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Neuronal nicotinic receptors: A perspective on two decades of drug discovery research

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ABSTRACT

Neuronal nicotinic acetylcholine receptors (nAChRs) have been a target for drug discovery efforts, primarily for CNS indications, for the past two decades. While nicotine and related natural products have been used for smoking cessation in various formulations (e.g., gum, spray, patches), it was only in 2006 with the launch of varenicline (Chantix™) by Pfizer for smoking cessation that a new chemical entity (NCE) originating from a rational medicinal chemistry effort targeting neuronal AChRs was approved. The current overview outlines the chronology of drug discovery efforts in nAChRs from the cloning of the receptor family in the 1980s, to initial research efforts at SIBIA, R.J. Reynolds and Abbott, to the current industry-wide interest in nAChR agonists as novel therapeutics for pain, schizophrenia and Alzheimer's Disease. Key events in the evolution of the nAChR field were the development of high throughput electrophysiological screening tools that provided the means to enable lead optimization efforts in medicinal chemistry and the discovery by John Daly at the NIH of the frog alkaloid, epibatidine, that provided the framework for the discovery of ABT-594, an $\alpha 4\beta 2$ agonist that is 200 times more potent than morphine as an analgesic. Over the next decade, it is anticipated that additional NCEs including antagonists and allosteric modulators (both positive and negative), interacting with various nAChR subtypes, will be advanced to the clinic in areas of high unmet medical need, e.g., pain, neurodegeneration, to provide novel medications with improved efficacy.

1. Introduction

For many years following the isolation of the nicotinic cholinergic receptor (nAChR) from the Torpedo electric organ [1–3], this receptor, a ligand-gated ion channel (LGIC), languished as a focus of interest for drug discovery although it remained a regular topic at the Gordon Conference on Medicinal Chemistry where numerous natural products, e.g. bungarotoxins, were discussed as molecular probes for the receptor but rarely as potential drugs.

Until the late 1980s, drug discovery interest in cholinergic transmission, which had been implicated as a key factor in the pathophysiology of Alzheimer's disease (AD) [4–6] had almost exclusively focused on the five members of the muscarinic GPCR family, the first to be cloned [7]. While some useful muscarinic receptor targeted therapeutics have been derived (e.g., atropine, ditropan, darafenacin, and tolterodine), these act peripherally. To date, CNS muscarinic agonist/mixed agonist/antagonist approaches have remained among the last to prove druggable using modern drug discovery

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techniques as a result of what appear to be unresolvable side effect liabilities.

While much of the biological research in the nAChR area focused on the electrophysiology of the electric organ and the isolated receptor [1,3,8], research efforts at the R. J. Reynolds group (now Targacept) had begun to lay the groundwork for chemistry-based pharmacological studies related to the effects of nicotine especially in the context of its presence as a key component of tobacco [9].Seminal work by Changeux and Edlestein [3], Heinemann and co-workers [13], Karlin [10], Katz [8], Lindstrom and co-workers [13], Patrick and co-workers [12,14], and others [1,2,7,9,11] focused on understanding the molecular structure and kinetic properties of nAChRs that were now recognized as a ligand-gated ion channel (LGIC)/receptor family. An unusual property of nAChRs, discussed further below, was the phenomenon of agonist-induced up-regulation of the receptor [8,10,15] that contrasted to the more traditional agonist-induced down-regulation seen with GPCRs.

2. The nAChR receptor family

With the advent of the molecular biology revolution in the late 1980s, Heinemann and co-workers [12,13] and Patrick [14] cloned and expressed nAChRs from a variety of species. The nAChR family [10,11,16,17] has as its basic motif, a functional pentamer consisting of five transmembrane spanning subunits around a central pore. Neuronal nAChRs are composed of combinations of α (α2–α6) and β (β2–β4) subunits, homomers of α subunits (α7 and α9) and α subunit heteromers (α9 and α10). The α8 subunit identified in avian brain has not been identified in mammals. Each subunit has four transmembrane (TM) segments, a long extracellular N-terminal domain, an

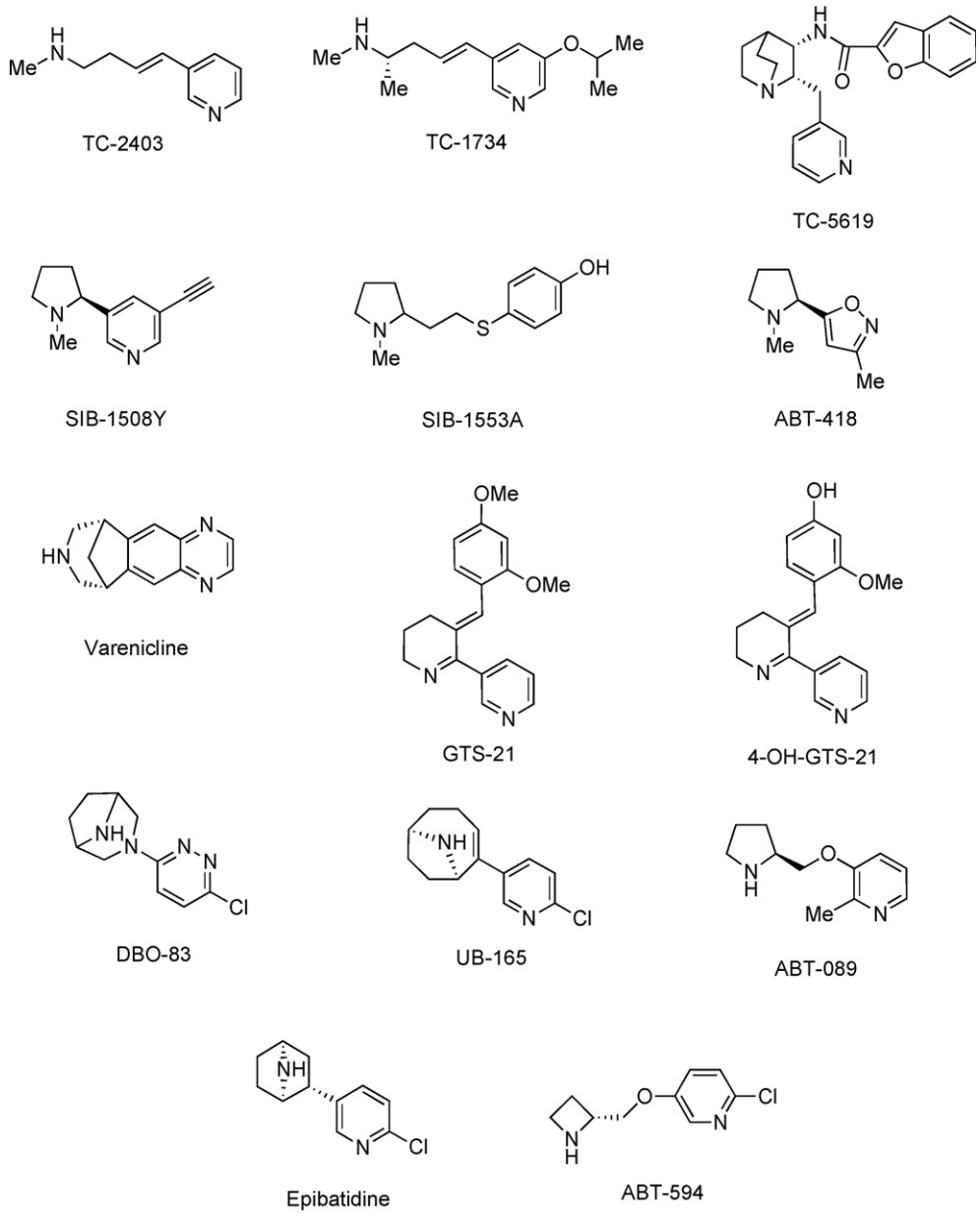

Fig. 1 – Early nAChR agonists.

intracellular loop between TMs 3 and 4 and a short C-terminal domain. A major advance in understanding the structure of nAChRs at less than 3 Á resolution was the identification and crystallization of an ACh binding protein, of unknown physiological function, secreted by glia [18].

The neuronal nAChRs of initial primary interest from a drug discovery perspective have been the α4β2 heteromer and the α7 homomer. At the former, ACh binds in a small pocket between the α4 and β2 subunits and in the latter, the binding site is defined by the adjacent α7 subunits [11,19]. Thus in the α4β2 construct, a maximum of 4 ACh orthotopic binding sites are possible while for the α7 homomer, there are potentially five ACh sites. From this consideration, α7 homomers may be considered more sensitive to agonist than the α2β4α7 heteromer [19]. Additionally, not all α4β2 heteromers may be anticipated to have similar pharmacology being dependent on the precise subunit ratio. For example, the (3)α4(2)β2 heteromer would be anticipated to be different in terms of both the number and structure of binding sites to the (2)α4(3)β2 heteromer. Moreover, substitution of the α5 subunit can further significantly alter the properties of the channel.

3. Applied research in the nAChR area

3.1. *R.J. Reynolds/Targacept*

The Winston-Salem, North Carolina-based tobacco company, R.J. Reynolds (RJR) had by virtue of its core business, a longstanding interest in the biological properties of nicotine. As already noted, its in house research group led by Don DeBethizy and Pat Lippiello was responsible for a number of peer-reviewed studies that elaborated on the basic pharmacology of nicotine. By the late 1980s, the RJR research group had added medicinal chemistry to its biology core, leading to the development of a proprietary quantum mechanics-based, computer-assisted QSAR modeling system, termed Pentad™ to reflect the pentameric structure of nAChRs. This was used to design new receptor subtype-selective ligands based on both compound and receptor structure and the biological properties of the former, especially their drug-like characteristics. The RJR research platform was spun out as Targacept in 2000 and since then has accumulated a library of some 6000 new chemical entities (NCEs) targeted to nAChRs and advanced a number of these NCEs into clinical trials including: TC-2403 (RJR-2403; Fig. 1) for ulcerative colitis; TC-2696, active at the α4β2 nAChR, for pain; TC-1734 (AZD3480; Fig. 1) in collaboration with AstraZeneca as monotherapy for mild-to-moderate AD [20] and cognitive deficits of schizophrenia and; TC-2216 for anxiety and depression. Other nAChR-selective NCEs in late preclinical development, include TC-5619 (Fig. 1), an α7 nAChR ligand with potential application across a number of indications.

3.2. *SIBIA*

Following cloning of nAChR subunits from various species by Patrick and Heinemann at the Salk Institute, the nAChR patent portfolio was licensed to SIBIA (Salk Institute Biomedical Industry Association), the commercial arm of the Institute. SIBIA, under the research leadership of Ken Lloyd, focused on developing nAChR agonists for CNS disorders in addition to developing programs in the NMDA receptor area. SIB-1508Y ([21] altinicline; Fig. 1) was targeted for Parkinson's disease (PD) and SIB-1553A [22] for AD (Fig. 1). Neither compound showed robust proof of concept in early clinical trials. In early 1999, however, both compounds were the subject of a collaboration agreement with Lilly. This was followed shortly after by the acquisition of SIBIA by Merck for $87 million which had a brief incarnation as Merck Laboratories San Diego until being closed in 2004. Neither Lilly or Merck are known to currently have any late stage nAChR targeted compounds in development.

3.3. *Abbott*

In 1989, Abbott had had a long-standing interest in muscarinic cholinergic receptor (mAChR) agonists as potential treatments for cognitive disorders that included AD. This interest, like that at many other drug companies, had been prompted by the considerable body of evidence for a decrease in cholinergic transmission in AD [5]. Unfortunately, the initial biological evaluation of the approximately 450 proprietary NCEs that had been synthesized by Abbott chemists did not lead to any fruitful discoveries.

In responding to the inevitable practicalities of the drug discovery environment, there still remained a priority within the Abbott R&D organization to identify *bona fide* drug candidates for AD. Necessity being the mother of invention, a decision was made by two of the authors (SPA and MW) to switch Abbott's drug discovery activities in AD to focus on nAChRs. Seventeen years later, this choice appears to have been well considered with a number of early clinical candidates/important research tools (ABT-418, ABT-594, ABT-089; Figs. 1 and 2) and more recent Phase II drug candidates (ABT-894, structure undisclosed) for the treatment of pain that emerged as the result of the efforts of the remaining author, MH and Mike Meyer in leading medicinal chemistry for nAChR ligands at Abbott.

At the time this project was initiated, it was a risky endeavor in that this LGIC target family was complex, access to stably transfected nAChR subunit constructs was limited until several years later [23] and there was a high level of skepticism from Abbott's Scientific Advisory Board (SAB) as to the target since, with the exception of Joe Coyle, a SAB member from Harvard, ''they had never heard of it'' and wondered ''if it's so good, why isn't Merck working on it?'' [NB- this was 8 years prior to Merck's acquisition of SIBIA]. In 1991, the SAB collectively expressed their skepticism. This 'watch and wait' approach is relatively common in larger pharmaceutical companies that promote a fast follow-on culture, and is one reason why other organizations did not embrace the wide spectrum of therapeutic opportunity afforded by nAChR pharmacology sooner. In 1993, when ABT-418 (Fig. 1) was nominated as a clinical candidate, the SAB offered their congratulations to the discovery team. In addition to the ongoing chemistry efforts [24], the recognition that electrophysiology was key to NCE characterization at LGICs led Abbott's Advanced Technology Group in collaboration with Daniel Bertrand at the University of Geneva to develop the first

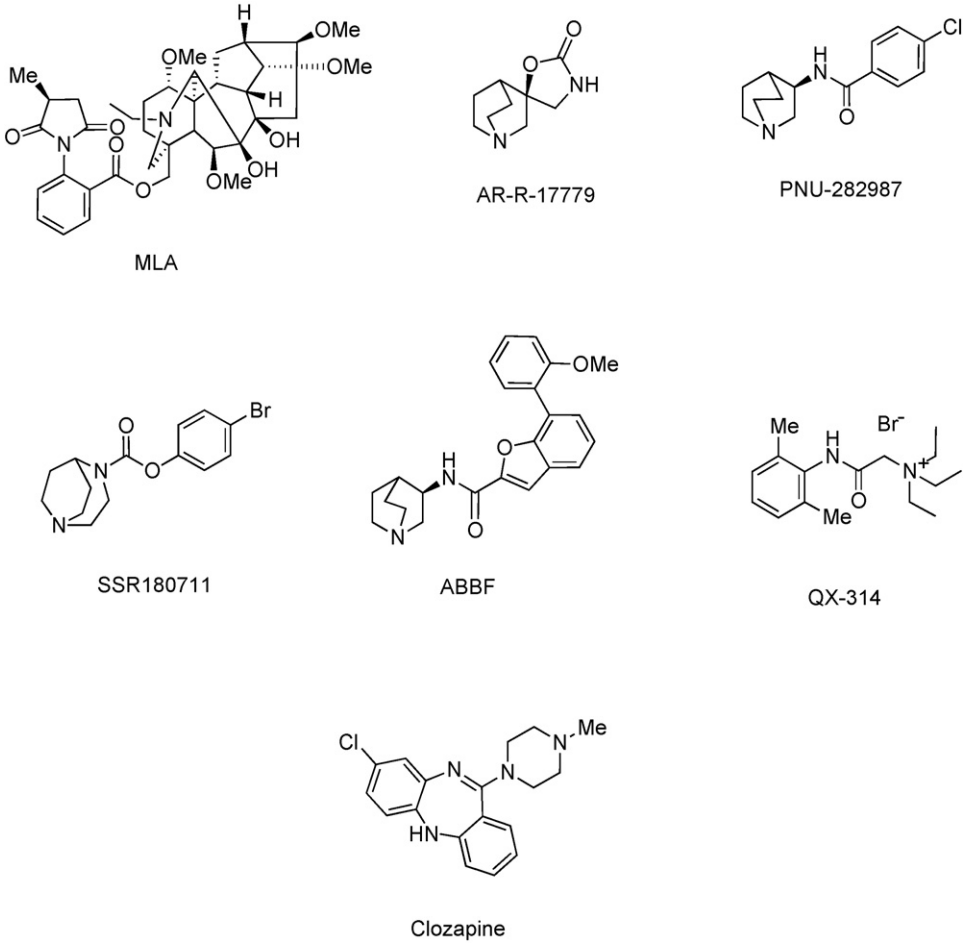

Fig. 2 – α7 nAChR agonists/partial agonists and antagonists.

automated oocyte recording device, Parallel Oocyte Electrophysiology Test (POET) [25].

3.4. *Pfizer*

The 2006 launch of varenicline (Chantix™; Fig. 1) represents the only approved CNS active nAChR derived from a rational medicinal chemistry effort—somewhat of an enigma given the relative paucity of Pfizer's contributions to the nAChR literature prior to 2006. Pfizer, like Abbott, had focused on nicotinic receptor-based drug discovery efforts in the 1990s. In contrast to Targacept, SIBIA and Abbott, who had invested in a diverse chemical and biological nAChR platform to understand and develop a broad-based approach to therapeutic targets involving nAChRs, the Pfizer objective led by Jim Heym and David Schultz, was exclusively focused on delivering a product for smoking cessation. The hypothesis was elegantly simple: partial agonists of α4β2 nAChRs should provide some of the beneficial properties of activating nAChRs (e.g., enhanced attention, weight control), have reduced side effects compared to nicotine while the intrinsic antagonist activity would result in less reinforcement of those properties to promote smoking cessation. While it can be debated whether restrained proprietary disclosure, or facile collegial exchange between academia and industry is the best approach to drive innovation in drug discovery, the fact remains that Pfizer was first to market in the last two decades of CNS research using a rational drug-design approach targeting nAChRs.

3.5. *Academic activities*

Concomitant with cloning activities on nAChRs at the Salk Institute, several other academic centers focused research on the nAChR receptor. At the University of Florida in the early 1990s, Bill Kem, Ed Meyer and Roger Papke worked on the anabaseine analog, GTS-21 (DXMBA; Fig. 1) in collaboration with the Japanese pharmaceutical company, Taiho. This selective α7 nAChR partial agonist [26,27] completed Phase I trials in 2000 and since then has had a long and chequered history in terms of its targeted therapeutic utility. It is currently being advanced by CoMentis (formerly Athenagen) for cognitive improvement in patients with schizophrenia, AD and ADHD based on its preclinical profile in numerous animal models. The major metabolite of GTS-21, 4-OH-GTS-21 (Fig. 1) was more active at the α7 nAChR than the parent [27].

DBO-83 (Fig. 1), a nicotinic agonist developed at the University of Milan by Barlocco and co-workers showed antinociceptive [28] and antiamnesic [29] activity in animal models. UB-165 (Fig. 1) is an α4β2 agonist emanating from Wonnacott and co-workers' research activities at the

University of Bath [30]. A key compound in the evolution of the therapeutic utility of nAChRs is the frog alkaloid, epibatidine (Fig. 1) discovered by Daly and co-workers [31] as a novel analgesic agent and is discussed further below.

3.6. Other efforts in drug discovery

With the preclinical proof of concept from SIBIA, R.J. Reynolds/Targacept and Abbott that drug-like, proprietary NCEs could be developed against nAChR subtypes, other pharmaceutical companies took note of the uptapped potential in the area and the broad possibilities in terms of therapeutic indications. Thus Astra, Bayer/En Vivo, Biogen, Cytomed/UCB, Lilly, Memory, NeuroSearch, Pharmacia, Pfizer and Sanofi (as Synthelabo) were all involved in NCE based research in the area. As noted below, these efforts have resulted in a number of NCEs active at nAChR subtypes that are both prototypic research tools and potential drugs.

4. Therapeutic indications

Given the profound effects of nicotine on CNS function both directly via nAChR modulation and via indirect potentiation of the release of a variety of key neurotransmitters including dopamine, gamma-aminobutyric acid, glutamate, serotonin, histamine and norepinephrine, a variety of indications for receptor subtype selective nAChR ligands have been postulated [32–34]. These are reviewed in detail below.

4.1. Smoking cessation

An obvious target for a novel nAChR agonist was for use in smoking cessation as a replacement for nicotine patches and gum that, together with behavioral therapy and the monoamine uptake blocker, buproprion, were the main treatment modalities. By designing a receptor subtype selective nAChR agonist or partial agonist to replace nicotine, the urge to smoke could theoretically be obviated while not activating those nAChRs stimulating autonomic function. ABT-418, the first NCE intentionally developed as a patch formulation built upon this concept and used positive results from a small Phase 2 study in smoking cessation as a proof of concept (PoC) to help support dose-selection for its study in AD patients. However following the failure of ABT-418 in AD (see Section 4.2), smoking cessation was considered as a possible alternative indication for NCEs from the extensive nAChR portfolio available at that time but this idea was discarded in 1997 as the commercial organization failed to reconcile the size of the then market in smoking cessation with the billions of dollars in revenue that an effective AD drug would produce. In the meantime, however, Pfizer who had also become involved in nAChR research, in part because of an in house interest and in part because of their acquisition of Pharmacia were investigating other potential uses of nAChR agonists. This culminated in 2006 with the FDA approval of varenicline (Fig. 1), an $\alpha4\beta2$ partial agonist derived from cytisine [35] for use in the treatment of smoking cessation. In a trial in which the effects of varenicline were compared with buproprion, a mixed monoamine uptake inhibitor approved for use in smoking cessation as Zyban™, the former showed a two-fold increase in smoking cessation with 23% of individuals taking varenicline stopping smoking compared with 10% in a control placebo group and 15% in the buproprion SR group [36]. The overall 1 year abstinence rate for veranicline remains the best of any approved smoking cessation therapy with 1.5 million patients treated to date including two notable individuals each of whom had smoked for 50 years and quit after taking varenicline. The compound has also shown promise in reducing ethanol consumption and seeking in animal models [87].

4.2. Cognition and attention

Based on acute studies showing efficacy for nicotine in the treatment of AD [37,38], the initial clinical candidate from the Abbott research effort in nAChRs was ABT-418 [Fig. 1; 39]. This NCE was advanced to trials in 1993 for proof of concept in AD having shown cognition enhancing activity in a number of preclinical models [40]. The resultant placebo controlled trial was run at two centers, one in Miami and one in Beverly Hills using more than 200 patients in five age-matched arms: placebo, three doses of ABT-418 selected on the basis of animal and human (proof of concept for smoking cessation) data and a dose of nicotine previously found to be effective in enhancing cognitive function in humans. Neither ABT-418 nor nicotine showed efficacy in this trial while the placebo response was larger than had been seen in similarly designed AD trials – possibly the result of the novel patch formulation. Contrary to many rumors, there were no adverse cardiovascular events attributable to ABT-418 in the targeted population in either Phase I or II studies. Following the disappointment of this trial, an acute study was conducted with ABT-418 in the original AD paradigm in which nicotine was found to be active [38] and where this $\alpha4\beta2$ agonist showed similar effects to nicotine, thus providing a proof of concept [41]. ABT-418 was also efficacious in a limited human trial in ADHD [42]. A second NCE, the agonist/partial agonist, ABT-089 [Fig. 1; 43] was also efficacious in ADHD [44]. A newer $\alpha4\beta2$ agonist entry into the cognition area is TC-1734 (ispronicline; Fig. 1) currently in Phase II for monotherapy for mild-to-moderate AD [19].

Nicotine can attenuate amyloid formation and neurotoxicity [45], the latter a key feature of the pathophysiology of AD [46]. Interestingly, the amyloid peptide, Aβ1-42 has potent interactions with nAChRs, with Ki values of 4 pM at the rat $\alpha7$ and 30 nM at the $\alpha4\beta2$ nAChR [47]. Additional studies demonstrated a non-competitive blockade of rat hippocampal $\alpha7$ nAChRs by rat Aβ1-42 (100 nM) that was more potent than rat Aβ1-40 or human Aβ1-40 at the same concentration while human Aβ10–1 was inactive [47,48]. This contrasted with the finding [49] that Aβ1-42 had agonist activity at $\alpha7$ nAChRs at 10 pM, an effect blocked by the $\alpha7$ agonist, 4-OH-GTS21 and the antagonist, methyllycaconitine (MLA; Fig. 2). Additionally, in the Tg2576 transgenic mouse model of AD, up-regulation of $\alpha7$ nAChRs in the dentate gyrus was temporally correlated with plaque accumulation and learning deficits [50] while antisense oligonucleotides to the $\alpha7$ nAChR blocked Aβ1-42-induced tau phosphorylation [51], further reinforcing a potential role for $\alpha7$ nAChRs in AD.

4.3. *Schizophrenia*

There is a considerable literature on the excessive smoking (''the most toxic and addictive form of nicotine delivery [52]) in schizophrenics that is viewed as a means to self-administer nicotine as a treatment for this neuropsychiatric disorder [53,54]. Work from Bob Freedman's group in Colorado had linked deficits in the gating of the hippocampal P_{50} auditory evoked potential to schizophrenia and also found that schizophrenics showed decreases in α7 nAChR mRNA [55,56]. Both nicotine [57] and the α7 partial agonist, GTS 21 [58] can ameliorate dysfunction in acoustic startle responses. These findings, together with evidence for a genetic relationship relating dysfunctional auditory gating in schizophrenics and α7 receptors [59,60] have focused considerable attention on modulators of this receptor as a treatment for the cognitive element of the disease [61,62,63].

The first α7 receptor ligands of note were GTS-21 [26] and AR-R 17779 [64] (Fig. 2) both partial agonists. Newer agents with known structures (Fig. 2) include PNU-282987 [65], SSR180711 [66] and ABBF [67]. Other compounds cited on competitive databases as α7 receptor agonists include MEM-3454 [68] and a follow on, MEM-63908, an alkaloid (+)205 [69] that has been described as an α7 receptor antagonist and QX-314 (Fig. 2), a quaternary lidocaine derivative that is not likely to penetrate the brain. Varenicline has been characterized as a full agonist at α7 receptors [70]. Additionally, the atypical antipsychotic clozapine (Fig. 2) has weak (Ki = 3.2 μM) α7 *antagonist* activity [71]. With the number of NCEs focused on the α7 receptor, reports describing initial positive data in Phase II trials, and an approved drug, varenicline, having α7 agonist activity, it appears that proof of concept in schizophrenia for these of agents in humans could be imminent.

4.4. *Pain*

Initial observations that nicotine might have analgesic activity dates back to 1932 [72]. However it was not until the discovery of what was eventually identified as the frog alkaloid, epibatidine [31; Fig. 1] that the role of α4β2 agonists in pain modulation was established. Alkaloids extracted from the skin of the frog, *Epipedobates tricolor* collected by Daly et al. [73] in 1974 led in the identification of a fraction that produced a Straub tail response in mice, characteristic of an opioid-like material. It was not until 1990 that NMR spectroscopy methods were developed that were sufficiently sensitive to identify the active ingredient, epibatidine, which was then synthesized by a number of laboratories and found to have extremely potent analgesic activity. Concomitant with these findings, Abbott scientists were also exploring the analgesic potential of nAChR ligands and in 1993, a chemist from Abbott's inflammation research group, Yat Sun Or, noted a news review in *Science* [74] identifying the structure of epibatidine but not its mechanism of action (MoA). Yat Sun immediately recognized that NCEs with similar structural motifs were being made at Abbott as part of its exploratory effort in nAChR-based analgesics. MW then immediately contacted Daly to see whether the MoA of epibatidine was known. John, in his usual gracious manner, indicated that a paper was in press on this topic and after being asked whether it was nicotinic receptor-mediated, agreed. While no formal collaboration was established with Daly, Abbott scientists led by MH, eventually identified ABT-594 (Fig. 1) an α4β2 agonist that was 200-times more potent than morphine as an analgesic [75]. This was published in *Science* in the first week of January 1998, a ''quiet news week'' because of the New Year holiday season that resulted in full media coverage, print, radio and cable TV. The latter and the concept of nicotinic analgesia being superior to opioids led the songwriter, Paul Simon to memorialize the event in song [76]. Contrary to later reports [77], Abbott's synthesis of ABT-594 was not directly based on Daly's research, nor was Abbott developing epibatidine [73]. Rather, Abbott researchers along with others in both academia and industry benefited significantly from the basic research efforts funded by the NIH. ABT-594 was discontinued in Phase II for undisclosed reasons. Nonetheless, Abbott has continued its efforts to identify an α4β2 analgesic ''more powerful than morphine'' [76] and like Targacept with TC-2696, continues clinical activities in the area with ABT-894 and related NCEs.

5. Challenges in nAChR-based research

It is now nearly 20 years since the nAChR family became a *bona fide* target for drug discovery and to date, despite many clinical entries, only one NCE, varenicline, a cytosine derivative has been approved as a nicotine replacement therapy. Many other potentially exciting indications are still being actively pursued with agonist ligands, either full or partial.

There is considerable emerging interest in both nAChR antagonists [78] and allosteric modulators of nAChRs [3,11,79]. As noted, clozapine (Fig. 2) has been described as an α7 receptor antagonist [71], a finding that is counterintuitive given the considerable evidence supporting *agonists* at this receptor as being beneficial in the treatment of the cognitive symptoms of schizophrenia. Certainly, clozapine remains the gold standard for the treatment of schizophrenia [80] suggesting that the physiological consequences of α7 receptor interactions may require some reappraisal with new data. As noted at the beginning of this overview, nAChRs are unique in that they rapidly desensitize by up-regulation of the receptor [8] leading to the possibility that such agents may be functional antagonists by virtue of this effect. If this were the case, and there is considerable debate on the topic, then an nAChR *antagonist* may in fact be a more rapidly acting *agonist* a concept that can be tested with selective antagonist ligands but where studies to date have been thought provoking. Interestingly, conotoxin-based nAChR antagonists have been identified [81]. Similarly, inhibitors of the dopamine transporter (DAT) like GBR-12909 (Fig. 3) can also block nAChRs [82].

On the allosteric front, negative allosteric modulators of nAChRs are well known [32] and include histrionicotoxin, PCP, MK-801 (Fig. 3), etc. and more recently, the cholinesterase inhibitor, galantamine (Fig. 3) has been classified as an allosteric potentiator at nAChRs [83]. Led by Vince Groppi's development of high throughput FLIPR technologies to assess ion channel modulation, robust subtype selective modulators of α7 function that include PNU-120596 (Fig. 3; [84]) and other NCEs that modulate nAChRs [85,86] have been identified including a modulator selective for the α7 receptor (Fig. 3).

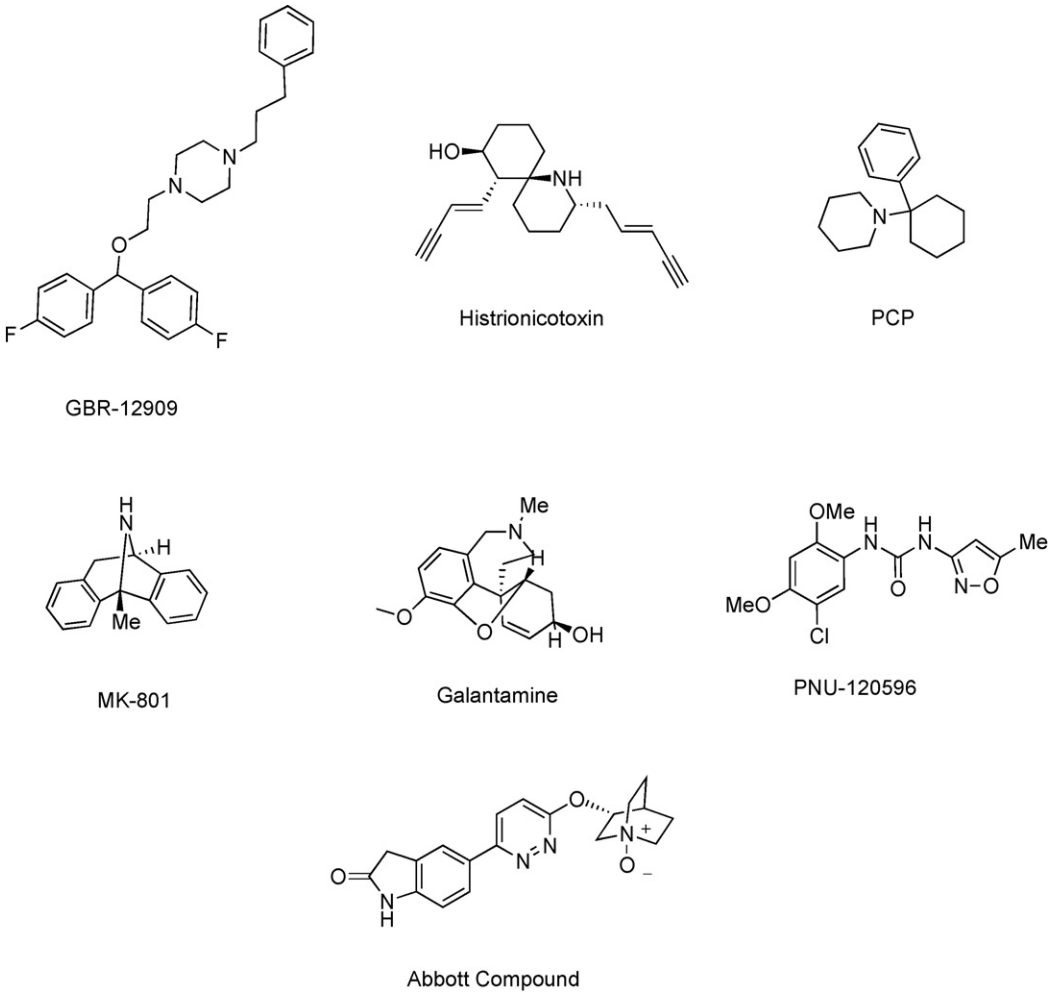

Fig. 3 – Antagonists and allosteric modulators.

While expanding the functional phenotype of nAChR agonists to the antagonist and allosteric modulator domains can only further add to the complexity of research in the field, it will aid in a more precise understanding of the nuances of nAChR function especially as this relates to disease conditions and will help efforts in drug discovery to identify novel, safe medications that represent incremental advances to drugs already available. The oft quoted ''200 times more potent than morphine with no addiction liability'' (at least as assessed in animal models) for ABT-594 represents a major advance in the area of analgesia where there is considerable unmet medical need and a huge market potential. Having demonstrated such remarkable efficacy in this one instance, the challenge to the medicinal chemist remains to understand the SAR of the nAChR substypes, including allosteric sites to derive the necessary therapeutic index for an approvable medication. In this context, it is to be expected that other modulators of nAChR function may have unanticipated efficacy in their targeted diseases.

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