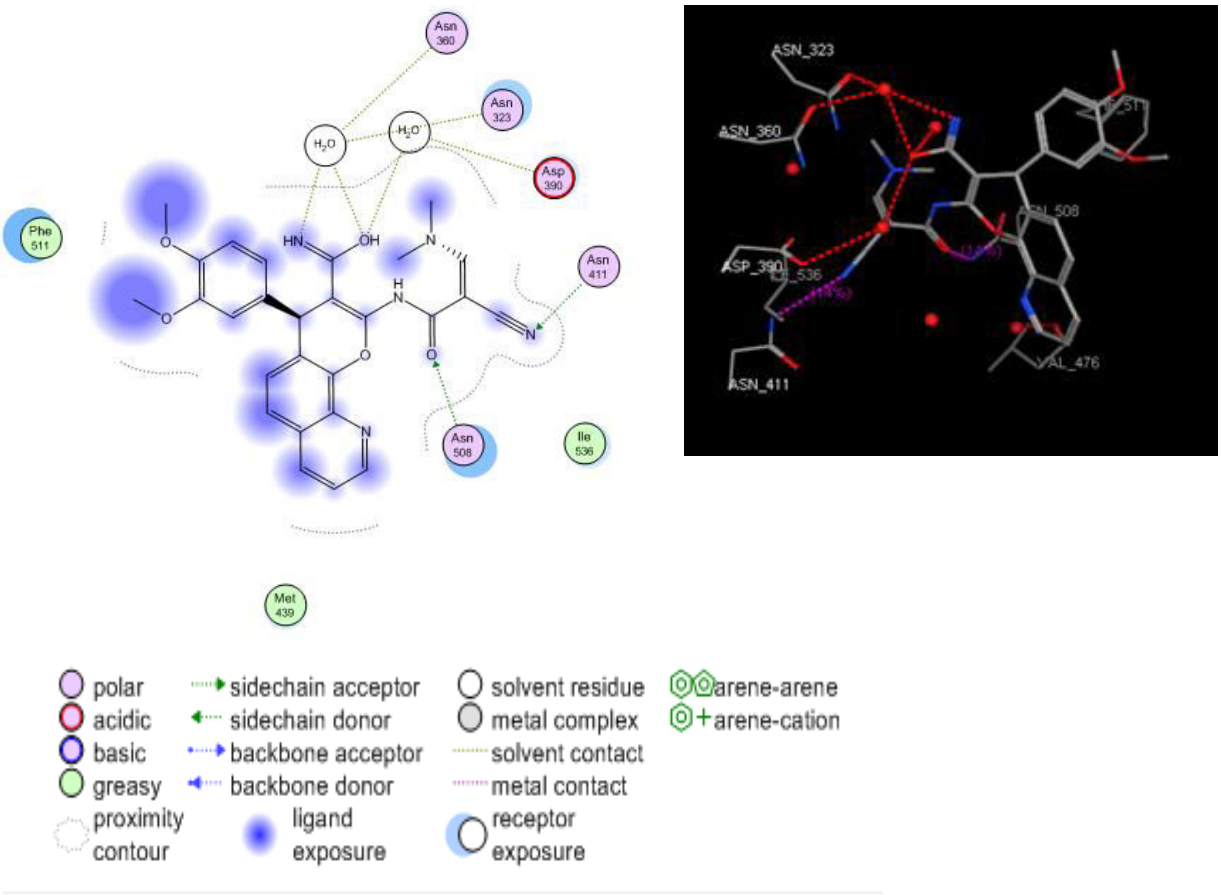


Fig. 5. Molecular docking modeling of compound 13 with Methionine Synthase : for lucidity, only interacting residues are displayed. Left: 2D model of the interaction between compound 13 and the Methionine Synthase binding site. Right: 3D model of the interaction between compound 13 and the Methionine Synthase binding site.

Acknowledgements:

This research was funded by project in the National Research Center, Giza, Egypt (code: P100225), titled "Synthesis, Docking and pharmacological evaluation of some new quinoline derivatives".

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