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Review

The Triggle effect

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ABSTRACT

Dr. David Triggle is considered a pioneer in the area of ion channel pharmacology. Over the course of his career, he made a number of particularly important contributions to our understanding of dihydropyridine interactions with L-type calcium channels. He also contributed his highly sought after expertise towards the drug discovery platform of the Canadian biopharmaceutical company, NeuroMed Pharmaceuticals (subsequently Zalicus). Here we briefly highlight his contributions to the field of calcium channel pharmacology, and then provide examples of his impact on NeuroMed.

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1. Introduction

The era of calcium antagonists can be traced back to the mid-1960s when German pioneer Dr. Albrecht Fleckenstein's group identified several classes of L-type calcium channel inhibitors with potent cardiovascular effects (for review see [1]). These novel inhibitors importantly included substituted dihydropyridines (DHPs) such as nifedipine, niludipine, and nimodipine, some of which are still used in the clinic today.

Over the course of his career, Dr. David Triggle made tremendous contributions towards understanding the biological and molecular actions of this important class of compounds that adds to many contributions to on channel pharmacology in general. His many contributions to the DHP field are too numerous to elaborate in detail here, but we would like to highlight just a handful that we view as particularly impactful.

One was a dissection of the drug structural determinants that conferred agonist versus antagonist activity onto certain types of DHPs [2]. Another important contribution was a detailed structure activity analysis of a large group of dihydropyridine derivatives that yielded important information about how these compounds interact with L-type calcium channels [3]. The use of permanently charged DHP analogs to probe the depth of the receptor site within the channel/plasma membrane complex was a particularly cleaver approach to delineate some of the key determinants of DHP action on these channels [4–6] which was later augmented by mutagenesis studies carried out by others (see below). Finally, the Triggle lab uncovered the reason for tissue selectivity of certain types of DHPs and was able to show that it derived from state dependent inhibition that, for some DHPs, favors block upon membrane depolarization [7]. It is important to note that David also recognized that the DHP pharmacophore can be utilized to develop ligands for many other types of ion channels and receptors (reviewed in [8,9]). This includes even T-type calcium channels which can be blocked preferentially over L-type calcium channels by certain types of DHPs [10–12].

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David Triggle's work on DHPs and other L-type calcium channel antagonists was complemented beautifully by work from the Catterall and Striessnig groups who identified individual amino acids in the L-type calcium channel α_1 subunit that are critically involved in drug interactions with phenylalkylamines [13–17], DHs [18–21], and benzothiazepines [22,23]. Together these seminal studies demonstrated overlap in the channel structural determinants for block by these distinct chemical classes, with hotspots for drug interactions located in the domains III and IV S5 and S6 regions.

2. Vancouver-based neuromed pharmaceuticals

In the late 1990s there was considerable interest developing within the traditional pharmaceutical industry to explore novel treatments aimed at underserved diseases of the nervous system with potentially large markets. At the same time, the more nimble biotechnology industry was rapidly evolving across North America, venture capital was plentiful, and some universities were well ahead of the curve encouraging entrepreneurialism within their computing, engineering and biomedical faculties. In this positive environment, NeuroMed Pharmaceutials Inc. was spun out of the laboratory of Terry Snutch at the University of British Columbia in 1998 as a private venture capital-funded biopharmaceutical company. Over the previous several years the Snutch lab had been intimately involved in the cloning and characterization of the biophysical, pharmacological and modulatory properties of the multiple types of voltage-gated calcium channels found in the mammalian nervous system. At its inception, NeuroMed was the only biopharmaceutical company worldwide focused exclusively on voltage-gated calcium channels as drug targets for the treatment of neurological diseases.

2.1. Building neuromed

The success of any start-up company requires a multitude of factors to align, including a proprietary, patentable technology, a downstream market opportunity, a competitive advantage, and access to capital. Most importantly, is the need to have a driven, highly engaged management team and advisors whose combined efforts will ensure success at all levels. At NeuroMed, Terry Snutch as founder, President and Chief Scientific Officer made the first hire Natalie Dakers as Chief Operating Officer and the first advisor John Swift as corporate counsel. Natalie had considerable experience as a technology and business development director at the University of British Columbia while John was a well-established serial entrepreneur as it related to legal and corporate faculties. Together, they ensured that the Company's initial corporate structure was founded on strong ethical and business principles and also that the initial business plan was sufficiently robust to attract high quality venture capital. After initially raising US$ 5 million to get the company off the ground and drive the advancement of the first new calcium channel blockers into preclinical development, it became apparent that additional corporate changes were required, most importantly having Natalie become Chief Executive Officer, and to hire a full time Chief Financial Officer in the form of Bruce Colwill. Together, in their first five years the team raised an outstanding US $ 52 million in venture capital that drove the Company's first investigation new drug application (IND) to the FDA and Phase 1 clinical trials.

2.2. Focus

The initial goal for Neuromed was to develop the first, orally available N-type calcium channel blocker to treat neuropathic pain, a vastly underserved therapeutic area in need of treatment options in addition to the weakly efficacious NSAIDs and the considerably stronger opioids albeit with their issues around addiction, tolerance and severe side effects (including death).

Spinally localized N-type channels had been validated as a target for neuropathic pain in animal models by two cone snail peptides, ω-conotoxin-GVIA and ω-conotoxin-MVIIA (also called SNX-111, ziconotide), with ziconotide having been approved to treat intractable pain in humans in Europe and the United States [24]. There were however significant issues targeting the N-type channel with ziconotide, including a very narrow therapeutic index generally reflective of off-target effects in the CNS and an equally limited patient population as it delivery required patients to have an existing implanted intrathecal pump.

The Company's proprietary technology integrated earlier work from the Snutch lab describing the first cloning of the N-type calcium channel together with critical structure-function studies on the effects of local anesthetics that Gerald Zamponi performed while a postdoctoral fellow in the Snutch lab [25,26]. A key to early successes was the notion that therapeutically efficacious and safe oral N-type channel blockers to treat pain would best come from electrophysiological screening assays identifying state-dependent blockers. While low throughput, patch clamp screening combined with rational drug design proved highly effective in fast-tracking the drug discovery process.

2.3. David triggle's role at neuroMed

David agreed to join the scientific advisory board (SAB) of NeuroMed in February 1999, providing a key piece for the young start-up drug discovery company and cementing the foundation of

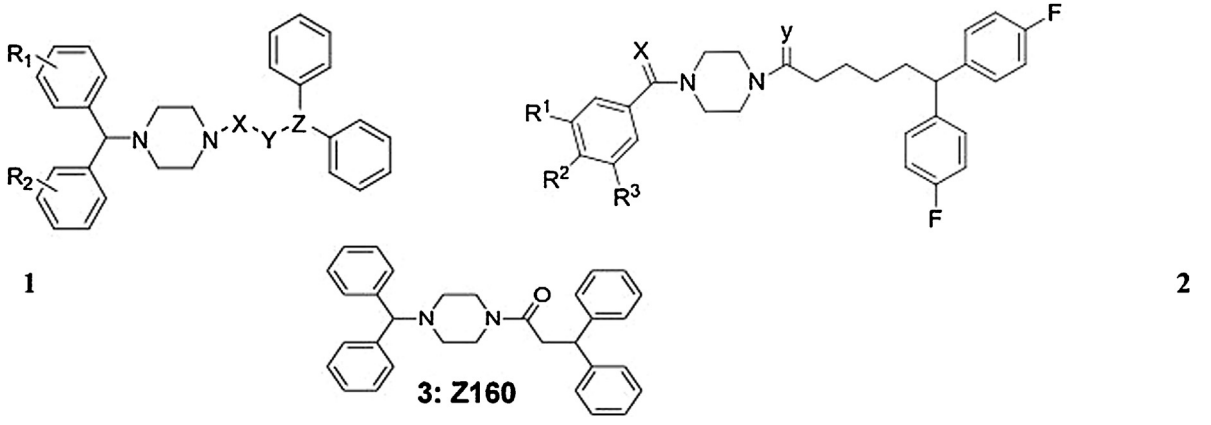

Fig. 1. Development of N-type channel blockers based on SAR studies.

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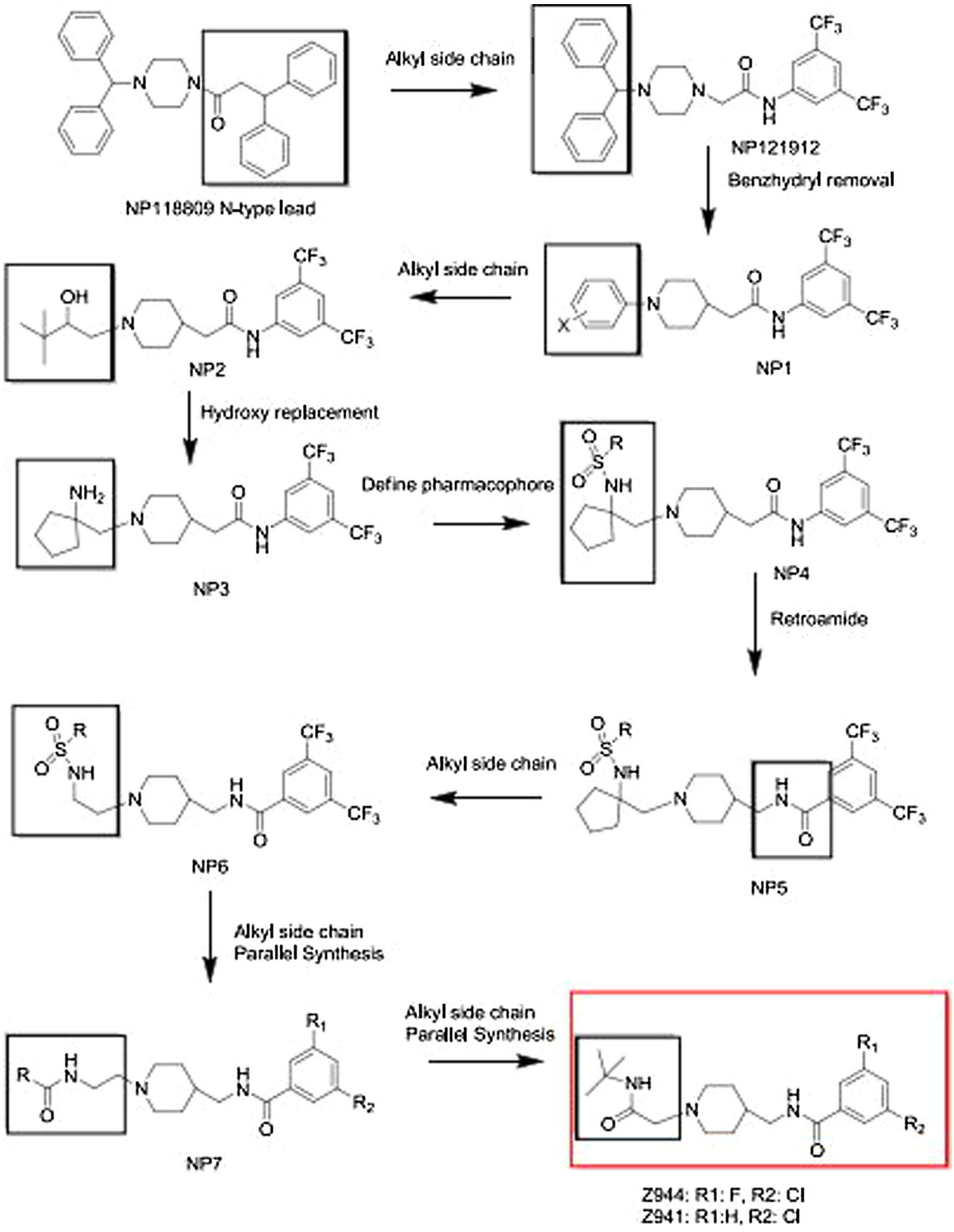

Fig. 2. T-type calcium channel blockers.

Reproduced from Ref. [31].

an experienced external advisory team including the medicinal chemists, the late Les Mitscher (University of Kansas) and David Dolphin (University of British Columbia), and ion channel experts Bill Catterall (University of Washington), Kurt Beam (University of Colorado) and Ed McCleskey (Vollum Institute, Oregon Health Sciences University)

David immediately and enthusiastically dived into his consultative role, and was especially interactive with Les Mitscher as together they effectively drove the NeuroMed team up to speed in the areas of drug design, synthesis, screening and preclinical development. In one aspect, while Dr. Mitscher immersed himself in the *de novo* rational design of N-type calcium channel blockers with the NeuroMed team, David focused on the druggablity of proposed compounds as they related to potential off-target activities in the cardiovascular system. An underappreciated issue raised by David both in person at SAB meetings and via email, and highly illustrative of the larger picture perspective that he brought, was early on to consider compound target affinity and selectivity

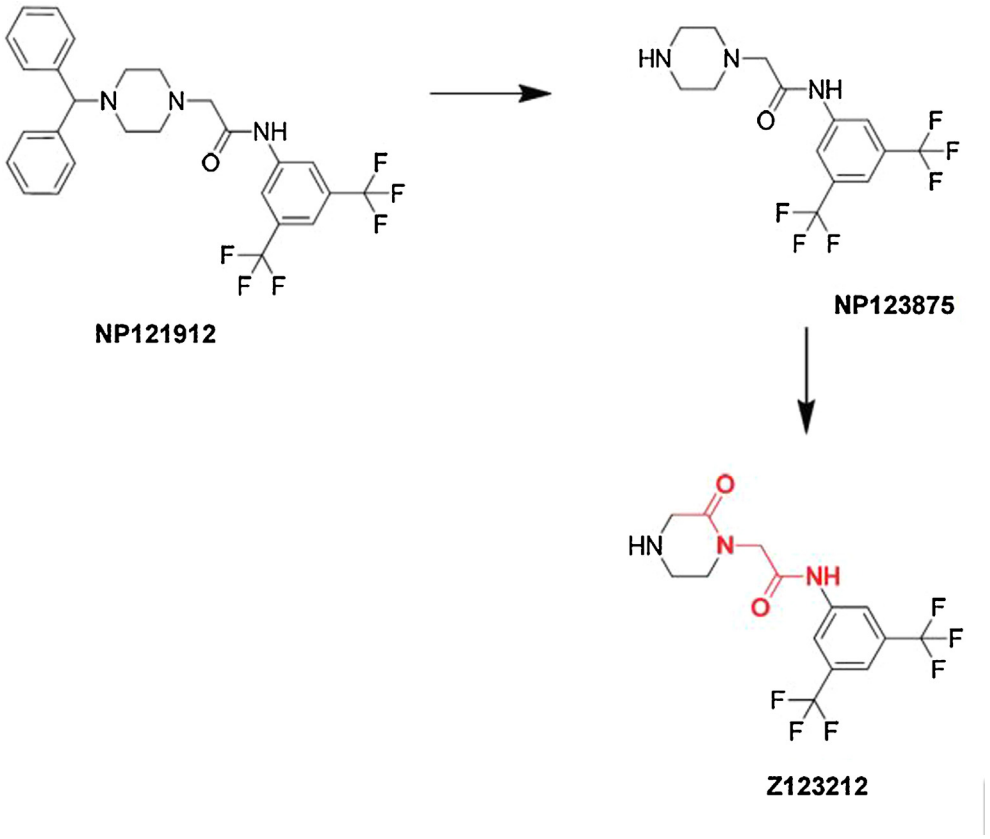

Fig. 3. Mixed T-type and sodium channels. The novel state-dependent compound Z123212 is derived from the piperazine-based T-type blockers NP123875 and NP121912 and contains a dipeptide backbone (highlighted in red).
Reproduced from Ref. [32].

using "worst case scenarios" that might occur under physiological and pathophysiological conditions in patients.

Indeed, with his typical modesty in an email commenting on the characteristics of a specific compound under consideration David noted "*I don't claim to be an expert by a long way – in fact I am not an electrophysiologist at all . . . .*" and then masterfully went on to point out "*You are probably blocking an inward rectifier, thus depolarizing the cell and inactivating Na+ channels and thus reducing Vmax and action potential amplitude. This could also be due to an effect on some background K^+ current. Regardless, this significantly alters excitability and behaves like a Na^+ channel blocker – this could be exacerbated (in vivo) if there is any myocardial disease or other conductive disorder.*" And with that he killed the compound on the spot!

In another aspect of his advisory role David was a strong proponent for not reinventing the wheel when it came to developing new classes of calcium channel blockers targeting the nervous system and therapeutic indications including neuropathic pain and epilepsy. David supported the notion that the previous several decades of drug development success illustrated that approximately 50% of all drugs could be described by only 32 chemical frameworks and 20 distinct sidechains [27]. While on the one hand this indicated that many current drugs represented a relatively small chemical diversity, it also illustrated a far-reaching set of proven pharmaceutically active "privileged structures" that could be further mined against new targets such as neuronal voltage-gated calcium channels [9,28].

The NeuroMed chemistry team took David's sage advice to heart and together with Les Mitscher's astute support much of the company's drug design work focused on druggable small organic backbones known to possess favorable characteristics including lipophilicity, oral bioavailability and CNS penetration.

The first clues towards deriving *de novo* N-type channel blockers came from earlier work demonstrating that certain piperidine-and morpholine-based local anesthetics and neuroleptics effectively blocked exogenously expressed neuronal calcium channels suggestive of a high affinity binding site [26]. Following this up with exhaustive pharmacophore-based structure-activity relationship (SAR) studies resulted in a number of promising series including Compounds **1** [27] and **2** [28] (Fig. 1); and after much preclinical off-target screening, safety pharmacology and animal efficacy, eventually describing the first orally available, high-affinity, state-dependent N-type channel blocker, Compound **3** Z160 (initially NP118809).

In single and multiple ascending dose phase 1 clinical studies Z160 exhibited a favorable tolerability profile, dose-dependent Cmax and no serious adverse effects, albeit low oral bioavailability requiring reformulation.

The Triggle-Mitscher-imbued rational design strategy also drove NeuroMed's (by then renamed Zalicus Pharmaceuticals) generation of the first small organic pure T-type calcium channel blockers, Z941 and Z944, one of which has exhibited promising efficacy in both animal models of epilepsy and in a human model of experimental pain [29–31]. Indeed, derivation of the novel T-type blockers utilized the Z160 core towards further directed SAR (Fig. 2).

Particularly impressive from these efforts was the high degree of selectivity that the T-type blockers exhibited over other types of voltage-gated ion channels (e.g., Z944 shows >100 fold higher affinity to T-type channels compared to the N-type calcium channel, >500 fold compared to the L-type calcium channel and

between ~80 to ~100 fold selectivity over voltage-gated sodium and potassium channels) [31].

In one other effort of significant note, David Triggle helped the team to design and generate a novel class of mixed T-type and sodium channels blockers efficacious in animal pain models. The agents appear to selectively stabilize slow-inactivated channel states and were found to mitigate off-target effects by preferentially attenuating hyperexcitable neurons while largely sparing normally firing neurons and other non-hyperexcited targets [32].

The novel state-dependent compound Z123212 is derived from the piperazine-based T-type blockers NP123875 and NP121912 and contains a dipeptide backbone (Fig. 3). *In vivo*, oral administration of Z123212 proved efficacious in reversing thermal hyperalgesia and tactile allodynia in a rat spinal nerve ligation model of neuropathic pain and also resulted in acute antinociception in a hotplate test. Further, at therapeutically relevant concentrations, Z123212 did not cause significant adverse effects including in the motor and cardiovascular systems [32].

Overall, the ultimate success of the drug discovery efforts at NeuroMed/Zalicus in part was due to David's keen insights, highly collaborative nature and willingness to make contributions at multiple levels to a small company. It goes without saying that this is just one pertinent example of his wider impact on the field of ion channel pharmacology.

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