Journal of Applied Pharmaceutical Science Vol. 7 (10), pp. 009-015, October, 2017
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2017.71002
ISSN 2231-3354

Synthesis and Antiproliferative Activity of Novel Neocryptolepine-Hydrazides Hybrids

Ahmed Abdel Hamid Sebeka[1], Amany M. A. Osman[1], Ibrahim El-Tantawy El Sayed[1*], Mohamed El Bahanasawy[1], Mohamed A. Tantawy[2]

[1]Chemistry Department, Faculty of Science, Menofia University, Shebin El-Koam, Menofia, Egypt.
[2]Medical Research Division, National Research Centre, Dokki, Giza, Egypt.

ARTICLE INFO

Article history:
Received on: 27/05/2017
Accepted on: 08/07/2017
Available online: 30/10/2017

Key words:
Synthesis, Neocryptolepine, Hydrazides, Anticancer activity.

ABSTRACT

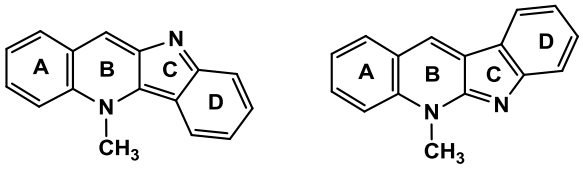
By combining the structural features of neocryptolepine and hydrazides, novel hybrid compounds **8a-d** and **9a-j** were synthesized using a simple and convenient method. Evaluation of these compounds against human hepatocellular carcinoma ($HepG_2$) cell line using MTT assay was performed. Compound **8c** was identified as the most active anticancer agent as it showed effectiveness at low concentration with IC_{50} 2.51 μM on the tested cell line when compared with reference drug 5-FU. It is worth to note that all tested compounds showed antiproliferative activity at very lower micro molar concentrated when compared with the reference drug except compounds **9a**, **9e**, and **9j** which were less active than 5-FU. The anticancer screening results of the tested compounds provide an encouraging framework that could lead to the development of potent new anticancer agents based on neocryptolepine core structure.

INTRODUCTION

Nitrogen-containing polycyclic structures based on privileged templates have drawn significant interest owing to their ability to imitate the natural product chemical probes that play an important role for the drug discovery process (Butler, 2004). In this context, the tetracyclic heteroaromatic compounds, cryptolepine **1** and neocryptolepine **2** (cf. Fig. 1) which are naturally occurred as indoloquinoline alkaloids isolated from the shrub *Cryptolepis sanguinolenta*, that is growing in west Africa, used for treatment of endemic disease such as malaria fever and various disorder of the body are important due to their wide spectrum of biological properties (Cimanga *et al.*, 1996; El Sayed *et al.*, 2009; 2011; 2012; Jonckers *et al.*, 2002; Lavrado *et al.*, 2010; Lu *et al.*, 2013; Prakash *et al.*, 2011; Mei *et al.*, 2013; Wang *et al.*, 2013, Wang *et al.*, 2012; Xu and Pieters 2013; Emam *et al.*, 2015; Okada *et al.*, 2016; Larghi *et al.*, 2015; Peng *et al.*, 2012) However, a direct comparison of the cytotoxicity of cryptolepine and neocryptolepine demonstrated that the latter was much less cytotoxic and high affinity towards DNA. (Bailly *et al.*, 2000; Guittat *et al.*, 2003).

In addition we aim to improve the biological activity of the parent compound **2** by substituting it with different side chains. As part of our ongoing program for developing anticancer agents based on a natural product scaffolds herein, we synthetized novel analogues of neocryptolepine compounds bearing sulfonyl- and carbonyl hydrazide moieties and evaluated their anticancer activity against the most common cancer in Egypt hepatocellular carcinoma (Ibrahim *et al.*, 2014).

**Corresponding Author*
Ibrahim El Tantawy E Sayed
Chemistry Department, Faculty of Science, Menofia University, Shebin El-Koam, Menofia, Egypt. Phone: +201096726008, Fax: +20482235689
E-mail: ibrahimtantawy @ yahoo.co.uk



1 **2**

cryptolepine **neocryptolepine**

Fig 1: Indoloquinolines from *Cryptolepis sanguinolenta*.

MATERIALS AND METHODS

General methods

All ^{1}HNMR experiments (solvent DMSO-d_6) were carried out with a 400MHz 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 at Medical Research Division, National Research Center, Cairo, Egypt. All reactions were followed by thin layer chromatography (TLC) on kiesel gel F254 precoated plates (Merck). Starting materials sulfonyl and carbonyl hydrazides were synthesized and purified according to literature procedures (Goyal *et al.,* 2016; Brandes, *et al.,* 2005; Bader *et al.,* 2008; Kumaraswamy *et al.,* 2008; Surendiran *et al.,* 2012; xie, *et al.,* 2012). Moreover, the compounds **3** and **4** were commercially available and purchased from Sigma-Aldrich. The required intermediates **5**, **6** and **7** were prepared by adopting the earlier reported procedures (Wang *et al.,* 2014).

Synthesis

Synthesis of Methyl 2-(methyl(phenyl) amino)-1H-indole-3-carboxylate (5)

1*H*-Methyl indole-3-carboxylate (**3**) (11.9 mmol) was dissolved in dry dichloromethane (50 mL) under argon atmosphere and the mixture was cooled to 0 ºC. 1,4-dimethyl piperazine (0.75 g, 6.56 mmol) and *N*-chlorosuccinimide (13.1 mmol) were mixed together and added then the reaction mixture allowed to stand at 0 ºC for 2h. A solution of trichloroacetic acid (3 mmol) and *N*-methyl aniline (**4**) (23.4 mmol) in dry dichloromethane (50 mL) was added dropwise to the reaction mixture at 0 ºC then the reaction was stirred to attain room temperature. The reaction mixture was washed with 10% aqueous $NaHCO_3$ (20 mL) then with 1.0 M aqueous HCl (20 mL) and finally with water and brine. The organic layer was dried by sodium sulphate anhydrous, filtered and evaporated under reduced pressure. The residue was purified using column chromatography with hexane/EtOAc (3:1) as eluent to afford the title compounds. Yield (2.13 g, 66%), m.p. 146–147 ºC, ^{1}H NMR ($CDCl_3$) δ = 3.35 (s, 3H, *N-CH*$_3$), 3.65 (s, 3H, *O-CH*$_3$), 6.70- 6.80 (m, 2H, Ar-H), 6.82 (t, 1H, Ar-H), 7.15-7.30 (m, 4H, Ar-H), 7.33 (m, 1H, Ar-H), 7.95 (m, 1H, Ar-H), 11.97 (s, 1H, NH).

5-Methyl-5,6-dihydro-5H-indolo[2,3-b] quinolin-11-one (6)

The ester **5** (4 mmol) in diphenyl ether (5 mL) was refluxed at (250 ºC) for 2h. brown solid was formed after cooling the reaction mixture to room temperature which was collected by filtration and washed with diethyl ether (100 mL) then dried under high vacuum to afford compound **6**. Yield (88%), m.p. >360 ºC, ^{1}H NMR (DMSO-d_6): δ = 3.98 (s, 3H, *N-CH*$_3$), 7.25 (m, 2H, Ar-H), 7.42 (d, *J = 7.9* Hz, 1H, Ar-H), 7.45 (d, *J = 7.0* Hz, 1H, Ar-H), 7.73 (m, 2H, Ar-H), 8.19 (d, *J = 7.0* Hz, 1H, Ar-H), 8.39 (d, *J = 7.*0 Hz, 1H, Ar-H), 12.07 (s, 1H, NH).

Synthesis of 11-Chloro-5-methyl-5H–indolo [2,3-b] quinoline (7)

Compound **6** (0.05 g, 0.18 mmol) was suspended in dry toluene (5 mL) then $POCl_3$ (5 mL) was added. The reaction mixture was refluxed overnight. The reaction mixture was cooled to room temperature then poured into ice. The reaction mixture was basified with a cold saturated solution of $NaHCO_3$ while keeping the internal temperature below 30 °C. The product was extracted with dichloromethane (3×30 mL). The combined organic layers were washed with water and brine, dried by anhydrous Na_2SO_4, and then evaporated under reduced pressure to afford the crude product **7**. The crude product was purified by column chromatography using EtOAc/hexane (1:1) to afford the pure product **7**. Yield (0.036 g, 75%), m.p. 310–312 ºC, ^{1}H NMR ($CDCl_3$) δ = 4.34 (s, 3H, *N-CH*$_3$), 7.30 (m, 1H, Ar-H), 7.51 (m, 1H, Ar-H), 7.73 (m, 3H, Ar-H), 8.36 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.44 (m, 1H, Ar-H), 8.87 (d, *J*= 8.4 Hz, 1H, Ar-H).

Synthesis of neocrypolpine deriaviatives using sulfonyl & carbonyl hydrazide derivatives (general procedure)

11-Chloro-5-methyl-5H-indolo [2, 3-b] quinoline **7** (1mmol) was added to dimethyl formamide (DMF) (10 mL) then added sulfonyl or carbonyl hydrazide derivatives (1mmol) in presence drops of triethyl amine (5 mmol). The reaction mixture refluxed at 135-155 °C until the starting materials were consumed as monitored by TLC (12-24h). The reaction mixture was poured into ice water, then the final product was extracted with chloroform, dried over anhydrous sodium sulfate then and the organic layer was evaporated to get afford the final product **8(a-d)** or **9(a-j)**, respectively.

4-Methyl-N'-(5-methyl-5H-indolo[2,3-b] quinolin-11-yl)benzenesulfonohydrazide(8a)

Yield (80%) brown solid, m.p. > 300 °C. IR (KBr) cm^{-1}: 3426(NH), 3049(-CH Arom.), 2925(-CH sym), 1613(C=C, Ar), 1320, 1135(S=O sym, asym respectively), 748(-CH asym). ^{1}H NMR (DMSO-d_6, 400 MHz) ppm: 3.99(s, 3H, *CH*$_3$), 4.33

(s, 3H, *N-CH$_3$*), 7.17-8.02(m, 12H, Ar-H), 11.99(br.s, 1H, NH). *EI-MS*, *m/z* ($C_{23}H_{20}N_4O_2S$) calcd, 416.5 $[M]^+$; found, 416.1.

4-Fluoro-N'-(5-methyl-5H-indolo [2, 3-b] quinolin-11-yl) benzenesulfonohydrazide (8b)

Yield (80%) yellow solid, m.p. > 300°C. IR (KBr) cm^{-1}: 3433(NH), 2929(-CH sym), 1604 (C=C, Ar), 1345, 1148(S=O sym, asym respectively), 1388(C-F), 750(-CH asym). ^{1}H NMR (DMSO-d_6, 400 MHz) ppm: 4.05(s, 3H, *N-CH$_3$*), 7.00-7.51(m, 12H, Ar-H), 11.89(br.s, 1H, NH). EI-MS, *m/z* ($C_{22}H_{17}N_4O_2FS$) calcd, 420.46 $[M]^+$; found, 420.12.

N'-(5-Methyl-5H-indolo[2,3-b]quinolin-11-yl)-4-(phenyldiazenyl)benzenesulfonohydrazide(8C)

Yield (70%) brown solid, m.p. 148 °C. IR (KBr) cm^{-1}: 3427(NH), 3055(-CH Arom.), 2924(-CH sym), 1613(C=C, Ar), 1570(N=N), 1295, 1159(S=O sym, asym respectively), 747(-CH asym). ^{1}H NMR (DMSO-d_6, 400 MHz) ppm: 4.34 (s, 3H, *N-CH$_3$*), 7.00-8.16 (m, 17H, Ar-H). EI-MS, *m/z* ($C_{28}H_{22}N_6O_2S$) calcd, 506.58 $[M]^+$; found, 506.20.

2,4,6-Triisopropyl-N'-(5-methyl-5H-indolo [2,3-b]quinolin-11-yl)benzenesulfonohydrazide (8d)

Yield (80%) orange solid, m.p. 210 °C. IR (KBr) cm^{-1}: 3417(NH), 3049(-CH Arom.), 2960(-CH sym), 1607 (C=C, Ar), 1309, 1198 (S=O sym, asym respectively), 747(-CH asym). ^{1}H NMR (DMSO-*d_6*, 400 MHz): 1.13-1.17(m, 18H, *CH$_3$*), 2.82(m, 3H, *CH*), 4.30(s, 3H, *N-CH$_3$*), 7.11-7.56(m, 10H, Ar-H). EI-MS, *m/z* ($C_{31}H_{36}N_4O_2S$) calcd, 528.71 $[M]^+$; found, 528.24.

N'-(5-Methyl-5H-indolo [2,3-b]quinolin-11-yl)isonicotinohydrazide (9a)

Yield (80%), orange solid, m.p. > 300 °C. IR (KBr) cm^{-1}: 3421 (NH), 3094 (-CH Arom.), 2933 (-CH sym), 1613 (C=O), 1518 (C=C, Ar), 743 (-CH asym). ^{1}H NMR (DMSO-*d_6* 400 MHz): 3.95(s, 3H, *N-CH$_3$*), 6.92-7.49(m, 8H, Ar-H), 7.77(s, 2H, Ar-H), 8.30-8.33(m, 2H, Ar-H), 10.43(br.s, 1H, NH), 11.72(br.s, 1H, NH). EI-MS, *m/z* ($C_{22}H_{17}N_5O$) calcd. 367.40 $[M]^+$; found, 367.12.

N'-(5-Methyl-5H-indolo [2,3-b]quinolin-11-yl)furan-2-carbohydrazide (9b)

Yield (80%) orange solid, m.p. > 300 °C. IR (KBr) cm^{-1}:- 3428 (NH), 3055(-CH Arom.), 2925 (-CH sym), 1615 (C=O), 1564 (C=C, Ar), 791 (-CH asym). ^{1}HNMR (DMSO-*d_6*, 400 MHz): 4.27(s, 3H, *N-CH$_3$*), 7.10-8.45(m, 11H, Ar-H). EI-MS, *m/z* ($C_{21}H_{16}N_4O_2$) calcd, 356.13 $[M]^+$; found, 356.17**.**

N'-(5-Methyl-5H-indolo [2,3-b]quinolin-11-yl)benzohydrazide (9c)

Yield (80%) orange solid, m.p. > 300 °C. IR (KBr) cm^{-1}: 3428(NH), 3047 (-CH Arom.), 2925(-CH sym), 1616 (C=O), 1592 (C=C, Ar). ^{1}H NMR (DMSO-d_6, 400 MHz): 4.08(s, 3H, *N-CH$_3$*), 7.00-8.03(m, 13H, Ar-H), 10.61(br.s, 1H, NH), 11.86 (br.s, 1H, NH). EI-MS, *m/z* ($C_{23}H_{18}N_4O$) calcd, 366.42$[M]^+$; found, 366.17.

N'-(5-Methyl-5H-indolo [2,3-b]quinolin-11-yl)-3-oxobutanehydrazide(9d)

Yield (80%) brown solid, m.p. 240 °C. IR (KBr) cm^{-1}: 3424 (NH), 2925 (-CH sym), 1614 (C=C, Ar), 1580 (C=O), 752 (-CH asym). ^{1}H NMR (DMSO-d_6, 400 MHz): 1.93(s, 3H, *CH$_3$*), 3.99(s, 2H, *CH$_2$*), 4.41(s, 3H, *N-CH$_3$*), 7.02-7.96(m, 8H, Ar-H), 12.09(br.s, 1H, NH). EI-MS, *m/z* ($C_{20}H_{18}N_4O_2$) calcd, 346.38 $[M]^+$ found, 347.13.

2-(4-Isobutylphenyl)-N'-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)propanehydrazide(9e)

Yield (80%) brown solid, m.p. 148 °C. IR (KBr) cm^{-1}: 3422 (NH), 3053 (-CH Arom.), 2952 (-CH sym), 1615 (C=O), 1571 (C=C, Ar), 748 (-CH asym). ^{1}H NMR (DMSO-d_6, 400 MHz): 0.83 (d, 6H, *2CH$_3$*), 1.03(t, 3H, CH_3) 1.25 (m, 1H, CH), 3.42 (d, 2H, CH_2, *J*= 8Hz), 4.14 (s, 1H, CH), 4.31(s, 3H, *N-CH$_3$*), 7.16-8.00(m, 12H, Ar-H). *EI-MS*, *m/z* ($C_{29}H_{30}N_4O$) calcd, 450.57 $[M]^+$; found, 450.28.

1-(3,4-Dihydro-1H-carbazol-9(2H)-yl)-2-(2-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)hydrazinyl)ethanone (9f)

Yield (80%) brown solid, m.p. 144 °C. IR (KBr) cm^{-1}: 3415 (NH), 3050 (-CH Arom.), 2925 (-CH sym), 1611 (C=C, Ar), 1519 (C=O), 748 (-CH asym). ^{1}H NMR (DMSO-d_6 400 MHz) 1.04 (t, 4H, 2*CH$_2$*), 2.67(s, 4H, 2*CH$_2$*) 3.97(s, 2H, *CH$_2$*), 4.24(s, 3H, *N-CH$_3$*), 6.88-7.47(m, 12H, Ar-H), 10.57(s, 1H, NH), 11.21(s, 1H, OH *tatomer*), 12.06(br.s, 1H, NH) *EI-MS*, *m/z* ($C_{30}H_{27}N_5O$) calcd, 473.57 $[M]^+$; found, 473.52.

1-Cyclopropyl-6-fluoro-N'-(5-Methyl-5H-indolo [2,3-b]quinolin-11-yl)-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-2-carbohydrazide(9g)

Yield (70%) brown solid, m.p. 192 °C. IR (KBr) cm-1:- 3433 (NH), 2925 (-CH sym), 1665 (C=O), 1621 (C=C, Ar), 1480(C-F), 752 (-CH asym). ^{1}H NMR (DMSO-d_6 400 MHz):- 1.13-1.17(m, 4H, 2*CH$_2$*), 3.58(s, 4H, 2*CH$_2$*) 3.70-3.73(m, 5H, 2*CH$_2$*+*CHN*) 4.29(s, 3H, *N-CH$_3$*), 6.97-8.10(m, 11H, Ar-H), 12.21(br.s, 1H. NH). ESIMS, *m/z* ($C_{33}H_{30}N_7FO_2$) calcd, 575.64 $[M]^+$; found, 575.47.

N'-(5-Methyl-5H-indolo [2,3-b]quinolin-11-yl)naphtho[2,1-b]furan-2-carbohydrazide(9h)

Yield (80%) black solid, m.p. > 300 °C. IR (KBr) cm^{-1}**:** 3428 (NH), 3054(-CH Arom.), 2926 (-CH sym), 1614 (C=O), 1582 (C=C Ar), 748 (-CH asym). ^{1}H NMR (DMSO-d_6 400 MHz):- 3.96(s, 3H, *N-CH$_3$*), 7.15-8.39(m, 15H, Ar-H), 12.07(br.s., 1H, NH). *EI-MS*, *m/z* ($C_{29}H_{20}N_4O_2$) calcd, 456.49 $[M]^+$; found, 456.37.

N'-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)benzofuran-2-carbohydrazide(9i)

Yield (85%) brown solid, m.p. 136 °C. IR (KBr) cm^{-1}: 3438 (NH), 3052(-CH Arom.), 2925 (-CH sym), 1609 (C=O), 1568 (C= C, Ar), 745 (-CH asym). ^{1}H NMR (DMSO-d_6 400

MHz):- 4.27(s, 3H, *N-CH*$_3$), 7.14-8.15(m, 13H, Ar-H). *EI-MS*, *m/z* ($C_{25}H_{18}N_4O_2$) calcd, 406.44 $[M]^+$; found, 406.25.

N'-(5-methyl-5H-indolo[2,3-b]quinolin-11-yl)-1H-indole-2-carbohydrazide(9j)

Yield (80%) orange solid, m.p. > 300 °C. IR (KBr) cm^{-1}**:** 3433 (NH), 2932 (-CH sym), 1639 (C=O), 1571 (C=C Ar), 749 (-CH asym). ^{1}H NMR (DMSO-d_6 400 MHz):- 4.31(s, 3H, *N-CH*$_3$), 7.15-8.15(m, 13H, Ar-H), 11.90(br.s, 1H, NH). *EI-MS*, *m/z* ($C_{25}H_{19}N_5O$) calcd, 405.45$[M]^+$; found, 405.18.

In vitro anticaner bioassay

Materials and methods

Cell Cultures

A human liver cancer cell line ($HepG_2$), was propagated in RPMI-1640 medium L-Glutamine (Lonza Verviers SPRL, Belgium, cat#12-604F) supplemented with 10% fetal bovine serum (FBS) (Seralab, UK, cat# EU-000-H). The cells were incubated in 5% CO_2 humidified at 37°C for growth.

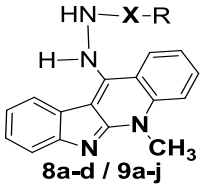
Evaluation of cell proliferation by MTT assay

The number of viable $HepG_2$ cells after treatment with different concentration of the compounds was evaluated by the MTT (3-[4,5-methylthiazol-2-yl]-2,5-diphenyl-tetrazoliumbromide) assay as reported previously with slight modification (Maurya *et al.*, 2011) . In brief, after evaluation of cell count and viability by trypan blue dye, $HepG_2$ cells ($1x10^4$cells/well) were seeded in a 96-well plate in triplicate and were allowed to adhere and spread for 24 h. The tested compounds were dissolved in 500µl Dimethyl sulfoxide (DMSO) to have stock solution of 100 mM, as the final concentration of DMSO in the culture medium never exceeded 0.2% (v/v) (Ranganathan *et al.*, 2015) and then various concentrations of tested compounds were prepared by further diluting in complete medium to have final concentration of 0.01, 0.1, 1, 10, and 100µM.

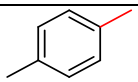
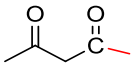
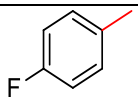
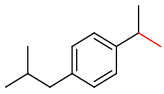
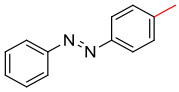
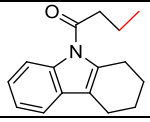
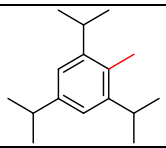
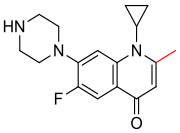
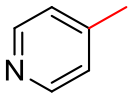
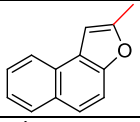
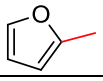
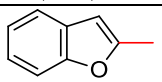
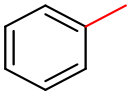
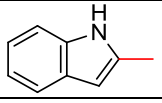
In the next day the medium was replaced with fresh medium with the indicated concentrations of tested compounds and cells were allowed to grow for 72 h. Four hours before completion of incubation, 10µl of MTT (5 mg/mL in PBS w/o Ca, Mg, Lonza Verviers SPRL Belgium, cat#17-516F) was added in each well. After completing the incubation, 100µl of Dimethyl sulfoxide (DMSO) was added to each well, the 96 well plates were centrifuged for 5 minutes at 4000 rpm to precipitate the formazan crystals. Color developed after the reaction was measured at 490 nm using Bio-Tekmicro plate reader. The experiment was conducted in triplicate.

Data were calculated as percent of cell viability by the following formula: % cell viability = (Mean absorbance in test wells / Mean absorbance in control wells) 100.The effect of tested compounds on the morphology of treated hepatocellular carcinoma cells was investigated by the light microscope and then photographed by SONY CYBER-SHORT (El-Far *et al.*, 2009).

Table 1: The target neocryptolepine derivative.



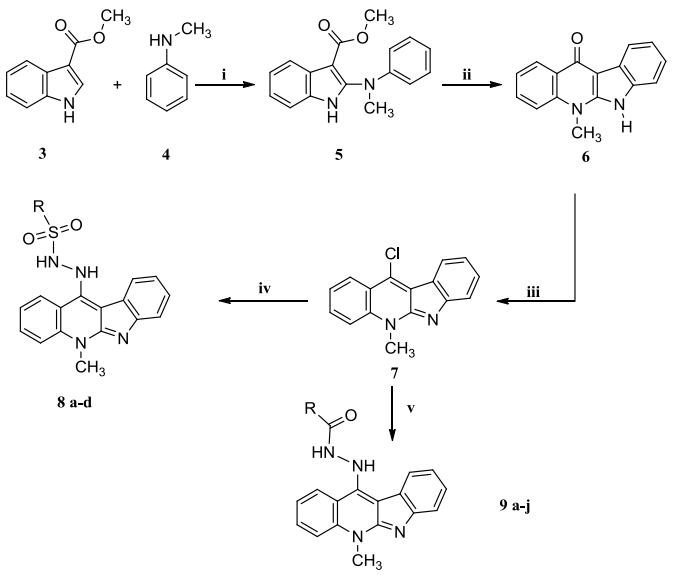
8a-d / 9a-j

Cpd. No.	R	IC_{50} (µM)	Cpd. No.	R	IC_{50} (µM)
8a		12.6	**9d**		11.5
8b		15.1	**9e**		73.95
8c		2.51	**9f**		4.24
8d		20	**9g**		5.02
9a		52.5	**9h**		10.71
9b		4.17	**9i**		28.11
9c		15.1	**9j**		40.95

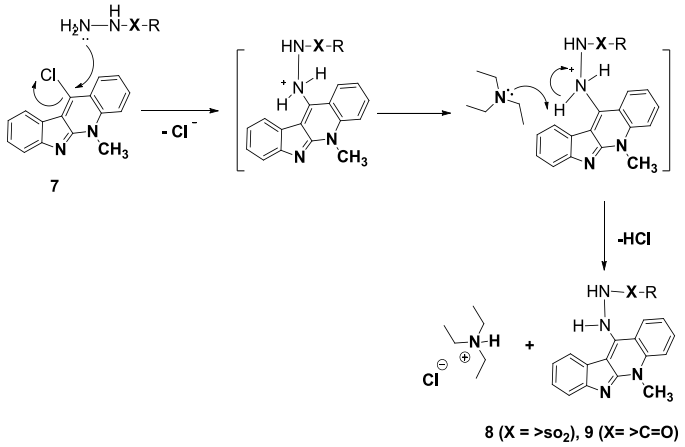
RESULTS AND DISCUSSION

Chemistry

The pathways to assemble the neocryptolepine core structure **2** have been developed starting from easily accessible intermediates as depicted in **Scheme 1**, this approach allowed us to synthesize new analogues with a varied substitution pattern at B ring. The 11-chloroneocryptolepines **7**, the key intermediate for the diversification of the parent natural compound, neocryptolepine **2** was initially obtained according to the route depicted in **Scheme 1**. The synthetic methodology for **7** was achieved starting from 1*H*-methyl indole-3-carboxylate **3** and *N*-methylaniline **4**. The intermediates 2-(phenylamino)-1*H*-indole- 3 -carboxylate **5**, obtained via chlorination with *N*-chlorosuccinimide (*NCS)* in the presence of 1,4-dimethylpiperazine followed by addition of aniline derivative **4** as trichloroacetate salt, was cyclized in boiling diphenyl ether to afford 5,6-dihydro-11*H*-indolo [2,3-b] quinolin-11-one **6** which, was dehydroxy chlorinated with $POCl_3$ to give 11-chloro-norneocryptolepine 7 in good yield as shown in **Scheme 1**.



Scheme 1: Synthesis of the neocryptolepine analogues containing substituted side-chains at *C-11*. Reagents and conditions:(i) a. *N*-chlorosuccinimide, 1,4-dimethylpiperazine, CH_2Cl_2, 0 ºC, 2 h. b. trichloroacetic acid, room temperature, 2 h. (ii) diphenyl ether, reflux, 3 h. (iii) $POCl_3$, toluene, reflux, 12 h. (iv).appropriate sulfonyl hydrazide, Et_3N, dry DMF, 135-155 ºC, 12-24 h . (v). appropriate carboxylic acid hydrazide, Et_3N, dry DMF, 135-155 ºC, 12-24 h.



Scheme 2: Proposed plausible mechanism.

It should be notable that the yield and the reaction time for the cyclization of the anilino derivative **5** to afford the teteracyclic indoloquinolone **6** are very crucial to the amount of the solvent, diphenyl ether used. It was observed that performing the reaction in less amount of solvent makes the cyclization step cleaner, faster, and higher yield. With the key compound **7** in hand, the sulfonylhydrazido neocryptolepine derivatives **8** and carbonylhydrazido neocryptolepine have been synthesized through the incorporation of basic nitogen side-chains at the C-11 position of the B ring of neocryptolepine scaffold with different sulfonyl or carbonyl hydrazide substitutions. The introduction of the sulfonamido side chain is expected to contribute to the DNA intercalation binding ability through hydrogen bonding motives. Furthermore, the incorporation of the ionisable amine functionality with different linker chain would promote the electrostatic interactions with the negatively charged phosphate groups of DNA backbone. In each case, the compounds were designed to incorporate structural features that might increase DNA binding affinity, cell viability, and cytotoxicity towards the cancer cells. The indoloquinoline scaffold was selected due to its well-documented DNA binding capability and to its DNA binding interactions through intercalation (Bailly *et al.*, 2000; Guittat *et al.*, 2003; Peczynska−Czoch *et al.*, 1994). Therefore, indoloquinolines may be considered promising scaffolds for the development of novel selective anticancer drugs. Therefore, the key intermediates 11-chloro neocryptolepine **7** was used further for the diversification of the neocryptolepine core at the C-11 position. Thus, the reaction of **7** with carbonylhydrazide derivatives in DMF under reflux and in presence of triethyl amine as a base catalyst yielded the corresponding 11-carbohydrazide neocryptolepine analogues **9** smoothly in very good yield according to the route depicted in **Scheme 1**. The NMR results for all products are in good agreement with the chemical structure of the compounds and well correspond with the bond formation of the terminal side-chain nitrogen to the sulfonyl or carbonyl group (*cf.* experimental part for details). The spectra of the known isolated products were in agreement with literature data. The plausible mechanism of this reaction involves the nucleophilic aromatic substitution (S_{NAr}) of the amino group at a chloride atom of **7** followed by elimination of chloride ion as HCl which reacts with triethyl amine to form triethylammonium chloride salt and the neocryptolepine conjugates **8** and **9** as shown in **Scheme 2.**

Anticancer Activity with structure–activity relationships (SARs)

The anticancer activity evaluation of the prepared compounds have been assessed against human liver cancer cell line ($HepG_2$) using the MTT assay. For comparison, 5-flurouracil (5-FU) was used as a reference anticancer drug. Dimethyl sulfoxide (DMSO) used as a control for the cancer cells. Cell viability was assessed using the MTT assay. The key results obtained for compounds **8a-d** and **9a-j** toward hepatocellular carcinoma (HepG2) cells are shown in Table 2, Figure 2**.**

Table 2: % Survival in HepG2 cells treated with different (Conc. µM) of Cpds.

% Survival in Hep G2 cells treated with different (Conc. µM) of Cpds.						
Cpd.	**0**	**0.01**	**0.1**	**1**	**10**	**100**
5FU	100	60.65	68.83	57.75	29.19	56.20
8a	100	89.63	95.63	90.90	54.80	1.62
8b	100	69.92	61.22	55.40	43.92	13.41
8c	100	64.24	49.39	52.85	22.43	4.63
8d	100	57.67	53.89	54.20	50.14	18.85
9a	100	100.00	100.00	100.00	69.86	56.54
9b	100	84.98	60.59	84.35	30.38	0.00
9c	100	67.42	59.69	58.08	46.77	39.83
9d	100	56.52	56.44	54.03	41.75	8.07
9e	100	100	100	95.74	68.65	49.23
9f	100	81.96	75.84	63.74	68.65	49.23
9g	100	98.63	87.72	71.56	44.41	8.93
9h	100	92.78	80.51	62.84	73.46	14.13
9i	100	100	84.75	61.46	92323	19.11
9j	100	97.57	81.62	75.74	48.56	51.23

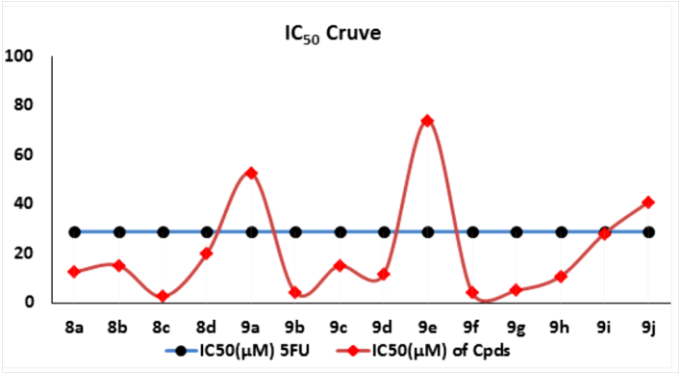


Fig 2: IC_{50} values of compounds against 5-FU.

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) 100. The cell viability was observed following 72 h of exposure to all compounds at doses of 0.01, 0.1, 1, 10 and 100 µM of compounds. The results revealed that compounds **8c, 9b, 9f** and **9g** with diphenylazo, furyl, tetracarbazolyl and ciprofloxacinyl substituents at the hydrazide side chain of C-11 position of the neocryptolepine core exhibited the highest activity against $HepG_2$ cell line with IC_{50}: 2.51, 4.17, 4.24 and 5.02 respectively, On the other hand, compounds **9h, 9d, 8a, 9c, 8b, and 8d** with naphthofuryl, oxobutanoyl, tolyl, phenyl, 4-fluorophenyl, 2,4,6-triisopropylphenyl moieties at the hydrazide side chain of C-11 exhibited strong activity against $HepG_2$ cell line with IC_{50}: 10.71, 11.50, 12.60, 15.10, 15.10, and 20 respectively, While compounds **9i, 9j, 9a** and **9e** with benzofuranyl, indoleyl, pyridinyl, (4-isobutylphenyl)ethyl substituents at the hydrazide side chain of C-11 showed lower potency when compared by 5-FU with IC_{50}: 28.11, 40.95, 52.50 and 73.95 respectively.

In summary, neocryptolepine has been confirmed as a useful lead compound for the development of new anticancer compounds. Our initial goal to prepare synthetic derivatives bearing hydrazide side chain with a higher anticancer activity could be achieved resulting in several compounds with a higher potency than the reference drug 5-FU.

CONCLUSION

In conclusion, a series of novel neocryptolepine–hydrazides hybrids bearing sulfonyl and carbonyl hydrazide moieties have been synthesized. The *in vitro* anticancer activity was evaluated on in vitro human liver cancer cell model (HepG2). The screening results suggested that the parent natural product neocryptolepine was remarkably influenced by various substituents on the B ring and at the N-terminal of the hydrazide moiety. Moreover, the data revealed that, among the tested compounds, four candidates (compounds **8c, 9b, 9f** and **9g**) showed high effectiveness on the HepG2, and could be considered as useful templates for further development to obtain more potent anticancer agent(s). Further studies to assess the effect of these compounds on other cancer cell biomarkers are currently underway in our lab.

ACKNOWLEDGMENT

Financial support and sponsorship: We would like to acknowledge the financial support of the Menoufia University throughout the project number Ib-C2013.

Conflict of Interests: There are no conflicts of interest.

REFERENCES

Bader, T.; Fazili, J.; Madhoun, M.; Aston, C.; Hughes, D.; Rizvi, S.; Seres, K.; Hasan, M. Fluvastatin inhibits hepatitis C replication in humans, Am. J. Gastroenterol. 2008, 103, 1383-1389.

Bailly C., Laine W., Baldeyrou B., De Pauw-Gillet M. C., Colson P., Houssier C., Cimanga K., Van Miert S., Vlietinck A., Pieters L. DNA intercalation, topoisomerase 11 inhibition and cytotoxic activity of the plant alkaloid neocryptolepine , Anti-Cancer Drug Des. 2000, 15, 191–201.

Brandes, J.L.; Kudrow, D.; Cady, R.; Tiseo, P.J.; Sun, W.; Sikes, C.R. Eletriptan in the early treatment of acute migraine: influence of pain intensity and time of dosing , Cephalalgia 2005, 25,735-742.

Butler M. S., the Role of Natural Product Chemistry in Drug Discovery. J. Nat. Prod. 2004, 67, 2141–2153. Cimanga K., Bruyne T. D., Pieters L., Claeys M., Vlietinck A., New alkaloids from Cryptolepis sanguinolenta, Tetrahedron Lett. 1996, 37, 1703-1706.

El Sayed I. E., Veken P. V., Dhooghe L., Hostyn S., Van Baelen G., Lemière G., Maes B. U. W., Cos P., Maes L., Joossens J., Haemers A., Pieters L., Augustyns K., Synthesis and Antiplasmodial Activity of Aminoalkyl- aminosubstituted Neocryptolepine Derivatives, J Med. Chem. 2009, 52, 2979-2988.

El Sayed I., El Kosy S. M., Hawata M. A., El Gokha A., Tolan A., Abd El-Sattar M., Synthesis and Antibacterial Activity of α-Aminophosphonates Bearing Neocryptolepine Moiety, J. of Am. Sci.,. 2011, 7, 357-361.

El Sayed I., Ramzy F., William S., El Bahana-sawy M., Abdel-Staar M. M., Neocryptolepine Analogues Containing N-Substituted Side-Chains at C-11: Synthesis and Antischistosomicidal Activity, Med. Chem. Res.2012, 21, 4219-4229.

El-Far M., , Elmegeed G. A., Eskander E. F., Rady H. M., Tantawy M. A.., Novel modified steroid derivatives of androstanolone as chemotherapeutic anti-cancer agents, Eur J Med Chem, 2009. 44. 3936-3946.

Emam S. M., El Sayed I.,Nassar N. Transition Metal Complexes of Neocryptolepine Analogues Part I: Synthesis, Spectroscopic Characterization, and In Vitro Anticancer Activity of Copper(II) Complexes, Spec-trochim.Acta A, 2015, 138, 942–953.

Goyal A., Sahewal J, Jain N., Jain S., Synthesis and pharmacological evaluation of some 2-(4-isobutylphenyl)-N-(4-oxo-2-arylthiazolidin-3-yl) propanamides Pharm Lett., 2016, 8, 275-280.

Guittat L., Alberti P., Rosu F., Van Miert S., Thetiot E., Pieters L., Gabelica V., E. Pauw D., Otta-viani A., Riou J. F., Mergny J. L., Interactions of cryptolepine and neocryptolepine with unusual DNA structures, Biochimie. 2003, 85, 535-547.

Ibrahim A. S., Khaled H. M., Mikhail N., Kamel H., Baraka H., Cancer incidence in egypt: results of the national population-based cancer registry program, J Cancer Epidemiol , 2014, 2014,437971.

Jonckers T. H. M., Van Miert S., Kanyanga C., Bailly C., Colson P., De Pauw-Gillet M. C., van Den H. H., Claeys M., Lemiere F., Esmans E. L., Rozenski J., Quirijnen L., . Maes L, Dommisse R., Lemière G. L. F., Vlietinck A., Pieters L., Synthesis, Cytotoxicity, and

Antiplasmodial and Antitrypanosomal Activity of New Neocryptolepine Derivatives, J. Med. Chem. 2002, 45, 3497- 3508.

Kumaraswamy M. N. , Chandrashekhar C. , Shivakumar H. , Prathima Mathias D. A. , Mahadevan K. M. , Vaidya V. P. Synthesis and Activity Evaluation of 2-(1-naphtho[2,1-b]furan-2-yl-carbonyl)-3,5-disubstituted-2,3-dihydro-1H-pyrazoles, Indian J Pharm Sci. 2008, 70, 715–720.

Larghi E.L., Bracca A.B.J., Aguilar A.A., Here-dia D.A., Pergomet J.L., Simonetti S.O., Kaufman T.S.; Neocryptolepine: A Promising Indoloisoquinoline Alkaloid with Interesting Biological Activity. Evaluation of the Drug and its Most Relevant Analogs., Curr top med chem, 2015, 15, 1683-1707.

Lavrado J., Moreira R., Paulo A., Indoloquinolines as scaffolds for drug discovery, Curr. Med. Chem. 2010, 17, 2348-2370.

Lu W. J., Świtalska M., Wang L., Yonezawa M., El Sayed I. E., Wietrzyk J., Inokuchi T., In vitro Antiproliferative Activity of 11-Aminoalkylamino-Substituted 5H-indolo[2,3-b]quinolines; Improving Activity of Neocryptolepines by Installation of Ester Substituent, Med. Chem. Res. 2013a, 22, 4492–4504.

Lu W. J., Wicht K. J., Wang L., Imai K., Mei Z. W., Kaiser M., El Sayed I. E., Egan T. J., Inokuchi T., Synthesis and antimalarial testing of neocryptolepine analogues: Addition of ester function in SAR study of 2,11-disubstituted indolo[2,3-b]quinolones, Eur. J. Med. Chem. 2013b, 64, 498-511.

Maurya D. K., Nandakumar N., and Devasagayam T. P. A., Anticancer property of gallic acid in A549, a human lung adenocarcinoma cell line, and possible mechanisms, J. Clin. Biochem. Nutr., 2011. 48, 85-90.

Mei Z. W., Wang L., Lu W. J., Pang C. Q., Maeda T., Peng W., Kaiser M., El Sayed I. E., In-okuchi T., Synthesis and In vitro Antimalarial Testing of Neocryptolepines: SAR Study for Improved Activity by Introduction and Modifications of Side Chains at C2 and C11 on Indolo[2,3-b]quinolines, J. Med. Chem., 2013, 56, 1431–1442.

Okada M., Mei Z.W., Hossain Md. I., Wang L., Tominaga T., Takebayashi T. , Murakami M., Yasuda M., Shigehiro T., Kasai T., Mizutani A. , Murakami H. , El Sayed I., Dan S., Yamori T., Seno M., Inokuch T. ; Synthesis and in vitro cancer cell growth inhibition evaluation of 11-amino-modified 5-Me-indolo[2,3-b]quinolines and their COMPARE analyses, Med Chem Res, 2016, 25, 879-892.

Peczynska−Czoch, W., Pognan, F., Kaczmarek, L., Boratynski, J. Synthesis and structure-activity relationship of methyl-substituted indolo[2,3-b]quinolines: novel cytotoxic, DNA topoisomerase II inhibitors, J. Med. Chem., 1994, 37, 3503−3510.

Peng W., Świtalsk M., Mei Z.W., Edazawa Y., Wang L., El Sayed I., Wietrzyk J., Inokuchi T.; Synthesis and in vitro antiproliferative activity of new 11-aminoalkylamino-substituted chromeno[2,3-b]indoles, Eur J. Med. Chem. 2012, 58, 441-451.

Prakash T. P., Perunninakulath S. P., Santosh G. T., Isolation, Biological Activities and Synthesis of Indoloquinoline Alkaloids: Cryptolepine, Isocryptolepine and Neocryptolepine, Curr. Org. Chem. 2011, 15, 1036-1057.

Ranganathan, S., Halagowder D., and Sivasithambaram N.D., Quercetin Suppresses Twist to Induce Apoptosis in MCF-7 Breast Cancer Cells, PLoS One, 2015. 10, e0141370.

Surendiran T. Synthesis of Novel N-acetyl-(3-substituted-1H-pyrazole-(4H)-5-on)-yl-1,2,3,4-tetrahydrocarbazole and its antimicrobial studies Der Pharmacia Sinica, 2012, 3, 156-159.

Wang L., Lu W.-J., Odawara T., Misumi R., Mei Z.-W., Peng W., El-Sayed I. E.-T., Inokuchi T., Improved Synthesis and Reaction of 11- Chloroneocryptolepines, Strategic Scaffold for Antimalaria Agent, and Their 6-Methyl Congener from Indole-3-carboxylate, J. Heterocyclic Chem. 2014, 51, 1106-1114.

Wang L., Świtalska M., Mei Z. W., Lu W. J., Takahara Y., Feng X. W., Sayed I. E., Wietrzyk J., Inokuchi T., Synthesis and in vitro antiproliferative activity of new 11-aminoalkylamino-substituted 5H- and 6H-indolo[2,3-b] quinolines; structure–activity relationships of neocryptolepines and 6-methyl congeners, Bioorg. Med. Chem. 2012, 20, 4820-4829.

Wang N., Imai K., Pang C. Q., Wang M. Q., Yonezawa M., Zhang Y., Nokami J., Inokuchi T., Regioselective N-Methylation of 6-Chloroindolo[3,2-c]quinolines and Their Amination Reactivity at the C-6 Position, Bull. Chem. Soc. Jpn. 2013, 86, 864-869.

Xie, S.O., Chen Y.S., Wand G.Q., Duan N.N. , Wen X.Y. , Cao T.Y. , Yin J., Wang W. , Hu G.Q , Huang W.L . Synthesis and antitumor evaluation of s-triazolothiadiazines and pyrazolo s-triazoles derived from ciprofloxacin, Acta Pharmaceut. Sin., 2012, 47, 66 -71.

Xu Y. J., Pieters L., Recent Developments in Antimalarial Natural Products Isolated from Medicinal Plants, Mini Reviews in Med. Chem. 2013, 13, 1056- 1072.

How to cite this article:

Sebeka AH, Osman AM, El Sayed IE, El-Bahanasawy M, Tantawy MA. Synthesis and Antiproliferative Activity of Novel Neocryptolepine-Hydrazides Hybrids. J App Pharm Sci, 2017; 7 (10): 009-015.