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Synthesis, Moleculer Docking and Anticancer Screening of Some Novel Tetrahydronaphthyl Thiazolyl Pyrazoles and other Related Derivatives

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CONDENSATION of 5-amino-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl]-1*H*-pyrazole-4-carbonitrile (3) with different reagents such as triethylorthoformate, ethyl chloroformate and/or hydroxyamine hydrochloride yielded the substituted pyrazoles 4-6. Cyclocondensation of 3 with formic acid, acetic anhydride, formamide and /or acetamide afforded the pyrimidine derivatives 8-11. Reaction of 3 with carbon disulphide, substituted isothiocynate, malononitrile, aldehydes and /or chloroacetylchloride yielded the corresponding derivatives 12-16. Also, hydrolysis of 3 with conc. sulfuric acid at different conditions gave 17 and/or 18. Docking studies of the newly prepared compounds as thymidylate synthase inhibitors have been done. Some of the newly prepared compounds were evaluated as anticancer agents against three human tumor cell lines (HCT116, HePG2, MCF7).

Keywords : Tetrahydronaphthylthiazole, Pyrazole, Pyrazolopyrimidine, Molecular docking, Anticancer screening, HCT116, HePG2 and MCF7.

Numerous compounds with biological activity have been investigated, however many of them are not suitable for the therapeutic use due to their toxic, carcinogenic and mutagenic properties. The use of chemotherapeutic drugs in cancer therapy involves the risk of the life threatening host toxicity. The search therefore continues to develop the drugs which selectively act on tumor cells[1,2] .

Various chemical and pharmaceutical activities were ascribed to 1,2,3,4-tetrahydronaphthalene derivatives especially those incorporated into heterocyclic systems[3]. It has been reported that this type of compounds possesses a wide variety of biological activities such as potent anti-HIV[4], antipoliovirus[5], antibacterial[6,7], hypotensive[8], anti-arrythmic[9], molluscicidal[10], antiplatelet aggregation[11] , anxiolytic and antidepressant[12,13] and as anticancer agents [14] .

The chemistry of 1,2,3,4-tetrahydronaphthalen-6-yl heterocycles especially those including thiazole moiety and/or nitrogen, oxygen or sulphur heterocycles such as pyrazoles, isoxazoles, thiadiazoles, pyrroles, pyridines and/or pyrimidines, has

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been of increasing interest since many of these compounds have found useful applications as chemotherapeutic agents of promising activities especially as anticancer[15, 16] , or antimicrobial[17] agents.

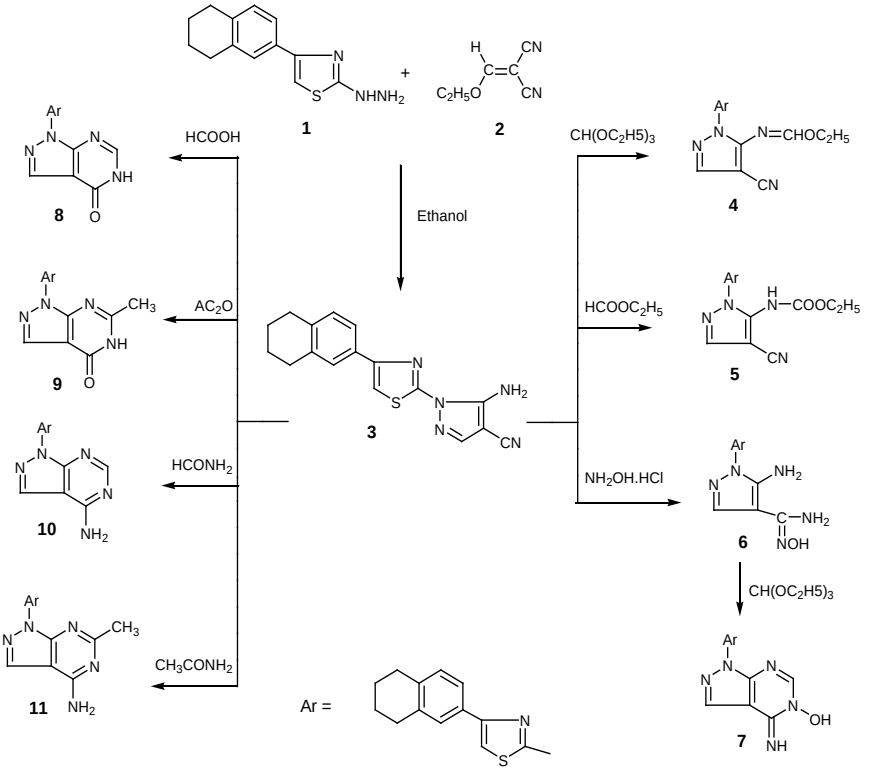
Folate metabolism is considered as an important target for the development of new anticancer agents due to its role in the biosynthesis of nucleic acid precursors[18,19] . The inhibition of folate-dependant enzymes such as thymidylate synthase which catalyses the reductive methylation of deoxyuridylate (dUMP) to thymidylate (dTMP) has also been recognized as an interesting target for drug discovery[20,21]. Classical antitumor agents that prevent this pathway have a disadvantage that they need an active transport mechanism to enter the cells which can cause tumor resistance if impaired[22,23]. Also, recent preclinical experiments on human liver carcinoma cell lines (HePG2) revealed that at higher doses of antifolate a loss of thymidylate synthase inhibition occurs and cytotoxic effects preserved[24] . So, a development of a new thymidylate synthase inhibitor that could be selective may be a good target for drug discovery.

Based on all these finding, the main goal of the present work was to design a new series of compounds structurally containing tetralin moiety incorporated with different heterocycles as a trial for the development of a new thymidylate synthase inhibitor that could be selective which may be a good target for drug discovery. For this target some docking of the newly synthesized compounds was done using Autodock Vina[25]. Moreover, the cytotoxic activities of ten a new selected compounds were screened against colon (HCT116), hepatocellular (HePG2) and breast (MCF7) carcinoma cell lines.

Results and Discussion

Chemistry

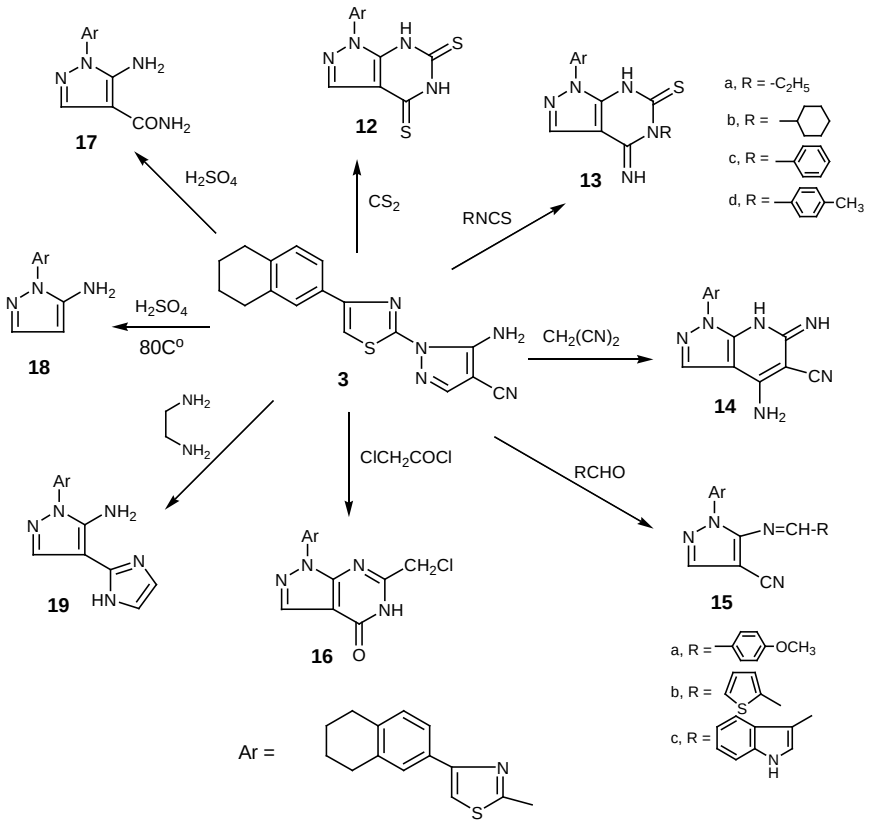
The synthetic approach was confined to two schemes to obtain the target compounds. Reaction of 1-(4-(1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl) hydrazine (1)[26] with ethoxymethylenemalononitrile (2) afforded the corresponding starting compound 4-cyano-5-aminopyrazole derivative 3. Condensation of 3 with either triethylorthoformate or ethyl chloroformate yielded the *N*-substituted-4-cyanopyrazoles 4 and 5, respectively. Also, condensation of compound 3 with hydroxyamine hydrochloride afforded N'-hydroxy-4-carboxamidine pyrazole derivative 6, that cyclized with triethylorthformate to give the pyrimidine derivative 7. Cyclocondensation of compound 3 with formic acid and/or acetic anhydride gave the corresponding pyrimidinone derivatives 8 and 9, respectively. While the cyclocondensation of compound 3 with formamide and /or acetamide gave the corresponding 4-amino pyrimidine derivatives 10 and 11, respectively (Scheme 1).



Scheme 1

On the other hand, the pyrazolo pyrimidine-dithione derivative 12 was obtained by cyclocondensation of 4-cyano-5-aminopyrazole derivative 3 with carbon disulphide in the presence of potassium hydroxide, while 4-imino-pyrazolo pyrimidine-thione derivatives 13a-d were obtained by cyclocondensation of compound 3 with isothiocynate derivatives. In addition, 4-imino-pyrazolo pyridine derivative 14 was obtained by refluxing of 3 with malononitrile in ethanol. Furthermore, condensation of derivative 3 with the appropriate aldehyde derivatives afforded the respective Schiff's bases 15a-c.

In addition, cyclocondensation of compound 3 with chloroacetylchloride yielded the pyrimidinone derivative 16. Also, reacting compound 3 with conc. sulfuric acid at 0-5^{o}C gave the corresponding carboxamide derivative 17 while the reaction at 80^{o}C afforded the corresponding aminopyrazole 18. Finally, compound 3 was reacted with diaminoethane to give the imidazolylpyrazole derivative 19 (Scheme 2).



Scheme 2

Experimental

Chemistry

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, using Vario Elementar and were found within ±0.5% of the theoretical values. Infrared spectra were recorded on a Jasco FT/IR-6100, Fourier transform, Infrared spectrometer at cm^{-1} scale using KBr disc technique at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ^1H NMR spectra were determined by using a JEOl EX-270 NMR spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. The mass spectra were measured with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure

to UV lamp at λ_{254} nanometer for few seconds. The chemical names given for the prepared compounds are according to the IUPAC system.

5-Amino-1- [4-(1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl]- 1H-pyrazole-4-carbonitrile (3)

A mixture of 1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl]hydrazine (2.45 g, 0.01 mol) and ethoxymethylenemalononitrile (1.22 g, 0.01 mol) in 30 ml absolute ethanol was refluxed for 3hr. Then poured onto ice/cold water, the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 3.

Yield 70%, mp. 141-143^{o}C; IR (KBr, cm^{-1}): 3233, 3142 (NH_2), 2929, 2853 (CH_2 tetralin protons) and 2210 (CN); ^{1}H NMR ($CHCl_3$): δ 1.57-1.82 (m, 4H, 2 (CH_2) tetralin), 2.56-2.80 (m, 4H, 2 (CH_2) tetralin), 4.60 (s, 2H, NH_2, D_2O exchangeable), 6.70-8.57 (m, 5H, CH-tetralin, thiazole, pyrazole protons); MS, m/z (%): 322 [M^{+1}] (100); Anal. For $C_{17}H_{15}N_5S$ (321.40): Calcd. C, 63.53; H, 4.70; N, 21.79; Found: C, 63.21; H, 4.33; N, 21.50.

5-(Ethoxymetheleneamino)-1-[4-(1,2,3,4- tetrahydronaphthalen-7-yl) thiazol-2-yl]-1H-pyrazole-4-carbonitrile (4)

A mixture of compound 3 (0.65 g, 0.002 mol) and triethyorthoformate (1.0 ml) in 20 ml acetic acid was refluxed for 5 hr. Then poured onto ice/cold water, the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 4.

Yield 64%, mp. 188-190^{o}C; IR (KBr, cm^{-1}): 2925, 2856 (CH_2 tetralin), 2218 (CN), 1684 (N=CH).; ^{1}H NMR ($CHCl_3$): δ 1.24 (t, 3H, -$CH_2\underline{CH_3}$), 1.60-1.79 (m, 4H, 2 (CH_2) tetralin), 2.56-2.79 (m, 4H, 2 (CH_2) tetralin),3.44 (q, 2H, -$\underline{CH_2}CH_3$), 7.06-7.91 (m, 6H, CH-tetralin, thiazole, pyrazole protons, N=CH); MS, m/z (%): 373 [M-4] (3) and 43 [C_3H_7] (100); Anal. For $C_{20}H_{19}N_5OS$ (377.46): Calcd. C, 63.64; H, 5.07; N, 18.55; Found: C, 63.44; H, 5.21; N, 18.31.

Ethyl 4-cyano-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazol-5-ylcarbamate (5)

A mixture of compound 3 (0.65 g, 0.002 mol), ethyl chloroformate (0.22 ml, 0.01 mol) and anhydrous sodium carbonate (1 g) in 20 ml tetrahydrofuran was refluxed for 5 hr. Then poured onto ice/cold water, the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 5.

Yield 68%, mp. 200-202^{o}C; IR (KBr, cm^{-1}): 3221 (NH), 2927, 2849 (CH_2 tetralin protons), 2215 (CN) and 1692 (CO amide); ^{1}H NMR ($CHCl_3$): δ 1.45 (t, 3H, -$CO_2CH_2CH_3$), 1.70-1.79 (m, 4H, 2 (CH_2) tetralin), 2.72-2.79 (m, 4H, 2 (CH_2) tetralin), 4.34 (q, 2H, -$CO_2CH_2CH_3$), 7.06-7.91 (m, 5H, CH-tetralin, thiazole, pyrazole protons), 8.70 (s, 1H, NH, D_2O exchangeable); MS, m/z (%): 390 [M-3]

(10), 63 [C_5H_3] (100); Anal. For $C_{20}H_{19}N_5O_2S$ (393.46): Calcd. C, 61.05; H, 4.87; N, 17.80; Found: C, 61.31; H, 4.61; N, 17.92.

5-Amino-1-[4-(1,2,3,4- tetrahydronaphthalen-7-yl) thiazol-2-yl]- N'-hydroxy-1H-pyrazole-4-carboxamidine (6)

A mixture of compound 3 (0.65 g, 0.002 mol) and hydroxyamine hydrochloride (0.14 g, 0.01 mol) in 20 ml absolute ethanol containing few drops of triethylamine was refluxed for 5 hr. Then poured onto ice/cold water, the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 6.

Yield 75%, mp.172-174^{o}C; IR (KBr, cm^{-1}): 3310, 3221, 3154 (2(NH_2), OH) and 2929, 2851 (CH_2 tetralin protons); MS, m/z (%): 354 [M-2] (3), 63 [C_5H_3] (100); Anal. For $C_{17}H_{18}N_6OS$ (354.43): Calcd. C, 57.61; H, 5.12; N, 23.17; Found: C, 57.24; H, 5.34; N, 23.30.

1-[4-(1,2,3,4- Tetrahydronaphthalen-7-yl) thiazol-2-yl]- 1H-4-imino-5-hydroxy-pyrazolo[3,4-d]pyrimidine (7)

A mixture of compound 6 (0.71 g, 0.002 mol) and triethyorthoformate (1.0 ml) in 20 ml acetic acid was refluxed for 5 hr then poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 7.

Yield 55%, mp. 187-189^{o}C; IR (KBr, cm^{-1}): 3387, 3210 (OH, NH) and 2925, 2858 (CH_2 tetralin protons); ^{1}H NMR (DMSO-d_6): δ 1.73-1.80 (m, 4H, 2 (CH_2) tetralin), 2.74-2.81 (m, 4H, 2 (CH_2) tetralin), 7.01-8.01 (m, 6H, CH-tetralin, thiazole, pyrazole, pyrimidine protons); MS, m/z (%): 365 [M+1] (3), 318 [M-(N_2H_2O)] (100); Anal. For $C_{18}H_{16}N_6OS$ (364.42): Calcd. C, 59.32; H, 4.43; N, 23.06; Found: C, 59.51; H, 4.22; N, 23.25.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (8)

A mixture of compound 3 (0.65 g, 0.002 mol) and 10 ml formic acid was refluxed for 3 hr then poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 8.

Yield 78%, mp. 167-169^{o}C; IR (KBr, cm^{-1}): 3212 (NH), 2923, 2850 (CH_2 tetralin) and 1702 (CO); MS, m/z (%): 348 [M-1] (2) and 45 [C_3H_9] (100); Anal. For $C_{18}H_{15}N_5OS$ (349.41): Calcd. C, 61.87; H, 4.33; N, 20.04; Found: C, 61.71; H, 4.68; N, 20.21.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl) thiazol-2-yl]- 6-methyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one (9)

A mixture of compound 3 (0.65 g, 0.002 mol), acidic anhydride (5 ml) and acetic acid (5 ml) was refluxed for 5 hr. The reaction mixture was evaporated

until its half volume then cold. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 9.

Yield 70%, mp. 149-151^{o}C; IR (KBr, cm^{-1}): 3192 (NH), 2925, 2855 (CH_2 tetralin) and 1705 (CO); ^{1}H NMR (DMSO-d_6): δ 1.70-1.85 (m, 4H, 2 (CH_2) tetralin), 1.88 (s, 3H, CH_3), 2.56-2.70 (m, 4H, 2 (CH_2) tetralin), 7.06-8.00 (m, 5H, CH-tetralin, thiazole, pyrazole protons); MS, m/z (%): 364 [M+1] (2) and 77 [C_6H_5] (100); Anal. For $C_{19}H_{17}N_5OS$ (363.44): Calcd. C, 62.79; H, 4.71; N, 19.27; Found: C, 62.59; H, 4.52; N, 19.38.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazolo[3,4-d]pyrimidin-4-amine (10)

A mixture of compound 3 (0.65 g, 0.002 mol) and formamide (10 ml) was refluxed for 1 hr. Cool, then, the formed precipitate was filtered, dried and recrystallized from ethanol to give the title compound 10.

Yield 65%, mp. 193-195^{o}C; IR (KBr, cm^{-1}): 3321, 3164 (NH_2), 2930, 2850 (CH_2 tetralin); MS, m/z (%): 348 [M^+] (100); Anal. For $C_{18}H_{16}N_6S$ (348.42): Calcd. C, 62.05; H, 4.63; N, 24.12; Found: C, 62.28; H, 4.71; N, 24.29.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl) thiazol-2-yl]-6-methyl-1H-pyrazolo[3,4-d] pyrimidin-4-amine (11)

A mixture of compound 3 (0.65 g, 0.002 mol) and acetamide (0.12 g, 0.002 mol) in 10 ml dimethylforamide was refluxed for 5 hr, then poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 11.

Yield 68%, mp. 201-203^{o}C; IR (KBr, cm^{-1}): 3289, 3148 (NH_2), 2925, 2849 (CH_2 tetralin); ^{1}H NMR (DMSO-d_6): δ 1.77-1.82 (m, 4H, 2 (CH_2) tetralin), 2.53 (s, 3H, CH_3), 2.74-2.80 (m, 4H, 2 (CH_2) tetralin), 7.06-8.00 (m, 5H, CH-tetralin, thiazole, pyrazole protons); MS, m/z (%): 362 [M^+] (100); Anal. For $C_{19}H_{18}N_6S$ (362.45): Calcd. C, 62.96; H, 5.01; N, 23.19; Found: C, 62.64; H, 5.22; N, 23.41.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazolo[3,4-d] pyrimidine -4,6(5H,7H)-dithione (12)

A mixture of compound 3 (0.65 g, 0.002 mol), potassium hydroxide (0.5 g /1 ml water) and dimethylsulfoxide (5 ml) was cooled at 0-5^{o}C. Then, carbon disulphide (1 ml) was added dropwise and the reaction mixture was stirred at room temperature for 2 hr. The reaction mixture was concentrated and poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 12.

Yield 60%, mp. 177-179^{o}C; IR (KBr, cm^{-1}): 3189, 3153 (2NH), 2920, 2855 (CH_2 tetralin) and 1151, 1180 (2C=S); MS, m/z (%): 395 [M-2] (16) and 364 [M-SH]

(100); Anal. For $C_{18}H_{15}N_5S_3$ (397.54): Calcd. C, 54.38; H, 3.80; N, 17.62; Found: C, 54.46; H, 3.64; N, 17.88.

5-Substituted- 4,5- dihydro-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl]- 4-imino-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (13a-d)

A mixture of compound 3 (0.65 g, 0.002 mol) and isothiocynate derivatives namely; ethylisothiocynate, cyclohexylisothiocynate, phenylisothiocynate and/or o-tolylisothiocynate (0.002 mol) in dioxane (20 ml) and pyridine (1 ml) was refluxed for 5 hr. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 13a-d.

5-Ethyl-4,5-dihydro-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl]-4-imino-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (13a)

Yield 68%, mp. 163-165^{o}C; IR (KBr, cm^{-1}): 3177, 3153 (2NH), 2925, 2848 (CH_2 tetralin) and 1150 (C=S); ^{1}H NMR (DMSO-d_6): δ 1.34 (t, 3H, -$CH_2\underline{CH_3}$), 1.72-1.78 (m, 4H, 2 (CH_2) tetralin), 2.72-2.80 (m, 4H, 2 (CH_2) tetralin), 3.56 (q, 2H, -$\underline{CH_2}CH_3$), 4.21 (s, 1H, NH, D_2O exchangeable), 7.02-7.98 (m, 5H, CH-tetralin, thiazole, pyrazole protons), 8.87 (s, 1H, NH, D_2O exchangeable); MS, m/z (%): 409 [M^+] (21), 65 [C_5H_5] (100) ; Anal. For $C_{20}H_{20}N_6S_2$ (408.54): Calcd. C, 58.80; H, 4.93; N, 20.57; Found: C, 58.66; H, 5.04; N, 20.34.

5-Cyclohexyl-4,5-dihydro-1- [4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl]-4-imino-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (13b)

Yield 62%, mp. 172-174^{o}C; IR (KBr, cm^{-1}): 3186, 3161 (2NH), 2924, 2850 (CH_2 tetralin) and 1159 (C=S); ^{1}H NMR (DMSO-d_6): δ 1.41-1.86 1 (m, 14H, 7 (CH_2) tetralin, cyclohexyl ring), 2.63-2.67 (m, 1H, N-cyclohexyl), 2.76-2.81 (m, 4H, 2 (CH_2) tetralin), 4.34 (s, 1H, NH, D_2O exchangeable), 7.03-7.89 (m, 5H, CH-tetralin, thiazole, pyrazole protons), 9.02 (s, 1H, NH, D_2O exchangeable); MS, m/z (%): 462 [M^+] (9), 77 [C_6H_5] (100); Anal. For $C_{24}H_{26}N_6S_2$ (462.63): Calcd. C, 62.31; H, 5.66; N, 18.17; Found: C, 62.54; H, 5.28; N, 18.36.

4,5-Dihydro-1-[4- (1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl]- 4-imino-5-phenyl-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (13c)

Yield 65%, mp. 181-183^{o}C; IR (KBr, cm^{-1}): 3171, 3150 (2NH), 2925, 2845 (CH_2 tetralin) and 1160 (C=S); MS, m/z (%): 455 [M-1] (11), 64 [C_5H_4] (100); Anal. For $C_{24}H_{20}N_6S_2$ (456.59): Calcd. C, 63.13; H, 4.42; N, 18.41; Found: C, 63.28; H, 4.22; N, 18.75.

4,5-Dihydro-1-[4-(1,2,3,4-tetrahydronaphthalen- 7-yl)thiazol-2-yl]- 4-imino-5-p-tolyl-1H-pyrazolo[3,4-d]pyrimidine-6(7H)-thione (13d)

Yield 71%, mp. 191-193^{o}C; IR (KBr, cm^{-1}): 3180, 3144 (2NH), 2926, 2851 (CH_2 tetralin) and 1152 (C=S); ^{1}H NMR (DMSO-d_6): δ 1.68-1.82 (m, 4H, 2 (CH_2) tetralin), 2.20 (s, 3H, CH_3), 2.70-2.73 (m, 4H, 2 (CH_2) tetralin), 4.14 (s, 1H, NH, D_2O exchangeable), 7.02-8.40 (m, 9H, CH-tetralin, thiazole, pyrazole protons, Ar-H), 9.21 (s, 1H, NH, D_2O exchangeable); MS, m/z (%): 472 [M+1] (4), 76 [C_6H_4] (100); Anal. For $C_{25}H_{22}N_6S_2$ (470.61): Calcd. C, 63.80; H, 4.71; N, 17.86; Found: C, 63.66; H, 4.85; N, 17.59.

4-Amino-6,7-dihydro-1- [4-(1,2,3,4- tetrahydronaphthalen-7-yl) thiazol-2-yl]-6-imino-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (14)

A mixture of compound 3 (0.65 g, 0.002 mol) and malononitrile (0.14 g, 0.002 mol) in 20 ml absolute ethanol containing few drops of piperdine was refluxed for 5 hr, then poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 14.

Yield 65%, mp. >300 ^{o}C; IR (KBr, cm^{-1}): 3343, 3198, 3149 (2NH, NH_2), 2921, 2851 (CH_2 tetralin) and 2198 (CN); ^{1}H NMR (DMSO-d_6): δ 1.51-1.71 (m, 4H, 2 (CH_2) tetralin), 2.69-2.79 (m, 4H, 2 (CH_2) tetralin), 7.01-8.10 (m, 5H, CH-tetralin, thiazole, pyrazole protons); MS, m/z (%): 361 [M-CN] (5), 56 [C_4H_8] (100); Anal. For $C_{20}H_{17}N_7S$ (387.46): Calcd. C, 62.00; H, 4.42; N, 25.30; Found: C, 62.13; H, 4.62; N, 25.54.

5-(Substituted amino)-1-(4-(1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl)-1H-pyrazole-4-carbonitriles (15a-c)

A mixture of compound 3 (0.65 g, 0.002 mol) and aldehyde derivatives namely; 4-methoxybenzaldehyde, 2-thiophene carboxaldehyde and/or 3-indolylcarboxaldehyde (0.002 mol) in 15 ml acetic acid was refluxed for 5 hr. Then, the reaction mixture was poured onto ice/cold water, the formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 15a-c.

5-(4-Methoxybenzylideneamino)-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazole-4-carbonitrile (15a)

Yield 73%, mp. 2105-217^{o}C; IR (KBr, cm^{-1}): 2924, 2850 (CH_2 tetralin), 2216 (CN) and 1645 (N=CH); ^{1}H NMR (DMSO-d_6): δ 1.71-1.85 (m, 4H, 2 (CH_2) tetralin), 2.70-.2.80 (m, 4H, 2 (CH_2) tetralin), 3.82 (s, 3H, OCH_3), 7.05-8.10 (m, 10H, CH-tetralin, thiazole, pyrazole protons, Ar-H, N=CH); MS, m/z (%): 440 [M^{+1}] (34), 56 [C_4H_8] (100); Anal. For $C_{25}H_{21}N_5OS$ (439.53): Calcd. C, 68.32; H, 4.82; N, 15.93; Found: C, 68.42; H, 4.69; N, 15.77.

5-[(Thiophen- 2-yl) methyleneamino]-1- [4-(1,2,3,4- tetrahydronaphthalen- 7-yl] thiazol- 2-yl)-1H-pyrazole-4-carbonitrile (15b)

Yield 68%, mp. 205-207^{o}C; IR (KBr, cm^{-1}): 2925, 2853 (CH_2 tetralin), 2216 (CN) and 1649 (N=CH); MS, m/z (%): 415 [M^+] (15), 77 [C_6H_5] (100); Anal. For $C_{22}H_{17}N_5S_2$ (415.53): Calcd. C, 63.59; H, 4.12; N, 16.85; Found: C, 63.37; H, 4.39; N, 16.92.

5-[(1H-Indol-3-yl)methyleneamino]-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl) thiazol-2-yl]-1H-pyrazole-4-carbonitrile (15c)

Yield 71%, mp. 224-226^{o}C; IR (KBr, cm^{-1}): 3111 (NH), 2926, 2853 (CH_2 tetralin), 2216 (CN) and 1654 (N=CH); MS, m/z (%): 448 [M^+] (8), 65 [C_5H_5] (100); Anal. For $C_{26}H_{20}N_6S$ (448.54): Calcd. C, 69.62; H, 4.49; N, 18.74; Found: C, 69.87; H, 4.66; N, 18.53.

6-(Chloromethyl)-1-[4-(1,2,3,4-tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazolo [3,4-d]pyrimidin-4(5H)-one (16)

A mixture of compound 3 (0.65 g, 0.002 mol) and chloracetylchloride (0.23 ml, 0.002 mol) in 20 ml dry benzene was refluxed for 5 hr. The formed precipitate was filtered, dried and recrystallized from ethanol to give the title compound 16.

Yield 71%, mp. 191-192^{o}C; IR (KBr, cm^{-1}): 3123 (NH), 2926, 2850 (CH_2 tetralin) and 1709 (CO); ^{1}H NMR (DMSO-d_6): δ 1.62-1.81 (m, 4H, 2 (CH_2) tetralin), 2.60-2.80 (m, 4H, 2 (CH_2) tetralin), 4.19 (s, 3H, -CH_2Cl,), 7.01-8.01 (m, 5H, CH-tetralin, thiazole, pyrazole protons); MS, m/z (%): 371, 369 [M-CO] (5, 2) and 27 [C_2H_3] (100); Anal. For $C_{19}H_{16}ClN_5OS$ (397.88): Calcd. C, 57.35; H, 4.05; N, 17.60; Found: C, 57.55; H, 4.35; N, 17.92.

5-Amino-1-[4-(1,2,3,4- tetrahydronaphthalen-7-yl) thiazol-2-yl]-1H-pyrazole-4-carboxamide (17)

Compound 3 (0.65 g, 0.002 mol) was added portionwise to 10 ml conc. sulfuric acid at 0-5^{o}C. Then , the reaction mixture was stirred for 15 min at room temperature and poured onto ice/cold water. The formed precipitate was filtered, washed several times with water, dried and recrystallized from ethanol to give the title compound 17.

Yield 73%, mp. 281-283^{o}C; IR (KBr, cm^{-1}): 3226, 3181, 3123 ($2NH_2$), 2925, 2855 (CH_2 tetralin) and 1671 (CO); MS, m/z (%): 339 [M^+] (21%) and 76 [C_6H_4] (100); Anal. For $C_{17}H_{17}N_5OS$ (339.41): Calcd. C, 60.16; H, 5.05; N, 20.63; Found: C, 60.40; H, 5.28; N, 20.51.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl)thiazol-2-yl]-1H-pyrazol-5-amine (18)

A mixture of compound 3 (0.65g, 0.002 mol) and 20 ml sulfuric acid (50%) was refluxed for 2 hr, cooled, poured onto ice/cold water. The formed precipitate was filtered, dried and recrystallized from ethanol to give the title compound 18.

Yield 65%, mp. >300^{o}C; IR (KBr, cm^{-1}): 3217 (NH_2), 2925, 2852 (CH_2 tetralin); MS, m/z (%): 296 [M^+] (100); Anal. For $C_{16}H_{16}N_4S$ (296.39): Calcd. C, 64.84; H, 5.44; N, 18.90; Found: C, 64.70; H, 5.31; N, 19.15.

1-[4-(1,2,3,4-Tetrahydronaphthalen-7-yl)thiazol-2-yl]-4-(1H-imidazol-2-yl)-1H-pyrazol-5-amine (19)

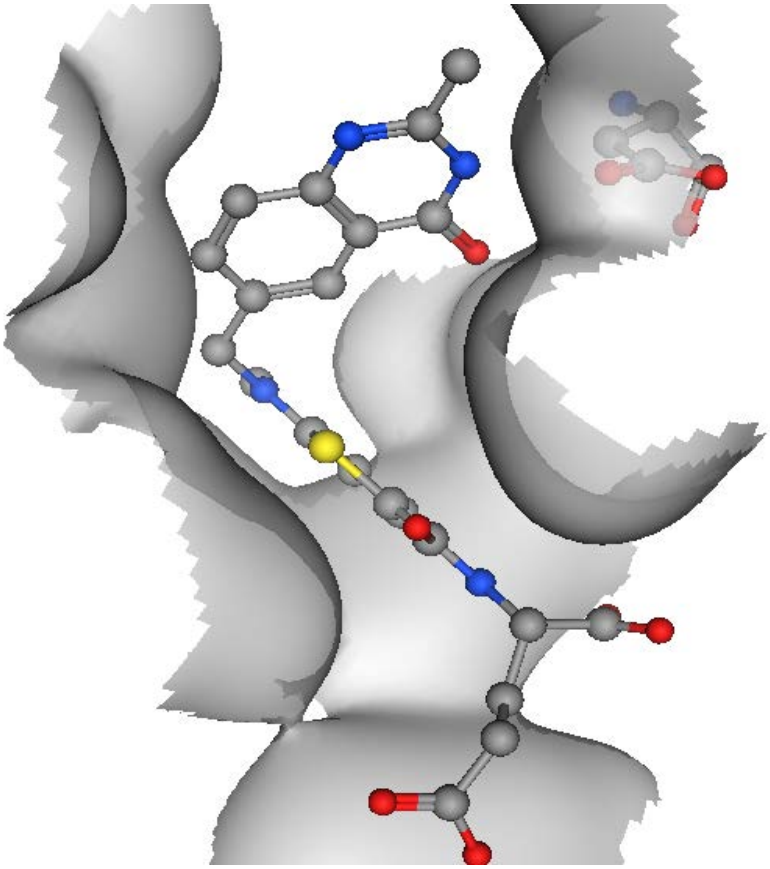
A mixture of compound 3 (0.65 g, 0.002 mol) and diaminoethane (5 ml) than carbon disulphide (1 ml) was added dropwise with stirring at room temperature. The reaction mixture was heated on water bath for 5 hr. The formed precipitate was filtered, dried and recrystallized from ethanol to give the title compound 19.

Yield 68%, mp. 154-156^{o}C; IR (KBr, cm^{-1}): 3217, 3147 (NH_2), 2924, 2852 (CH_2 tetralin); MS, m/z (%): 362 [M^+] (100); Anal. For $C_{19}H_{18}N_6S$ (362.45): Calcd. C, 62.96; H, 5.01; N, 23.19; Found: C, 62.80; H, 5.27; N, 23.25.

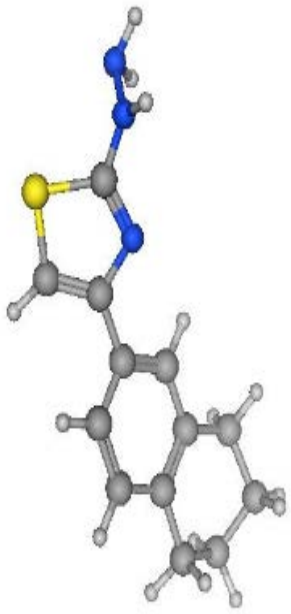
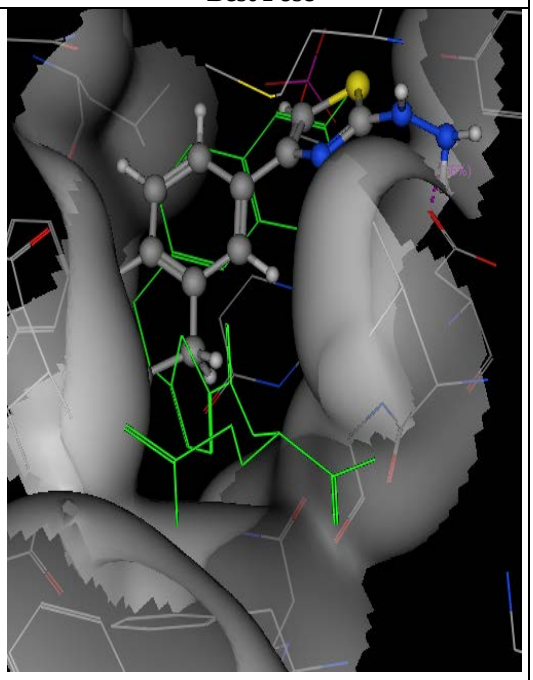
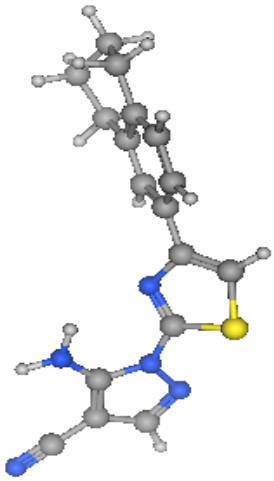
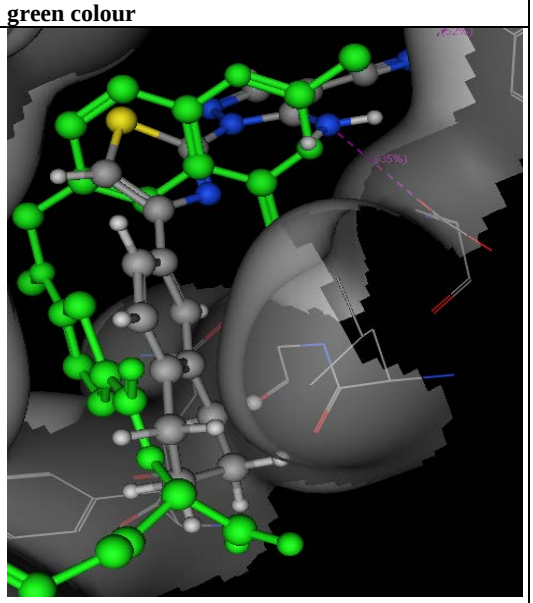
Molecular docking

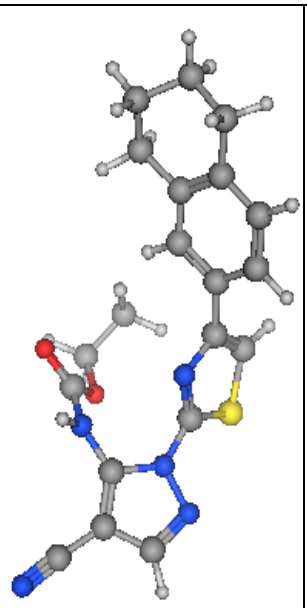
Preparation of protein and ligands for docking

The crystal structure of Thymidylate Synthase complexed with Raltitrexed was downloaded from protein data bank (http://www.pdb. org/pdb/home/ home.do) with pdb code = 1HVY and the site in which the inhibitor was complexes was identified and the surrounding important residues such as Tyr 258, Asp 218, Leu 221, Gly 222, Asn 226, Phe 225, Phe 80, Glu 87, IIe 108, TRP 109, Ala 312, Asn 112 and Leu 192 were recognised. Both the protein and ligands were saved as pdbqt format. Docking was performed as in the Autodock vina and MOE (Table 1) . Binding affinities were calculated and the highly ranked compounds were selected and docking was repeated for these selected compounds to confirm their affinity. The docking results have shown that 8 compounds could be potential anticancer agents due to their promising binding and fitting in Thymidylate Synthase. The best conformations and docking poses are shown below.

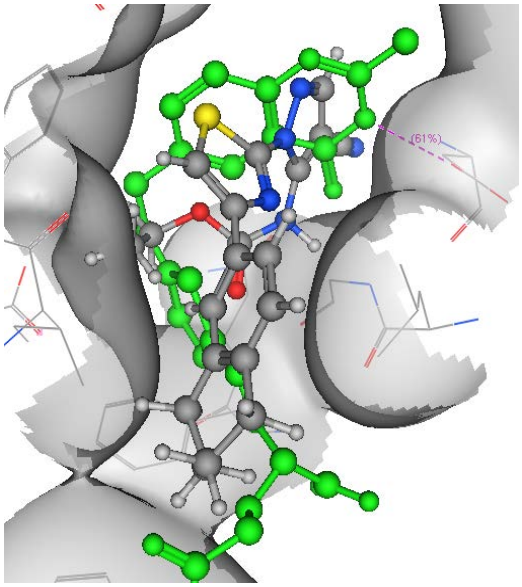


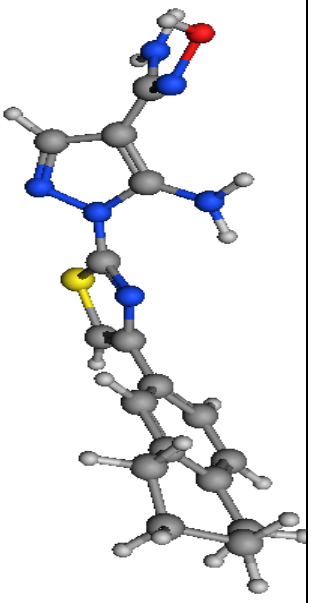
Raltitrexed binding site. Asp 218 is shown close to the binding site.

Best conformation	Best Pose
 **compound** 1	 **Raltitrexed is used as a reference and is shown in green colour**
 **compound 3**	

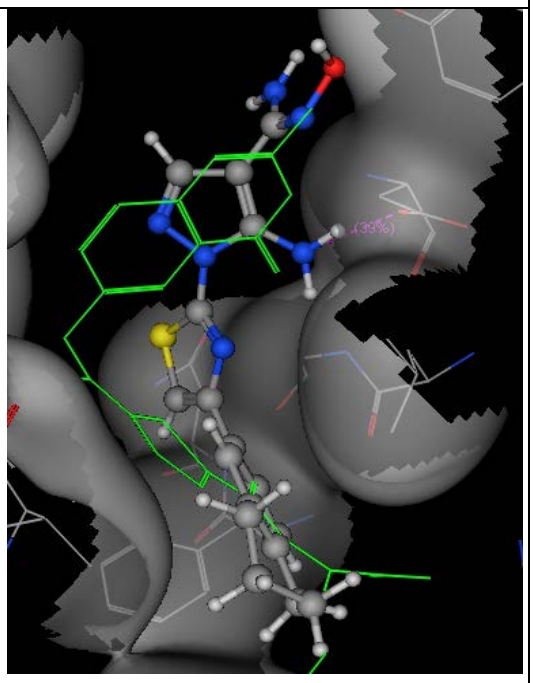


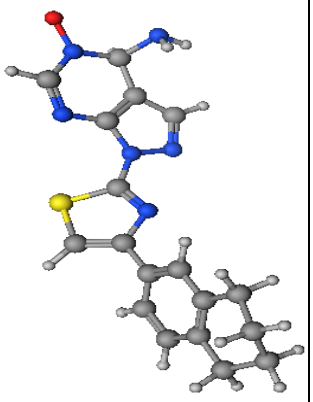
compound 5





compound 6





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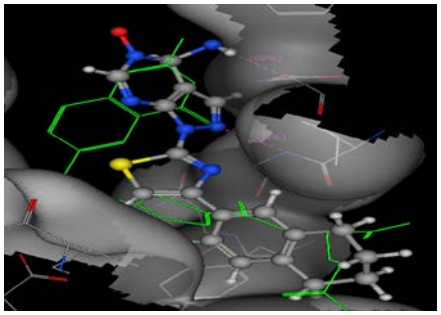
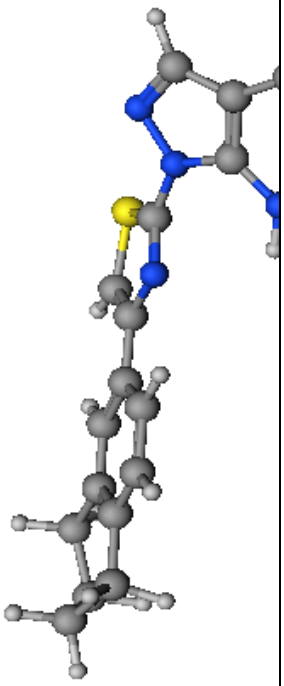
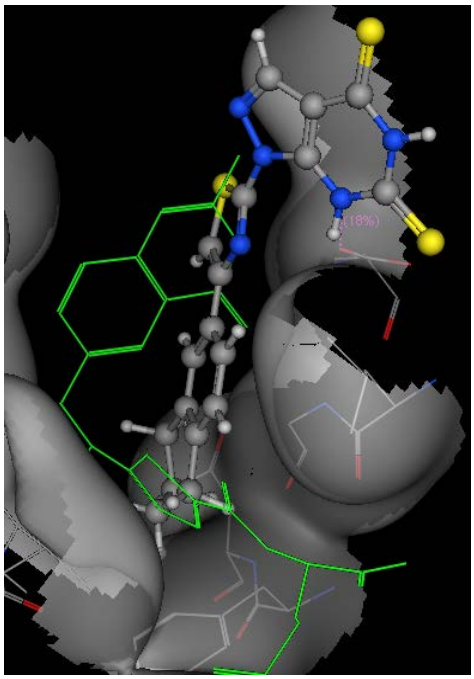
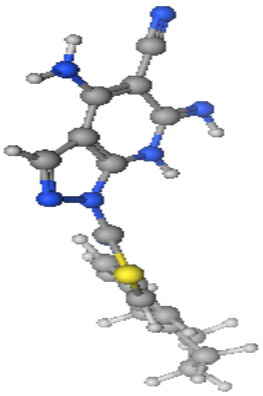
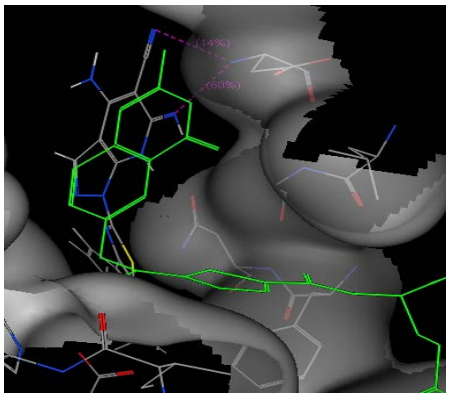




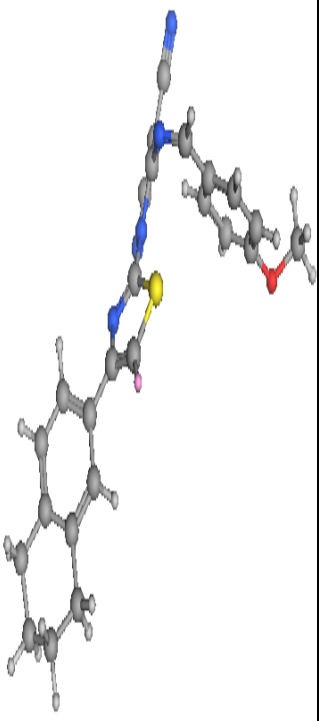
compound 12

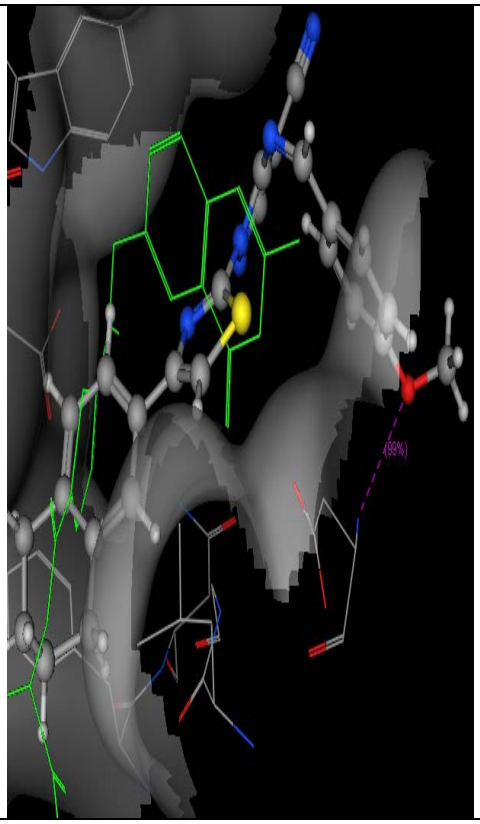






compound 14





compound 15a

TABLE 1. Docking results of the synthesized compounds .

Compound	MOE Affinity Kcal/mol	Autodock vina Affinity Kcal/mol	Main Residue	Bond strength
1	--10.80	-7.5	Tyr 258	66 %
2				
3	-9.98	-6.90	Tyr 258, Asp 218	35 %
4	-8.5	-5.98	No action	No action
5	-10.98	-8.70	Asn 226	61.5 %
6	-10.70	-7.34	Asp 218	39 %
7	-12.80	-7.56	Tyr 258, Asp 218	58 %
8	-9.88	-4.5	Phe 225	23 %
9	-9.1	-6.3	Phe 225	13%
10	-9.5	-5.8	Glu 87	No action
11	-9.0	-6.12	No action	No action
12	-11.65	-6.74	Asp 218	39 %
13a	-9.2	-6.90	Trp 109	34%
13b	-9.0	-5.12	No action	No action
13c	-7.78	-4.98	No action	No action
13d	-8.34	-5.34	No action	No action
14	-10.45	-6.54	Asp 218	60 %
15a	-10.97	-7.77	Asp 218	68 %
15b	-9.2	-5.43	Leu 221	26 %
15c	-8.70	-4.22	No action	No action
16	-7.98	-5.50	No action	No action
17	-8.46	-6.90	No action	No action
18	-9.65	-6.84	No action	No action

Calculated affinity: Kcal/mol

According to the docking results, eight compounds showed high affinities to Thymidylate Synthese enzyme. Compounds 1, 3, 5, 6, 7, 12, 14 and 15a have MOE affinities -10.80, -9.98, -10.98, -10.70, -12.80, -11.65, 10.49 and -10.97 kcal/mol, respectively and Autodock Vina affinities -7.5, -6.90, -8.70, -7.34, -7.56, -6.74, -6.54 and 7.77, respectively.

Most of the tested derivatives showed interaction with Asp218 of the enzyme. It has been found that the hydrazine group of compound 1 was involved in H-bonding with Asp218. While compound 3 exhibited two binding conformations,

one with Tyr258 and the other with Asp218, via the amino and cyano groups. The cyano and amino groups of compounds 5 and 7 participated in H-bonding with Asn 226 and Asp218, respectively. In case of derivative 6, it exhibited two H-bonding interaction with Asp218 and Tyr258 via NH_2 and NOH groups. Finally, compounds 12, 14 and 15a showed conformation that poses H-bonding between Asp218 and NH, CN groups, respectively.

Biological Evaluation

Material and methods

Cytotoxic effect on human cell line (HePG 2 - MCF 7 - HCT 116)

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [27] .

All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA). Cells were suspended in RPMI 1640 medium for HePG2- MCF7 and HCT116 – DMEM for A549. The media are supplemented with 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000µg/ml Streptomycin Sulfate and 25µg/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37ºC under 5% CO_2.

Cells were batch cultured for 10 days, then seeded at concentration of 10x103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37ºC for 24 hr under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 ug/ml). After 48 hr of incubation, medium was aspirated, 40ul MTT salt (2.5 µg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 µL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. A positive control which composed of 100µg/ml was used as a known cytotoxic natural agent which gives 100% lethality under the same conditions[28] .

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

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

In vitro cytotoxic screening

Ten of the newly synthesized tetralin derivatives (3, 4, 5, 6, 7, 12, 14, 15a, 16, 19) were selected as representative examples for cytotoxic activity evaluation against colon (HCT116), hepatocellular (HePG2) and breast (MCF7) carcinoma cell line (Table 2 and Fig.1-3).

TABLE 2. LC_{50} (µg/ml) of the tetralin derivatives on colon cell line (HCT116), human liver carcinoma cell line (HePG2) and human Caucasian breast adenocarcinoma (MCF7) .

Comp. No	HCT116-48 hr	HePG2- 48 hr	MCF7- 48 hr
	LC_{50} (µg/ml)	LC_{50} (µg/ml)	LC_{50} (µg/ml)
3	46.1	50.8	44.3
4	55.9	30.8	30.7
5	--------	--------	--------
6	52.1	45.3	50
7	--------	69.9	59.1
12	74.5	68.4	55.1
14	--------	--------	--------
15a	--------	--------	64.5
16	--------	--------	--------
19	35.5	40.7	51.9
Doxorubicin	37.7	21.4	26.1
DMSO	--------	--------	--------
Negative control	--------	--------	--------

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

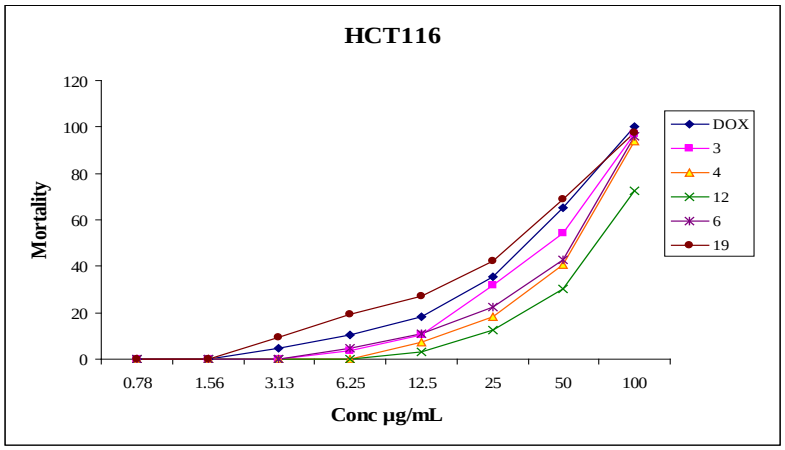


Fig. 1. Cytotoxicity screening of tetralin derivatives against HCT116.

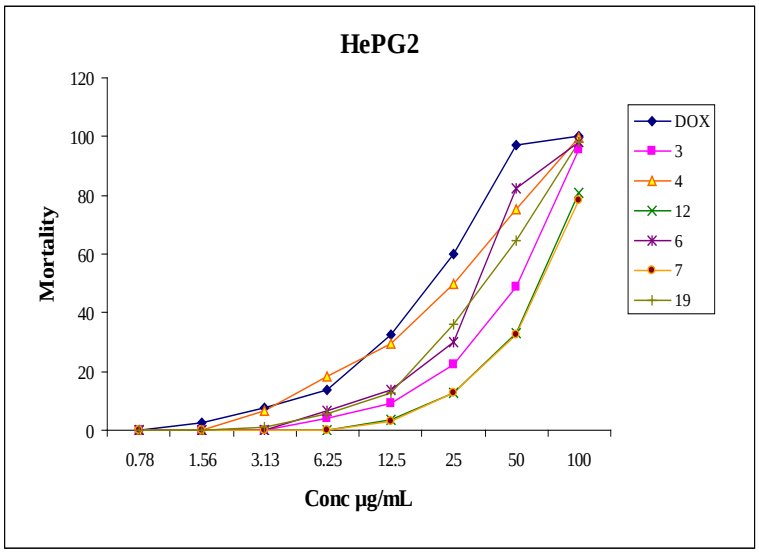


Fig. 2. Cytotoxicity screening of tetralin derivatives against HePG2.

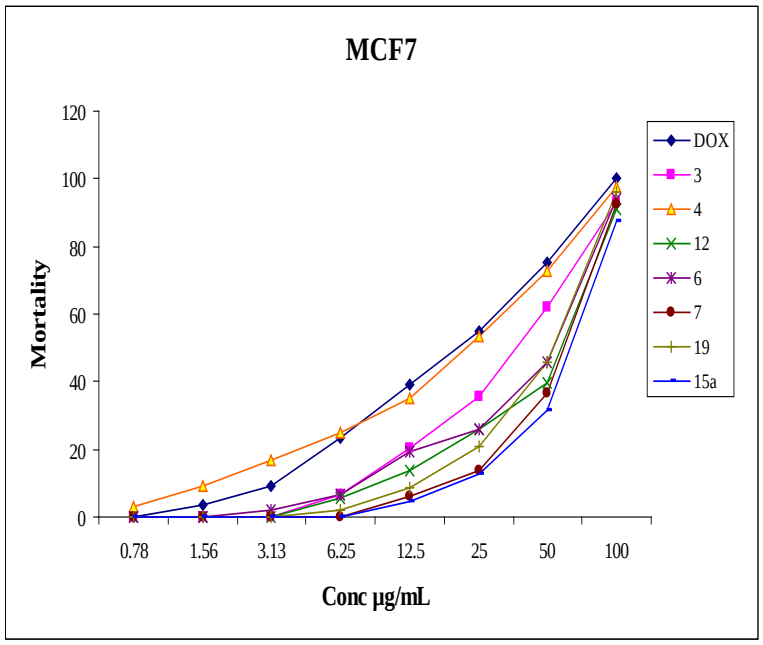


Fig. 3. Cytotoxicity screening of tetralin derivatives against MCF7.

Structure activity relationship

Ten of the newly synthesized derivatives (3, 4, 5, 6, 7, 12, 14, 15a 16, 19) were selected as representative examples for cytotoxic activity evaluation against colon (HCT116), hepatocellular (HePG2) and breast (MCF7) carcinoma cell lines.

According to the resultant data of HCT116 cell lines: it has been noticed that the parent compound 3 carrying 4-cyano-5-aminopyrazole moiety attached to thiazolo-tetralin ring showed efficient cytotoxic activity (LC_{50}: 46.1µg/ml). The conversion of the same cyano group to imino hydroxyl group 6 significantly decreased the potency to be less than the parent compound (LC_{50}: 52.1µg/ml). While, the replacement of the cyano group with imidazole ring 19 intensified the potency to be the most active one of the tested derivative (LC_{50}: 35.5µg/ml). Also, the replacement of 5-amino group of the pyrazole ring with imino ethoxy substituent 4 or the formation of the fused pyrimido-pyrazole ring system 12 (LC_{50}: 55.9 and 74.5 µg/ml) greatly inhibited the cytotoxic activity of the parent compound. Unfortunately, the rest of the examined compounds showed complete loss of activity.

In case of (HePG2) carcinoma cell lines, the data exhibited that six out of the ten tested derivatives exhibited cytotoxic activity. The attachment of imino ethoxy group to the pyrazole ring instead of the amino group 4 greatly increased the activity (IC_{50}: 30.8 µg/ml) to be more potent than the parent 3 (LC_{50}: 50.8 µg/ml). Also, an increase of the cytotoxic efficacy, but to a less extent, was observed due to the conversion of the cyano group to imino hydroxyl group 6 (LC_{50}: 45.3 µg/ml) or imidazole ring 19 (LC_{50}: 40.7 µg/ml). A remarkable decrease in the activity was assigned due to the presence of the fused (substituted) pyrimido-pyrazole ring in conjugation with thiazolo-tetralin ring system as the derivatives 7 and 12 (LC_{50}: 69.9 and 68.4µg/ml) , respectively.

With respect to the obtained data of MCF7 cell lines: it is observable that the number of the active derivatives was larger than the other two types of carcinoma cell lines. About seven compounds exhibited cytotoxic activity against breast carcinoma cell lines. As the above HePG2, compound 4 inhibited the cancerous cell lines at (LC_{50}: 30.7 µg/ml), which is greatly less than that of the parent compound 3 of (LC_{50}: 44.3 µg/ml). While the other derivatives 6, 7, 12, 15a and 19 showed observable drops in the cytotoxic activity.

These variations in the cytotoxic activities due to variation in the types of the substituents conjugated to the pyrazolo-thiazolotetralin core should be taken in our considerations while designing and synthesizing novel anticancer agents of high efficiency and selectivity.

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anticancer screening. Also, thanks to Dr. Mohamed Abdou Khedr, Faculty of pharmacy, Helwan University for molecular docking studies.

References

1 **Ali, M.M. and Hassan, S.A.**, *Int J Cancer Res* **3**, 103 (2007).

2 **Tsuchiya, T., Takagi, Y. and Yamada, H.,** *Bioorg Med Chem Lett*, **10**, 203 (2000).

3 **Fathalla, O.M., Anwar, M.M, Haiba, M.E. and Nofal, S.M.,** *Acta Pol Pharm* - Drug Research, **66**, 259 (2009).

4 **Hara, H., Fujihashi, T., Sakata, T., Kaji, A. and Kaji, H.,** AIDS Res. Hum. Retroviruses **13**, 695 (1997).

5 **Stigliani, J.L., Boustie, J., Amoros, M., Montanha, J., Payard, M. and Girre, L.**, *Pharm Pharmacol Commun* **4,** 65 (1998)

6 **Ferreante, A., Auliera, J., Lewis, K. and Klibanov, A.M.**, *Proc Natl Acad Sci* USA **92**, 7617 (1995).

7 **Nabih, I., Zayed, A., Kamel, M.M. and Motawie, M.S.,** *Egypt J. Chem* **29**, 101 (1986).

8 **Hussain, R.A., Dicky, J. and Rosser, M.P.**, *J. Nat. Prod*. **58**, 1515 (1995).

9 **Chalina, E.G. and Chakarova, L.,** *Eur J. Med Chem* **33**, 975 (1998).

10 **Kamel, M.M. and Michael, J.M.,** *Egypt J. Bilharziasis* **10**, 121 (1988).

11 **Cimetiere, B., Dubuffet, T. and Muller, O.,** *Bioorg Med Chem Lett* **8,** 1375 (1998).

12 **Rogoo, Z., Skuza, G. and Klodzinska, A.,** *Pol J Pharmacol* **56,** 519 (2004).

13 **Kitamuraa, Y., Arakib, H., Shibatac, K., Gomitac, Y., Tanizakid, Y.,** *Eur J. Pharmacol* **481**, 75 (2003).

14 **Amin, K.M., El-Zahar, M.I., Anwar, M.M., Kamel, M.M. and Mohamed, M.H.,** *Acta Pol Pharm - Drug Research*, **66**, 279 (2009).

15 **Soliman, E.A., El-Zahar, M.I., El-Masry, A.H. and Gohar, R.S.,** *Egypt J. Chem* **51**, 701 (2008).

16 **Lawrence, W., Purohit, A. and Barry, A.V.L.,** *J. Med Chem* **41,** 1068 (1998).

17 **Sikkena, J. and Bont, J.A.,** *Appl Environ Microbiol* **59**, 567 (1993).

18 **Chan, D.C.M. and Anderson, A.C.,** *Curr Med Chem* **13**, 377 (2006).

19 **Hawser, S., Lociuro, S. and Islam, K.,** *Biochem Pharmacol*, **71,** 94 (2006).

20 **Blakley, R.L.,** Dihydrofolate Reductase. In Folate and Pterines, R.L. Blakley, S.J. Benkovic, Eds; Wiley-Interscinence: New York **1**, 191 (1984).

21 **MacKenzie, R.E.,** Biogenesis and interconversion of Substituted Tetrahydrofolates. In Folates and Pterins Chemistry and Biochemistry, R. L. Blakley, S. J. Benkovic, Eds.; Wiley: New York 1, 255 (1984).

22 **Assaraf, Y.G.,** *Cancer Metastasis Rev* **26**, 153 (2007).

23 **Matherly, L.H. and Hou, Z.,** *Vitam Horm* **79**, 145 (2008).

24 **Nikolaos, T.**, *Clin Cancer Res* **2**, 227 (1996).

25 **Trott, O. and Olson, A.J.**, *J. Comput Chem* **31,** 455 (2010).

26 **El-Zahar, M.I., Abd El-Karim, S.S. and Anwar, M.M.,** *S Afr J. Chem*., **62**, 189 (2009).

27 **Mosmann, T.,** *J. Immunol Methods* **65**, 55 (1983).

28 **Thabrew, M.I., Hughes, R.D. and McFarlane, I.G.,** *J. Pharm Pharmacol.* 49, 1132 (1997).

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تشييد و دراسات المرسى و التقيم البيولوجى كمضادات للاورام لبعض مشتقات رباعى هيدرونفثيل ثيازول بيرازول

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تم فى هذا البحث اجراء تفاعل مركب 5-امينو-1- (4(4،3،2،1- رباعى هيدرونفثالين-2-يل) ثيازول-2- يل)-*H*1- بيرازول- 4 - كربونيتريل (3) مع كواشف مختلفة مثل ثلاثى ايثيل اورثوفورمات ، ايثيل كلورو فورمات و/او هيدركسيل امين هيدروكلوريد ليعطى مشتقات البيرازل المقابلة (4-6). وايضا تفاعل تكاثف المركب 3 مع حمض الفورمك، انهيدريد حمض الاستيك، فورماميد و/او اسيتاميد ليعطى مشتقات البيرميدين المقابلة (8-11). تم مفاعلة المركب 3 مع ثنائى كبريتيد الكربون، مشتقات ايزوثيوسيانات، مالونونيتريل ، بعض الالدهيدات و/او كلورالاسيتيل كلوريد ليعطى المشتقات المقابلة (12-16) و ايضا التحلل المائى لمركب 3 بواسطة حمض الكبرتيك تحت ظروف مختلفة اعطى مركبات 17، 18.

تم دراسة المرسى لهذه المركبات الجديدة كمثبطات لانزيم ثيميديلات سينساز و ايضا تم التقييم لبعض من هذه المركبات كمضادات لاورام القولون و الكبد و الثدى.