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Synthesis and *in vitro* Antiproliferative Activity of 11-Substituted Sulfonamide Analogues of Neocryptolepine

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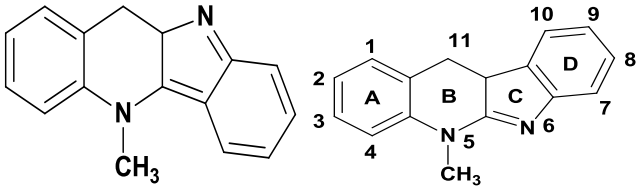
Abstract
The present study describes the synthesis and antiproliferative evaluation of several neocryptolepine analogues carrying sulfonamide side chain at C11. The key intermediate compound amine 7 was prepared by nucleophilic aromatic substitution (S_{NAr}) of 11-chloroneocryptolepines 5 with 4, 4'-diaminodiphenylmethane 6. The 11-diamino derivatives 7 were further reacted with arylsulfonly chlorides 8 to afford the corresponding 11-sulfonamide neocryptolepine analogues 9. Some of the prepared neocryptolepine derivatives showed a strong antiproliferative activity against the breast, colon and hepatocellular carcinoma cell lines. Among them, compound 9c was the most cytotoxic with a mean IC_{50} value of 8.95 μM, 14.24 and 11.76 μM against the breast, colon and hepatocellular carcinoma cell lines respectively.

Key words: 5-me-indolo [2, 3-*b*] quinolones, neocryptolepines, antiproliferative activity

Introduction

The tetracyclic indoloquinoline ring systems constitute important structural motifs in natural products exhibiting numerous biological activities (Kumar *et al*. 2008; Lavrado *et al*. 2010; Pavatkar *et al*. 2011) [11, 12]. For example, cryptolepine (I, indolo [3, 2-*b*]quinoline) and neocryptolepine (II, indolo[2,3-*b*]quinoline) are representative alkaloids isolated from the roots of the African plant *Cryptolepis sanguinolenta* (Cimanga *et al*. 1996, 1997[3, 4]; Paulo *et al*. 2000) [16]. Notably,an aqueous macerate ordecoction of

This plant is used in traditional medicine against malaria (Ansah *et al*. 2009).These two alkaloids, which only differ in the respective orientation of their indole and quinoline components, display potent antiparasitic properties (Cimanga *et al*. 1997 [4]; Kirby *et al*. 1995 [10]; Wright *et al*. 1996) [21]. Due to the linearly arranged planar tetracyclic structure, cryptolepine (I**)** and neocryptolepine (II**)** are DNA-intercalating agents and inhibit topoisomerase II, showing a high level of cytotoxicity (Guittat *et al*., 2003 [8]; Jonckers *et al*. 2002 [9]; Bailly *et al*. 2000 [2]; Dassonneville *et al*. 2000) [5].



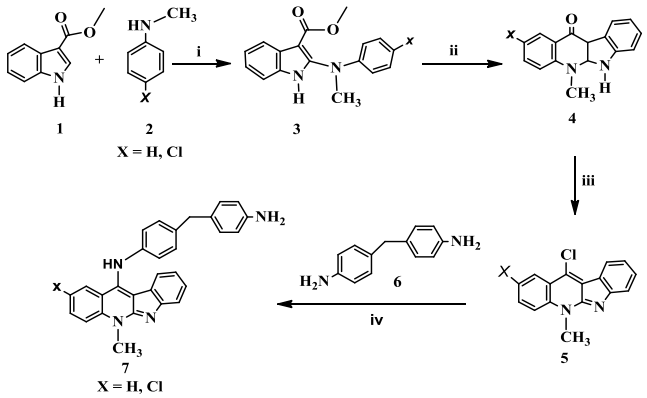
Cryptolepine (I) Neocryptolepine (II)

Indoloquinolines from *Cryptolepis sanguinolenta*

Our previous structure activity relationship (SAR) study about the antiproliferative activity of the 5-methyl-indolo [2, 3-*b*]quinoline derivativ es revealed that the ω-aminoalkylamino substituent at C11 is an important element for their bioactivity. For example, the 3-amino propylamino group on 2 (R^1 = H) could increase the antiproliferative activity against the human leukemia cell line MV4-11 about 20 times compared to that the of 11-chloro precursor 1 (R^1 = H) (Wang *et al*., 2012; Lu *et al*., 2013) [13, 14]. Based on these findings, in this work, we discuss further the effect of aminosulfonamide substituent at C11 in the 5-methyl-indolo [2, 3-*b*] quinolone core by diversifying the side-chain structure, *i.e*., changing the length and branching of the linker between the two nitrogen atoms, etc. We also examined whether a sulfonamide group residing on the spacer would exert influence on the antiproliferative activity.

Results and Discussion

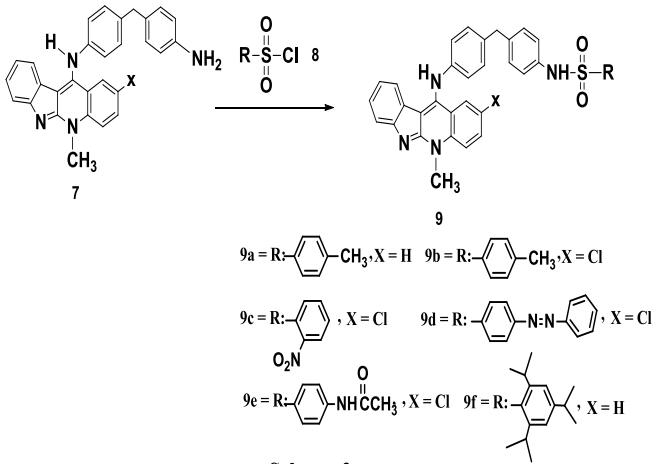
Synthesis: The synthetic strategy for target compounds 11-Substituted Sulfonamide analogues of Neocryptolepine 9 is based on the nucleophilic aromatic substitution (S_NAr) reaction of 11-chloroneocryptolepines 5 with 4,4'-diaminodiphenylmethane 6 as shown in below. The 11-chloroneocryptolepines 5, the key intermediates for the diversification, were derived starting with substituted *N*-methylanilines 2 and methyl-1*H*-indole-3- carboxylate 1 in three steps in good yields (Wang *et al*., 2014). The amination of 5 with 6 using triethyl amine in dimethyl formamide (DMF), under heating, yielded the corresponding 11-aminated compounds 7 smoothly in good yields.



Synthesis of the amino neocryptolepine analogues containing N-substituted side-chains at C-11.

Reagents and conditions: (i) a. N-chlorosuccinimide, 1,4-dimethylpiperazine, CH_2Cl_2, 0 °C, 2-16 h. b. trichloroacetic acid, *N*-methylaniline 2, room temperature, 2 h. (ii) diphenyl ether, reflux, 3 h. (iii) $POCl_3$, toluene, reflux, 6-12 h. (iv) amine 6, DMF, 120 °C, 4 h.

Subsequently, a series of sulfonamide derivatives 9 were prepared in high yields by further modification of the free terminal amine group of 7 with arylsulfonyl chlorides 8 in dry DMF at room temperature in presence of triethyl amine as a base catalyst via nucleophilic aromatic substitution (S_{NAr}) reaction as depicted in below.



The NMR results for all new 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 group (*cf.* experimental part for details). The spectra of the known isolated products were in agreement with literature data[xx]

Antiproliferative Activity

The anticancer activity evaluation of the prepared compounds have been assessed against the breast (MCF-7), colon (HCT-116) and hepatocellular ($HepG_2$) carcinoma cell line. For comparison, doxorubicin (DOX) 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 9a, 9b, 9c, 9d, 9e toward the three cell lines are shown in Table 1 and dose-survival curves in figures 1-6. 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 most of the tested compounds showed a strong to moderate activity (*cf.* Table1). Compounds 9c with sulfonamide moiety combined with nitro nito group at the amine side chain of C-11 position and of the neocryptolepine core exhibited the highest activity against breast cancer (MCF-7) cell line with IC_{50}: 8.95 µM. SONY CYBER-SHORT (El-Far *et al.* 2009).

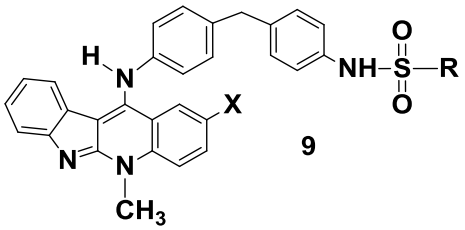


Table 1: Antiproliferative activity of 9 against human cancer cell lines

Cpd. No.	R, x	IC_{50}(µM) HePG2	IC_{50}(µM) HCT-116	IC_{50}(µM) MCF-7
DOX	----------	4.50±0.2	5.23±0.3	4.17±0.2
9a	CH_3, H	67.97±3.9	60.25±3.6	56.65±3.3
9b	CH_3, Cl	32.10±2.3	28.09±2.0	36.67±2.5
9c	O_2N, Cl	11.76±1.1	14.24±1.3	8.95±0.9
9d	N=N, Cl	16.81±1.4	18.10±1.5	23.51±1.8
9e	NHCCH3, Cl	16.81±1.4	18.10±1.5	23.51±1.8

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

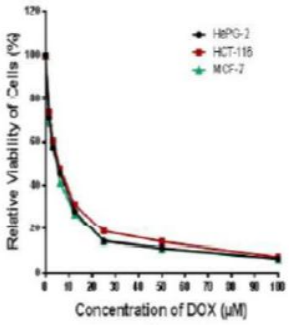


Fig. 1

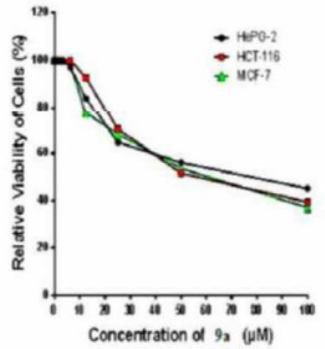


Fig. 2

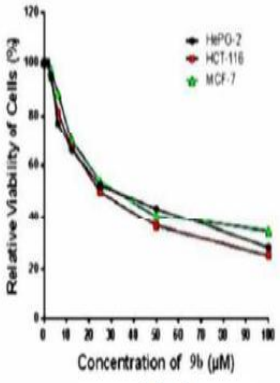


Fig. 3

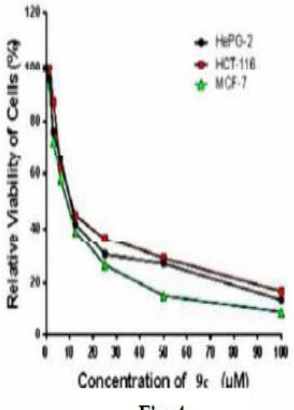


Fig. 4

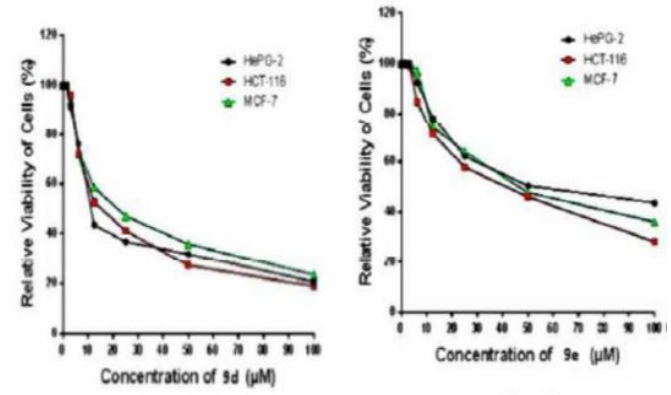


Fig 1-6: Dose-survival curves for compounds 9a, 9b, 9c, 9d and 9e as well as for the reference drug Doxorubicin (DOX).

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 sulfonamide side chain with a higher anticancer activity could be achieved resulting in some compounds with a higher potency. Further studies to assess the effect of these compounds on other cancer cell biomarkers are currently underway in our lab.

Experimental

General methods

All ^{1}HNMR experiments (solvent DMSO-d_6) were carried out with a 300MHz varian 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 was performed at Cairo University, Egypt. The biological activity analysis was carried out at central laboratory, Faculty of Pharmacy, Mansoura University, Egypt. All reactions were followed by thin layer chromatography (TLC) on kiesel gel F254 precoated plates (Merck). The amine **6** and arylsulfonly chlorides were commercially available and purchased from Sigma-Aldrich. The required intermediates **3**, **4** and **5** were prepared by adopting the earlier reported procedures (Wang *et al.* 2014) [20].

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

1*H*-methyl indole-3-carboxylate **1** (2.08 g, 11.9 mmol) was dissolved in dry dichloromethane (50 mL) under argon atmosphere and the mixture was cooled to 0 ºC. 1,4-dimethylpiperazine (0.75 g, 6.56 mmol) and *N*-chlorosuccinimide (1.75 g, 13.1 mmol) were mixed together and added to the reactiom mixture then the reaction mixture allowed to stand at 0 ºC for 2h. A solution of trichloroacetic acid (0.5 g, 3 mmol) and *N*-methyl aniline 2a or 4-chloro-N-methyl aniline (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 and was further stirred overnight at 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.

Methyl-2-(phenyl) (methyl)amino)-1*H*-indole 3carboxylate 3a

Yield (2.13 g, 66%), m.p. 146–147 ºC, ^{1}H NMR ($CDCl_3$) □ = 3.35 (s, 3H), 3.65 (s, 3H), 6.7- 6.8 (m, 2H), 6.82 (t, *J=7.3*Hz, 1H) 7.15-7.30 (m, 4H), 7.33 (m, 1H), 7.95 (m, 1H), 11.97 (s, 1H, NH).

Methyl-2-(4-chlorophenyl) (methyl)amino)-1*H*-indole- 3-carboxylate 3b

Mp = 179-180 ºC; IR (neat) νmax: 3190, 3020, 1619,1454, 1328 cm^{-1}- ^{1}H NMR (300 MHz, CDCl3) □ ppm: 8.69 (s, 1H), 8.13 (d, *J* = 8.2 Hz, 1H), 7.26-7.22 (m, 3H), 7.14 (d, *J* = 8.9 Hz, 2H), 6.70 (d, *J* = 8.9 Hz, 2H), 3.78 (s, 3H), 3.36 (s, 3H); MS: 315 [M + H]+.

5-methyl-5,6-dihydro-5*H*-indolo[2,3-*b*]quinolin-11-one 4

The ester 3 (1.12 g, 4 mmol) in diphenyl ether (5 mL) was refluxed at (250 ºC) for 2h. A brawn solid was formed after cooling the reaction mixture to room temperature which was collected by filtration the washed with diethyl ether (100 mL) then dried under high vacuum to afford compound **4a**. Yield (0.93 g, 88%), m.p. >360 ºC, ^{1}H NMR (DMSO-d_6): □ = 3.98 (s, 3H), 7.25 (m, 2H), 7.42 (d, *J = 7.9* Hz, 1H), 7.45 (d, *J = 7.0* Hz, 1H), 7.73 (m, 2H), 8.19 (d, *J = 7* Hz, 1H), 8.39 (d, *J = 7* Hz, 1H), 12.07 (s, 1H, NH).

2-Chloro-5-methyl-5,6-dihydro-11H-indolo[2,3-b]quinolin-11-one 4b

Mp = >370 ºC ;**^{1}H NMR** (400 MHz; DMSO-d6): δ 12.12 (s, 1H), 8.27 (dd, *J* = 2.6, 0.3 Hz, 1H), 8.20-8.17 (m, 1H), 7.77 (d, *J* = 9.1 Hz, 1H), 7.71 (dd, *J* = 9.0, 2.6 Hz, 1H), 7.47-7.44 (m, 1H), 7.28 (td, *J* = 63). 7.5, 1.5 Hz, 1H), 7.23 (td, *J* = 7.3, 1.3 Hz, 1H), 3.94 (s, 3H)

Synthesis of 11-chloro-5-methyl-5H-indolo [2,3-b]quinoline 5a

Compound **4** (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 water layer was extracted with dichloromethane (3 × 30 mL). The combined organic layer was washed with water and brine, dried by anhydrous Na_2SO_4, and then evaporated under reduced pressure afforded 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 ($CDCl_3$) □ = 4.34 (s, 3H), 7.30 (m, 1H), 7.51 (m, 1H), 7.73 (m, 3H), 8.36 (d, *J* = 8.4 Hz, 1H), 8.44 (m, 1H), 8.87 (d, *J*= 8.4 Hz, 1H).

Synthesis of 2,11-dichloro-5-methyl-5H- indolo[2,3-b]quinoline 5b

Mp = 211-213 ºC; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: δ 8.37-8.35 (m, 2H), 7.75-7.67 (m, 3H), 7.59-7.55 (m, 1H), 7.28 (td, *J* = 7.4, 0.8 Hz, 1H), 4.38 (s, 3H). 13C NMR (101 MHz; CDCl3):

δ 154.4, 135.2, 134.8, 131.4, 130.4, 128.5, 125.5, 125.2, 124.1, 123.2, 120.9, 120.3, 117.6, 115.9, 33.8; **MS**): 301 $[M + H]^+$.

General procedure for the synthesis of 11-aminoneocryptolepines 7:

To 11-Chloroindoloquinolines **5** (0.3 mmol) and 4,4'-diaminodiphenylmethane 6 (0.35 mmol) in dry dimethylformaide (DMF) excess triethyl amine (1.5 mmol) was added, the reaction mixture was heated 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 crystallization from MeOH to yield pure yellowish solid products.

N-(3-(4-aminobenzyl)phenyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine 7a:

Yield: (0.06 g, 75%), yellow solid, m.p:266 -269°C, ^{1}H NMR (400 MHz, DMSO) ppm: 3.69 (s, 2H, C*H*$_2$), 4.28 (s, 3H, N-C*H*$_3$), 4.84 (s, 2H, N*H*$_2$), 6.46-6.49 (m, 1H, Ar), 6.61-6.69 (m, 2H, Ar), 6.79-6.82 (m, 1H, Ar), 6.86-6.91 (m, 2H, Ar), 7.02-7.07 (m, 3H, Ar), 7.18-7.25 (m, 1H, Ar), 7.44-7.48 (m, 2H, Ar),7.82-7.87 (m, 2H, Ar), 7.94-7.97 (m, 1H, Ar), 8.51 (d, *J*=8 Hz, 1H, Ar), 9.38 (s, 1H, N*H*). Ms (EI, 70 eV): *m/z*: 429 ($[M+1]^+$, 26.55%).

N-(4-(4-aminobenzyl)phenyl)-2-chloro-5-methyl-5H-indolo[2,3-b]quinolin-11-amine 7b:

Yield: (0.06 g, 75%), yellow solid, m.p: > 300 °C, ^{1}H NMR (400 MHz, DMSO) ppm: 3.82 (s, 2H, C*H*$_2$), 4.29 (s, 3H, N-C*H*$_3$), 4.74 (s, 2H, N*H*$_2$), 6.48-6.51 (m, 1H, Ar), 6.66-6.70 (m, 2H, Ar), 6.78-6.83 (m, 1H, Ar), 6.86-6.91 (m, 2H, Ar), 7.02-7.07 (m, 2H, Ar), 7.18-7.25 (m, 1H, Ar), 7.44-7.48 (m, 2H, Ar),7.82-7.87 (m, 2H, Ar), 7.94-7.97 (m, 1H, Ar), 8.51 (d, *J*=8 Hz, 1H, Ar), 9.38 (s, 1H, N*H*). Ms (EI, 70 eV): *m/z*: 464 ($[M+1]^+$, 10.83%).

General Procedure for the Synthesis of Compounds9a-f:. N-(3-(4-aminobenzyl)phenyl)-5-methyl-5H-indolo[2,3-b]quinolin-11-amine 7 (0.12 mmol) was completely dissolved in dry DMF (2 mL), and then a mixture of arenesulfonyl chloride 8 (1.2 equiv) in dry DMF (1 mL) were added drop by drop with stirring, and finally 2.0 equiv of triethylamine was added and the reaction was carried out at room temperature for 24 h. TLC monitoring was used to ensure the completion of reaction. The crude product was purified by crystallization from toluene to yield pure products as yellowish-orange solids.

4-methyl-N-(4-(4-((5-methyl-5H-indolo[3,2-b]quinolin-11-yl)amino)benzyl)phenyl)benzenesulfonamide 9a:

Mp: 201-213, Yield:78% IR (KBr) 3389, 3055, 2932, 1626, 1568, 1489, 1445, 1420, 1308, 1281, 1246, 1153, 1092, 878, 860, 743, 691 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.25 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 4.29 (s, 3H), 6.23-8.22 (m,19H, Ar-H), 8.84 (Brm, 1H, NH), 10.35 (brs, 1H, NH). 456($C_{24}H_{16}N_4O_4S$, M, 26.93%),

N-(4-(4-((2-chloro-5-methyl-5H-indolo[3,2-b]quinolin-11-yl)amino)benzyl)phenyl)-4-methylbenzenesulfonamide 9b:

yield (80%) as a yellow solid, m.p. >220°C

^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.25 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 4.29 (s, 3H), 6.23-8.22 (m,19H, Ar-H), 8.84 (Brm, 1H, NH), 10.35 (brs, 1H, NH).

N-(4-(4-((5-methyl-5H-indolo[3,2-b]quinolin-11-yl)amino)benzyl)phenyl)-2-nitrobenzenesulfonamide 9c:

yield (78%) as a yellow solid, m.p. >220°C, IR (KBr) 3393, 3055, 2924, 2874, 2359, 1738, 1626, 1574, 1489,1443, 1424, 1314, 1283, 1242, 1144, 1103, 1080, 878, 862, 814, 745 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 3.30 (s, 2H,, CH_2), 4.29 (s, 3H), 6.25-8.22 (m,20H, Ar-H), 8.91 (br. d, 1H, Ar-H), 10.11 (br. d, 1H, NH), 10.30 (br. S, 1H, NH). MS: 613($C_{35}H_{27}N_5O_4S$, M, 1.13%),

N-(4-(4-((2-chloro-5-methyl-5H-indolo[3,2-b]quinolin-11-yl)amino)benzyl)phenyl)-4-(phenyldiazenyl)benzenesulfonamide 9d:

yield (78%) as a yellow solid, m.p.187-191°C, (KBr) 3335, 3208, 3055, 2938, 1622, 1595, 1568, 1539, 1512, 1485, 1441, 1422, 1406, 1310, 1277, 1242, 1190, 1142, 1103, 1072, 955, 858,748, 733 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 3.82 (s, 2H, CH_2), 4.26 (s, 3H), 4.29 (s, 3H, CH_3), 6.63-8.20 (m, 24H, Ar-H), 8.90 (br.d, 1H, NH), 10.33 (br. s, 1H, NH). MS: 709 (M^+, 2.50%).

N-(4-(N-(4-(4-((2-chloro-5-methyl-5H-indolo[3,2-b]quinolin-11-yl)amino)benzyl)phenyl)sulfamoyl)phenyl)acetamide 9e:

yield (84%) as a yellow solid, m.p. 220-225 °C. IR (KBr) 3341, 3048, 3024, 2969, 2930, 1694, 1620, 1591, 1557, 1489, 1443, 1406, 1314, 1275, 1227, 1177, 1144, 891, 758, 718, 692 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 2.48 (s, 3H, CH_3CO), 3.84 (s, 2H, CH_2), 4.27 (s, 3H), 6.51-8.22 (m, 19H, Ar-H), 8.60 (d. 2H, Ar-H), 9.88 (br. s, 1H, NH), 10.09 (br. s, 1H, NH). MS: 660 (M^+, 3%).

2,4,6-Triisopropyl-N-(4-(4-((5-methyl-5H-indolo[3,2-b]quinolin-11-yl)amino)benzyl)phenyl)benzenesulfonamide 9f:

yield (86%) as a brown solid, m.p. **189-193** °C**,** IR (KBr) 3360, 3312, 2953, 2920, 2870, 1622, 1593, 1568, 1520, 1489, 1443, 1416, 1400, 1288, 1250, 1196, 1065, 1022, 841, 750 cm^{-1}, ^{1}H NMR (300 MHz, DMSO-d6) δ ppm: 1.01 (d,12H, 4CH_3), 1.22 (d, 6H, 2CH_3), 2.81 (septet, 3.30 (s, 2H, CH_2), 3.89 (s, 2H) 4.29 (s, 3H), 4.58 (septet, 1H), 6.23-8.28 (m, 16H, Ar-H), 8.85 (d. 2H, Ar-H), 10.11 (br. s, 1H, NH), 10.47(br. s, 1H, NH). MS: 694 (M^+, 5.23%).

In vitro anticaner bioassay Materials and methods Cell Cultures

A human breast (MCF-7), colon (HCT-116) and hepatocellular ($HepG_2$) cancer cell lines were 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) [23]. 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

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