SciBX
Science-Business eXchange

Truly boronic

By Michael J. Haas, Senior Writer

Researchers at **Tufts University** have shown that peptide boronic acid inhibitors of dipeptidyl peptidase-4 could provide effective, low-toxicity treatments for diabetes.[1] The findings challenge the long-held assumption that boronic acids are too toxic for therapeutic use. **Arisaph Pharmaceuticals Inc.** has already in-licensed the related IP and has ARI-2243, a boronic acid inhibitor of dipeptidyl peptidase-4, in Phase I testing to treat diabetes.

Other companies contacted by *SciBX* said the findings make the case for taking another look at boronic acids as therapeutics, but they noted that the high potency makes selectivity a key issue, because that potency increases the likelihood of off-target effects at therapeutic doses.

Boronic acid redux

Dipeptidyl peptidase-4 (DPP-4) is expressed in many mammalian cells and tissues and plays a role in glucose metabolism. The serine protease degrades glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP), two gastrointestinal hormones secreted in response to the intake of food and involved in regulating insulin and glucose production.

Two non–boronic acid DPP-4 inhibitors are already approved for type 2 diabetes: Januvia sitagliptin, a selective DPP-4 inhibitor from **Merck & Co. Inc.**, and Galvus vildagliptin, a nonselective DPP-4 inhibitor from **Novartis AG**. Two other non–boronic acid DPP-4 inhibitors are in registration for the indication: Onglyza saxagliptin from partners **Bristol-Myers Squibb Co.**, **AstraZeneca plc** and **Otsuka Pharmaceutical Co. Ltd.** and alogliptin from **Takeda Pharmaceutical Co. Ltd.**

However, dipeptide boronic acids were among the first—and are still among the most potent—inhibitors of DPP-4, according to William Bachovchin, leader of the Tufts team and CSO of Arisaph. In 1991, he and colleagues at Tufts reported the discovery of dipeptide boronic acids with binding affinities for DPP-4 that extended into the picomolar range.[2] But he said that long-held—though unproven—assumptions about the intrinsic toxicity of boronic acids have largely precluded their commercial development.

Indeed, the only marketed boronic acid drug is Velcade bortezomib, a proteasome inhibitor from **Millennium Pharmaceuticals Inc.** that is approved to treat multiple myeloma (MM) and mantle cell lymphoma. Millennium, a unit of Takeda, markets Velcade in the U.S., whereas **Johnson & Johnson** markets the drug elsewhere.

In a paper in the *Journal of Medicinal Chemistry*, the Tufts team compared DPP-4 inhibitors to test whether boronic acids were indeed more toxic than other classes of molecules.

The group chose three dipeptide boronic acid inhibitors of DPP-4: Val-boroPro talabostat, Ala-boroPro and Glu-boroAla. Talabostat and Ala-boroPro were potent but nonselective. The molecules had low picomolar binding affinities for DPP-4 and low nanomolar binding affinities for DPP-8 and DPP-9. Talabostat also inhibits fibroblast activation protein (FAP).

The third compound, Glu-boroAla, was more selective, with a low nanomolar binding affinity for DPP-4 but micromolar binding affinities for DPP-8 and DPP-9.

Talabostat was being developed by Point Therapeutics Inc., but a Phase III trial was halted last year when the talabostat arm showed lower overall survival than the control arm. Point reverse merged with **DARA BioSciences Inc.** later that year.

All three compounds lowered blood glucose in mice and rats at least as well as two non–boronic acid DPP-4 inhibitors—one selective, the other nonselective—examined in an earlier study at Merck Research Laboratories.[3]

The Tufts team also found that the maximum tolerated dose for Glu-boroAla in rats was comparable to the two non–boronic acid DPP-4 inhibitors.

The university team concluded that dipeptide boronic acids can be potent inhibitors of DPP-4 and are not intrinsically more toxic than non–boronic acid inhibitors of DPP-4.

Bachovchin told *SciBX* that in his team's unpublished experiments in animal models, dipeptide boronic acids showed greater efficacy than sitagliptin, as measured by lower levels of glucose and hemoglobin A1c.

"It is always encouraging to see science push the conventional boundaries and challenge perceptions of what is achievable," said Chris Claiborne, senior director of medicinal chemistry at Millennium. Boronic acids do not have any intrinsic limitations as potential therapeutics, and "this publication will enhance interest in the use of boronic acid derivatives to selectively target proteases for therapeutic areas outside oncology," he said.

Dispelling a bad rap

Other companies and academics contacted by *SciBX* agreed that the *JMC* report is a major step forward in dispelling the misconceptions about boronic acid toxicity. The consensus is that the data could revive therapeutic interest in the class, if off-target activity against other serine proteases can be curbed.

Historically, boronic acid has not been considered a likely pharmacophore, due in part to numerous reports in the 1950s and 1960s of infants who died as a result of accidental ingestion of boric acid.[4,5]

In the 1980s and 1990s, **E.I. du Pont de Nemours and Co.** held patents on boronic acids as inhibitors of elastase, thrombin and other proteolytic enzymes, but "people assumed that they must be toxic because du Pont didn't do anything with them," Bachovchin said.

However, Charles Kettner, who co-discovered du Pont's boronic acid elastase inhibitors in the 1980s, told *SciBX* that the compounds exhibited no obvious adverse effects in animals or in cell culture. du Pont was not interested in developing the compounds, he said, because the company

did not have the appropriate biological resources at the time. Indeed, du Pont did not shy away from developing boronic acids when the company expanded its focus in the late 1980s to include pharmaceuticals.

Kettner was a research fellow when he retired from Bristol-Myers Squibb in 2003.

"du Pont was ahead of its time with its work," said John Kozarich, chairman and president of ActivX Biosciences Inc., a subsidiary of **Kyorin Pharmaceutical Co. Ltd.** "It was done before human genome studies revealed how broad the class of serine proteases is and how many thrombin homologs there are."

He estimated that there are 300 serine proteases or serine hydrolases—another indication that selectivity is the main issue for boronic acids.

"In reality, du Pont's compounds just weren't selective enough," Kozarich said. "There is a lot of potential in using boronic acids. They have many possible targets—serine proteases and serine hydrolases—that play roles in many diseases, such as diabetes, atherosclerosis, cancer and others."

"This publication will enhance interest in the use of boronic acid derivatives to selectively target proteases for therapeutic areas outside oncology."

—Chris Claiborne, Millennium Pharmaceuticals Inc.

The key question, he said, is whether boronic acids can be both selective and safe enough for chronic use. "Emerging evidence like this paper says yes," Kozarich concluded.

In August, ActivX completed a Phase IIa trial of KRP-104, a selective non–boronic acid DPP-4 inhibitor, to treat type 2 diabetes. The company has not disclosed a timeline for additional studies of the compound.

"It would be hard to find a better pharmacophore for protease inhibitors than boronic acid," said David Campbell, VP of drug discovery and preclinical sciences at **Phenomix Corp.** "But because it's so good, you have to devote a lot of time to selectivity" to avoid off-target effects on structurally related serine proteases.

For example, a drug candidate with nanomolar activity should typically have binding affinities for other molecules (off-target selectivity) that are 10–100 times lower than its affinity for the intended target, he said. But with a picomolar drug, off-target binding that is 100-fold lower puts such activity in the nanomolar range—still in the preferred range for drugs—making off-target effects likely even at therapeutic doses.

Thus, the off-target selectivity of a picomolar-active compound needs to be several orders of magnitude lower than the compound's binding affinity for its target.

"It really comes down to what the off-target proteins are and what the selectivity index for the compound is," Campbell said. "These are the same issues you have with any compound, but it arises more often with boronic acids because they are often so potent."

Earlier this year, Phenomix completed a Phase IIb study of its non–boronic acid DPP-4 inhibitor, dutogliptin (formerly PHX1149). Phase III testing is expected to start this year.

Making a comeback

Although selectivity is usually essential for drugs, off-target effects are not always toxic or unwelcome. A case in point is the boronic acid that Arisaph has in the clinic.

"ARI-2243's effect goes beyond DPP-4 inhibition," said Bachovchin. "It lowers blood glucose even in DPP-4 knockout animals. So, we are looking for the second mechanism that explains this enhanced efficacy."

Bachovchin said ARI-2243, which was not studied in the *JMC* paper, is not a dipeptide boronic acid but rather a new class of boronic acid. He declined to describe the structure.

"We are also looking at the selectivity and mechanisms of interactions between boronic acids and other enzymes such as DPP-2, FAP, DPP-8, DPP-9 and prolyl endopeptidase," Bachovchin said. These studies will be the subject of future papers.

Arisaph has other boronic acids in preclinical development to treat cancer and atherosclerosis.

Tufts holds international patents for the use of boronic acids to treat diabetes, cancer and atherosclerosis and has out-licensed them to Arisaph.

REFERENCES

1. Connolly, B. *et al. J. Med. Chem.*; published online Sept. 11, 2008; doi:10.1021/jm800390n
   Contact: William W. Bachovchin, Tufts University, Boston, Mass. e-mail: william.bachovchin@tufts.edu
2. Flentke, G. *et al. Proc. Natl. Acad. Sci. USA* **88**, 1556–1559 (1991)
3. Lankas, G. *et al. Diabetes* **54**, 2988–2994 (2005)
4. Goldbloom, R. & Goldbloom, A. *J. Pediatr.* **43**, 631–643 (1953)
5. Wong, L. *et al. Can. Med. Assoc. J.* **90**, 1081–1023 (1964)

COMPANIES AND INSTITUTIONS MENTIONED

Arisaph Pharmaceuticals Inc., Boston, Mass.
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
DARA BioSciences Inc., Raleigh, N.C.
E.I. du Pont de Nemours and Co. (NYSE:DD), Wilmington, Del.
Johnson & Johnson (NYSE:JNJ), New Brunswick, N.J.
Kyorin Pharmaceutical Co. Ltd. (Tokyo:4560), Tokyo, Japan
Merck & Co. Inc. (NYSE:MRK), Whitehouse Station, N.J.
Millennium Pharmaceuticals Inc. (NASDAQ:MLNM), Cambridge, Mass.
Novartis AG (NYSE:NVS; SWX:NOVN), Basel, Switzerland
Otsuka Pharmaceutical Co. Ltd. (Tokyo:4768), Tokyo, Japan
Phenomix Corp., San Diego, Calif.
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502), Osaka, Japan
Tufts University, Boston, Mass.