Synthesis and Antiproliferative Activity of Chlorinated Maprotiline Analogues

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Abstract

A novel chlorinated tetracyclic compound 13 of the class ethanoanthracene, as analogue of maprotiline, was prepared via multistep syntheses. The tetracyclic key intermediates 4 and 5 with its [2.2.2] system were built via a Diels-Alder reaction between acrolein and 1,8-dichloroanthracene.The synthesized chlorinated maprotiline analogues 6 , 7 and 13 as well intermediates 4 and 5 exerted antiproliferative activity against cancer cell lines A549 and HepG2 at low micromolar concentrations. In addition, the intermediates 4 and 5 exerted high antiproliferative activity against HCT cell line. Interestingly, the intermediate 4 was the most active against all treated cell lines.

Introduction

Designing and synthesis of new agents, that selectively target the cancer cells whilst ignoring the normal cells, are imperative challenges for developing of save and effective anticancer drugs. Maprotiline **1** is a tetracyclic, with a secondary amine side chain, second generation antidepressant. It has a broad spectrum of activity in various types of depression and even effective in the treatment of therapy-resistant depression [1-3]. Maprotiline strongly blocks norepinephrine reuptake and has a weak effect on serotonin and dopamine reuptake. The multi-drug resistance (MDR) is considered one of the major problems in anticancer drugs treatment. The anti-multi-drug resistance (MDR) effect of maprotiline **1** on malaria strain plasmodium falciparum and cancer cell lines was reported [4-7]. In 2010, Cloonan *et al.* proved that maprotiline possesses potent selective antiproliferative activity against Burkitts Lymphoma (BL) independently of its classical target norepinephrine transporter but no target was identified, however cell death investigations was suggested [6]. Later the mechanism action of maprotiline as antiproliferative agent against drug-resistant Burkitts Lymphoma (BL) cell line was identified and found to induce autophagic cell death which doesn't involve poly(ADP-ribose) polymerase (PARP) cleavage, caspases or DNA fragmentation [7] On the other hand, series compounds structurally related to maprotiline structure exerted a potent effect on BL cell lines [8,9] and thus this class of compounds may hold medicinal applications.

Halogen bond, as an useful molecular design tool, has attracted interest in an experimental and theoretical chemistry aiming at improving drug-target binding affinity; it could be employed during ligand design to overcome drug resistance [10], to increase lipophilicity and to prolong the lifetime of the drug, thereby improving bioavailability. Halogenated compounds are important inhibitors against proteins those are involved in carcinogenesis [11-13]. A high-throughput drug screening study reported that about 50% of compounds contain halogens [14]. The insertion of a chlorine atom in the maprotiline related compounds could be provide a big improvement aiding in the development of more potent analogues, since the introduction of halogen atom into an organic molecule causes dramatic impact in its biological profiles.

Based on the above, we synthesized novel compounds related in structure to maprotiline of the class ethanoanthracene containing chlorine atoms, substituted in 11-position by a strain chain alkyl of one and

three carbon atoms connected by amino group, **Fig. 1**, and then evaluated their antiproliferative activities in vitro against three carcinoma cell lines; the lung carcinoma cell line A549, the hepatocellular carcinoma HepG2 cell line and the colorectal carcinoma HTC-116 cell line.

Results And Discussion

Chemistry

We previously reported a synthetic route of the maprotiline analogues 1-(4,5-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)-*N*-methylmethanamine (**6**) and 1-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)-*N*-methylmethanamine (**7**) [15,16]. In our continuation interest in Diels-Alder reaction [17-21], we developed a synthetic approach toward chlorinated tetracyclic maprotiline analogue 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)-*N*-methylpropan-1-amine (**13**). Referring to the **scheme 1**; The reduction of the commercially available starting material 1,8-dichlroroanthraquinone (**2**) to afford 1,8-dichloroanthracene (**3**) was the first step toward the forward synthesis of the target compounds **6**, **7** and **13**. In 1973, House *et al.* reported that 1,8-dichloroanthracene **3** was prepared by reducing 1,8-dichloroanthraquinone **2** with zinc powder in aqueous ammonia followed by an acidic treatment [22]. Later Zhao *et al.* reported that reduction could be accomplished with $NaBH_4$ in isopropanol [23]. In this work, 1,8-dichloroanthracene **3** was obtained in a good yield according to House *et al.* method [22]. The 1,8-dichloroanthracene **3** reacted with acrolein via Diels-Alder [4+2] cycloaddition reaction in dichloromethane at room temperature in the presence of boron trifluoride etherate as catalyst affording a mixture of the intermediate isomers 4,5-dihalo-9,10-dihydo-9,10-ethanoanthracene-11-carbaldehyde (**4**) and 1,8-dihalo-9,10-dihydo-9,10-ethanoanthracene-11-carbaldehyde (**5**). The obtained isomers **4** and **5** is due to the fact that both the 1,8-dichloroanthracene (diene) **3** and acrolein (dienophile) are unsymmetrical reactants. The mixture of carbaldehyde isomers **4** and **5** was chromatographed on silica gel with the eluent system ethyl acetate: petroleum ether (1:10) and carbaldehyde **4** was eluted first. The maprotiline analogues **6** and **7** were obtained by direct reductive amination of their respective carbaldehydes **4** and **5** respectively. The reductive amination of carbaldehydes **4** and **5** was carried out separately by treating with 3 molar equivalents of a commercially available solution of methylamine in methanol in the presence of Pd-C as heterogeneous catalyst and stirred for 4 hours at room temperature under H_2 (balloon). After filtration of the reaction mixture through a pad of celite and evaporation of the solvent, the corresponding amine analogues **6** and **7** were obtained. The carbaldehyde **5** was further recruited and converted by Wittig homologation using (carbethoxymethylene)triphenylphosphorane into two carbon homologated α,β unsaturated ester isomers namely; *Z*-ethyl 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propenoate (**8**) and *E*-ethyl 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propenoate (**9**). The Wittig reaction was smoothly carried out at room temperature for 5 hours in dichloromethane affording 97% of α,β-unsaturated ester isomers **8** and **9**. These isomers were easily separated by silica gel column chromatography with the eluent system ethyl acetate: petroleum ether (1:10). The NMR *J* coupling and chemical shift of the vinylic protons were employed to distinguish between the *Z* **8** and *E* **9** isomers. The ratio of the isomers, as deduced from integration of the vinylic

proton signals, is approximately 1:2. The 1H-NMR spectrum of the *Z* **8** showed double doublet signal at δ 5.47 ppm with coupling constants *J* = 11.3, 9.5 Hz integrated for the proton assigned for olefinic proton (-CH=CH-) and a doublet signal at δ 5.61 ppm with coupling constant *J* = 11.7 Hz integrated for the proton assigned for olefinic proton (-CH=CH-) attached to ester group (-COO-CH_2-CH_3). Whereas these signals of the *E* **9** appeared at δ 6.36 ppm as double doublet with coupling constants *J* = 15.4, 9.5 Hz and at δ 5.75 ppm as doublet with coupling constant *J* = 15.4 Hz. The α,β unsaturated esters **8** and **9**, products of the Wittig reaction, were then subjected to hydrogenation to reduce the double bond by stirring for 24 hours at room temperature in ethanol in presence of Pd/C under H_2 (balloon). After filtration of the reaction mixture through a pad of celite and solvent was removed in vacuo, the saturated ester Ethyl 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propanoate **10** was obtained in an excellent yield of 92 %. Reduction of ester **10** with reducing agent diisobutylaluminium hydride (DIBAL) at room temperature gave the alcohol 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propan-1-ol (**11**) in 53 % yield, which was completely oxidized using pyridinium chlorochromate (PCC) at room temperature in dichloromethane to give the desired aldehyde 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propanal (**12**). An attempt to transform the ester **10** to the aldehyde **12** in one step according to the known literature procedure [24] using DIBAL at -78 ^{0}C resulted in a mixture of aldehyde **12** and its corresponding alcohol **11**. Direct reductive amination of the aldehyde **12** by the same procedure applied to synthesize the chlorinated maprotiline analogues **6** and **7** led to the desired chlorinated maprotiline analogue **13**. In fact, the reductive amination using Pd-C in methanol led to obtain chlorinated normaprotiline **13** and normaprotiline with no chlorine atoms, so in case dechlorination of aromatic rings was mainly occurred, the chlorinated normaprotiline 2 was solely obtained according to the literature [25]. The overall yield of the synthesis of the target (76) using DIBAL at – 78 ^{0}C and at room temperature was 19 % and 15.3 % respectively.

In vitro anticancer activity evaluation

The cancer cell lines were incubated with serial dilution of each tested compounds (from 313 pg ML^{-1} to 5 mg ML^{-1}) in a 96-well plate for 4 days, and then investigated for growth inhibition by MTT test (MTT= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Maprotiline **1** was used as a positive control. Previous studies reported that maprotiline showed a potential antiproliferative activity against BL lymphoma cell line DG-75 [6,7]. A number of 9,10-dihydro-9,10-ethanoanthracenes exhibited a potent antiproliferative activity through inducing apoptosis and caspase activation in BL cell lines as well displayed activity in multi-drug resistant (MDR) cells. Furthermore many of those compounds were more active than maprotiline [8]. Our results showed that all tested maprotiline analogues as well intermediates were able to inhibit the growth of cancer cell lines A549 and HepG2 at low micromolar concentrations. In addition, the intermediates compounds **4** and **5** were also able to inhibit the growth of a third cell line (HCT). The IC_{50} values of all tested compounds including maprotiline, are given in **Table 1**. The chlorinated maprotiline analogues **6, 7** and **13** exhibited a potent antiproliferative activity against A549 cell line with IC_{50} values 25.5, 18.9 and 7.8 µg/mL respectively as well as against HepG2 cell line with IC_{50} values 12.66, 13.8 and 4.44 µg/mL while these maprotiline analogues had no effect against HCT cell

line. The results showed that intermediates compounds **4** and **5** with formyl group were more potent than compounds **6, 7** and **13** against A549 and HepG2 cell lines, since the IC_{50} of compounds **4** and **5** were 1.1 and 3.71 against A549 and 0.12 and 0.65 µg/mL against HepG2 respectively. That's mean, the sensitivity of the treated cancer cells to the compounds **4** and **5** were six times higher than maprotiline **1** in case of the breast cancer cell line A459, and forty times higher in case of hepatocyte carcinoma cell line HepG2. Furthermore, The IC_{50} of compounds **4** and **5** against HCT were 0.4 and 0.7 µg/mL respectively. Importantly, the compound **4** with formyl group above on chlorine atom was the most potent against all three tested cancer cell lines. These results indicated to a direct or an indirect biological role of the chemical nature of formyl group and its position in compounds **4** and **5**. Further investigations for these compounds are suggested.

Table 1. The IC_{50} of the Tested Compounds

Substance	A549	HepG2	HCT
	IC_{50} value µg/mL	IC_{50} value µg/mL	IC_{50} value µg/mL
4	1.1(0.2)±	0.12 (±0.03)	0.4 (±0.1)
5	3.71 (±0.8)	0.65 (±0.1)	0.7 (±0.1)
6	25.5 (±5.2)	12.66 (±4.4)	ND
7	18.9 (±2.5)	13.8 (±0.6)	ND
13	7.8 (±1.25)	4.44 (±0.41)	ND
Maprotiline	6.1 (±1.16)	5.15 (±0.77)	ND

ND=Not determined

Experimental

Synthetic procedures

4.1.1. Synthesis of: 1,8-dichloroanthracene (**3**), 4,5-dichloro-9,10-dihydro-9,10-ethanoanthracene-11-carbaldehyde (**4**), 1,8-dichloro-9,10-dihydro-9,10-ethanoanthracene-11-carbaldehyde (**5**), 1-(4,5-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)-*N*-methylmethanamine (**6**) and 1-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)-*N*-methylmethanamine (**7**).

The compounds **3-7** were synthesized according to [15,22] and their characterizations were also recorded in supplementary information.

4.1.2. Synthesis of *Z*-ethyl 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propenoate (**8**) and *E*-ethyl 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propenoate (**9**)

(Carbethoxymethylene)triphenylphosphorane (2 g, 5.75 mmol) was added to a solution of the aldehyde **5** (1.2 g, 4 mmol) in (36 mL) CH_2Cl_2. The reaction mixture was stirred at room temperature for 5 h. The solvent was removed and the residue was purified via flash column chromatography on silica gel using (Ethyl acetateA/Petroleum ether, 1:5) to afford separable isomers **8** and **9** (1.45 g, 97 %) in ratio of 1:2 respectively as yellow oil.

Compound **8**: IR (KBr): v = 3066, 2927, 2860, 1716, 1641, 1575, 1456, 1190, 1029, 771, 759,594 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.20 (t; *J* = 7.3, 3H, -O-CH_2-CH_3), 1.24-1.29 (m; 1H, H-12) 2.11-2.14 (m; 1H, H-12), 3.76 (m; 1H, H-11), 4.08 (q; *J* = 7.3, 2H, -O-CH2-CH_3), 4.18 (d; *J* = 2.9, 1H, H-10), 5.29 (t; *J* = 2.9, 1H, H-9), 5.47 (dd; *J* = 11.3, 9.5, 1H, -CH=CH-COO-), 5.61 (d; *J* = 11.7, 1H, -CH=CH-COO-), 6.96-7.19 (m; 6 H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ =14.2, 33.1, 36.5, 36.9, 49.7, 60.0, 119.6, 122.1, 123.7, 126.6, 126.8, 127.0, 129.3, 129.5, 139.5, 140.3, 142.3, 145.2, 152.2, 166.0 ppm; MS (EI): m/z (%) = 372 (10) [M^+], 367 (5), 248 (65), 246 (100), 176 (18), 131 (5), 69 (12); HRMS (EI): Calcd. For $C_{21}H_{18}O_2Cl_2$ [M^+] 372.0684, Found 372.0683.

Compound **9**: IR (KBr): v = 3066, 2979, 2935, 2898, 1718, 1650, 1577, 1456, 1446, 1369, 1271, 1180, 1039, 985, 769, 740, 703, 590 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ = 1.22 (t; *J* = 7.3, 3H, -O-CH2-CH_3), 1.22-1.24 (m; 1H, H-12) 2.04-2.07 (m; 1H, H-12), 2.71 (m;1H, H-11), 4.12 (q; *J* = 7.3, 2H, -O-CH2-CH_3), 4.20 (d; *J* = 2.2, 1H, H-10), 5.35 (t; *J* = 2.5, 1H, H-9), 5.75 (d; *J* = 15.4, 1H, -CH=CH-COO-), 6.36 (dd; J = 15.4, 9.5, 1H, -CH=CH-COO-), 7.04-7.24 (m; 6 H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 14.2, 32.0, 36.8, 40.9, 49.7, 60.3, 121.5, 121.9, 123.8, 126.7, 126.9, 127.0, 127.1, 129.3, 129.8, 139.5, 140.2, 141.6, 145.1, 150.5, 166.2 ppm; MS (EI): m/z (%) = 372 (41) [M^+], 367 (19), 248 (62), 246 (100), 176 (29); HRMS (EI): Calcd. For $C_{21}H_{18}O_2Cl_2$ [M^+] 372.0684, Found 372.0683.

4.1.3. Synthesis of ethyl 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propanoate (**10**)

In a two-necked round-bottomed flask (0.37 g of 10% Pd/C) was wetted with ethanol and the flask was evacuated, and flushed with hydrogen two times, then a solution of (1.3 g, 3.5 mmol) unsaturated ester **8** and **9** in (40 mL) ethanol was added to the reaction mixture. The mixture was stirred for 24 h at room temperature under H_2 (balloon). The reaction mixture was filtered through a pad of celite and the solvent was removed in vacuo to afford the corresponding **10** (1.2 g, 92%) as yellow oil.

IR (KBr): v = 3020, 2933, 2900, 1733, 1460, 1375, 1261, 1176, 1029, 754, 559 cm^{-1}; ^1HNMR ($CDCl_3$, 400 MHz): δ = 1.12-1.16 (m; 2H, H-/1), 1.24 (t; *J* = 7.3, 3H, -O-CH_2-CH_3), 1.46-1.51 (m; 1H, H-12), 1.86-1.93 (m; 1H, H-11), 1.97-2.04 (m; 1H, H-12), 2.31 (t; *J* = 8.0, 2H, H-/2), 4.08 (q; *J* =7.3, 2H, -O-CH_2-CH_3), 4.15 (d; *J* = 2.2, 1H, H-10), 5.29 (t; *J* = 2.5, 1H, H-9), 6.99-7.25 (m; 6 H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ =14.1, 31.1, 32.4, 34.3, 37.9, 44.1, 48.7, 60.2, 122.9, 123.2, 123.3, 125.2, 125.4, 125.5, 125.5, 125.8, 140.4, 143.2, 143.7, 144.2, 173.4 ppm; MS (EI): m/z (%) = 374 ([M^+], not recorded), 331 (11), 329 (25), 295 (12), 248 (58), 246 (100), 212 (46), 178 (45).

4.1.4. Synthesis of 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propan-1-ol (**11**)

To a solution of saturated ester **10** (600 mg, 1.6 mmol) in CH_2Cl_2 (6 mL), DIBAL (7 mL) was added. The reaction mixture was stirred for 5 h at room temperature. Then the reaction mixture was quenched with Methanol (1 mL) followed by the addition of ethyl acetate (30 mL) and saturated aqueous of NH_4Cl (10 mL). The quenched reaction mixture was filtered through suction funnel and extracted with CH_2Cl_2 and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified via flash column chromatography on silica gel using ethyl acetate / hexane (1:3) to afford **11** (280 mg, 53 %) as a milky viscous oil.

IR (KBr): v = 3577, 3336, 2970, 2933, 2860, 1456, 1055, 756, 567cm^{-1}; ^{1}HNMR ($CDCl_3$, 400 MHz): δ = 0.87-0.98 (m; 2H, H-/1), 1.13-1.28 (m; 1H, H-12), 1.53-1.63 (m; 1H, H-11), 1.84-1.93 (m; 2H, H-/2), 1.97-2.08 (m; 1H, H-12), 3.47 (t; *J* = 6.6, 2H, -CH_2OH), 4.04 (d; *J* = 2.2, 1H, H-10), 4.16 (t; *J* = 2.5, 1H, H-9), 7.01-7.17 (m; 6 H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 30.6, 32.2, 34.6, 38.2, 44.3, 48.9, 62.8, 122.9, 123.1, 123.3, 125.2, 125.3, 125.4, 125.5, 125.6, 140.7, 143.3, 143.8, 144.4 ppm; MS (EI): m/z (%) = 332 ([M^+], not recorded), 295 (7), 264 (12), 212 (29), 178 (100), 1152 (4).

4.1.5. Synthesis of 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)propanal (**12**)

To a solution of alcohol **11** (250 mg, 0.75 mmol) in CH_2Cl_2 (6 mL), PCC (250 mg, 1.2 mmol) was added. The reaction mixture was stirred for 2 h at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified via flash column chromatography on silica gel using ethyl acetate / hexane (1:3) to afford **12** (250 mg, 100 %) as a colorless oil.

IR (KBr): v = 3020, 2935, 2862, 2812, 1726, 1460, 1172, 1026, 760, 754, 559 cm^{-1}; ^{1}HNMR ($CDCl_3$, 400 MHz): δ = 1.12-1.25 (m; 2H, H-/1), 1.43-1.50 (m; 1H, H-12), 1.84-1.90 (m; 1H, H-11), 1.97-2.04 (m; 1H, H-12), 2.41-2.45 (m, 2H, H-/2), 4.10 (d; *J* = 2.2, 1H, H-10), 4.25 (t; *J* = 2.9, 1H, H-9), 7.09-7.25 (m; 6 H, ArH), 9.69 (t; *J* = 1.4, 1H, CHO) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 28.3, 34.5, 37.9, 41.9, 44.1, 48.7, 123.0, 123.2, 123.4, 125.6, 125.5, 125.6, 125.6, 125.9, 140.2, 143.1, 143.7, 144.0, 202.1 ppm; MS (EI): m/z (%) = 330 ([M^+], not recorded), 321 (7), 319 (11), 311 (12), 289 (7), 251 (6), 225 (8), 204 (11), 201 (21), 199 (51), 197 (92), 181 (28), 165 (100), 151 (38), 149 (15).

4.1.6. Synthesis of 3-(1,8-dichloro-9,10-dihydro-9,10-ethanoanthracen-11-yl)-N-methylpropan-1-amine (**13**)

In a two-necked round-bottom flask (100 mg, 10% Pd/C) was wetted with dichloromethane and the flask was evacuated, flushed with hydrogen two times, then a solution of (110 mg, 0.33 mmol) aldehyde **12** in (5 mL) methanol was added to the reaction mixture followed by the addition of (0.7 mL , 2 M) solution of methylamine in methanol. The mixture was stirred for 4 h at room temperature under H2 (balloon). The reaction mixture was filtered through a pad of celite and the solvent was removed in vacuo to yield (90 mg, 79 %) of the corresponding amine **13** as white powder, mp: 178 ^{0}C.

IR (KBr): v = 3414, 2935, 2864, 1471, 1399, 1171, 1034, 804, 752, 551, 466 cm^{-1}; ^{1}HNMR ($CDCl_3$, 400 MHz): δ = 0.79-0.87 (m; 2H, H-/1), 1.0-1.10 (m; 2H, H-/2), 1.2 (s; 3H, N-CH3), 1.73-1.79 (m; 1H, H-11), 1.85-1.92 (m; 2H, H-12), 2.68-2.72 (m; 2H, H-/3), 3.18 (d; *J* = 1.8, 3H, N-CH3), 4.03 (d; *J* = 2.2, 1H, H-10), 4.13 (t; *J* = 2.2, 1H, H-9), 6.97-7.02 (m; 3H, ArH), 7.13-7.18 (m; 3H, ArH) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ = 23.8, 32.8, 32.9, 34.4, 37.7, 44.1, 48.5, 49.2, 122.9, 123.3(2x), 125.2, 125.4, 125.5, 125.6, 125.8, 140.3, 143.2, 143.6, 144.0 ppm; MS (EI): m/z (%) = 346 (18) [M^+ + H], 336 (19), 335 (31), 334 (75), 332 (100), 326 (5), 318 (11), 298 (11), 286 (5), 284 (9); HRMS (EI): Calcd. For $C_{20}H_{22}NCl_2$ [M^+] 346.1129, Found 346.1128.

MTT assay protocol

In vitro antiproliferative activities of the synthesized chlorinated maprotiline analogues **6**, **7** and **13** as well intermediates **4** and **5** were demonstrated by determining the IC_{50} values against three cancer cell lines; the lung carcinoma cell line A549, the hepatocellular carcinoma HepG2 cell line and the colorectal carcinoma HTC-116 cell line (**Table 2.**). Growth inhibitions were measured in 96-well plates. Aliquots of 120 µL of the suspended cells (50,000 mL^{-1}) were given to 60 µL of a serial dilution of the tested compound. After 5 days of incubations, growths were determined the MTT assay. Briefly, 20 µL MTT (5 mg/mL in PBS) was added to each well, and the plate was incubated for 2 h at 37 ^{0}C, and 5% CO_2-atmosphere in the cell incubator. Then the supernatant was discarded and 200 µL of isopropanol/ HCl was added to each well. The absorbance was read at 550 nm using a microplate reader (Thermo Scientific, USA). The viability of the cells was calculated by dividing the absorbance average of the treated cells by the absorbance average of the control cells multiply 100%. The IC_{50} value was defined as a concentration that inhibits 50% of cell growth. The activities of the cells were plotted against the concentration of the drugs, and the IC_{50} values were calculated from the regression curves.

Table 2. Cancer Cell Lines

No.	Cell line	ATCC-No.	Disease or organ
1	A549	A549 (ATCC® CCL-185™)	Lung Carcinoma
2	HepG2	HepG2 [HEPG2] (ATCC® HB-8065™)	Hepatocellular carcinoma
3	HTC-116	HCT 116 (ATCC® CCL-247™)	Colorectal Carcinoma

Conclusion

In conclusion, a simple, economical and flexible synthetic route of tetracyclic chlorinated maprotiline analogue **13** was reported. This analogue and intermediates were found to exert potent antiproliferative activities against three carcinoma cell lines; A549, HepG2, and HTC-116. Among of the tested

compounds, the intermediate **4** was the most active against all treated cell lines. Further investigations of these compounds, in particular compounds **4** and **5**, are suggested.

Abbreviations

MDR: multi-drug resistance; BL: Burkitts Lymphoma; PARP: poly(ADP-ribose) polymerase; NMR: Nuclear magnetic resonance ; DIBAL: diisobutylaluminium hydride; PCC: Pyridinium Chlorochromate; IC50: half maximal inhibitory concentration; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; HTC-116: Hepatoma tissue culture-116; HepG2: human hepatocellular carcinoma; A549: Carcinomic human alveolar basal epithelial cell line; PBS: Phosphate Buffered Saline; Pd/C: Palladium on carbon; DNA: Deoxyribonucleic Acid

Declarations

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Author contributions

MAS, AIA, KET and UK designed research; MAS and UK carried out the synthetic experiments; YAE, TAM performed the anticancer evaluation studies. MAS, UK, YAE, TAM, MSAG and analyzed the data; All authors wrote the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The manuscript does not contain studies with animal subjects.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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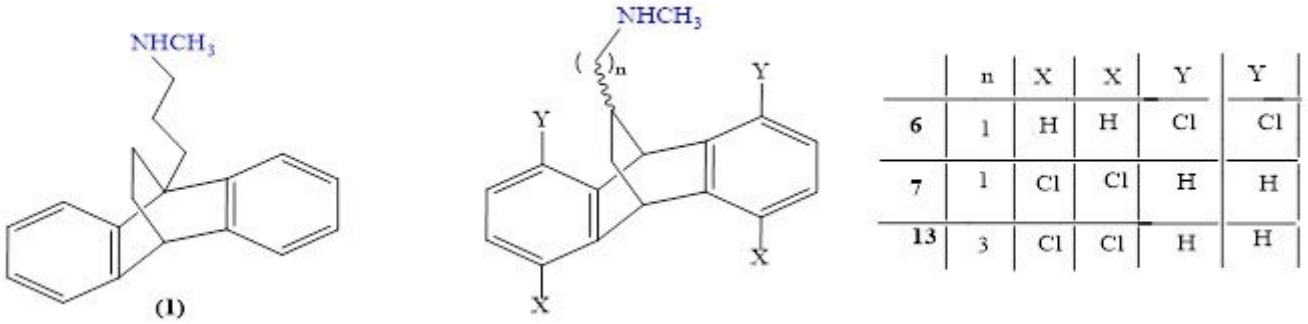

Figure 1

Maprotiline and chlorinated tetracyclic analogues.

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