Bioorganic & Medicinal Chemistry Letters 24 (2014) 2728–2733

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and biological effects of new hybrid compounds composed of benzylguanidines and the alkylating group of busulfan on neuroblastoma cells

Thomas Hampel [a,†], Marietta Bruns [b,†], Melanie Bayer [b], Rupert Handgretinger [b], Gernot Bruchelt [b,*], Reinhard Brückner [a,*]

[a] Institut für Organische Chemie, Albert-Ludwigs-Universität Freiburg, Albertstraße 21, D-79104 Freiburg, Germany
[b] Children's University Hospital, Hoppe-Seyler-Str.1, D-72076 Tübingen, Germany

ARTICLE INFO

Article history:
Received 24 March 2014
Revised 8 April 2014
Accepted 9 April 2014
Available online 18 April 2014

Keywords:
Neuroblastoma
meta-Iodobenzylguanidine (**mIBG**)
Alkylating agent
Busulfan

ABSTRACT

^{131}Iodine-labelled (*meta*-iodobenzyl)guanidine ([^{131}I]-**mIBG**) and busulfan [butane-1,4-diylbis(methanesulfonate)] are well-established pharmaceuticals in neuroblastoma therapy. We report the design, synthesis, and testing of hybrid molecules—**mBBG** and **pBBG**—which combine key structural features of (*meta*-iodobenzyl)guanidine and busulfan: they contain a benzylguanidine moiety for accumulating in neuroblastoma cells via the noradrenaline transporter and, in the *meta*- or *para*-position, respectively, one of the two identical alkylating motives of busulfan for killing cells. Uptake and toxicity of hybrids **mBBG** and **pBBG** in human neuroblastoma cells compared favorably to their ancestors [^{131}I]-**mIBG** and busulfan.

Neuroblastoma is a malignant tumor of the sympathetic nervous system in childhood with a poor prognosis in stage IV.[1] It usually originates from the adrenal gland. It is the most abundant extracranial solid tumor in childhood making up for 8–10% of all cancers and for 15% of the corresponding mortality.[2] Approximately 1.1 children out of 100,000 under the age of 15 are diagnosed with neuroblastoma. 90% of those who are diagnosed are younger than 10 years, one third is diagnosed in the first year of life.[1,3]

A majority of the cytotoxic drugs currently used in cancer therapy acts non-specifically. Such chemotherapeutics[4] often cause severe side effects and/or long-term damages. The latter are especially severe concerning the treatment of infants. The aim of our project is to develop cytotoxic compounds with a higher specificity than their predecessors. This objective shall be reached by targeting a particular structure in neuroblastoma cells, namely the noradrenaline transporter. A substance with an enhanced specificity for such cells of the sympathetic nervous system is (*meta*-iodobenzyl)guanidine (**mIBG**) [formula: Scheme 1].

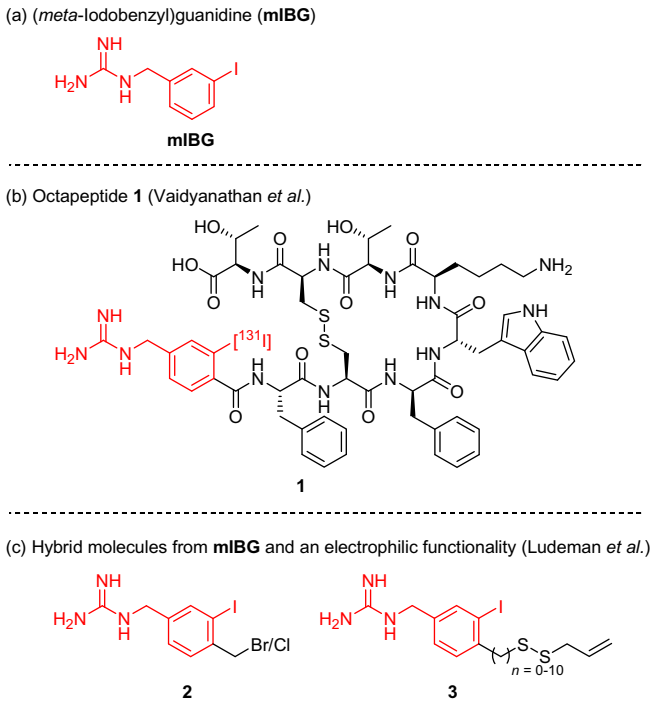
An **mIBG** equipped with a radiolabel was first synthesized by Wieland et al. in 1979.[5] In 1984/1985 radio-labelled **mIBG** was introduced independently both in Heidelberg[6] and Tübingen[7] for scintigraphic imaging of neuroblastoma. Following this discovery [^{123}I]-**mIBG** (β^+-emitter, $\tau_{1/2}$ = 13 h) has been used around the world as a routine diagnosis of neuroblastoma and also for an ensuing therapy. The major therapeutical agent, which was developed from the mentioned discovery and which has been used ever since, is [^{131}I]-**mIBG** (β^--emitter, $\tau_{1/2}$ = 8.0 d).[8] Radio-labelled analogs of [^{131}I]-**mIBG** containing iodine-125[9] (β^+-emitter, $\tau_{1/2}$ = 59 d) or astatine-211[10] (mainly α-emitter, $\tau_{1/2}$ = 7.2 h) were prepared as well. They represent therapeutical alternatives for special manifestations of neuroblastoma.[9,10]

The uptake of **mIBG** in neuroblastoma cells is the basis for the aforementioned activities. It was first described in one of our laboratories in cell culture experiments.[11] Later it was shown that this uptake occurs via the noradrenaline transporter.[12] **mIBG** was also shown to be taken up by OCT-expressing cells (OCT: organic cationic transporter).[13] This process is a well known side effect in scintigraphy of neuroblastoma.[8a,14] Very recently, it turned out that the uptake of **mIBG** in OCT-expressing cells can be reduced by glucocorticoids.[15]

Even nonradioactive benzylguanidines—which would not per se be expected to affect cell life [exceptions: a nitro-substituted **mIBG** (Ref. 16) and unsubstituted **mIBG** (Refs. 17–19; see also the next

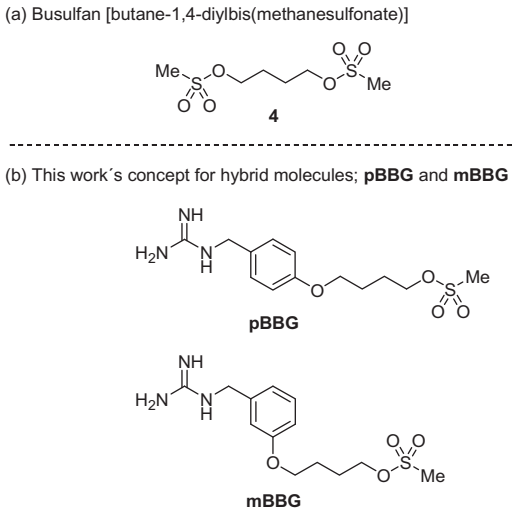
* Corresponding authors. Tel.: +49 (0) 7071 298 4710; fax: +49 (0) 707 129 5482 (G.B.); tel.: +49 (0) 761 203 6029; fax: +49 (0) 761 203 6100 (R.B.).
E-mail addresses: gernot.bruchelt@med.uni-tuebingen.de (G. Bruchelt), reinhard.brueckner@organik.chemie.uni-freiburg.de (R. Brückner).
† These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bmcl.2014.04.030
0960-894X/



Scheme 1. Benzylguanidine motives in **mIBG** (a), in octapeptide **1**[21f] (b), and in the iodine-containing alkylating agents **2**[22] and **3**[22] (c).

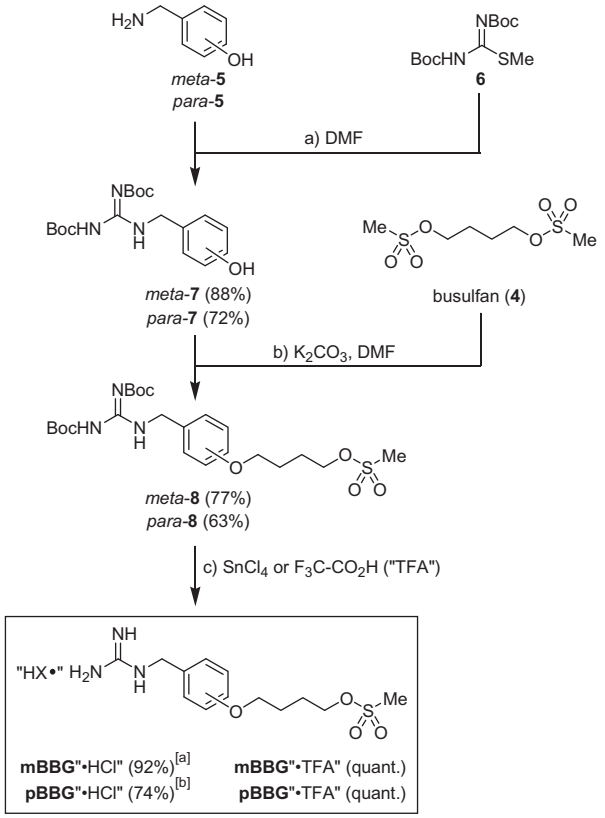
paragraph]—should be modifiable so that they kill neuroblastoma cells. Such a concept has been pursued by at least two groups:[20] Vaidyanathan et al. developed modified—yet always iodine-containing—analogues of **mIBG**,[21] such as the octapeptide **1**[21f] [Scheme 1]. Its cytotoxic effect is most likely due the redox active S—S bond. Ludeman et al. proposed 'A Method for Treating Neuroblastoma' based on a sizable number of **mIBG**s.[22] Each of these molecules contains an electrophile—in substituents of widely varied structures [e.g., Scheme 1 (c)]—for attacking the mitochondrial glutathione of tumor cells. Without an exception the Ludeman compounds display an iodine-substituent in an unvaried **mIBG** substructure.[16,22]



Scheme 2. The bifunctional alkylating agent busulfan (**4**[25]) (a). Our concept for a specific therapy of neuroblastoma (b): hybrid molecules **pBBG** and **mBBG** composed of the alkylating motive of busulfan (**4**) and of the common benzylguanidine motive both of **pIBG** and **mIBG**.

The simplest nonradioactive **mIBG** is the parent compound itself, namely **mIBG**. Intriguingly, it is active against neuroblastoma cells: associated effects are a sharp decrease of the ATP/ADP ratio[18] and aberrances in glycolysis and oxidative phosphorylation.[17–19] Recently, the HR-NBL1[23] trial of the European SIOP[24] Neuroblastoma Group demonstrated that busulfan [**4**;[25] formula: Scheme 2 (a)] and melphalan (={4-[*N*,*N*-bis(2-chloroethyl)amino]phenyl}alanine) combined make an excellent myeloablative therapy for high-risk neuroblastoma.[26] It is superior to the so-called 'CEM protocol', which is combined treatment with carboplatin, etoposide, and melphalan. The 'CEM protocol' represented the recommended neuroblastoma therapy before it was replaced by the combination busulfan (**4**)/melphalan.[27]

What we hoped would turn out to be anti-neuroblastoma agents were hybrids of the alkylating motive of busulfan [**4**;[25] formula: Scheme 2 (a)] and a benzylguanidine. The alkylating group should be installed in the benzene ring such that a *meta*- (**mBBG**) or *para*-substituted benzylguanidine (**pBBG**) results [formulas: Scheme 2 (b)]. We hoped that the respective hybrid molecules (**mBBG** and **pBBG**) would combine the cytotoxicity of busulfan with the ability of (*meta*-iodobenzyl)- or (*para*-iodobenzyl)guanidines of intruding neuroblastoma cells by means of the noradrenaline transporter. Neither **mBBG** nor **pBBG** contains an iodine atom. This makes our design novel compared to the Ludeman systems[22] and simpler: introducing two substituents into benzene (as



Scheme 3. Synthesis of the hybrid molecules **pBBG** and **mBBG** by deprotection of the *N*,*N*′-Boc-protected precursors *para*-**8** or *meta*-**8** either by HCl or by TFA in the final step. Conditions: (a) **5** (1.0 equiv), **6** (1.1 equiv), DMF, room temperature, 16–24 h; 88% (*meta*-**7**), 72% (*para*-**7**). (b) **7** (1.0 equiv), **4** (3.0–3.1 equiv), K_2CO_3 (5.1–5.2 equiv), DMF, room temperature, 20 h; 63% (*para*-**8**), 77% (*meta*-**8**). (c) $SnCl_4$ (2.0–2.4 equiv), EtOAc, room temperature, 3 h; 92% (**mBBG**"·HCl")[a], 74% (**pBBG**"·HCl")[b]. (c) F_3CCO_2H (TFA)/CH_2Cl_2 1:1 (v:v), room temperature, 1.5 h; quant. [a]Different batches contained 0–20% of an S_N2 product, in which the methanesulfonate group was replaced by an unknown nucleophile.[36] [b]Contained 9% of a S_N2-product where the methane sulfonate group was replaced by an unknown nucleophile.[36]