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Synthesis and Antiproliferative activity of novel Acridine-biotin conjugates

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Abstract
Acridine-biotin conjugates 7 were synthesized in good yields by coupling aminoalkylaminoa acridines 5 with biotin 6. The key starting diamines 4 were synthesized by reaction of 9-chloroacridine 3 with diamines 4. The structure of the synthesized compounds were elucidated by the spectroscopic tools and showed results consistence with the proposed structures. Furthermore, the antiproliferative activity for the synthesized compounds were evaluated against hepatocellular (HepG2), colon (HCT-116) and breast (MCF-7) carcinoma cell lines using colorimetric MTT assay. The screening results showed that compound 7l exhibits the best activity with IC_{50}: 5.99, 8.84 and 7.80 µM against HepG2, HCT-116 and MCF-7 cancer cell lines respectively.

Keywords: chloroacridine, biotin, diamines, anticancer, doxorubicin

Introduction
Acridine containing analogues are one of the more studied chemotherapeutic agents with broad spectrum of activities,such as antimalarial [1], antiprotozoal [2], antibacterial [3], anticancer and DNA-intercalating agents [4]. These compounds are characterized by the presence of planner tricyclic fused aromatic system, and one or two flexible substituted pharmacophoric groups at different core position on the acridine skeleton. The acridine derivatives known as DNA-intercalators, such as nitracrine 1 [5], m-AMSA 2 [6], DACA 3 [7] and others
DNA intercalative agents as proflavine 4 [8] and ellipticine 5 [9] exhibit cytotoxic activity and some of them have been found to be clinically useful (Fig. 1).

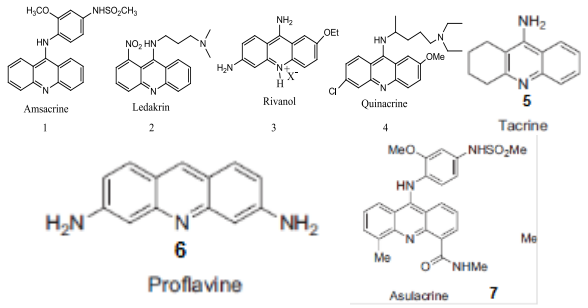
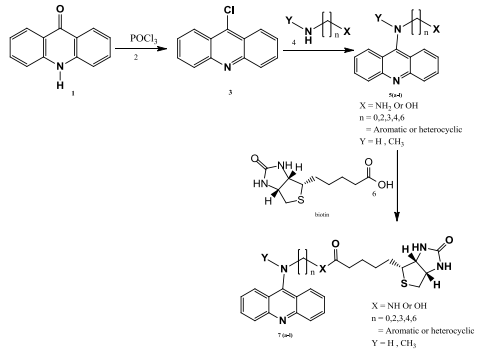


Fig 1: General formula of known anticancer agents.

Amsacrine 2 is the best-known compound of 9-anilinoacridines series. It was one of the first DNA-intercalating agents to be considered as a topoisomerase II inhibitor. The significant clinical use of several of these compounds is limited by problems such as side effects, drug resistance and poor bioavailability, which have encouraged further modifications to these compounds. At present, almost all the reported antitumor agents in the acridine series have been derived from pattern compounds and they have incorporated changes in the substituents or heterocyclic system modifications. SAR studies of acridine-based DNA-intercalating agents suggest that the mode of binding is important and the chromophore will locate to give maximum overlap with the DNA base pairs. The intercalative binding appears to be a necessary but not sufficient condition for the antiproliferative activity [10].
Recent work has based on conjugation of acridine moiety with biotin aiming to develop a selective anticancer agents. 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 [11]. The aim of this work is to synthesize new acridine conjugations of biotin and screening their anticancer properties.

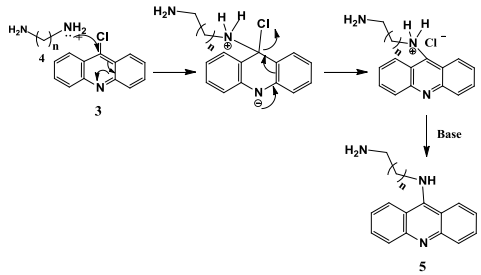
Result and discussion
Chemistry
The pathways to assemble the 9-aminoacridine core structure 5a-i have been developed starting from easily accessible intermediates as depicted in Schemes 1, this approach allowed us to synthesize new analogues with a varied substitution pattern at 9-position. The 9-chloroacridine 2, the key intermediate for further diversification was initially obtained according to the route depicted in Scheme 1. The synthetic methodology for 2 was achieved starting from commercially available starting material acridin-9(10H)-one 1 which, was dehydroxychlorinated with $POCl_3$ 2 to give 9-chloroacridine 3 in good yield. Then 3 was condensed with various diamines as listed in Scheme 1 to afford the 9-aminoalkylacridine derivatives 5a-i *via* nucleophilic aromatic substitution (S_{NAr}) reaction, in which the nucleophilic nitrogen the amino group attack the sp^2 carbon at 9-position of the acridine core followed by the displacement of chloride atom in a

nucleophilic aromatic substitution mechanism as depicted in Schemes 1 and 2.



Scheme 1: Synthesis of Acridine-Biotin Conjugates

The Nucleophilic aromatic substitution of the chloride atom at position C-9 of the 9-chloroacridine core by the appropriate aminoalkyl amines proceeds through the addition of the amino group (:Nu–) to form a resonance-stabilized anion with a new C– N bond as shown Scheme 2.



Scheme 2: Mechanism of aromatic nucleophilic substitution of 5a-I

Further 9-aminoacridine 5a-i was reacted with biotin 6. The carboxylic acid group of biotin underwent condensation with 9-aminoacridine deivatives 5a-i in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 1-hydroxylbenzotriazole (HOBt) as peptide coupling agent at room temperature to form the new hybrids 7a-l after purification in good yields. The analytical data for all compounds were in good agreement with the proposed structures.

Antiproliferative Activity

The anticancer activity evaluations of the prepared compounds have been assessed against the breast (MCF-7), colon (HCT-116) and hepatocellular (HepG2) carcinoma cell line. For comparison, doxorubicin (DOX) was used as a reference anticancer drug doxorubicin (DOX) was used. Dimethyl sulfoxide (DMSO) used as a control and a solvent for the cancer cells. Cell viability was assessed using the MTT assay. The key results obtained for compounds 7 toward the three cell lines are shown in Table 1 and dose-survival curves in figures 1. 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. Table 1). The screening results showed that compound 7l exhibits the best activity with IC_{50}: 5.99, 8.84 and 7.80 µM against HepG2, HCT-116 and MCF-7 cancer cell lines respectively.

Table 1: Antiproliferative activity of 7a-l against human cancer cell lines

	In vitro Cytotoxicity IC50 (µM)•		
	HePG2	HCT-116	MCF-7
DOX	4.50±0.2	5.23±0.3	4.17±0.2
7A	49.12±2.7	37.24±2.6	51.07±3.0
7B	87.49±4.1	49.54±3.3	72.73±3.9
7C	20.77±1.5	17.24±1.4	13.32±1.3
7D	13.35±1.0	11.37±1.2	10.19±1.1
7E	83.24±3.9	66.54±3.7	57.87±3.5
7F	33.67±2.1	30.09±2.5	19.88±1.6
7G	45.76±2.4	28.00±2.1	39.24±2.3
7H	>100	85.55±4.4	93.28±4.8
7I	54.06±2.9	46.60±2.8	40.57±2.6
7J	28.70±1.8	18.46±1.6	20.26±1.8
7K	68.33±3.2	83.33±4.2	68.55±3.7
7L	5.99±0.4	8.84±0.9	7.80±0.7

Experimental

General methods

All ^{1}HNMR experiments (solvent DMSO-d_6) 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., Biotin was obtained as commercial available at sigma – aldrich as a pure product, 9-chloroacridine was synthesized as in litreature]All reactions were followed by thin layer chromatography (TLC) on kiesel gel F254 precoated plates (Merck).

Synthesis of 9-chloro acridine

Acridin-9(10H)-one (1) (0.005 mol) was dissolved in phosphorus oxy chloride(22 ml) .The reaction mix was refluxed, at 90 -110°C for 3 hr, The reaction mixture was neutralized by pouring it on ice gradually with stirring then adding Sod. Bicarbonate gradually with stirring until neutralized (PH = 7) then filtrate, dry and recrystallized by ethanol [ ]

General procedure for synthesis 5(a-l)

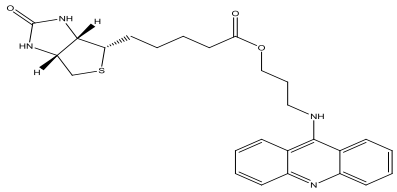
9-Chloroacridine (3) (0.001 mol) and appropriate excess amines (1.5 eq.) was dissolved in methanol in presence of 1.5 eq. Triethylamine. The reaction mixture was refluxed 3- 6 hr

at 70°C - 80°C until the starting material s were consumed as monitored by TLC. The reaction mixture was poured into ice water and left it in refrigerator. The solid formed was isolated by filtration and washed by cold water (200 ml), to afford 5(a-l)

General procedure for synthesis Acridine- Biotin hybrids 7(a-l)

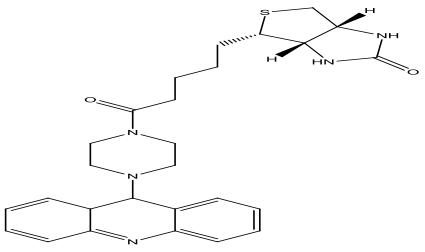
Compounds (3a-I) (0.01 mol) and HOBT (0.001 mol) were dissolved in CH2CL2 then added EDCI (0.001 mol), Biotin (0.001 mol) was added to the mixture at 0°C then left it on stirring (24 – 72 h) until the starting materials were consumed as monitored by TLC and evaporate the solvent to afford ppt. dried and crystallized by ethanol.

3-(acridin-9-ylamino)propyl-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (7a)



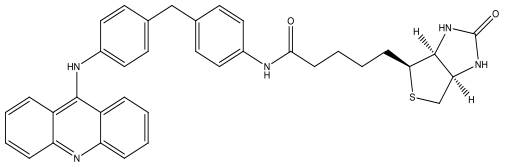
Yield (88%) yellow solid, m.p. 168˚C – 170°C. IR (KBr) cm^{-1}: 3300(NH), 2927(-CH asym),
1684 (C=O), 1632 (C=C, Ar), 748(-CH sym). ^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.31 (br.m, 2H, *CH_2*), 1.48(br. m, 2H, *CH_2*), 1.57(br. m, 2H, *CH_2*), 2.17(br.t, 2H, *CH_2*), 2.57 (br. s, 2H, *CH_2*), 2.81(br.d, 2H, CH2, J= 5.2Hz), 3.08 (br. m, 2H, *CH_2*), 3.50 (br. m, 2H, *CH_2*), 4.11 (br. m, H, *CH*), 4.28 (br. m, H, *CH*), 6.32 (br. s, H, *NH*), 6.39 (br. s, H, *NH*), 7.21-8.64 (br. m, 8H, *Ar-H*), 11.73 (br.s, H, NH). EIMS, m/z ($C_{26}H_{30}N_4O_3S$) calcd, 478.61 $[M]^+$; found, 478.34.

(3aS,4S,6aR)-4-(5-(4-(8a,9-dihydroacridin-9-yl)piperazin-1-yl)-5-oxopentyl)tetrahydro-1H-thieno[3,4-d]imidazol-2(3H)-one (7b)



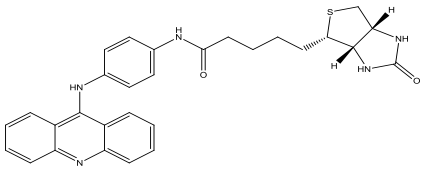
Yield (82%) yellow solid, m.p. 165°C – 170°C. IR (KBr) cm-1: 3300(NH), 2926(-CH sym), 1689(C=O). 1630 (C=C, Ar), 1345, 746(-CH asym). 1H NMR (DMSO-d6, 400 MHz) ppm: Mixture of diastereomers: 1.31(br. t, 2H, CH2), 1.47(br. t, 2H, CH2), 1.59(br. t, 2H, CH2), 2.18(br. t, 2H, *CH_2*), 2.59 (br. s, 2H, *CH_2*), 2.81(br. m, 2H, *CH_2*), 2.94 (br.s, 4H, 2CH2), 3.60 (br. m, H, *CH*), 3.73 (br. s, 4H, 2CH_2), 4.08(br.s, 4H,2 CH2), 4.28 (br. m, H, *CH*), 6.33 (br. s, H, *NH*), 6.40 (br. s, H, *NH*), 7.21-8.62 (br. m, 8H, *Ar-H*), 11.80 (br.s, H, NH OH). EIMS, m/z ($C_{27}H_{33}N_5O_2S$) calcd, 491.65 $[M]^+$; found, 490.02.

N-(4-(4-(acridin-9-ylamino)benzyl)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7C)



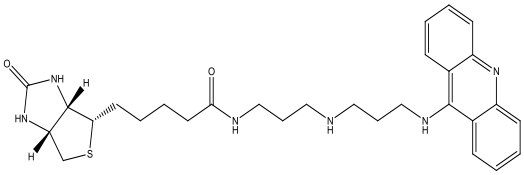
Yield (79%) green solid, m.p. 175°C – 178°C. IR (KBr) cm^{-1}: 3299(NH), 2919(-CH sym), 1683(C=O), 1632(C=C, Ar), 753(-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.30(br. m, 2H, CH_2), 1.46(br. m, 2H, *CH_2*), 1.58(br. m, 2H, *CH_2*), 2.18(br. t, 2H, *CH_2*), 2.55(br. s, 2H, *CH_2*), 2.80(br. m, 2H, *CH_2*), 3.10 (br. m, 2H, CH_2), 3.86 (br. m, H, *CH*), 4.10(br. m, H, CH), 4.28 (br. m, H, *CH*), 6.33 (br. s, H, *NH*), 6.40 (br. s, H, *NH*), 7.03-8.10 (br. m, 8H, *Ar-H*),11.13 (br. s, H, *NH*), 12.08 (br. s, H, NH OH). EIMS, m/z ($C_{36}H_{35}N_5O_2S$) calcd, 601.76 $[M]^+$; found, 600.99.

N-(4-(acridin-9-ylamino)phenyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7d)



Yield (80%) brown solid, m.p. 158°C -160°C. IR (KBr) cm^{-1}:3300(NH), 2920(-CH sym), 1687(C=O), 1632 (C=C, Ar), 744(-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.31(br. m, 2H, *CH_2*), 1.49(br. m, 2H, *CH_2*), 1.59(br. m, 2H, *CH_2*), 2.18(br. t, 2H, *CH_2*), 2.55 (br. d, 2H, *CH_2, J*= 12Hz), 3.08(br. m, 2H, *CH_2*), 3.30 (br. d, 2H, *CH_2, J*= 12Hz), 4.10 (br. m, H, *CH*), 4.28 (br. m, H, *CH*), 6.40 (br. s, 2H, 2*NH*), 7.21-8.62 (br. m, 12H, *Ar-H*),11.13 (br. s, H, *NH*), 11.81 (br. s, H, *NH OH*).EIMS, m/z ($C_{22}H_{29}N_5O_2S$) calcd, 511.64 $[M]^+$; found, 511.52.

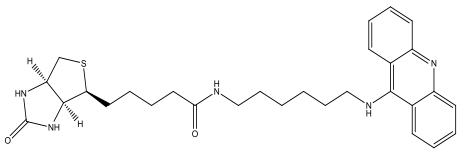
N-(3-((3-(acridin-9-ylamino)propyl)amino)propyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7e)



Yield (85%), green solid, m.p. 160°C – 163°C. IR (KBr) cm^{-1}: 3048 (NH), 2944 (-CH sym), 1692 (C=O), 1641(C=C, Ar), 741(-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.31(br. m, 2H, *CH_2*), 1.46 (br. m, 2H, *CH_2*),

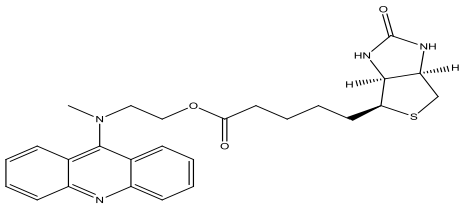
1.57 (br. m, 6H, 3CH_2), 2.18(br. t, 2H, CH_2), 2.41 (br. s, 2H, CH_2), 2.57 (br. s, 2H, CH_2), 3.08(br. m, 2H, CH_2), 3.65 (br. t, 2H, CH_2), 4.12 (br. m, H, *CH*), 4.28 (br. m, H, *CH*), 6.33 (br. s, H, *NH*),6.40 (br. s, H, *NH*), 7.21-8.21 (br. m, 8H, *Ar-H*), 9.10 (br. s, H, *NH*), 11.77 (br. s, H, *NH OH*). EIMS, m/z ($C_{29}H_{38}N_6O_2S$) calcd. 534.72 $[M]^+$; found, 538.45.

N-(2-((2-(acridin-9-ylamino)ethyl)amino)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7f)



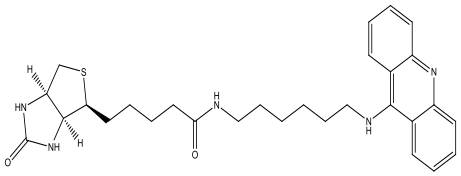
Yield (87%) green solid, m.p. 138°C – 140°C. ^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.31 (br. m, 2H, CH_2), 1.49 (br. m, 2H, CH_2), 1.57 (br. m, 2H, CH_2), 2.18 (br. t, 2H, CH_2), 2.63 (br. s, 2H, CH_2), 2.81(br. m, 2H, CH_2), 3.05 (br. m, 2H, CH_2),3.13 (br. m, 2H, CH_2), 3.75 (br. s, H, *CH*), 4.12 (br. d, H, *CH*), 4.28 (br. m, H, *CH*), 6.33 (br. s, H, *NH*), 6.40 (br. s, H, *NH*), 7.21-8.62 (br. m, 8H, *Ar-H*),9.10 (br. s, H, *NH*), 11.75 (br. s, H, *NH OH*).EIMS, m/z ($C_{27}H_{34}N_6O_2S$) calcd, 506.66 $[M]^+$; found, 506.60.

2-(acridin-9-yl(methyl)amino)ethy-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanoate (7g)



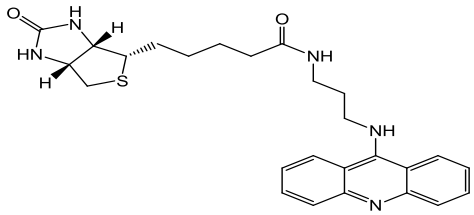
Yield (87%) pale yellow solid, m.p. 168°C – 170°C. IR (KBr) cm^{-1}: 3249(NH), 2927(-CH sym), 1691 (C=O), 1641 (C=C, Ar), 739 (-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.30 (br. t, 2H, CH_2), 1.48 (br. m, 2H, CH_2), 1.57 (br. m, 4H, 2CH_2), 2.17 (br. t, 2H, CH_2), 2.57 (br. s, H, CH_3), 2.78 (br. d, 2H, CH_2),2.80 (br. m, 2H, CH_2) 3.08 (br. m, 2H, CH_2), 3.80 (br. m, 2H, CH_2), 4.09 (br. s, H, *CH*), 4.28 (br. s, H, *CH*), 6.33 (br. s, H, *NH*), 6.40 (br. s, H, *NH*), 7.21-8.21 (br. m, 8H, *Ar-H*), 8.62 (br. s, H, *NH*), 11.81 (br. s, H, *NH OH*)**.** EIMS, m/z ($C_{26}H_{30}N_4O_3S$) calcd, 478.61 $[M]^+$; found, 477.46.

N-(6-(acridin-9-ylamino)hexyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7h)



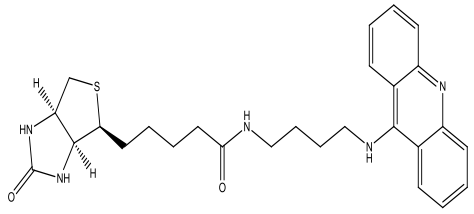
Yield (80%) green solid, m.p. 178°C – 180°C. IR (KBr) cm^{-1}: 3249 (NH), 2934 (-CH sym), 1692 (C=O,), 1640 (C=C Ar), 740 (-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.28 (br. t, 4H, 2CH_2), 1.46 (br. m, 4H, 2CH_2), 1.58 (br. m, 4H, 2CH_2), 2.17 (br. t, 2H, CH_2),2.80 (br. d, 2H, CH_2) 3.07 (br. m, 2H, CH_2), 4.12 (br. m, H, *CH*), 4.31 (br. m, H, *CH*), 6.32 (br. s, H, *NH*), 6.39 (br. s, H, *NH*), 7.21-8.21 (br. m, 8H, *Ar-H*), 8.63 (br. s, H, *NH*), 11.74 (br. s, H, *NH OH*). EIMS, m/z ($C_{29}H_{37}N_5O_2S$) calcd, 519.70 $[M]^+$ found, 519.67.

N-(3-(acridin-9-ylamino)propyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7i):



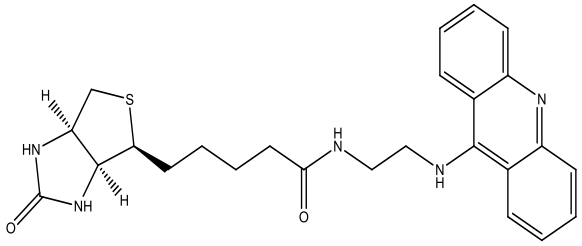
Yield (77%) yellow solid, m.p. 163°C – 165°C. IR (KBr) cm^{-1}: 3301 (NH), 2923 (-CH sym), 1687(C=O), 1632 (C=C, Ar), 761 (-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 1.29 (br. m, 2H, CH_2), 1.47 (br. m, 4H, 2CH_2), 1.58 (br. m, 2H, CH_2), 2.18 (br. m, 2H, CH_2), 2.54 (br. s, 2H, CH_2), 2.57 (br. s, 2H, CH_2),2.78 (br. d, 2H, CH_2),2.82 (br. d, 2H, CH_2), 3.08 (br. m, 2H, CH_2),3.54 (br. t, 2H, CH_2), 4.11 (br. s, H, *CH*), 4.28 (br. s, H, *CH*), 6.32 (br. d, H, *NH*), 6.39 (br. d, H, *NH*), 7.16 - 8.24 (br. m, 8H, *Ar-H*), 8.63 (br. d, H, *NH*), 11.74 (br. s, H, *NH OH*). EIMS, m/z ($C_{26}H_{31}N_5O_2S$) calcd, 477.62 $[M]^+$; found, 477.74.

N-(4-(acridin-9-ylamino)butyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7j)



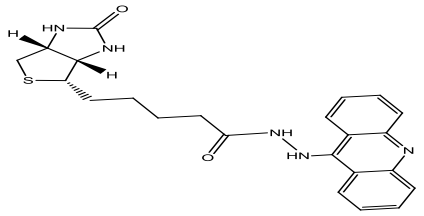
Yield (87%) brown solid, m.p. 132°C – 134°C. IR (KBr) cm^{-1}::- 3240 (NH), 2936 (-CH sym), 1694 (C=O), 1641 (C=C, Ar), 743 (-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 0.97 (br. t, 2H, CH_2), 1.29 (br. m, 2H, CH_2), 1.49 (br. m, 4H, 2CH_2), 1.67 (br. m, 2H, CH_2), 2.17 (br. t, 2H, CH_2), 2.27 (br. t, 2H, CH_2), 2.61 (br. s, 2H, CH_2), 3.02 (br. m, 2H, CH_2),3.50 (br. s, 2H, CH_2), 4.11 (br. m, H, *CH*), 4.28 (br. m, H, *CH*), 6.00 (br. d, H, *NH*), 6.37 (br. d, H, *NH*), 7.10 - 8.21 (br. m, 8H, *Ar-H*), 8.66 (br. s, H, *NH*), 11.90 (br. s, H, *NH OH*). EIMS, m/z ($C_{26}H_{32}N_5O_2S$) calcd, 478.63 $[M]^+$; found, 479.24.

N-(2-(acridin-9-ylamino)ethyl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanamide (7k)



Yield (89%) green solid, m.p. 142°C – 144°C. IR (KBr) cm-1:- 3290 (NH), 2935 (-CH sym), 1701 (C=O), 1631 (C=C, Ar), 748 (-CH asym).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 0.95 (br. t, 2H, *CH_2*), 1.38 (br. m, 8H, 4*CH_2*), 2.17 (br. t, 4H, 2*CH_2*), 2.80 (br. s, 2H, *CH_2*), 3.01 (br. s, 2H, *CH_2*),3.18 (br. s, 2H, *CH_2*),3.20 (br. s, 2H, *CH_2*),3.50 (br. s, 2H, *CH_2*), 4.10 (br. m, H, *CH*), 4.28 (br. m, H, *CH*), 6.00 (br. d, H, *NH*), 6.38 (br. d, H, *NH*), 7.23 - 8.21 (br. m, 8H, *Ar-H*), 8.70 (br. s, H, *NH*), 12.00 (br. s,H,*NH OH*).ESIMS, m/z ($C_{25}H_{29}N_5O_2S$) calcd, 463.60 $[M]^+$; found, 464.19.

N'-(acridin-9-yl)-5-((3aS,4S,6aR)-2-oxohexahydro-1H-thieno[3,4-d]imidazol-4-yl)pentanehydrazide (7l)



Yield (86%) brown solid, m.p. 128°C – 130°C. IR (KBr) cm^{-1}**:** 3275 (NH), 2924 (-CH sym), 1701 (C=O), 1631 (C=C Ar), 748 (-CH asym).).^{1}H NMR (DMSO-d_6, 400 MHz) ppm: Mixture of diastereomers: 0.95 (br. t, 2H, *CH_2*), 1.10 (br. m, 2H, *CH_2*), 1.31 (br. m, 2H, *CH_2*), 1.49 (br. m, 4H, 2*CH_2*), 2.18 (br. t, 2H, *CH_2*), 2.27 (br. t, 2H, *CH_2*), 2.78 (br. m, H, *CH*), 3.06 (br. m, 2H, *CH_2*),3.28 (br. s, 2H, *CH_2*), 4.10 (br. s, H, *CH*), 4.27 (br. s, H, *CH*), 6.06 (br. d, H, *NH*), 6.37 (br. d, H, *NH*), 6.94 - 8.23 (br. m, 8H, *Ar-H*), 10.56 (br. s, H, *NH*), 11.91 (br. s, H, *NH OH*). EIMS, m/z ($C_{23}H_{25}N_5O_2S$) calcd, 435.54 $[M]^+$; found, 436.39.

In vitro antiproliferative activity

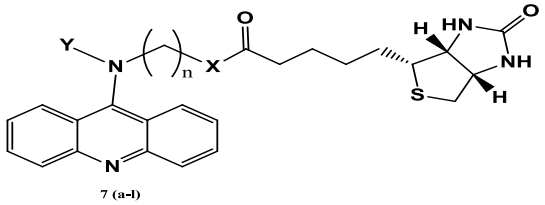
Materials and methods

A human breast (MCF-7), colon (HCT-116) and hepatocellular (HepG2) 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% CO2 humidified at 37°C for growth.

Evaluation of cell proliferation by MTT assay

The number of viable HepG2 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, HepG2 cells (1x104cells/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.



7 (a-l)

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

Conclusions

In this study, we have synthesized and characterized a series of new biotin – acridine hybrids, including compounds 7 a-I, their anticancer activity were evaluated by antiproliferative screening *in vitro* against a human breast (MCF-7), colon (HCT-116) and hepatocellular (HepG2) cancer cell lines. Results have shown that the most promising active hybrid 7I which showed IC50 of 5.99, 8.84 and 7.80 µM against hepatocellular carcinoma, colon and breast respectively. It was provide that the introduction of biotin with acridine with short linker improved the antiproliferative activity and selectivity towards cancer cell lines

Further studies on the modification of acridine and use different active cores are still ongoing.

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