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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF NEW INDOLOQUINOLINE – BIOTIN HYBRIDS

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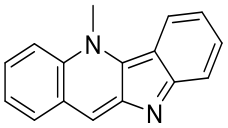
ABSTRACT

A series of indoloquinoline- biotin hybrids conjugates **7a-f** were designed and synthesized in good yields to improve and develop higher potency and selective anti-tumor agents. Their antiproliferative evaluation activities against HePG2, HCT-116 and MCF-7 cell lines in vitro were tested using colorimetric MTT assay. These neocryptolepines (2-substituted-5-methyl-indolo[2,3-b]quinolones) were synthesized via nucleophilic aromatic substitution (S_{NAr}) of the Cl leaving group of 11-chloroneocryptolepines with diamines which further hybrids by reacting the 11-(aminoalkylamino) derivatives with biotin. The result of the present work indicated also that 7e, 7f were good potent compared with doxorubicin and ultimately would be appreciated as potential anticancer agents.

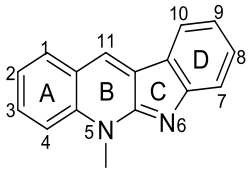
KEYWORDS: Chloroneocryptolepine, Biotin, aminoalkylamino-substituted, antiproliferative activity, doxorubicin.

INTRODUCTION

Indoloquinolines, tetracyclic ring systems, which establish significant structural motifs in naturally occurring products are demonstrating diverse and multiple biological activities.[1-3] For instance, the two major alkaloids that are isolated from the roots of western African plant called Cryptolepis sanguinolenta are chemically identified as cryptolepine(**I**, indolo[3,2-b]quinoline) and neocryptolepine(**II**, indolo[2,3-b]quinoline) as shown below.[4-7] These alkaloids that only differ in the specific orientation of indole ring with respect to quinoline moiety, are exhibiting strong antiparasitic activities.[5,7-9] Particularly in traditional medicine, an aqueous marinade of the roots of Cryptolepis sanguinolenta is used to treat malaria.[10] The high antimalarial cytotoxicity could be explained with regard to the linearly organized planar structure of cryptolepine (I) and neocryptolepine (II) which enhances their intercalation within DNA and eventually inhibits topoisomerase II.[11-15]



Cryptolepine (I)



Neocryptolepine (II)

Our previous structure-activity relationship (SAR) study of the antiproliferative activity of the 5-methyl-indolo[2,3-b]quinoline derivatives showed that derivatization of the later with aminoalkylamino at C11 position is an essential element for enhancing the bioactivity. For instance, the 3-aminopropylamino group increased the antiproliferative activity against the human leukemia MV4-11 cell line approximately 20 times compared with that the of the 11-chloro precursor.[16-20] On the other hand in the last two decades several efforts have been made to overcome of high toxicity and poor selectivity of treatment of cancer by chemotherapy. Among the different approaches, the so called vitamin - mediated drug targeting has recently emerged as a novel and valuable strategy. Indeed, the linkage of cytotoxic drugs to selected vitamins, leading to vitamin-drug conjugates, would result in specifically delivering great amounts of the targeted drug at high doses to cancer cells. Among vitamins, biotin seems to be the most promising

targeting agent.[21] Previous study showed that the synthesized biotin-doxorubicin hybrid, in which the free amine group of doxorubicin was derivatized with a photocleavable biotinylated spacer. They found that the active drug is released only after the internalization of the hybrid into cancer cells and the subsequent activation of the photocleavable group *via* exposure to UV at 350 nm, thus reducing the cytotoxic effects on normal cells.[22]

Based on our previously published findings, herein we are considering whether the potency of biotin analogues against cancer cell lines could be improved their bioavailability to obtain better activity by linking to the neocryptolepine moiety. Thus, the goal of this work, a series of biotin-indoloquinoline hybrids **7a**–**f** were designed and synthesized. Their potencies as an antitumor agent were evaluated by antiproliferative screening.

EXPERIMENTAL SECTION

General Methods

All ^{1}HNMR experiments (solvent DMSO-d6) were carried out with a 400 MHz varian and Bruker Avance at the main chemical warfare laboratories, Egypt. Chemical shifts are reported in part per million (ppm) relative to the respective solvent or tetramethylsilane (TMS). The mass spectroscopy experiments were recorded on thermos scientific trace 1310 gas chromatograph at Fungi National Centre, Al- Azhar University and IR spectroscopy & Melting points (m.p) were performed at Cairo University, Egypt. The biological activity analysis was carried out The biological activity analysis was carried out at central laboratory, Faculty of Pharmacy, Mansoura University, Egypt., 11-chloroneocryptolpine was synthesized as in literature.[19]

All reactions were followed by thin layer chromatography (TLC) on kiesel gel F254 precoated plates (Merck).

General procedure for the synthesis of 11-aminoneocryptolepines 6 derivatives

11-Chloroindoloquinolines 1 (0.3 mmol)and an excess of the appropriate aminoalkylamine (3.0 mmol) were heated together at 120 °C for 4 h. Thin Layer Chromatography (TLC)monitoring was used to ensure the completion of the reaction. The resulting brown crude oil was purified by flash chromatography using AcOEt/2M ammonia in MeOH(9:1,v/v) as an eluent to yield pure yellowish solid products.

General Procedure for the Synthesis of biotin-Indoloquinoline Hybrids 7a-f

biotin **3** (102 mg), EDCI (39 mg) and HOBt (27.6 mg) were dissolved in CH_2Cl_2 with stirring for 1 h, then appropriate substituted 5-methyl-5H-indolo[2,3-b]quinoline was added with stirring together at room temperature for 6 h. TLC checked the completion of the reaction. The reaction mixture was washed by brine, dried over anhydrous MgSO4. After concentrated under vacuum, the crude products were purified by flash chromatography using AcOEt-MeOH (1:10 V/V) as the eluent to yield pure 3a-f as solids.

N-(4-(4-((5-methyl-5,11a-dihydro-4aH-indolo[2,3-b]quinolin-11-yl)amino)benzyl)phenyl)-5-biotin hybrid (7a)

Yield (65%) olive solid, m.p. 174°C –177°C. IR (KBr) cm^{-1}: 3298(NH), 2919(-CH sym), 1616-1701(C=O), 1261(CH_3).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.31 m (2H, CH_2), 1.45 br. m (2H, CH_2), 1.58 br. m (2H, CH_2), 2.18 br. t (*J* = 16 Hz, 2H, CH_2) 2.79 m(2H, CH_2), 3.72 m(2H, CH_2), 3.86 d (*J* = 17.6 Hz, CH), 4.06 s (3H, CH_3), 4.11 d (*J* = 7.4 Hz, CH), 4.28 d (*J* = 7.4 Hz, CH), 6.33 d (*J* = 12 Hz, 2H, Ar-H) 6.46 d (*J* = 8 Hz, 2H, Ar-H), 6.78-6.91 m, (5H, Ar-H), 6.08-796 m (7H, Ar-H) 9.97 s (NH) 10.66 s (NH). MS, m/z calcd for $C_{39}H_{38}N_6O_2S$ [M + H]+. Calculated: 654.82, found: 654.88.

N-(4-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)cyclohexa-1,3-dien-1-yl)-5-biotin hybrid (7b)

Yield (65%) brown solids, m.p.132°C –134°C. IR (KBr) cm^{-1}: 3325(NH), 1627,1701(C=O), 1261(CH_3).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.03 m (2H, CH_2), 1.14 br. m(4H, CH_2), 1.68 t (*J* = 4 Hz, 2H, CH_2), 2.29 m (2H, CH_2), 3.o8 br. m (1H, CH), 4.06 s (3H, CH_3), 4.11 d (*J* = 7.4 Hz, CH), 4.28 d (*J* = 7.4 Hz, CH), 5.54 d (*J* = 6.4 Hz, 2NH) 6.33-6.39 m (3H, Ar-H) 7.16 d (*J* = 1.6 Hz, 2H, Ar-H), 7.45-7.47 m (7H, Ar-H). MS, m/z calcd for $C_{32}H_{32}N_6O_2S$ [M + H]+. Calculated: 564.70, found: 564.56.

2-(methyl(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)ethyl 5- biotin hybrid (7c)

Yield (84%) brown solids, m.p.101°C –134°C. IR (KBr) cm^{-1}: 3309,3360 (NH), 1689,1612(C=O), 1269(CH3).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.28-1.40 br. m(2H, CH_2), 1.44-1.59 br. m (4H, CH_2), 2.17 t (*J* = 9.6 Hz, 2H, CH_2), 2.51-2.84 m(6H, CH_2), 3.04- 3.63 br. m(6H, CH_3), 3.80 t (*J* = 6.4 Hz, 2H, CH), 4.11 ddd (*J* = 4.8, 4,7.2 Hz,2CH,) 6.54 d (*J* = 6.4 Hz, 2NH) 7.28-7.45 m, (3H, Ar-H) 7.58-8.03 m (5H, Ar-H). MS, m/z calcd for $C_{29}H_{33}N_5O_3S$ [M + H]$^+$. Calculated: 530.68, found: 531.19.

2-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)ethyl 5- biotin hybrid (7d)

Yield (84%) white solids, m.p.80°C–83°C. IR (KBr) cm^{-1}: 3309,3360 (NH), 1687,1689(C=O), 1269(CH_3).^{1}H NMR (DMSO-d6, 400 MHz) ppm: Mixture of diastereomers: 1.01-1.05 m (2H, CH_2), 1.28-1.59 br. m (4H,CH_2), 2.18 t (*J* = 7.2 Hz, 2H, CH_2), 2.54-2.82 ddd (*J* = 12.4, 4.8, 12.4 Hz, 4H, CH_2), 3.55 d (*J* = 4.8 Hz, 1H, CH), 4.12 s (CH_3), 4.28 t (*J* = 7.2 Hz, 4H, CH_2),4.74 m (6H, CH_2) 6.32 d (*J* = 2.8 Hz,2H,CH) 7.36 s (1H, Ar-H) 7.48-7.51 t (*J* = 7.3 Hz, 1H, Ar-H) 7.67 m (3H, Ar-H), 7.94 m (3H, Ar-H) MS, m/z calcd for $C_{32}H_{31}N_5O_3S$ [M + H]+. Calculated: 604.76, found: 605.17.

N-(2-((2-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)ethyl)amino)ethyl)-5- biotin hybrid (7e)

Yield (78%) brown solids, m.p.127°C –129°C. IR (KBr) cm-1: 3309,3360 (NH), 1685,1627(C=O), 1319(CH_3).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereoers: 1.30-1.43(m, 2H, CH_2), 1.47-1.60 (br. m, 4H,CH_2), 2.16(t, *J* = 7.2 Hz, 2H, CH_2), 2.18-2.57(m, 4H, CH_2), 2.78- 2.82(m,2H, CH_2), 3.05- 3.15 (m, 2H, CH_2), 3.39- 3.53 (m, 1H,CH) 3.78 (m,3H, CH_3) 4.11 (t, *J* = 4.2 Hz,6H, CH_2) 4.28 (t, *J* = 7.3 Hz, 2H, CH) 6.40 (d, *J* = 6.4 Hz, 2NH) 7.2 (d, *J* = 4.2 Hz,1H Ar-H), 7.40 (m, 3H, Ar-H) 7.80-7.89 (m, 4H, Ar-H) MS, m/z calcd for $C_{32}H_{42}N_8O_2S$ [M + H]+. Calculated: 602.79, found: 602.45

N-(3-(4-(3-((5-methyl-5H-indolo[2,3-b]quinolin-11-yl)amino)propyl)piperazin-1-yl)propyl)-5-biotin hybrid (7f)

Yield (78%) white solids, m.p.150°C –153°C. IR (KBr) cm^{-1}: 3421,3402,3309 (NH), 1620,1689(C=O), 1265(CH_3).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereoers: 1.01-1.04(m, 2H, CH_2), 1.29-1.43 (br. m, 4H,CH_2), 1.44-1.61 (br. m, 4H,CH_2), 2.16(t, *J* = 4.2 Hz, 4H, CH_2), 2.54 (d, *J* = 12.8 Hz,8H, CH_2 Piperazine), 3.58(d, *J* = 4.8 Hz, 4H, CH_2), 3.78(d, *J* = 4.8 Hz, 1H, CH)3.98 (s, CH_3), 4.09- 4.14 (m, 4H,CH_2) 4.11 (q, *J* = 4.8 Hz,2H, CH) 6.32 (d, *J* = 4 Hz, 2NH) 7.28-7.40 (m, 3H, Ar-H), 7.60 (d, *J* = 8.4 Hz,2H, Ar-H) 7.83 (d, *J* = 8.4 Hz, 3H, Ar-H). MS, m/z calcd for $C_{36}H_{48}N_8O_2S$ [M + H]+. Calculated: 656.36, found: 556.41.

Cytotoxicity assay

Cell line

Hepatocellular carcinoma (HEPG-2), Mammary gland (MCF-7) and Colorectal carcinoma (HCT-116). The cell lineswere obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. Doxorubicin was used as a standard anticancer drug for comparison.

Chemical reagents

The reagents RPMI-1640 medium, MTT. and DMSO (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK).

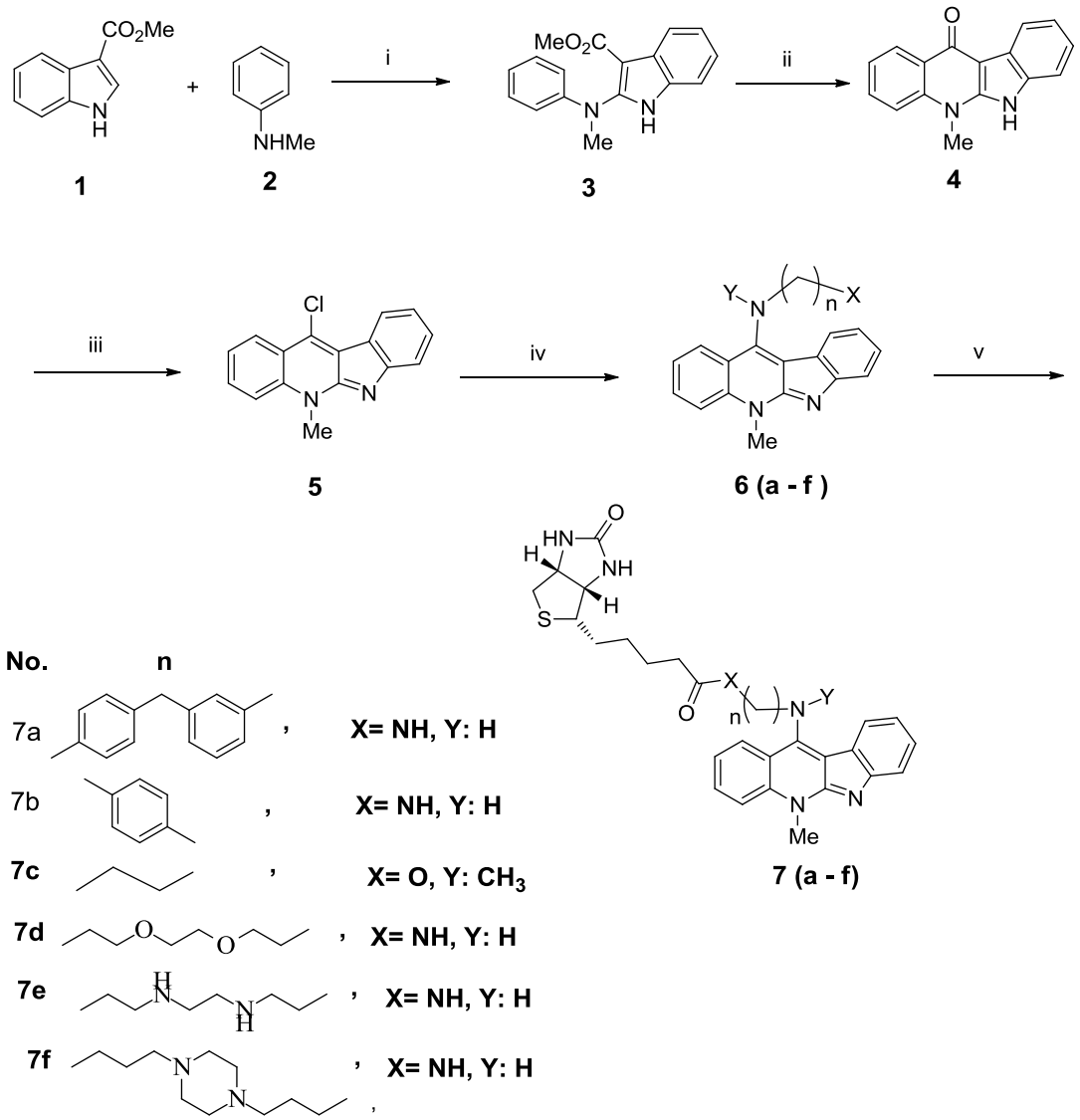
MTT assay

The cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell line were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37 C in a 5% Co2 incubator. The cell lines were seeds in a 96-well plate at a density of 1.0x104 cells/ well at 37 ^{o}C for 48 h under 5% Co2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800, USA). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.[24-25]

RESULTS AND DISCUSSION

Chemistry

The construction of neocryptolepine conjugates is outlined in Scheme 1 below. The starting key compounds **(5)** were prepared according to previous procedures in three steps. Accordingly, methyl-1*H*-indole-3-carboxylates was chlorinated in the *N*-chlorosuccinimide followed by the coupling with *N*-methylaniline in the presence of 1,4-dimethylpiperazine. Cyclization of the coupling product was affected by heating at 200 ^{o}C in diphenyl ether. The third step was the chlorination of the quinolone ring with $POCl_3$.[19] The synthetic strategy for the synthesis 5-methyl-5*H*-indolo[2,3-*b*]quinolines **(6)** was built on the nucleophilic aromatic substitution (S_NAr) reaction of 11-chloro-5-methyl-5*H*-indolo[2,3-b]quinoline (**5**) with an appropriate amine (Scheme 1).[16] However, amination of (**5**) was achieved using an excess amount of diamines in hot DMF furnished the anticipated 11-amino compounds **(6)** smoothly. Subsequently, The carboxylic acid group of the biotin underwent condensation with the free terminal amino or hydroxyl group of aminoalkylamino-indoloquinoline intermediates in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCl) and 1-hydroxybenzotriazole (HOBt) at room temperature to form satisfactory yields via target compounds **7a-f**. After the appropriate purification, these hybrids were confirmed by spectroscopic tools and evaluated by the biological activity screening. The NMR and spectroscopic tools results for all products are in good agreement with the chemical structure of the compounds.



Scheme 1: Reagents and conditions: (i) a, N-Chlorosuccinimide, 1,4-dimethylpiperazine, CH_2Cl_2, ^oC, 2 h; b,trichloroacetic acid, RT, 2 h; (ii) diphenyl ether, reflux, 1-3 h; (iii) $POCl_3$, toluene, reflux. 6-12 h; (iv) appropriate amine, DMF, 135 ^oC.

Biological evaluation

***In vitro* antiproliferative activity**

In the last few decades, human cancer cell lines have aggregated an accessible, easily usable set of biological models to examine cancer biology [25]. The synthesized Indoloquinoline – Biotin Hybrids **(7a-f)** were tested by antiproliferative activity against the breast (MCF-7), colon (HCT-116) and hepatocellular (HepG2) carcinoma cell line using MTT colorimetric assay. For comparison, doxorubicin (DOX) was used as a reference anticancer drug. Dimethyl Sulfoxide (DMSO) used as a control for the cancer cells. IC_{50}-concentration of tested compounds leading to 50% inhibition of cell proliferation was obtained in a standard 24h MTT assay. Results from three separate experiments were recorded and the percentage of viable cells was calculated as percent of cell viability by the following formula % cell viability = (Mean absorbance in test wells / Mean absorbance in control wells) X 100. The key results obtained for compounds **7a-f** toward the three cell lines are shown in **Table 1**.

The cell viability was observed following 24h of exposure to all compounds at doses of 0.01, 0.1, 1, 10 and 100 µM of compounds. The results revealed that most of the tested compounds showed a strong to moderate activity (Table 1) Therefore, various kinds of amino groups were introduced to assess their antiproliferative activity and varying substituent R_1at C11.Compound **7b** with phenylenediamine at C**11** turned out to be the most powerful antiproliferative activities active hybrids against a human hepatocellular (HepG2), colon (HCT-116) and breast (MCF-7) cancer cell lines with IC_{50} value of 9.10 µM, 8.29 µM and 7.97 µM respectively. Besides, **7e** and **7c** possessed strong antiproliferative activities against three cell lines. On the other hand, **7d** and **7f** showed weak antiproliferative activities against the tested cell lines.

Table 1: Antiproliferative activity of of the biotin-indoloquinoline-hybrids against human tumor cells.

Compounds	In vitro Cytotoxicity IC_{50} (μM)•		
	HePG2	HCT-116	MCF-7
DOX	4.50±0.2	5.23±0.3	4.17±0.2
7a	41.24±2.8	28.14±2.0	36.84±2.6
7b	9.10±1.0	8.29±0.8	7.97±0.9
7c	20.15±1.7	23.47±1.9	25.15±1.8
7d	61.26±3.4	36.22±2.3	69.90±3.9
7e	14.00±1.3	16.20±1.5	12.99±1.3
7f	65.70±3.7	78.81±4.2	53.55±3.1

• IC50 (μM): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic).
• DOX: Doxorubicin.

CONCLUSIONS

A series of neocryptolpine-biotin hybrid including compounds **7a-f** was synthesized which was characterized by the functionalization of C-11with branched aminoalkylamino chains of different linker lengths between the two nitrogen atoms. The antiproliferative activities were evaluated by in vitro against a human breast (MCF-7), colon (HCT-116) and hepatocellular (HepG2) cancer cell lines. All compounds in this series was provide that the introduction of biotin over the 11-chloro-substituted precursors with different linker improved the antiproliferative activity and selectivity towards cancer cell lines.Results have shown that the most promising active hybrid **7b** which showed IC_{50} of 9.10 μM, 8.29 μM and 7.97 μM respectively against hepatocellular carcinoma, colon and breast respectively.Further studies on the modification of Nocryptolpine hybrid and use different active cores are still ongoing.

Abbreviations

EDCI 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride HOBt 1-hydroxybenzotriazole.

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