# High-Throughput Screening for Next-Generation Analgesics: Charting Non-Opioid Pain Targets and Assay Innovations

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**DOI: https://dx.doi.org/10.51244/IJRSI.2026.1307000150**

**Received: 20 July 2026; Accepted: 25 July 2026; Published: 04 August 2026**

## ABSTRACT

The growing burden of acute and chronic pain, coupled with the limitations of opioid-based therapies, has intensified the search for safer and more effective non-opioid analgesics. High-throughput screening (HTS) has emerged as a transformative strategy in analgesic drug discovery, enabling the rapid identification and characterization of novel compounds targeting peripheral pain pathways while minimizing central nervous system liabilities. This short communication highlights recent advances in HTS technologies and their role in accelerating the development of next-generation analgesics. We discuss the expanding landscape of non-opioid molecular targets, including voltage-gated sodium (NaV) channels, transient receptor potential (TRP) channels, non-opioid G protein-coupled receptors, and neuroimmune signalling pathways, alongside innovative screening platforms such as automated patch-clamp electrophysiology, human induced pluripotent stem cell (hiPSC)-derived nociceptor models, multi-electrode arrays, all-optical electrophysiology, and label-free mass spectrometry. We further examine the complementary roles of target-based and phenotypic screening approaches and consider the growing integration of artificial intelligence and machine learning in virtual screening, hit prioritization, and lead optimization. Collectively, these advances are reshaping the analgesic discovery pipeline by improving the physiological relevance, scalability, and predictive power of preclinical screening. As clinically validated non-opioid therapies continue to emerge, the convergence of advanced screening technologies and computational approaches offers a promising framework for the discovery of safer, more effective analgesics to address the global burden of pain.

**Keywords**: High-throughput screening (HTS), Non-opioid analgesics, Pain drug discovery, Human induced pluripotent stem cells (hiPSCs), Phenotypic screening

The global management of acute and chronic pain stands at a critical juncture. For decades, pharmacotherapy for moderate-to-severe pain has been dominated by classic μ-opioid receptor (μ-OR) full agonists [1]. While highly effective at blunting nociceptive signalling, standard opioids carry profound liabilities including rapid tolerance, dose-limiting respiratory depression, dependence, and high potential for misuse [2]. The resulting public health crisis has underscored an urgent, unmet clinical imperative: the discovery and development of potent, non-addictive analgesics that decouple robust pain relief from central reward mechanisms and respiratory risks [3].

Historically, the analgesic drug discovery pipeline suffered from severe translational bottlenecks. Preclinical campaigns relied heavily on low-throughput animal behavioural models or rodent cell cultures that frequently failed to recapitulate complex human pain biology, leading to high attrition rates in Phase II and Phase III clinical trials [4]. Furthermore, early target-based screening was heavily constrained by an over-reliance on isolated GPCR pathways and simplified heterologous expression systems, which often lack the complex

signalling machinery, endogenous co-factors, and native excitability characteristics of human sensory neurons [5, 6]

To overcome these barriers, the field of pain drug discovery is undergoing a paradigm shift driven by modern High-Throughput Screening (HTS) platforms. Rather than relying on isolated single-target binding assays, modern HTS has evolved into high-content, physiologically relevant systems capable of evaluating vast compound libraries across a broad spectrum of non-opioid targets. Screening campaigns are increasingly expanding beyond μ-ORs to interrogate peripheral voltage-gated sodium channels, transient receptor potential (TRP) channels, non-opioid GPCRs, and neuro-immune signalling pathways [7]. Simultaneously, advances in human induced pluripotent stem cell (hiPSC) technology now enable automated, high-density screening directly on patient-derived sensory neurons, effectively bridging the translational gap between *in vitro* assays and human nociception [8]. When combined with high-throughput automated patch-clamp (APC), all-optical electrophysiology, and high-density microelectrode arrays (MEA), these platforms permit real-time functional evaluation of neuronal excitability at unprecedented scale [4].

The clinical feasibility of targeting peripheral, non-opioid pain mechanisms has been recently validated by the discovery and clinical advancement of suzetrigine (VX-548), a highly selective $Na_v1.8$ inhibitor that acts directly on peripheral nociceptors without central nervous system liabilities[3, 7]. Building on this momentum, this review provides a comprehensive overview of how modern HTS paradigms are reshaping the landscape of pain research. We examine the expanding repertoire of non-opioid molecular targets, evaluate next-generation screening technologies from hiPSC nociceptive models to optical electrophysiology and discuss how integrating machine learning and phenotypic screening can accelerate the pipeline for non-addictive analgesics.

This short communication is based on a narrative review of recent literature on high-throughput screening technologies for analgesic drug discovery. Publications were identified through searches of PubMed, Scopus and Web of Science using combinations of keywords including "high-throughput screening", "analgesics", "pain drug discovery", "non-opioid targets", "Nav channels", "TRP channels", "GPCR", "hiPSC", and "phenotypic screening". Priority was given to peer-reviewed articles published within the last decade, together with landmark studies and recent clinical reports relevant to emerging non-opioid analgesics.

The modern landscape of analgesic drug discovery has expanded significantly beyond classical mu-opioid receptor (μ-OR) activation, driven by a deeper understanding of peripheral and central nociceptive sensitization. High-Throughput Screening (HTS) campaigns now systematically target diverse molecular families that modulate neuronal excitability without engaging central reward circuitry [6].

Among these, peripheral voltage-gated sodium channels $Na_v$ represent a primary focal point. Subtypes $Na_v1.7$, $Na_v1.8$, $Na_v1.9$ are predominantly expressed in primary afferent nociceptors within dorsal root ganglia (DRG). Selective blockade of these isoforms inhibits action potential generation and propagation in peripheral sensory nerves while avoiding central nervous system liabilities [7, 9]. Concurrently, transient receptor potential (TRP) ion channels notably TRPV1, TRPA1, and TRPM8 have emerged as key targets due to their role as polymodal sensors of thermal, chemical, and mechanical noxious stimuli [10]. Modern functional HTS platforms utilize automated fluorescent imaging and high-throughput electrophysiology to identify state-dependent blockers and negative allosteric modulators capable of desensitizing these channels during chronic inflammatory states.

Beyond ion channels, target profiling increasingly encompasses non-opioid G-protein coupled receptors (GPCRs) and neuro-immune signalling networks. Screening campaigns targeting cannabinoid type2 $CB_2$ receptors, adenosine $A_2A/A_3$ receptors, and chemokine receptors (such as CCR2 and CX3CR1) aim to suppress microglial activation and neuroinflammation without inducing psychotropic side effects or physical dependence [5]. Furthermore, high-throughput kinase profiling against targets like p38 mitogen-activated protein kinase (p38 MAPK) and tropomyosin receptor kinase A (TrkA) offers avenues to halt downstream nociceptive sensitization [3]. By diversifying screening libraries across these complementary ion channel, GPCR, and enzymatic pathways, modern HTS establishes a robust foundation for multi-target and non-addictive analgesic drug discovery. The growing diversity of molecular targets has created new technical demands, requiring

screening platforms capable of accurately measuring diverse electrophysiological, biochemical, and cellular responses.

The expansion of non-opioid drug target libraries has necessitated parallel advancements in high-throughput assay instrumentation. Classical fluorescent dye-based platforms, while capable of screening large chemical libraries, often produce high false-positive rates and fail to capture complex functional electrophysiology[11, 12]. To bridge this gap, second-generation automated patch-clamp (APC) systems now enable parallel planar patch recordings from 384- to 768-well plates, generating gigaseal-quality whole-cell recordings across thousands of single cells daily[13]. These APC platforms allow precise voltage-clamp profiling of state-dependent channel blockers against pain-relevant targets, such as $Na_V$ and transient receptor potential (TRP) channels, directly accelerating hit-to-lead transitions in early drug discovery[11, 13].

In parallel, all-optical electrophysiology ("Optopatch") platforms have emerged as a high-content alternative for functional neuronal screening [14]. By co-expressing genetically encoded voltage indicators with light-activated ion channels within human induced pluripotent stem cell (hiPSC)-derived sensory neurons, Optopatch enables contactless, optical stimulation and recording of action potential dynamics across dense neuronal populations at throughputs exceeding half a million cells per day [14, 15]. This spatial parallelism permits phenotypic screening of compound libraries against activity-dependent neuronal firing and network hyperexcitability in native human cellular contexts without requiring physical microelectrode attachment [8, 15].

Complementing optical methods, multi-electrode array (MEA) platforms provide non-invasive extracellular recordings of network-level spontaneous and evoked action potentials in cultured hiPSC-nociceptors [4, 16]. MEA-based phenotypic screens allow researchers to evaluate how candidate analgesics dampen inflammatory or neuropathic hyperexcitability phenotypes in real time across multi-well formats [4, 16]. Finally, label-free bioanalytical screening has been transformed by ambient mass spectrometry [17]. Technologies such as ultra-high-throughput desorption electrospray ionization mass spectrometry (DESI-MS) process samples directly from complex biological matrices at acquisition rates exceeding 1 sample per second [17, 18]. By quantifying native enzyme substrates and small-molecule metabolites without requiring radiolabels or fluorescent tags, DESI-MS facilitates rapid ligand-binding assays and enzymatic profiling for neuro-immune and enzymatic pain targets [18]. Together, the integration of high-density APC, optogenetic electrophysiology, MEA recording, and label-free DESI-MS establishes a robust technological foundation for evaluating novel analgesic candidates at scale. While these platforms have greatly expanded experimental capabilities, their value ultimately depends on the screening strategy employed.

Table 1. Comparison of High-Throughput Screening Platforms for Analgesic Drug Discovery

| **Screening Platforms** | **Advantages** | **Limitations** | **Relative Costs** | **Best Applications** |
|---|---|---|---|---|
| Automated Patch Clamps (APC) | Gold-standard electrophysiology; precise ion-channel measurements | Expensive instrumentation; specialised expertise | High | $Na_V$ and TRP channel screening |
| Multi-Electrode Arrays (MEA) | Measures neuronal network activity; long-term recordings | Lower single-cell resolution | Moderate-High | Phenotypic screening of neuronal excitability |
| All-Optical Electrophysiology (Optopatch) | Very high throughput; non-invasive functional recording | Requires genetic engineering and specialised imaging | High | hiPSC-derived nociceptor screening |
| DESI-MS | Label-free; rapid metabolite and | Very high equipment cost; limited functional | Very High | Enzyme assays and metabolic profiling |

| | | | | |
|---|---|---|---|---|
| | enzyme analysis | information | | |
| Fluorescence-based assays | Fast, inexpensive, scalable | Higher false-positive rates; indirect functional readouts | Low | Primary high-throughput screening |

Each screening platform offers distinct advantages and trade-offs. Automated patch-clamp provides the highest electrophysiological precision for ion-channel studies but requires costly instrumentation and specialised expertise. In contrast, fluorescence-based assays are inexpensive and highly scalable but often sacrifice physiological accuracy. Phenotypic platforms such as MEA and Optopatch provide greater biological relevance by capturing neuronal network activity, although they involve higher technical complexity and operating costs. Consequently, the choice of screening technology should be guided by the biological target, experimental objectives, throughput requirements, and available resources.

The paradigm shift in analgesic discovery is defined by an evolving synergy between target-based and phenotypic screening approaches. Target-based High-Throughput Screening (HTS) has historically dominated industrial drug discovery, leveraging recombinant expression systems to rapidly identify high-affinity small-molecule modulators against isolated receptors or ion channels[19, 20]. While target-centric campaigns excel at delivering precise structure-activity relationships, their clinical translation in pain research has frequently stalled because single-target modulation fails to account for the redundant, polygenic signalling networks that drive pathological nociception [6, 21].

Conversely, phenotypic HTS evaluates drug candidates based on their functional capacity to reverse disease-relevant cellular behaviours in complex bioassays, irrespective of a predefined single molecular mechanism [22]. In analgesia discovery, phenotypic screening increasingly utilizes "pain-in-a-dish" models composed of human induced pluripotent stem cell (hiPSC)-derived nociceptors [8]. By measuring multi-parameter functional outputs such as the attenuation of spontaneous firing, reduction of inflammatory hyperexcitability, or normalization of action potential waveforms phenotypic assays capture multi-target polypharmacology within native human cellular architecture [4, 19]

Despite these advantages, phenotypic campaigns present distinct downstream challenges, notably the labour-intensive requirement for target deconvolution and mechanism-of-action determination prior to clinical optimization [22]. Consequently, modern screening strategies are converging on integrated hybrid frameworks. By combining initial ultra-high-throughput target-based screening with early phenotypic validation in hiPSC-nociceptor models, drug discovery programs can efficiently reconcile target specificity with translational physiological relevance [8, 20]. The integration of hybrid experimental workflows also provides a foundation for computational methods that further improve screening efficiency.

The future of high-throughput screening (HTS) in pain drug discovery relies on the synergistic integration of physical assays with artificial intelligence (AI) and machine learning (ML) [23]. Virtual ultra-HTS platforms can now screen giga-scale chemical libraries containing billions of make-on-demand molecules against target structures, dramatically expanding the chemical space evaluated prior to physical synthesis[24, 25]. When coupled with structural biology breakthroughs in cryo-electron microscopy, predictive ML algorithms rapidly prioritize non-opioid hits with optimized binding kinetics and subtype selectivity [26].

Despite these computational and bioanalytical advances, significant translational hurdles remain. High-throughput human induced pluripotent stem cell (hiPSC) assays require rigorous standardization across cell lines and differentiation protocols to ensure reproducible electrophysiological phenotypes[6, 22]**.** Furthermore, translating *in vitro* functional attenuation of nociceptor hyperexcitability into clinical efficacy demands robust biomarker integration and refined patient stratification strategies in early-phase trials [3].

Table 2. Examples of Non-Opioid Analgesics Advancing from High-Throughput Screening to Clinical Development

| Compound | Molecular Target | Developmental Status | Clinical Significance |
|---|---|---|---|
| Suzetrigine (VX-548) | $Na_V1.8$ | FDA approved/Clinical use | First-in-class oral non-opioid analgesic for acute pain |
| Resiniferatoxin | TRPV1 | Clinical Trials | Investigated for severe cancer pain and osteoarthritis |
| CNTX-4975 | TRPV1 | Phase II/III clinical studies | Long-acting treatment for osteoarthritis pain |
| Olorinab (APD371) | CB2 Receptor | Clinical Trials | Evaluated for visceral pain disorders |

These examples demonstrate that advances in high-throughput screening are increasingly translating into clinically relevant non-opioid analgesics. Nevertheless, the overall success rate remains low, as many promising preclinical candidates fail because of inadequate efficacy, unexpected toxicity, poor pharmacokinetic properties, or difficulties reproducing preclinical findings in heterogeneous patient populations. Addressing these translational barriers will require closer integration of physiologically relevant screening models, predictive biomarkers, and carefully designed clinical trials.

Ultimately, modern HTS is shifting the analgesic landscape away from central μ-opioid receptor full agonism toward diversified peripheral targets, human-relevant cellular models, and AI-accelerated workflows. As clinical proofs-of-concept continue to emerge, the evolution of high-throughput screening stands as the premier frontier for delivering potent, non-addictive therapies for acute and chronic pain.

**Ethical Approval**

Ethical approval was not required because this article is based solely on published literature and does not involve human participants, animals, or primary experimental data.

**Data Availability**

No new datasets were generated or analysed during this study. Data sharing is therefore not applicable.

**Conflict Of Interest**

The authors declare no conflict of interest.

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