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Synthesis and antiproliferative action of a novel series of maprotiline analogues

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ABSTRACT

The synthesis of a diverse library of compounds structurally related to maprotiline, a norepinephrine reuptake transporter (NET) selective antidepressant which has recently been identified as a novel *in vitro* antiproliferative agent against Burkitt's lymphoma (BL) cell lines is reported. A series of 9,10-dihydro-9,10-ethanoanthracenes were synthesised with modifications to the bridge of the dihydroethanoanthracene structure and with alterations to the basic side chain. A number of compounds were found to reduce cell viability to a greater extent than maprotiline in BL cell lines. In addition a related series of novel 9-substituted anthracene compounds were investigated as intermediates in the synthesis of 9,10-dihydro-9,10-ethanoanthracenes. These compounds proved the most active from the screen and were found to exert a potent caspase-dependant apoptotic effect in the BL cell lines, while having minimal effect on the viability of peripheral blood mononuclear cells (PBMCs). Compounds also displayed activity in multi-drug resistant (MDR) cells.

1. Introduction

Burkitt's lymphoma (BL) is a rare but aggressive B-cell malignancy that was first documented in 1958 by Dennis Burkitt [1]. There are three main forms of BL, the sporadic form found in developed countries, the more common endemic form found in the malarial belt of equatorial Africa and an HIV-associated form [2,3]. BL is the most frequent childhood cancer in equatorial Africa while in developed countries, the sporadic form accounts for 1–2% of adult lymphomas. The disease can manifest as tumours in the jaw and facial bones, kidneys, ovaries and abdomen. Endemic BL is usually associated with the oncogenic Epstein–Barr virus (EBV) [2,3]. EBV acts to interrupt cellular pathways that regulate cell proliferation and prevent apoptosis of the cell. In this way, EBV maintains proliferation of the tumour cells [4,5].

BL malignancies proliferate rapidly and as such require intensive combination chemotherapy treatments including a combination of cyclophosphamide, doxorubicin, vincristine (oncovin) and prednisone (CHOP) and more recently rituximab, a monoclonal antibody which targets the CD20 antigen on the surface of malignant and normal B-lymphocytes. Rituximab, in conjunction with chemotherapeutic drugs such as vincristine, doxorubicin, methotrexate and cyclophosphamide can allow up to 60% survival rates in children [6,7]. However, due to a growing incidence of HIV-associated BL and increased resistance to treatments there is a vital need to develop more potent, selective and economical treatments for this disease.

Antidepressants are a class of compounds used to treat the symptoms of depression [8,9] which target the monoamine transporters: NET, serotonin reuptake transporter (SERT) and dopamine reuptake transporter (DAT) by mimicking the effects of naturally occurring neurotransmitters. Different types of antidepressants vary in their affinities for different transporters. Recent discoveries of the presence of these transporters in some malignancies [10] originally led to the study of monoamine transporter ligands as pro-apoptotic agents including citalopram, fluoxetine, tricyclic antidepressants (TCA) imipramine and clomipramine [11–15] and amphetamine related compounds such as MDMA (ecstasy) and fenfluramine. BL cells have also been shown to overexpress the monoamine transporters SERT and NET to various degrees.

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However their involvement in the antiproliferative effect of monoamine transporter ligands has been disputed and is unlikely to play an important role [16].

Maprotiline is an atypical antidepressant compound, characterised by its tetracyclic structure and secondary amine side chain. Maprotiline was first patented in 1969 by Wilhelm and Schmidt and a subsequent publication reported its synthesis [17]. Maprotiline selectively targets NET over SERT and DAT transporters [18] (norepinephrine selective reuptake inhibitor, NSRI) but is also known to have moderate effects on β-noradrenergic receptors, α-adrenergic and muscarinic receptors and histaminic receptors [19,20]. Side effects from the use of maprotiline include seizures, drowsiness, sweating, headaches, arrhythmia and memory impairment [19]. Although maprotiline is not used clinically as an antidepressant due to the emergence of more efficient drugs such as selective serotonin reuptake inhibitors (SSRI), other effects of maprotiline have recently been discovered.

Previous research from our groups identified the antidepressant maprotiline (Fig. 1) as a potential antiproliferative agent against BL cell lines, in particular, the resistant lymphoma cell line DG-75 had an EC_{50} value in the low micromolar range following a treatment time of 72 h with maprotiline [16,21]. It was found that maprotiline (and the SSRI fluoxetine) induce Type II autophagic cell death in the resistant DG-75 cell line [21]. This antiproliferative effect was not observed for other NSRIs nisoxetine and reboxetine and is thought to occur independently of the NET transporter. Also, when the normal activity of the transporters was blocked with nisoxetine, the autophagic death induced by maprotiline was not prevented [16].

Maprotiline-induced anti-multi-drug resistance (MDR) effects in both cancer cell lines and the malarial strain *Plasmodium falciparum* have recently been reported [22,23]. MDR is a major problem in drug treatment of all cancers. Proteins such as P-glycoprotein (P-gp) and breast cancer resistant protein (BCRP) are drug efflux pumps commonly overexpressed in many cancers and are responsible for eliminating therapeutic drugs from a target cancer cell. Maprotiline has previously been shown to sensitise resistant malarial strains and resistant cancer cell lines overexpressing P-gp towards anti-malarial and chemotherapeutic drugs [24,25]. A study on strains of *P. falciparum* known to be resistant and sensitive to the anti-malarial drug chloroquine found several functional moieties of 9,10-dihydro-9,10-ethanoanthracene compounds including aromatic groups, the nature of a basic side chain and a cationic charge were important for an anti-MDR effect. It was found that most of the successful compounds contained amine substituted ethanoanthracene structures compared to compounds which contained amide side chains which were not as potent [25,26]. The anti-MDR effect is thought to have an inhibitory effect on the P-gp mediated efflux pump but the exact mechanism of activity is unknown. Further studies demonstrated the ability of these compounds to inhibit anti-MDR activity in a leukaemic MDR cell line [24].

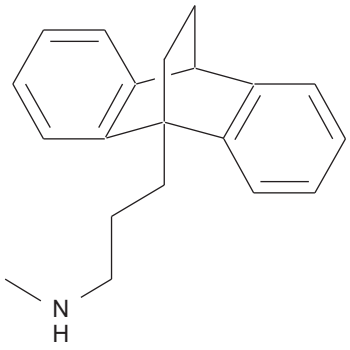

Fig. 1. Maprotiline **1**.

Based on this evidence, it was decided to design a library of analogues related in structure to maprotiline **1** (Fig. 1), with modifications to both the bridgehead of the 9,10-dihydro-9,10-ethanoanthracene structure and to the C-9 substituent. These compounds were then evaluated in a series of malignant cell lines including BL cell lines, MUTU-I and DG-75 cell lines, and MDR cells overexpressing P-gp and BCRP proteins, in order to investigate the structure–activity relationships of these maprotiline analogues and to attempt to improve potency.

2. Chemistry

In the present work, modifications of the dihydroethanoanthracene bridgehead structure and also to the C-9 basic substituent of the maprotiline were investigated to produce a varied library of compounds for evaluation. Synthesis of novel maprotiline analogues was achieved as illustrated in Schemes 1–4. The initial approach required formation of the dihydroethanoanthracene and dihydroethenoanthracene structures from an anthracene precursor, by way of a Diels–Alder reaction, to give products with varied functional groups on the bridgehead (Series 1 and 2). An alternative route involved building the basic side chain from anthraldehyde, followed by a Diels–Alder reaction to form the bridged dihydroethanoanthracene structure (Series 4). A further series of related anthracene compounds were also prepared to allow the effects of the presence or absence of the ethylene bridge on the biochemical activity of the products to be assessed (Series 3).

2.1. Series 1

9,10-Dihydro-9,10-ethanoanthracenes **2** and **3** were obtained *via* a Diels–Alder reaction of the diene anthracene with diethyl fumarate and ethyl acrylate [27]. Esters **2** and **3** were then hydrolysed to give the corresponding carboxylic acids **4** and **5** which were then coupled to a series amines (EDCI/HOBt) to provide the 11-substituted- and 11,12-disubstituted-dihydroethanoanthracene amides (**6**–**20**) (Scheme 1). In the ^{1}H NMR spectrum of **7**, the alkyl protons H11 and H12 appear as a singlet at δ 4.30 ppm, integrating for two protons. Interestingly, the alkyl protons H9 and H11 do not show coupling to each other to give the expected doublet, perhaps a result of a small dihedral angle calculated as 65.9° [28]. Instead, both signals appear as singlets; however, the H–H COSY spectrum indicates the presence of coupling interaction between the two protons. This small angle would predict a small coupling constant of approximately <1.0 Hz, which was not observed in the ^{1}H NMR spectrum. The appearance of protons H9 and H11 as singlets in the ^{1}H NMR spectrum is in agreement with literature reports of related 9,10-dihydroethanoanthracene compounds [24,25].

The ^{1}H NMR spectrum for the novel amide **9** shows the diastereotopic methylene protons $H12_a$ and $H12_b$ resonating as two signals at δ 1.95 ppm and δ 2.15 ppm. The signal at δ 2.15 ppm appears as a multiplet, while the signal at δ 1.95 ppm ($H12_a$) resonates with coupling to the diastereotopic $H12_b$ (J = 11.9 Hz). *Cis* coupling with H11 (J = 17.8 Hz) and coupling to H10 (J = 2.5 Hz) is also clearly seen in the H–H COSY spectrum. A multiplet at δ 2.96 ppm represents the alkyl proton H11 due to the interaction with both diastereotopic H12 protons and H9. H10 is found as a singlet at δ 4.89 ppm. Interestingly, even though coupling was observed for $H12_a$ with H10 (J = 2.5 Hz), this is not obvious from its singlet signal. Similarly, H9, which is further downfield than H10 due to its relative proximity in space to the amide group, does not show any coupling to H11, as it resonates as