World Journal of Chemistry 4 (2): 100-108, 2009
ISSN 1817-3128

Synthesis, Anticancer and Antiviral Activities of diazacyclopenta[b]-phenanthrene, diazabenzo[a]anthracene and dihydrobenzo[h]quinazoline Derivatives Using 2-Thiophen-2-ylmethylene-3,4-dihydro-2H-naphthalen-1-one as Starting Material

[1]Salwa F. Mohamed, [2]Hamdy Kh. Thabet, [3]El-Sayed E. Mustafa, [4]Mohamed M. Abdalla and [1]Samir H. Shafik

[1]Applied Organic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt
[2]Chemistry Department, Faculty of Science, El-Azhar University, Cairo, Egypt
[3]Microbial Chemistry Department, National Research Centre, Dokki, Cairo, Egypt
[4]Research Unit, Univet Pharmaceutical Co., Egypt

Abstract: A series of diazacyclopenta[b]phenanthrene, diazabenzo[a]anthracene and dihydrobenzo[h]quinazoline derivatives 3-15 were synthesized using 2-thiophen-2-ylmethylene-3,4-dihydro-2H-naphthalen-1-one 2 as a starting material. The biological screening showed that many of these obtained compounds have good anticancer and antiviral activities. The structure assignments of the new compounds based on chemical and spectroscopic evidence. The detailed synthesis, spectroscopic data and pharmacological properties are reported.

Key words: α,β-unsaturated ketone, Diazacyclopenta[b]phenanthrene, Diazabenzo[a]anthracene and dihydrobenzo[h]quinazoline, Anticancer and antiviral activities

INTRODUCTION

There are increasing interests in the chemistry of fused pyrimidines, many of these compounds have pharmacological properties such as analgesic, anti-arrhythmic, anticancer, antipyretic and anti-inflammatory agents [1-14]. In addition, the heterocyclic nitrogen derivatives exhibited a general ionophoric potency for divalent cations [15] and used a novel thiocyanate-selective membrane sensor [16]. Recently, in a previous work we reported that certain of newly substituted pyrimidines exhibited antiparkinsonian [17-19], antitumor [20], antimicrobial [21], anti-inflammatory [22,23] activities. In view of these observations and in continuation of our previous work in pyrimidine chemistry, we have herein synthesized of some new derivatives containing quinazoline moiety. Some of the synthesized compounds were screened for their anticancer and antiviral activities.

MATERIALS AND METHODS

Melting points were determined on open glass capillaries using an Electrothermal IA 9000 digital melting point apparatus. Elemental analyses were performed on Elementar, Vario EL, Microanalytical Unit, National Research Center, Cairo Egypt and were found within ±0.4% of the theoretical values. Infrared spectra (ν, cm^{-1}) were recorded on Carlzeise Spectrophotometer model "UR 10" spectrophotometer using the KBr disc technique. ^{1}H-NMR spectra were recorded on Varian Gemini 270 MHz spectrometer (DMSO-d_6) and the chemical shifts are given in ä (ppm) downfield from tetramethylsilane (TMS) as an internal standard. The mass spectra were measured using a Finnigan SSQ 7000 mass spectrometer. Follow up of the reactions and checking the purity of the compounds was made by TLC on silica gel-aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany).

2-Thiophen-2-ylmethylene-3,4-dihydro-2H-naphthalen-1-one (2): A mixture of 1-tetralone 1 (7.4 g, 0.05 mole) and 2-thiophenaldehyde (5.6 g, 0.05 mole) in absolute ethanol was stirred at 40°C. After cooling to the room temperature, ethanolic KOH (5 ml, 4%) was added to the reaction mixture with stirring. The obtained solid was filtered off, washed with dilute ethanol, dried and crystallized to give

Corresponding Author: Dr. Salwa F. Mohamed, Applied Organic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

2. Yield 89 %, m.p. 95°C (EtOH); IR: 1659 (C=O) characteristic to α,β-unsaturated ketone; ^{1}H-NMR (DMSO-d_6): 2.97, 3.09 (2t, 4H, $2CH_2$ of naphthalinone moiety), 7.19-7.93 (m, 8H, Ar-H + thiophene-H + methylidine-H). MS *m/z* (%): 240 (M^+, 12) corresponding the molecular formula $C_{15}H_{12}OS$ and base peak at 187 (100).

4-Thiophen-2-yl-3,4,5,6-tetrahydro-1H-benzo [h] quinazoline-2-thione (3): Thiourea (0.76 g, 0.01 mole) was added to a boiling solution of compound 2 (2.4 g, 0.01 mole) in ethanol (100 ml) containing potassium hydroxide (1 g), the reaction mixture was refluxed for 2 hrs., then allowed to cool and the solid formed was filtered off and crystallized to give 3. Yield 85 %, m.p. 248-250°C (dioxane); IR: 3145 cm^{-1} (2NH), 1198 cm^{-1} (C=S). The IR spectrum is also devoid of the band corresponding to ν(C=O) present in the IR spectrum of 2. ^{1}H-NMR (DMSO-d_6): 1.90-2.85 (m, 4H, $2CH_2$ of quinazoline ring), 5.30 (s, 1H, pyrimidine proton), 7.00-7.80 (m, 7H, Ar-H + thiophene-H) and 9.30, 9.90 (2s, 2H, 2NH, exchangeable with D_2O). MS *m/z* (%): 298 (M^+, 43) corresponding the molecular formula $C_{16}H_{14}N_2S_2$ and base peak at 82 (100).

2-(4-(thiophen-2-yl)-5,6-dihydrobenzo[h]quinazolin-2-ylthio) acetic acid (4): A mixture of 3 (2.98 g, 0.01 mole) and chloroacetic acid (0.94 g, 0.01 mole) in ethanolic KOH (50 ml, 2 %) was heated under reflux for 2 hrs. The reaction mixture was acidified with acetic acid; the solid obtained was filtered off, dried and crystallized to give 4. Yield 82%, m.p. 180 °C (MeOH/H_2O); IR: 1712 (C=O); ^{1}H-NMR (DMSO-d_6): 2.87, 3.18 (2t, 4H, $2CH_2$ of quinazoline ring), 4.02 (s, 2H, CH_2), 7.22-7.49 (m, 4H, Ar-H), 7.70 (d, 1H, Ar-H), 7.84 (d, 1H, C_3 thiophene), 8.18 (d, 1H, C_5 thiophine), 12.77 (s, 1H, OH, exchangeable with D_2O). MS *m/z* (%): 354 (M^+, 2) corresponding to the molecular formula $C_{18}H_{14}N_2O_2S_2$, base peak at 263 (100).

7-Thiophen-2-yl-5,7-dihydro-6H-10-thia-7a,11-diaza-cyclopenta[b]phenanthren-8-one(6)

Method A: A mixture of compound 3 (2.98 g, 0.01 mole), chloroacetic acid (0.94 g, 0.01 mole) and fused sodium acetate (6 g, 0.075 mole) in glacial acetic acid (30 ml) and acetic anhydride (10 ml) was refluxed for 3 hrs. The reaction mixture poured onto cold water, the formed solid was filtered off, dried and crystallized from ethanol to give 6 in yield 86%.

Method B: A mixture of compound 3 (2.98 g, 0.01 mole), ethylchloroacetate (1.22 g, 0.01 mole) and (1.12 g, 0.02 mol) of KOH in (50 ml) ethanol was heated under reflux for 10 hrs. The separated solid was collected by filtration, dried and crystallized to give 6. Yield 55%, m.p. 233-235°C; IR: 1720 cm^{-1} corresponding to (C=O). The spectrum also devoid of the bands corresponding to ν(NH) and ν(C=S) previously identified in the IR spectra of their parent compound 3, ^{1}HNMR (DMSO-d_6): 2.09-2.78 (m, 4H, $2CH_2$ of quinazoline ring), 4.10 (dd, 2H, CH_2 of thiazole), 6.01 (s, 1H, pyrimidine proton), 6.97-7.79 (m, 7H, Ar-H + thiophene-H), MS *m/z* (%): 338 (M^+, 100, base peak) corresponding to the molecular formula $C_{18}H_{14}N_2OS_2$.

9-(3,4-Dimethoxy-benzylidene)-7-thiophen-2-yl-5,7-dihydro-6H-10-thia-7a,11-diaza- cyclopenta [b] phenanthren-8-one (7)

Method A: A mixture of compound 3 (2.98 g, 0.01 mole), chloroacetic acid (0.94 g, 0.01 mole) and fused sodium acetate (6 g, 0.075 mole) in glacial acetic acid (30 ml), acetic anhydride (10 ml) mixture and 3,4-dimethoxybenzaldehyde (1.66 g, 0.01 mole) was refluxed for 3 hrs. The reaction mixture was cooled and poured onto cold water; the solid formed was filtered off and crystallized from methanol to give 7 in yield 79%.

Method B: A mixture of compound 6 (3.38 g, 0.01 mole), equimolecular amount of 3,4-dimethoxybenzaldehyde (1.66 g, 0.01 mole) in glacial acetic acid (30 ml), acetic anhydride (10 ml) mixture and fused sodium acetate (3 g, 0.0375 mole) was refluxed for 1 hr., allowed to cool. The reaction mixture poured onto cold water, the solid formed was filtered off and crystallized from methanol to give 7. Yield 48%, m.p. 130-132°C; IR: 1711 cm^{-1} characteristic (C=O) of α,β-unsaturated ketone [this shift to lower frequency is due to conjugation with exocyclic double bond], ^{1}H-NMR ($CDCl_3$): 2.33 (t, 2H, CH_2 of quinazoline ring), 2.85 (t, 2H, CH_2 of quinazoline ring), 3.92 (s, 6H, $2OCH_3$), 6.03 (s, 1H, pyrimidine proton), 6.90-7.27 (m, 10H, Ar-H + thiophene-H), 7.67 (s, 1H, CH=C), 7.97 (d, 1H, C_5 thiophene), MS *m/z* (%): 486 (M^+, 45) corresponding to the molecular formula $C_{27}H_{22}N_2O_3S_2$ and base peak at 338 (100).

Synthesis of cyclopenta [b] phenanthren-8-one derivatives (8a,b): Aromatic amine (aniline or *p*-chloroaniline (0.01 moles) was dissolved in concentrated hydrochloric acid (3 ml) and water (2 ml) cooled to 0°C and treated with sodium nitrite (0.7g, 5 ml water). The diazotized amine was added gradually with stirring to cooled solution of compound 6 (3.38 g, 0.01 mole) in ethanol (20 ml) in the presence of sodium

acetate. The reaction mixture was kept in refrigerator for ½ hr. and then diluted with water. The solid formed was collected by filtration, dried and crystallized to give compound 8a,b.

9-(phenyl-hydrazono)-7-thiophen-2-yl-5,7-dihydro-6H-10-thia-7a,11-diaza-cyclopenta[b]phenanthren-8-one (8a): Yield 65%, 192°C (EtOH); IR: 3422, 1715cm^{-1} corresponding to ν(NH) and ν(C=O) respectively, ^{1}H-NMR (DMSO-d_6): 2.05-2.72 (m, 4H, 2CH_2 of quinazoline ring), 5.98 (s, 1H, pyramidine proton), 7.11-7.75 (m, 12H, Ar-H+ thiophene), 12.38 (s, 1H, NH exchangeable with D_2O). MS *m/z* (%): 442 (M^+, 3) corresponding to the molecular formula $C_{24}H_{18}N_4OS_2$ and base peak at 338 (100).

9-(4-chlorobeznyl-hydrazono)-7-thiophen-2-yl-5,7-dihydro-6H-10-thia-7a,11-diaza-cyclopenta [b] phenanthren-8-one 8b: Yield 60%, 215°C (EtOH); IR: 3422, 1715cm^{-1}corresponding to ν(NH) and ν(C=O) respectively. ^{1}H-NMR (DMSO-d_6): 2.00-2.80 (m, 4H, 2CH_2 of quinazoline ring), 5.98 (s, 1H, Pyrimidine proton), 6.92-7.78 (m, 11H, Ar-H+ thiophene-H),10.79 (s, 1H, NH exchangeable with D_2O). MS *m/z* (%): 476 (M^+, 51) corresponding the molecular formula $C_{24}H_{17}ClN_4OS_2$ and base peak at 238 (100%).

2-(4-(thiophen-2-yl)-5,6-dihydrobenzo[h]quinazolin-2-ylthio) propanoic acid (9): A mixture of compound 3 (2.98 g, 0.01 mole) and á–bromopropionic acid (1.52 g, 0.01 mole) in ethanolic potassium hydroxide (5 ml, 2%) was heated under reflux for 2 hr. The reaction mixture acidified with acetic acid, the solid formed was collected by filtration and crystallized to give compound 9. Yield 63 %, m.p. 125°C (MeOH/H_2O); IR: 3423, 1723 cm^{-1} corresponding to ν(OH) and ν(C=O), respectively; ^{1}H-NMR (DMSO-d_6): 1.60 (d, 3H, CH_3), 2.60, 3.10 (2t, 4H, 2CH_2 of quinazoline ring), 4.47 (q, 1H, CH), 7.20-8.23 (m, 7H, Ar-H + thiophene-H), 12.60 (b, 1H, OH, exchangeable with D_2O); MS *m/z* (%): 368 (M^+, 2) corresponding to molecular formula $C_{19}H_{16}N_2O_2S_2$ and base peak at 352 (100%).

9-Methyl-7-thiophen-2-yl-5,7-dihydro-6H-10-thia-7a,11-diaza-cyclopenta[b]-phenanthren-8-one (11): A mixture of compound 3 (2.98 g, 0.01 mole), α-bromopropionic acid (1.52 g, 0.01 mole) and fused sodium acetate (6 g, 0.075 mole) in glacial acetic acid (30 ml) and acetic anhydride (10 mL) was refluxed for 3 hrs. The reaction mixture was poured onto cold water; the solid formed was filtered off, washed with water and crystallized to give 11. Yield 75 %, m.p. 135°C (EtOH); IR: 1721 cm^{-1} corresponding to ν(C=O); ^{1}H-NMR (DMSO-d_6): 1.40 (d, 3H, CH_3), 2.07-2.72 (m, 4H, 2CH_2 of quinazoline ring), 4.37 (q, 1H, CH of thiazole), 6.01 (s, 1H, CH of pyrimidine), 6.97-7.79 (m, 7H, Ar-H + thiophene-H); MS *m/z* (%): 352 (M^+, 100, as base peak) corresponding to molecular formulae $C_{19}H_{16}N_2OS_2$.

3-(4-(thiophen-2-yl)-5,6-dihydrobenzo[h]quinazolin-2-ylthio) propanoic acid (12): A mixture of compound 3 (2.98 g, 0.01 mole) and â–bromopropionic acid (1.52 g, 0.01 mole) in ethanolic potassium hydroxide (5 ml, 2%) was heated under reflux for 2 hrs. The reaction mixture was acidified with acetic acid, the solid formed was collected by filtration and crystallized to give 12. Yield 60 %, m.p. 95°C (MeOH/H_2O); IR: 3398, 1725 corresponding to ν(OH) and ν(C=O), respectively; ^{1}H-NMR (DMSO-d_6): 2.90-3.28 (m, 8H, 4CH_2), 7.07-7.85 (m, 7H, Ar-H + thiophene-H) and 9.09 (s, 1H, OH). MS *m/z* (%): 368 (M^+, 2) corresponding to molecular formula $C_{19}H_{16}N_2O_2S_2$.

7-Thiophen-2-yl-5,7,9,10-tetrahydro-6H-11-thia-7a,12-diazabenzo[a]anthracen-8-one (14): A mixture of compound 3 (2.98 g, 0.01 mole), β-bromopropionic acid (1.52 g, 0.01 mole) and fused sodium acetate (6 g, 0.075 mole) in glacial acetic acid (30 ml) and acetic anhydride (10 mL) mixture was refluxed for 3 hrs. The reaction mixture was poured onto cold water; the solid formed was filtered off, washed with water and crystallized to give 14. Yield 78 %, m.p. 150°C (EtOH); IR: 1681 cm^{-1} corresponding to ν(C=O); ^{1}H-NMR (DMSO-d_6): 2.33 (m, 2H, CH_2), 2.80(t, 2H, CH_2), 3.35(s, 4H, 2CH_2), 6.43 (s, 1H, CH of pyrimidine), 6.96-7.67 (m, 7H, Ar-H + thiophene-H). MS *m/z* (%): 352 (100, as base peak) corresponding to molecular formulae $C_{19}H_{16}N_2OS_2$.

2-Hydrazinyl-4-(thiophen-2-yl)-5,6-dihydrobenzo [h] quinazoline (15): A mixture of compounds 14 (0.01 mole) and excess hydrazine hydrate in absolute ethanol (20 ml) was refluxed to 30 hr. After cooling the mixture was poured onto cold water and acidified with acetic acid. The solid formed was filtered off, washed with water and crystallized to give 15. Yield 55%, m.p. 100-102°C (EtOH); IR: 3285, 3170 corresponding to ν(NH, NH_2); MS *m/z* (%): 294 (M^+, 19) corresponding to the molecular formula $C_{16}H_{14}N_4S$ and base peak at 159 (100).

4,5,6,7-Tetrachloro-2-(4-(thiophen-2-yl)-5,6-dihydrobenzo [h]quinazolin-2-yl-amino)isoindoline-1,3-dione (16): A mixture of compound 15 (2.94 g, 0.01 mole) and 3,4,5,6-tetrachlorophthalic anhydride (2.85 g, 0.01 mole) in glacial acetic acid (50 ml) was heated under reflux for 6 hrs. The reaction mixture was evaporated under reduced pressure; the obtained residue was solidified with ether, filtered off and crystallized to give 16. Yield 67%, m.p. >300°C (AcOH); IR: 1749 cm^{-1} ν(C=O); MS *m/z* (%): 563 (M^++1, 5%) corresponding to the molecular formula $C_{24}H_{12}Cl_4N_4O_2S$ and base peak at 213 (100).

Biological Screening *In vitro* Cytotoxicity: The cytotoxicity of the newly synthesized tested compounds against cancer cell lines *in vitro* was performed with the MTT assay according to the Mosmann's method [24]. The MTT assay is based on the reduction of the soluble 3-(4,5-methyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into a blue-purple formazan product, mainly by mitochondrial reductase activity inside living cells. The cells used in cytotoxicity assay were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. Cells suspended in the medium (2x 104/mL) were plated in 96-well culture plates and incubated at 37 °C in a 5% CO2 incubator. After 12 h, the test sample (2 μL) was added to the cells (2Ý 104) in 96-well plates and cultured at 37°C for 3 days.

The cultured cells were mixed with 20 μL of MTT solution and incubated for 4 h at 37°C. The supernatant was carefully removed from each well and 100μL of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a microplate reader using a test wavelength of 570 nm. The results were expressed as the IC_{50}, which is the concentration of the drugs inducing a 50% inhibition of cell growth of treated cells when compared to the growth of control cells. Each experiment was performed at least 3 times. There was a good reproducibility between replicate wells with standard errors below 10%.

HIV Inhibitor Activities (Reverse Transcriptase Inhibitors) with Therapeutic Windows: Cells and viruses. The established human cells, laboratory-derived virus isolates (including drug-resistant virus isolates) and low-passage clinical virus isolates used in these evaluations have previously been described in detail [25]. These cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin (100 U/ml) and streptomycin (100 μg/ml). Fresh human cells were obtained from the American Red Cross (Baltimore, Md.).

Antiviral and Cross-resistance Assays: The inhibitory activities of the compounds against HIV were evaluated by microtiter anti-HIV assays with CEM-SS cells or fresh human peripheral blood mononuclear cells (PBMCs); these assays quantify the ability of a compound to inhibit HIV-induced cell killing or HIV replication. Quantification was performed by the tetrazolium dye XTT assay (CEM-SS, 174×CEM, MT2 and AA5 cell-based assays), which is metabolized to a colored formazan product by viable cells, RT assay (U937- and PBMC-based assays) and/or p24 enzyme-linked immunosorbent assay (monocyte-macrophage assays). Antiviral and toxicity data are reported as the quantity of drug required inhibiting virus-induced cell killing or virus production by 50% (EC_{50}).

***In Vitro* Assays of Anti-HIV Activity:** Purified RT assays Each of the newly synthesized compounds were tested for RT inhibitory activity against purified recombinant HIV-1 RT using the cell-free Quan-T-RT assay system (Amersham Corp., Arlington Heights, IL), which utilizes the scintillation proximity assay (SPA) principle, as previously described in detail [26].

In the assay, a DNA/RNA template is bound to SPA beads via a iotin/streptavidin linkage. The primer DNA is a 16-mer oligo(T), which has been annealed to a poly(rA) template. The primer-template is bound to a streptavidin-coated SPA bead. [^{3}H]TTP (thymidine-5'-triphosphate) is incorporated into the primer by reverse transcription. In brief, [^{3}H]TTP, at a final concentration of 0.5 μCi/sample, was diluted in RT assay buffer (49.5 mM Tris-HCl, pH 8.0, 80 mM KCl, 10 mM $MgCl_2$, 10mM dithiothreitol, 2.5 mM EGTA, 0.05% Nonidet P-40) and added to annealed DNA/RNA bound to SPA beads. The compound being tested was added to the reaction mixture at 0.001–100 μM concentrations. Addition of 10 mU of recombinant HIV RT and incubation at 37°C for 1 h resulted in the extension of the primer by incorporation of [^{3}H]TTP. The reaction was stopped by addition of 0.2 ml of 120 mM EDTA. The samples were counted in an open window using a Beckman LS 7600 instrument and $IC_{50[RT]}$ values (concentration at which the compound inhibits recombinant RT by 50%) were calculated by comparing the measurements to untreated sample.

Hepatitis C Virus (HCV) NS3-4A Protease Inhibitor Activities: The success of human immunodeficiency virus (HIV) protease inhibitors in treating HIV-infected patients has raised the hope that inhibitors of HCV NS3-4A serine protease could also become effective therapy options for hepatitis C patients.

Cells: Parental Huh-7 and HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS), 2 mM l-glutamine and nonessential amino acids. Stable Huh-7 cells containing the self-replicating, subgenomic HCV replicon, which was identical in sequence to the I_{377}neo/NS3-3'/wt replicon described by Lohmann *et al.* [27] were selected and maintained in the presence of 0.25 mg/ml G418 (Invitrogen, Carlsbad, CA) and were used for anti-HCV assays. Peripheral blood mononuclear cells (PBMC) were isolated from fresh donor blood and cultured in RPMI-1640 medium (JRH Biosciences).

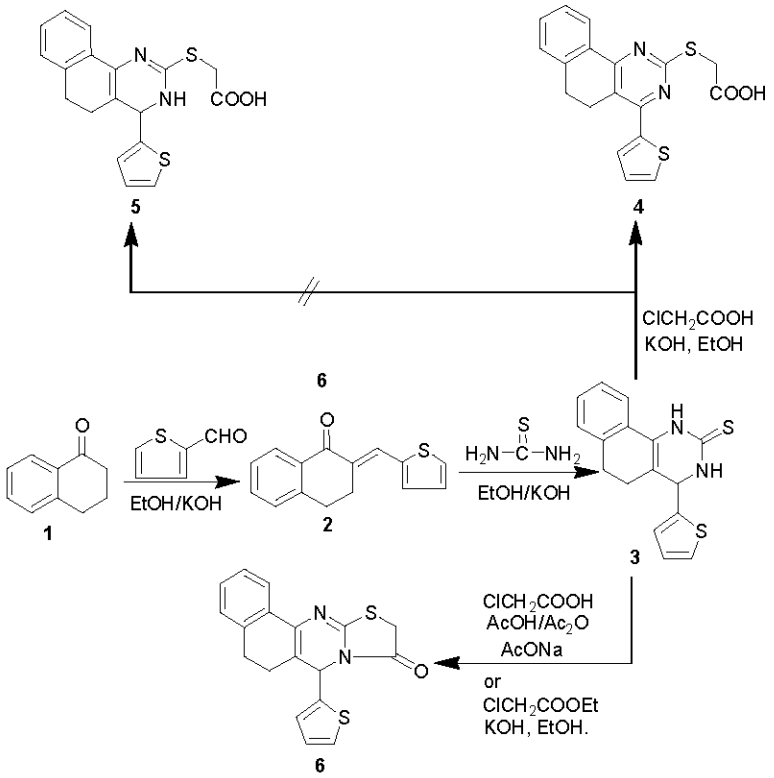
Determination of Anti-HCV Activity and Cytotoxicity: Determination of 50% inhibitory concentration (IC_{50}), 90% inhibitory concentration (IC_{90}) and 50% cytotoxic concentration (CC_{50}) of all the tested compounds in HCV replicon cells was performed as described[31]. Preclinical profile of VX-950, a potent, selective and orally bioavailable inhibitor of hepatitis C virus NS3-4A serine protease.). Briefly, 1×10^4 replicon cells per well were plated in 96-well plates. On the following day, replicon cells was incubated at 37°C for the indicated period of time with antiviral agents serially diluted in DMEM plus 2% FBS and 0.5% dimethyl sulfoxide (DMSO). Total cellular RNA was extracted using an RNeasy-96 kit (QIAGEN, Valencia, CA) and the copy number of HCV RNA was determined using a quantitative RT-PCR (QRT-PCR) assay. Combination of a hepatitis C virus NS3-NS4A protease inhibitor and alpha interferon synergistically inhibits viral RNA replication and facilitates viral RNA clearance in replicon cells. Each datum point represents the average of five replicates in cell culture. The cytotoxicity of the tested compounds was measured under the same experimental settings using a tetrazolium (MTS)-based cell viability assay. For the cytotoxicity assay with human hepatocyte cell lines, 1×10^4 parental Huh-7 cells per well or 4×10^4 HepG2 cells per well were used. To determine cytotoxicity of the tested compound against resting PBMC, 1×10^5 cells per well were incubated with the tested compound in RPMI-1640 medium (no serum) for 48 h and the cell viability was determined by the MTS-based assay. To determine cytotoxicity of f the tested compound against proliferating PBMC, 1×10^5 cells per well in RPMI-1640 medium were added to a 96-well plate, which was precoated with anti-human CD3 antibody (Accurate Chemical and Scientific Corporation, Westbury, NY). The cells were incubated with f the tested compound and anti-human CD28 antibody (Pharmingen/BD Biosciences, San Jose, CA) for 72 h at 37°C and the cell growth was determined by [^{3}H]thymidine update between the 48th and 72nd h.

RESULTS AND DISCUSSION

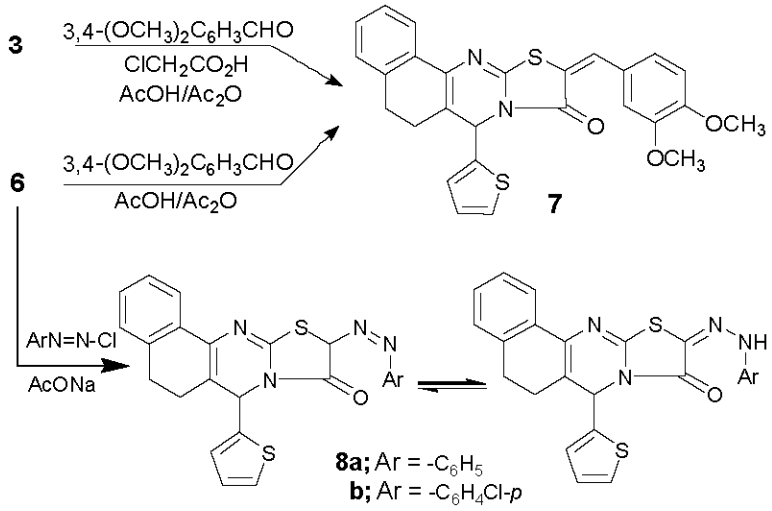
1-Tetralone (1) was reacted with 2-thiophenaldehyde in ethanolic potassium hydroxide solution to yield 2-thiophen-2-ylmethylene-3,4-dihydro-2H-naphthalen-1-one (2), which was reacted with thiourea in ethanolic potassium hydroxide solution to yield 4-thiophen-2-yl-3,4,5,6-tetrahydro-1H-benzo[h]quinazoline-2-thione (3). The alkylation of 3 with chloroacetic acid in ethanolic potassium hydroxide solution afforded unexpected product (4) rather than the target compound dihydrobenzoquinazoline 5. The aimed thiazolopyrimidine 6 was synthesized by reaction of 3 with chloroacetic acid in the presence of fused sodium acetate in a mixture of glacial acetic acid/acetic anhydride or ethyl chloroacetate in EtOH/KOH solution (Scheme 1).

The presence of active methylene group in compound (6) could be confirmed by condensation with 3,4-dimethoxybenzaldehyde in the presence of sodium acetate in a mixture of glacial acetic acid/acetic anhydride to yield arylmethylene 7, which was prepared directly from the reaction of compound 3 with 3,4-dimethoxybenzaldehyde and chloroacetic acid in the presence of fused sodium acetate in glacial acetic acid/acetic anhydride mixture. Thiazolopyrimidine 6 was condensed with aryldiazonium salts, namely, benzenediazonium chloride or *p*-chlorobenzene diazonium chloride in the presence of sodium acetate to give the corresponding diazacyclopenta[b]-phenanthren-8-one derivatives (8a,b), respectively (Scheme 2).

Thus when 2-thiopyrimidine 3 was reacted with α-bromopropionic acid or β-bromopropionic acid in ethanolic potassium hydroxide solution afforded the unexpected oxidizing products 9 and 12, respectively,

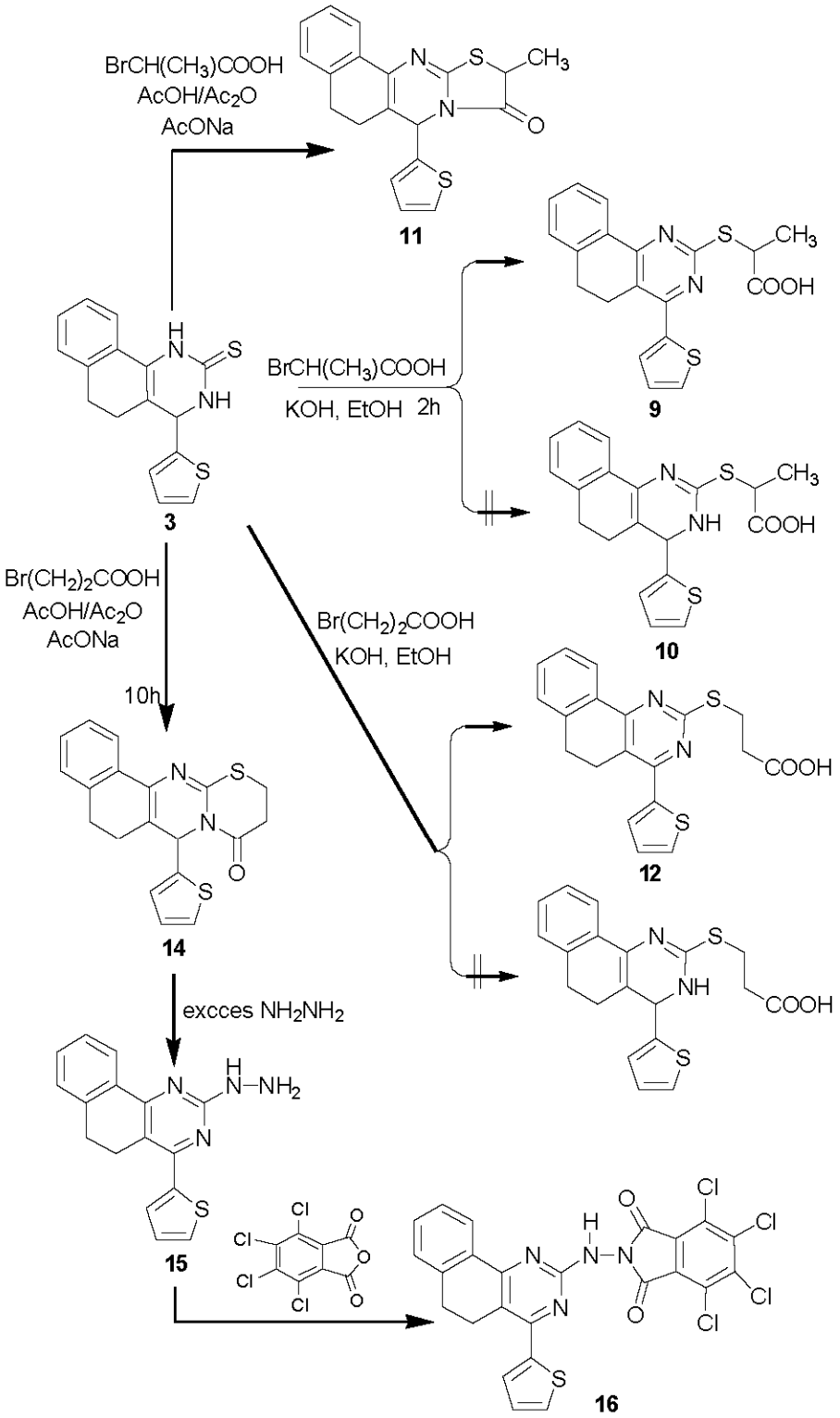


Scheme 1:



Scheme 2:

rather than the aimed products 10 and 13. The aimed product 11 and 14 were synthesized by refluxing compound 3 with the same reagents in a mixture of glacial acetic acid/ acetic anhydride in the presence of fused sodium acetate. Also, the compound 14 was reacted with hydrazine hydrate to give the corresponding hydrazino derivative 15, according to previous reported methods [28, 29]. The hydrazino compound 15 was condensed with 3,4,5,6-tetrachloro-phthalic anhydride to yield 4,5,6,7-tetrachloro-2-(4-thiophen-2-yl-3,4,5,6-tetrahydrobenzo[h] quinazolin-2-ylamino)iso-indole-1,3-dione (16) (Scheme 3).


BrCH(CH3)COOH
AcOH/Ac2O
AcONa
11
BrCH(CH3)COOH
KOH, EtOH 2h
9
10
3
Br(CH2)2COOH
AcOH/Ac2O
AcONa
10h
Br(CH2)2COOH
KOH, EtOH
12
14
excces NH2NH2
15
16

Scheme 3:

Table 1: The activity of the tested compounds on some cancer cell lines

	IC50							
	Leukaemia			Melanoma		Neuroblast		Normal cell Carcinoma
Product No.	HL60	U937	K562	G361	SK-MEL-28	GOTO	NB-1	W138
2	5.4 E-03	4.4 E-03	1.4 E-03	1.5 E-03	5.2 E-03	7.5 E-03	4.7 E-03	6.4 E-03
3	3.5 E-03	6.4 E-03	6.4 E-03	6.8 E-03	7.9 E-03	9.7 E-03	9.7 E-03	9.7 E-03
4	8.3 E-03	8.7 E-03	7.2 E-03	8.8 E-03	7.7 E-03	6.6 E-03	8.5 E-03	9.8 E-03
6	9.9 E-03	4.5 E-03	7.7 E-03	8.8 E-03	4.5 E-03	6.2 E-03	6.7 E-03	8.8 E-03
7	7.7 E-03	7.8 E-03	6.5 E-03	6.5 E-03	7.6 E-03	4.5 E-03	7.6 E-03	8.7 E-03
8a	7.3 E-03	7.7 E-03	8.6 E-03	8.8 E-03	9.7 E-03	9.0 E-03	5.7 E-03	7.5 E-03
9	5.6 E-03	7.7 E-03	8.6 E-03	8.7 E-03	8.0 E-03	9.0 E-03	8. 0 E-03	9.0 E-03
11	1.3 E-03	7.1 E-03	2.4 E-03	2.4 E-03	4.2 E-03	8.6 E-03	6.8 E-03	8.5 E-03
12	-	-	-	-	-	-	-	-
14	2.6 E-03	7.3 E-03	1.5 E-03	8.4 E-03	4.8 E-03	4.8 E-03	4.6 E-03	6.9 E-03
15	6.6 E-03	7.7 E-03	6.3 E-03	6.4 E-03	6.5 E-03	5.6 E-03	7.6 E-03	8.9 E-03

From Table 1:

- All the tested compounds except compound 12 showed regarding activity against Leukemia HL60. The most active compound was 11.
- All the tested compounds except compound 12 showed regarding activity against Leukemia U937. The most active compound was 2.
- All the tested compounds except compound 12 showed regarding activity against Leukemia K562. The most active compound was 2.
- All the tested compounds except compound 12 showed regarding activity against Melanoma G361. The most active compound was 2.
- All the tested compounds except compound 12 showed regarding activity against Melanoma SK-MEL-28. The most active compound was 11 and 15.
- All the tested compounds except compound 12 showed regarding activity against Neuroblast GOTO. The most active compound was 7.
- All the tested compounds except compound 12 showed regarding activity against Neuroblast NB-1. The most active compound was 14.
- All the tested compounds except compound 12 showed regarding activity against normal cell carcinoma W138.

Table 2: Cytotoxicity of some new compounds on some new cancer cell lines

	IC50				
	cervical carcinoma	ovarial carcinoma	CNS cancer	non-small-cell lung cancer	colon adeno-carcinoma
Product No.	KB	SK OV-3	SF-268	NCl H460	RKOP27
2	4.9 E-03	5.9 E-03	3.0 E-03	2.4 E-03	3.3 E-03
3	5.6 E-03	5.4 E-03	6.7 E-03	7.6 E-03	9.8 E-03
4	3.9 E-03	4.7 E-03	5.8 E-03	5.9 E-03	5.0 E-03
6	3.9 E-03	3.8 E-03	4.9 E-03	6.8 E-03	9.8 E-03
7	8.5 E-03	7.0 E-03	9.0 E-03	6.9 E-03	7.4 E-03
8a	9.8 E-03	5.6 E-03	7.6 E-03	6.5 E-03	7.6 E-03
9	4.8 E-03	5.9 E-03	4.0 E-03	4.0 E-03	9.8 E-03
11	3.8 E-03	7.8 E-03	4.8 E-03	9.8 E-03	9.0 E-03
12	9.0 E-03	7.0 E-03	4.0 E-03	4.0 E-03	4.8 E-03
15	6.7 E-03	7.0 E-03	5.8 E-03	4.7 E-03	8.5 E-03

From Table 2:

- All the tested compounds except compound 2 showed regarding activity against cervical carcinoma. The most active compounds were 11 and 4.
- All the tested compounds except compound 2 showed regarding activity against CNS cancer. The most active compounds was 6, 11 and 9.
- All the tested compounds except compound 2 showed regarding activity against colon adenocarcinoma.
- All the tested compounds except compound 2 showed regarding activity against ovarial carcinoma. The most active compound was 6.
- All the tested compounds showed regarding activity against non-small-cell lung cancer. The most active compound was 2.

Table 3: HIV Inhibitor activities (reverse transcriptase inhibitors) with Therapeutic windows

Product No.	EC50/Mm	IC50/µM	Therapeutic index
2	0.00525	6.1	33000
3	0.00722	2.6	32122
4	0,00084	17.4	784,532
6	$10^{\prime 6}$	14.1	5.16×10^{7}
7	$10^{\prime 5}$	2.2	6.44×10^{6}
8a	0.00298	8.8	99897
9	0,00011	5.1	229 600
11	$10^{\prime 6}$	13.2	134,567
12	$4.9 \times 10^{\prime 4}$	3.2	7.81×10^{5}
15	0.00034	5.3	553.333

From Table 3: Regarding the anti-HIV activities all the tested compounds showed potent activities the most active compound was 6.

Table 4: Hepatitis C Virus (HCV) NS3-4A Protease Inhibitor Activities

Product	IC 50 (±SD)μM After 48 Hours	IC 90 (±SD)μM After 48 Hours	CC50 (±SD)μM	Window Index
2	0.318±0.042	2.78±0.077	2738±8.5	2201.450
4	0.188±0.022	0.501±0.033	516±4.5	2723.450
6	0.196±0.023	0.516±0.031	531±3.4	2623.660
7	0.235±0.026	0.717±0.043	689±6.5	2589.857
9	0.320±0.041	2.95±0.088	2840±4.6	2100.230
11	0.155±0.021	0.422±0.025	238±2.3	2743.450
15	0.267±0.034	1.34±0.054	987±5.4	2445.340

From Table 4: Regarding the anti-HCV activities all the tested compounds showed potent activities the most active compounds were 11, 4 and 6.

Biological Activity: Some of the new synthesized compounds were tested for their cytotoxicity on the different cancer cell lines and virus. The obtained results were summarized in the pervious Tables (1-4).

REFERENCES

1. Zigeuner, G., W.B. Lintschinger, A. Fuchsgruber and K. Kollmann, 1976. Monatsh. Chem., 107: 171.
2. Mathes, R.A. and F.D. Stewart, 1950. J. Am. Chem. Soc., 72: 1879.
3. Khodairy, A. and H. Abdel-Ghany, 2000. Phosphorus, Sulfur, Silicon and Related Elements, 162: 259.
4. Khodairy, A., 2003. Phosphorus, Sulfur, Silicon and Related Elements, 178: 893.
5. Abass, M., 2003. Phosphorus, Sulfur, Silicon and Related Elements, 178: 1413.
6. Khatoon, S. and K. Yadav, 2004. Phosphorus, Sulfur, Silicon and Related Elements, 179: 345
7. El-Sharief, A.M., J.A.A. Sh., N.A. Micky, M.M. Shmeiss and G. El-Gharieb, 2003. Phosphorus, Sulfur, Silicon and Related Elements, 178: 439.
8. Moustafa, H.M., A. Khodairy and A.M.M. El-Saghier, 2003. Phosphorus, Sulfur, Silicon and Related Elements, 178: 1211.
9. Bakhite, E.A., A. Abdel-Rahman and E.A. Al-Taifi, 2004. Phosphorus, Sulfur, Silicon and Related Elements, 179: 513.
10. Pathak, U.S., M.B. Devani, C.J. Shishoo, R.R. Kulkarni, V.M. Rakholia, V.S. Bhadti, S. Ananthan, M.G. Dave and V.A. Shah, 1986. Ind. J. Chem. B., 25B: 489.
11. Swelam, S.A., O.S. Abd El-Salam and M.E.A. Zaki, 1999. J. Serb. Chem. Soc., 64: 655.
12. El-Gazzar, A.B.A., M.I. Hegab, S.A. Swelam and A.S. Aly, 2002. Phosphorus, Sulfur, Silicon and Related Elements, 177: 123.
13. Amr, A.E., M.I. Hegab, A.A. Ibrahiem and M.M. Abdulla, 2003. Monatsh. Chem., 134: 1395.
14. Amr, A.E., H.H. Sayed and M.M. Abdulla, 2005. Arch. Pharm. Chem. Life Sci., 338: 433.
15. Hassan, S.S.M., M.H. Abou-Ghalia, A.E. Amr and A.H.K. Mohamed, 2003. Talanta, 60: 81.
16. Hassan, S.S.M., M.H. Abou-Ghalia, A.E. Amr and A.H.K. Mohamed, 2003. Anal. Chem. Acta., 482: 9.
17. Abd El-Latif, N.A., E. Amr and A.A. Ibrahiem, 2007. Monatsh. Chem., 138: 559.
18. Ouf, N.H., A.E. Amr and A.A. Fayed, 2008. Monatsh. Chem., 139: 281.
19. Shalaby, A.F.A., M.M. Abdulla and A.E. Amr, 2007. Monatsh. Chem., 138: 1019.
20. Amr, A.E., A.M. Mohamed, S.F. Mohamed, N.A. Abdel-Hafez and A.G. Hammam, 2006. Bioorg. Med. Chem., 14: 5481.
21. Amr, A.E., 2000. Ind. J. Heterocycl. Chem., 10: 49.
22. Ouf, N.H. and A.E. Amr, 2008. Monatsh. Chem., 139: 579.
23. Amr, A.E., N.M. Sabry and M.M. Abdulla, 2007. Monatsh. Chem., 138: 699.
24. Buckheit, R., W. Jr., T.L. Kinjerski, V. Fliakas-Boltz, J.D. Russell, T.L. Stup, L.A. Pallansch, W.G. Brouwer, D.C. Dao, W.A. Harrison, R.J. Schultz, J.P. Bader and S.S. Yang, 1995. Antimicrob. Agents Chemother, 39: 2718.
25. a) Bosworth, N. and P. Towers, 1989. Nature, 341: 167; b) Zarling, J.M., P.A. Moran, O. Haffar, M. Diegel, D. E. Myers, V. Kuelbeck, J.A. Ledbetter and F.M. Uckun, 1991. Int. J. Immunopharmacol., 13: 163.
26. Lohmann, V., F. Korner, J. Koch, U. Herian, L. Theilmann and R. Bartenschlager, 1999. Science 285: 110.
27. Lin, K., A.D. Kwong and C. Lin, 2004. Antimicrob. Agents Chemother, 48: 4784.
28. Salama, M.A. and L.A. Almotabacani, 2004. Phosphorus, Sulfur, Silicon and Related Elements. 179: 305.
29. El-Shahat, M., 2007 M. Sc. Thesis, Zagazig university, Faculty of Science, Chem. Department.