Synthesis and Antiproliferative Activity of Chlorinated Maprotiline Analogues

Mujeeb A. Sultan (✉ Alhosami1983@yahoo.co.uk)

Aljanad university for science and technology

Usama Karama

king saud university

Abdulrahman I. Almansour

King saud university

Kamal Eldin El-Taher

king saud university

Yasser A. Elnakady

king saud university

Talal Abdulaziz Mohaya

king Saud University

Mansour S. A. Galil

Aljanad University fro science and technology

Research article

Keywords: Multistep syntheses; Diels-Alder reaction, Chlorinated maprotiline analogues; Antiproliferative activity

Posted Date: December 17th, 2019

DOI: <https://doi.org/10.21203/rs.2.18805/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

A novel chlorinated tetracyclic compound **13** of the class ethanoanthracene as analogue of maprotiline was prepared via multistep syntheses. The tetracyclic key intermediate **5** with its [2.2.2] system was built nicely and economically in one-step via a Diels-Alder reaction between acrolein and 1,8-dichloroanthracene, followed by Wittig C-2 homologation. The synthesized chlorinated maprotiline analogues **6**, **7** and **13** as well intermediates **4** and **5** exerted antiproliferative activity in cancer cell lines A549 and HePG2 at low micromolar concentrations. In addition, the intermediates **4** and **5** were also had high activity against HCT cell line. Interestingly, the intermediate **4** was the most active against all cell lines studied.

Introduction

Designing and synthesis of new agents that target the processes of cancer cells selectively whilst ignoring the normal cells of the body is imperative challenge 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 block 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 possess 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 does not involve poly(ADP-ribose) polymerase (PARP) cleavage, caspases or DNA fragmentation (**7**). On the other hand, series compounds structurally related to maprotiline exert a potent caspase-dependant apoptotic effect on BL cell lines (**8**) and thus this class of compounds may hold an diverse medicinal application.

Halogen bond, an useful molecular design tool, has attracted increased interest in experimental and theoretical chemistry aiming at improving drug-target binding affinity; it could use for designing ligand to overcome drug resistance (**9**) and 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 (**10-12**). It has been reported that about 50% of compounds in high-throughput drug screening contains halogens (**13**). The insertion of a chlorine atoms in the maprotiline related compounds could be provide a big improvement aiding in the development of more potent analogues, since introduction of the halogen atom into an organic molecule cause dramatic impact in its biological profiles.

Based on the above, we decided to synthesis a 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, two and three carbon atoms both of which are substituted by amino group for use in medicine, particularly as potential anticancer agents as well as certain new intermediates, **Fig. 1**, and evaluated in vitro their anti-cancer activity on three carcinoma cell lines, including, 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 recently reported a synthetic route to obtain 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**) (14, 15) and in our continuation interest in Diels-Alder reactions as well tetracyclic compounds (16-19) 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-dichloroanthraquinone (**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 (20). Later Zhao et al., reported that reduction could be accomplished with NaBH₄ in isopropanol (21). In this work, 1,8-dichloroanthracene **3** was obtained in good yield according to House et al. method (20). 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 to afford a mixture of the intermediate isomers 4,5-dihalo-9,10-dihydro-9,10-ethanoanthracene-11-carbaldehyde (**4**) and 1,8-dihalo-9,10-dihydro-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) being unsymmetrical. The mixture of carbaldehyde isomers 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 H₂ (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-CH₂-CH₃). 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 H₂ (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 (22) using DIBAL at -78 °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**. The overall yield of the synthesis of the target (76) using DIBAL at -78 °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 compound (from 313 $\mu\text{g mL}^{-1}$ to 5 $\mu\text{g mL}^{-1}$) in a 96-well plate for 4 days, and then tested for growth inhibition by MTT-Test. Maprotiline **1** had been 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 were exhibited potent antiproliferative activity through inducing apoptosis and caspase activation in BL cell lines as well some of these compounds displayed activity in multi-drug resistant (MDR) cells. Furthermore many of these compounds were more active than maprotiline (8). Our results showed that all tested maprotiline analogues as well intermediates were able to inhibit the growths of the 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 IC₅₀ values of all compounds, and that from maprotiline, are given in Table 1. The chlorinated maprotiline analogues **6**, **7** and **13** were found to exhibit a potent antiproliferative effect on A549 cell line with IC₅₀ values 25.5, 18.9 and 7.8 $\mu\text{g/mL}$ respectively as well as against HePG2 cell line with IC₅₀ values 12.66, 13.8 and 4.44 $\mu\text{g/mL}$ while these maprotiline analogues had no effect on 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, the IC₅₀ of compound **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 sensitivities of the treated cancer cells to **4** and **5** were **6** times higher than maprotiline in case of the breast cancer cell line A459, and even 40 times higher in case of hepatocyte carcinoma cell line HepG2. Furthermore, The IC₅₀ of compounds **4** and **5** against HCT were 0.4 and 0.7 µg/ml respectively. Importantly the intermediates compound **4** with formyl group above on chlorine atom was the most potent against all three tested cancer cell lines. This result shows a direct or an indirect role of the formyl group and its position in the biological activity of the two compounds **4** and **5**. Further investigations for these compounds are suggested.

Table 1. The IC₅₀ of the Tested Compounds

Substance	A549 IC ₅₀ value µg/ml	HepG2 IC ₅₀ value µg/ml	HCT 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 (14, 20) and characterization is also recorded in supplementary data.

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) CH₂Cl₂. 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 acetate/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): ν = 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-CH₂-CH₃), 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-CH₃), 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 $\text{C}_{21}\text{H}_{18}\text{O}_2\text{Cl}_2$ [M^+] 372.0684, Found 372.0683.

Compound **9**: IR (KBr): ν = 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-CH₃), 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-CH₃), 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 $\text{C}_{21}\text{H}_{18}\text{O}_2\text{Cl}_2$ [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 H₂ (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): ν = 3020, 2933, 2900, 1733, 1460, 1375, 1261, 1176, 1029, 754, 559 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz): δ = 1.12-1.16 (m; 2H, H-/1), 1.24 (t; J = 7.3, 3H, -O-CH₂-CH₃), 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-CH₂-CH₃), 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 CH₂Cl₂ (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 NH₄Cl (10 ml). The quenched reaction mixture was filtered through suction funnel and extracted with CH₂Cl₂ and the combined organic layers were washed with brine, dried over MgSO₄, 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): ν = 3577, 3336, 2970, 2933, 2860, 1456, 1055, 756, 567 cm⁻¹; ¹H NMR (CDCl₃, 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, -CH₂OH), 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; ¹³C NMR (CDCl₃, 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 CH₂Cl₂ (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): ν = 3020, 2935, 2862, 2812, 1726, 1460, 1172, 1026, 760, 754, 559 cm⁻¹; ¹H NMR (CDCl₃, 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; ¹³C NMR (CDCl₃, 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 °C.

IR (KBr): ν = 3414, 2935, 2864, 1471, 1399, 1171, 1034, 804, 752, 551, 466 cm⁻¹; ¹H NMR (CDCl₃, 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; ¹³C NMR (CDCl₃, 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 C₂₀H₂₂NCl₂ [M⁺] 346.1129, Found 346.1128.

MTT assay protocol

In vitro anti-cancer activity was demonstrated by determining the IC₅₀ values in three cancer cell lines, including 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 μ L of the suspended cells (50,000 mL⁻¹) were given to 60 μ L of a serial dilution of the inhibitor(S). After 5 days of incubations, growths were determined the MTT assay (MTT= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Briefly, 20 μ L MTT (5 mg/mL in PBS) were added to each well, and the plates were incubated for 2 h at 37°C, and 5% CO₂-atmosphere in the cell incubator. The supernatants were then discarded and 200 μ L of isopropanol/ HCl were added to each well and mixed to dissolve the formazan crystals. The absorbance was then 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 IC₅₀ values were defined as sample concentration inhibiting 50% of cell growth. The activities of the cells were plotted against the concentration of the drugs, and the IC₅₀ values were calculated from the regression curve.

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 synthesis route of tetracyclic chlorinated maprotiline analogue was described. The key cyclisation step was achieved through Diels-Alder reaction at room

temperature followed by Wittig C-2 homologation. This analogue and intermediates were found to exert potent anticancer activity. In particular, in vitro anticancer activity was demonstrated by determining the IC50 values in three carcinoma cell lines, including, the lung carcinoma cell line A549, the hepatocellular carcinoma HePG2 cell line, and the colorectal carcinoma HTC-116 cell line.

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

Acknowledgements

The authors would like to express their appreciation to Chemistry Department, College of Science, King Saud University, Riyadh, Saudi Arabia, as well as great thanks to Aljanad University for Science and Technology, Taiz, Republic of Yemen.

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.

Funding

The research was partially funded by King Saud University, Deanship of Scientific Research, College of Science Research Center.

Availability of data and materials

All data and materials are available on request (Mujeeb A. Sultan)

Competing interests

The authors declare that they have no competing interests.

Author details

¹ Department of Pharmacy, Faculty of Medical Sciences, Al-Janad University for Science and Technology, Taiz, Republic of Yemen. ² Chemistry Department, College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia. ³ Department of Pharmacology, College of Pharmacy, King Saud University, P. O. Box 2457, Riyadh 11451, Saudi Arabia. ⁴ Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11415, Saudi Arabia. ⁵ King Abdelaziz City for Science and Technology, P.O. Box. 6086, 11442 Riyadh, Saudi Arabia. ⁶ Chemistry Department, Faculty of applied science, Taiz University, Republic of Yemen. ⁷ Al-Janad University for Science and Technology, Taiz, Republic of Yemen.

References

1. Morishita S, Arita S. The use of maprotiline for major depression: a clinical report of 62 cases. *J Appl Res.* 2004;4,252-6.
2. Wilhelm M, Schmidt P. Synthese und Eigenschaften von 1-Aminoalkyl-dibenzo [b, e] bicyclo [2.2. 2] octadienen. *Helvetica chimica acta.* 1969;52(6):1385-95.
3. Kielholz P. Treatment for therapy-resistant depression. *Psychopathology.* 1986;19(Suppl. 2):194-200.
4. Szabó D, Szabó G, Ocsovszki I, Aszalos A, Molnár J. Anti-psychotic drugs reverse multidrug resistance of tumor cell lines and human AML cells ex-vivo. *Cancer letters.* 1999;139(1):115-9.
5. Bitonti AJ, Sjoerdsma A, McCann PP, Kyle DE, Oduola A, Rossan RN, et al. Reversal of chloroquine resistance in malaria parasite *Plasmodium falciparum* by desipramine. *Science.* 1988;242(4883):1301-3.
6. Cloonan SM, Drozgowska A, Fayne D, Williams DC. The antidepressants maprotiline and fluoxetine have potent selective antiproliferative effects against Burkitt lymphoma independently of the norepinephrine and serotonin transporters. *Leukemia & lymphoma.* 2010;51(3):523-39.
7. Cloonan SM, Williams DC. The antidepressants maprotiline and fluoxetine induce Type II autophagic cell death in drug-resistant Burkitt's lymphoma. *International Journal of Cancer.* 2011;128(7):1712-23.
8. McNamara Y, Bright S, Byrne A, Cloonan S, McCabe T, Williams D, et al. Synthesis and antiproliferative action of a novel series of maprotiline analogues. *European journal of medicinal chemistry.* 2014;71:333-53.
9. Xu Z, Yang Z, Liu Y, Lu Y, Chen K, Zhu W. Halogen bond: its role beyond drug–target binding affinity for drug discovery and development. *Journal of chemical information and modeling.* 2014;54(1):69-78.
10. Scholfield MR, Zanden CMV, Carter M, Ho PS. Halogen bonding (X-bonding): A biological perspective. *Protein Science.* 2013;22(2):139-52.
11. Zimmermann MO, Lange A, Wilcken R, Cieslik MB, Exner TE, Joerger AC, et al. Halogen-enriched fragment libraries as chemical probes for harnessing halogen bonding in fragment-based lead discovery. *Future medicinal chemistry.* 2014;6(6):617-39.

12. Wilcken R, Liu X, Zimmermann MO, Rutherford TJ, Fersht AR, Joerger AC, et al. Halogen-enriched fragment libraries as leads for drug rescue of mutant p53. *Journal of the American Chemical Society*. 2012;134(15):6810-8.
13. Mu X, Wang Q, Wang L-P, Fried SD, Piquemal J-P, Dalby KN, et al. Modeling organochlorine compounds and the σ -hole effect using a polarizable multipole force field. *The Journal of Physical Chemistry B*. 2014;118(24):6456-65.
14. Karama U, Sultan MA, Almansour AI, El-Taher KE. Synthesis of chlorinated tetracyclic compounds and testing for their potential antidepressant effect in mice. *Molecules*. 2016;21(1):61.
15. Karama US, Sultan MAS, Tahir KEHE, Almansour AI. Antidepressant compounds. Google Patents; 2015.
16. Karama U, Sultan MA, Ghabour HA, Fun HK, Warad IK. Crystal structure of 4, 5-dichloro-anthracen-9(10H)-one, C₁₄H₈Cl₂O. *Talanta*. 1991;38:571-88.
17. Sultan MA, Karama U. Substituent Effects on Regioselectivity of the Diels-Alder Reactions: Reactions of 10-Allyl-1, 8-dichloroanthracene with 2-Chloroacrylonitrile, 1-Cyanovinyl Acetate and Phenyl Vinyl Sulfone. *Journal of Chemistry*. 2016;2016.
18. Sultan MA, Karama U, Almansour AI, Soliman SM. Theoretical Study on Regioselectivity of the Diels-Alder Reaction between 1, 8-Dichloroanthracene and Acrolein. *Molecules*. 2016;21(10):1277.
19. Al-Saedi A, Karama U, Sultan MA, Farooqui M. *Journal of Chemical and Pharmaceutical Research*, 2016, 8 (2): 279-284. *Journal of Chemical and Pharmaceutical Research*. 2016;8(2):279-84.
20. House HO, Koepsell D, Jaeger W. Derivatives of 1, 8-diphenylanthracene. *The Journal of Organic Chemistry*. 1973;38(6):1167-73.
21. Zhao W, Tang Q, Chan HS, Xu J, Lo KY, Miao Q. Transistors from a conjugated macrocycle molecule: field and photo effects. *Chemical Communications*. 2008(36):4324-6.
22. Nicolaou K, Ninkovic S, Sarabia F, Vourloumis D, He Y, Vallberg H, et al. Total syntheses of epothilones A and B via a macrolactonization-based strategy. *Journal of the American Chemical Society*. 1997;119(34):7974-91.

Figures

Figure 1

Maprotiline and chlorinated tetracyclic analogues.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplemantrayData.pdf](#)
- [GraphicalAbstract.pdf](#)
- [Scheme1.jpg](#)