EMBO reports

analysis

Not boring at all

Boron is the new carbon in the quest for novel drug candidates

The element boron is not renowned among biologists, short of a few specialists who know that it is an essential nutrient for plants and an element in boromycin—an antibiotic compound produced by *Streptomyces*. Yet, on the whole, molecular biologists and, in particular, those in drug development seem to have little use for carbon's left-hand neighbour in the periodic table. This is about to change. Currently, boron is largely produced in Turkey and the USA, and is used in a wide range of products, including glass, detergents, fire retardants, fibres to reinforce plane fuselages and body armour, and in superhard materials. Now, both researchers and the pharmaceutical industry are showing an increasing interest in boron as an alternative to carbon in drug design.

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A series of recent scientific and commercial developments indicate that boron-based compounds are interesting drug candidates against all disease categories and might even speed up drug development. Pharmaceutical companies have already increased their boron research, particularly GlaxoSmithKline (GSK; Brentford, UK), which announced a US$2.5 billion investment in the US company Anacor (Palo Alto, CA, USA) in November 2008. Anacor was founded in 2002 to develop boron-based antibacterial drugs, but has since expanded into antivirals and other targets with its boron-based platform. Co-founders Lucy Shapiro and Stephen Benkovic began collaborating in 2001 to look for novel inhibitors of several newly identified bacterial target sites that, they thought, could lead to more effective antibiotics. "They randomly inserted boron and got good activity," said David Perry, CEO of Anacor. This serendipitous discovery led to the formation of Anacor a year later. "We were lucky," Perry conceded. "At that stage we had no idea what the broader potential of boron was. We saw ourselves just as an antibiotic company, but as we learned more, we realised that boron included in small molecules could be used in probably every single disease area."

The evidence so far supports this claim—the first clinically tested boron-based drug is Velcade® (bortezomib; Millennium Pharmaceuticals, Boston, MA, USA), which is used to treat multiple myeloma—the cancer of plasma cells. The Japanese company Takeda (Osaka, Japan), which acquired Velcade through its takeover of Millennium in 2008, has reported that sales of the drug passed the US$1 billion mark in November 2008 after various regulatory approvals followed successful phase III clinical trials. The US Food and Drug Administration (FDA; Bethesda, MD, USA) extended the use of Velcade to newly diagnosed myeloma patients in June 2008; previously, Velcade had been confined to severe cases in which there was no option other than to risk a new drug. Velcade was also approved by the European Medicines Agency (EMEA; London, UK) in September 2008 for use in combination with two other drugs for newly diagnosed multiple myeloma patients.

Apart from breaking new ground for boron, Velcade is also one of the first proteasome inhibitors to be approved for full clinical use. Such drugs—whether they include boron or not—are in their infancy, partly because the exact mechanisms involved are still unclear. The proteasome is a large barrel-shaped complex found in both the cytoplasm and the nucleus of eukaryotic cells, where it degrades unwanted and misfolded proteins. Various studies (Almond & Cohen, 2002) have shown that proteasome inhibitors are potential cytotoxins, as they prevent the degradation of pro-apoptotic proteins. Although other mechanisms are almost certainly involved, a crucial mechanism behind the immortality of many tumour cells is the degradation of proteins that would otherwise cause apoptosis. In the case of Velcade, a single boron atom within the molecule binds to the catalytic site of the most common type of proteasome, called 26S, and blocks its action.

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Although Velcade has nothing to do with Anacor or GSK, Perry interprets its approval and growing use as the final proof that boron itself poses no fundamental risk to humans; safety will be determined by the design of individual drugs, just as has been the case with traditional carbon-based compounds. Meanwhile, Anacor hopes to make its commercial debut with a boron-based drug called AN2690®, which they hope to use to treat onychomycosis, a fungal infection that affects fingers and toes, causing the nail to become brittle and discoloured with soreness of the surrounding skin. Although rarely serious, onychomycosis can cause pain and accounts for half of all nail abnormalities, so any treatment has considerable market potential; phase III clinical trials are scheduled to begin early in 2009.

According to Zbigniew Lesnikowski, from the Laboratory of Molecular Virology and Biological Chemistry at the Polish Academy of Sciences in Lodz, Poland, this sudden interest is grounded in the fact that boron has both useful similarities and important differences to carbon. Lesnikowski convened a workshop on boron chemistry, organized by the European Science Foundation (ESF; Strasbourg, France) in 2008, to explore its therapeutic potential. Boron is immediately to the left of carbon in the periodic table and forms small compounds of a size appropriate to target key binding sites, just as carbon does. Indeed, several important carbon-based compounds have boron-based equivalents. "From a chemist's point of view, one of the most fascinating aspects of boron is that it forms a very complex series of hydrides like carbon does," Lesnikowski commented.

However, as Lesnikowski noted, there are also some interesting differences between boron and carbon compounds. For example, boron hydrides are composed of cages and clusters, rather than the chains and rings formed by carbon. The structure of these compounds, boranes, reflects the nature of the boron atom itself; it is the smallest of the metalloids, which are hybrid metal/non-metals with properties of both. In this regard, boron behaves similar to a metal when forming oxides such as boric oxide (B_2O_3) and salts such as boron sulphate ($B_2(SO_4)_3$); however, similarly to non-metals, it also forms acids such as boric acid (H_3BO_3). As such, boron is a trivalent metal, but, unlike a true metal, it has a strong affinity for electrons owing to its vacant p-orbital—a dumbbell-shaped region around the atomic nucleus. This makes boron, and many of its compounds, electron-deficient, which leads to rather unusual structures.

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Carbon compounds involve conventional covalent bonds in which constituent atoms share pairs of electrons, which lead to relatively rigid ring and chain structures, whereas boron compounds form cage and cluster-like structures with more geometric variability. Furthermore, boron compounds have unique charge distributions that allow them to form the non-covalent bonds necessary for the transient connections with protein docking sites that are required for many biochemical reactions. They also exhibit unique hydrophobic behaviour, which also provides the basis for several interesting interactions with organic molecules.

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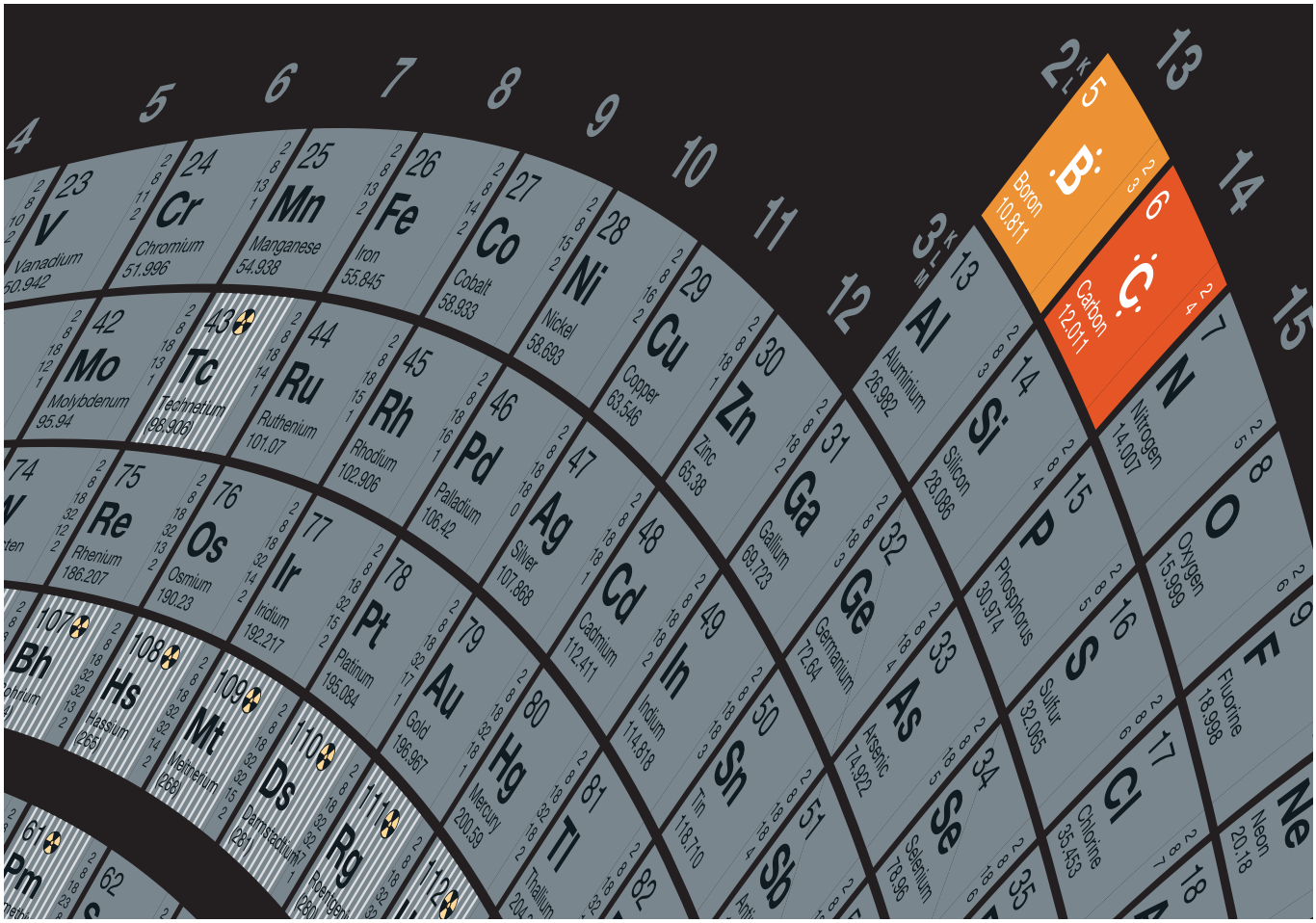
These structural and electronic aspects of boron compounds feed into expectations that boron could lead to a new generation of drugs that would be able to interfere with target molecules not readily accessible to carbon-based compounds. This, as Perry noted, is the reason for the excitement around boron chemistry at the moment; in particular, Anacor hopes that its boron-based antibiotics could overcome the growing problems of bacterial drug resistance that is reducing the efficacy of penicillin in particular. Although bacteria could just as easily develop resistance to boron-based drugs, the case for boron is that it could be directed against new proteins—such as AN2690, which targets leucyl-transfer RNA synthetase, an essential enzyme in protein synthesis—and would be less likely to trigger the development of resistance. At the very least, boron's novel chemistry will broaden the angles of attack, and should thus provide new classes of drug for use in the ongoing arms race against pathogens.

In the immediate future, however, candidate boron drugs will not exploit the exotic structural features of the element, according to Katherine Widdowson, group director of antibacterial chemistry at GSK. The immediate attraction of boron is its ability to form strong covalent bonds with hydroxyl groups, which could reduce the size and complexity needed to dock with target proteins. "I think that one of the big promises of boron, especially in the anti-bacterial area, is that smaller molecules can be used. The boron molecules we're working on currently are structurally quite a bit simpler than our non-boron molecules in other programmes," she said. This, in turn, could reduce both the cost of boron-based antibacterials and the time needed to develop them; it would not reduce the time and costs for clinical testing, but the ability to use smaller, less complex molecules could decrease the cost of production and time during the early discovery stages.

However, boron-based compounds that form covalent bonds will probably not be used to inhibit host proteins, for example, in anti-inflammatory drugs, because such covalently labelled sites can trigger the generation of antibodies. The altered site might thus no longer be recognized as 'self' by the host's immune system. This does not matter when the target is a bacterial protein, as the aim is to eliminate the bacteria anyway. Although the emphasis is on antibacterials at present, the same advantages should apply to antivirals; indeed, of the four specific therapeutics being worked on by Anacor, three are antibacterials and one is an antiviral drug intended to treat hepatitis C.

The utility of boron is not limited to drug design; boron neutron capture therapy (BNCT) involves another, very different, application of boron compounds. Work on this therapy actually began around 30 years ago, but has only recently reached the stage of phase I clinical trials in the USA and Japan, where a lot of pioneering work in the field has been carried out. BNCT exploits the nuclear reaction that occurs when boron-10, a stable isotope of boron, is irradiated by low-energy neutrons. On capturing a neutron, boron fissures into helium-4 alpha particles—two protons and two neutrons—and lithium-7 nuclei, which both recoil with high kinetic energy, but quickly run out of steam after about 15 μm—roughly the diameter of a single cell. BNCT therefore works as a two-compound therapy: the boron and the slow neutrons are each relatively harmless to cells but, when combined, they produce a highly cytotoxic effect. Furthermore, the nuclear reaction only takes place inside those cells that have accumulated boron compounds and therefore does not damage the surrounding tissue. BNCT has been experimentally tested primarily to treat malignant brain tumours, and recurrent head and neck cancer; however, despite some successful outcomes, BNCT has not entered routine clinical use.



The first main challenge for BNCT is to develop boron compounds that are taken up selectively by tumour cells, while only accumulating in low doses in healthy cells. Early trials with sodium borocaptate and boronophenylalanine have already shown selective uptake by tumour cells (Altieri *et al*, 2008). One of the more promising groups of compounds for use in the BNCT treatment of brain tumours is boron-containing nucleosides—precursors to nucleotides. These are required for DNA synthesis during cell division and therefore accumulate in tumour cells, which undergo mitosis, but not in normal brain cells, which do not generally divide in adults. "Hence, a boron nucleoside will be incorporated into the dividing cells where there is active transport more so than in 'resting' cells," explained Raymond Schinazi, Director of the Laboratory of Biochemical Pharmacology at Emory University (Atlanta, GA, USA). Emory uses a refitted nuclear reactor at the nearby Georgia Institute of Technology to generate neutron beams for its BNCT research programme. Schinazi pointed out that BNCT could be used against various solid tumours but, for the immediate future, he sees its application only in cases where conventional radiotherapy is unsuccessful.

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Yet, Schinazi commented, in order to become widely and routinely used in hospitals, BNCT must overcome the challenge of generating neutron beams with a portable low-energy neutron source. At present, the technique requires a specially refitted nuclear reactor, which is not accessible or affordable for most clinics. BNCT is therefore available only in a few dedicated units; mostly nuclear research laboratories that have modified their research reactors. According to Yasuyuki Endo, from the Laboratory of Organic and Medicinal Chemistry at Tohoku Pharmaceutical University in Japan, smaller neutron accelerators are now being developed. "When the neutron accelerator is available, BNCT will be used widely in hospitals," he predicted.

Among those boron compounds that have attracted the interest of researchers working in BNCT are the carboranes. Endo explained that these represent promising pharmacophores that could provide the molecular skeleton for a range of drugs that bind to protein sites by hydrophobic interaction. Hydrophobic contact might be a better way to describe

the action of such skeletons, as the effect is to cause molecules to bind to each other at specific sites, rather than just to coalesce. Unlike oil molecules in water, which are, in effect, squeezed together by the attraction of water molecules for each other, hydrophobic bonding would occur only locally, at specific sites on the respective molecules. This creates the potential for drugs to bind to desired target proteins at selected sites in a highly specific manner.

The use of carboranes, which contain both carbon and boron, provides a link between the use of boron in radiotherapy and its biochemical applications. Endo is convinced that both avenues will eventually prove successful, even if further work is required to prove the general safety of boron in drug development. Above all, the unique characteristics of the carborane cages should help drug developers to design compounds that are more efficient at reaching their targets because it should allow drug designers to create molecules with highly specific shapes and charge distributions. "Although detailed toxicity testing and metabolic studies are needed for the development of commercial drugs, the carboranes will be new structural components for drug design as hydrophobic pharmacophores," Endo concluded.

Boron is far from being a boring trace element known only to specialists. The similarities it shares with carbon—the most important structural element in organic chemistry—and the important chemical and electronic differences between the two make boron an exciting candidate for a wide range of applications. For the time being, only some pharmaceutical and biotechnology companies, as well as a handful of biomedical scientists, are exploring the diversity and utility of boron-based compounds. Yet, once their research shows the true potential of carbon's over-looked neighbour, boron might well find its way into the catalogue of useful elements for standard research.

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