Appendix A

ACS APPLIED MATERIALS & INTERFACES

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Research Article

Logic "AND Gate Circuit" Based Mussel Inspired Polydopamine Nanocomposite as Bioactive Antioxidant for Management of Oxidative Stress and Neurogenesis in Traumatic Brain Injury

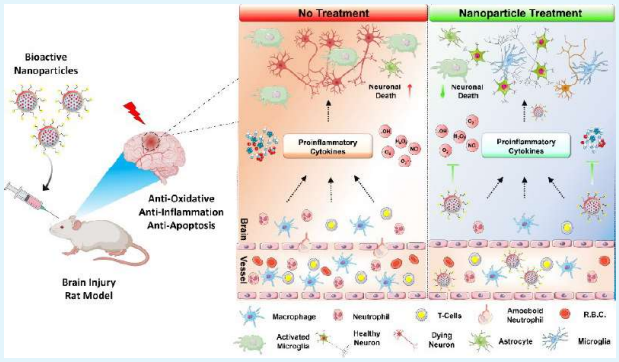
Shubham Garg, Aniket Jana, Juhee Khan, Sanju Gupta, Rajsekhar Roy, Varsha Gupta, and Surajit Ghosh*

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ABSTRACT: In the intricate landscape of Traumatic Brain Injury (TBI), the management of TBI remains a challenging task due to the extremely complex pathophysiological conditions and excessive release of reactive oxygen species (ROS) at the injury site and the limited regenerative capacities of the central nervous system (CNS). Existing pharmaceutical interventions are limited in their ability to efficiently cross the blood-brain barrier (BBB) and expeditiously target areas of brain inflammation. In response to these challenges herein, we designed novel mussel inspired polydopamine (PDA)-coated mesoporous silica nanoparticles (PDA-AMSNs) with excellent antioxidative ability to deliver a new potential therapeutic GSK-3β inhibitor lead small molecule abbreviated as Neuro Chemical Modulator (NCM) at the TBI site using a neuroprotective peptide hydrogel (PANAP). PDA-AMSNs loaded with NCM (i.e., PDA-AMSN-D) into the matrix of PANAP were injected into the damaged area in an *in vivo* cryogenic brain injury model (CBI). This approach is specifically built while keeping the logic AND gate circuit as the primary focus. Where NCM and PDA-AMSNs act as two input signals and neurological functional recovery as a single output. Therapeutically, PDA-AMSN-D significantly decreased infarct volume, enhanced neurogenesis, rejuvenated BBB senescence, and accelerated neurological function recovery in a CBI.



KEYWORDS: *Antioxidant, Neuroprotection, Traumatic brain injury (TBI), Polydopamine, Mesoporous silica nanoparticles*

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INTRODUCTION

Treatment of Traumatic Brain Injury (TBI) is a challenging task due to its multifaceted complex condition that causes significant global mortality annually.[1] It is worthwhile to mention that TBI itself accounts for the highest incidences among all common neurological disorders.[2] Every year, an astonishing almost 50 million individuals suffer the deleterious consequences of TBI.[2] The substantial costs associated with the clinical treatment of TBI and the resulting socio-economic challenges have imposed a huge burden on the healthcare system and family and on society at large. The complex pathophysiology of TBI can be categorized into two distinct features: primary injury and secondary injury. Primary damage is followed by extended secondary damage, which encompasses several factors, namely the disruption of the blood-brain barrier (BBB),[3] glutamate excitotoxicity,[4] oxidative stress, overproduction of reactive oxygen species (ROS), reactive nitrogen species (RNS),[5] reactive gliosis,[6] and the activation of neuroinflammation.[7] This, in turn, triggers subsequent processes that ultimately result in a persistent neurological disorder, including activation of microglia and astrocytes at the lesion site,[8] hindering the integration and generation of new neurons, preventing tissue and axonal development.[9] In this sequence of events, primary injury can be avoided only; however, secondary injury presents a potential avenue for therapeutic intervention to preserve brain tissue. Unfortunately, despite an extensive array of clinical trials, no available therapeutic has shown efficacy in improving the pathophysiology of TBI.[10] Hence, there is an urgent need for the development of potential therapeutics that can target secondary injury-related pathophysiology in TBI.

However, in recent years, transplantation of induced pluripotent stem cells (iPSCs),[11] mesenchymal stem cells (MSCs),[12,13] and neural stem cells (NSCs)[14] have emerged as potent and promising therapeutic approaches in the domain of regenerative medicine. However, challenges such as low cell viability, immunological rejection, and the inability to precisely quantify cell viability could impact the accurate evaluation of

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Discovery of gallic acid-based mitochondriotropic antioxidant attenuates LPS-induced neuroinflammation

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A B S T R A C T

Mitochondria are complex organelle that plays a pivotal role in energy metabolism, regulation of stress responses, and also serve as a major hub for biosynthetic processes. In addition to their well-established function in cellular energetics, it also serves as the primary site for the origin of intracellular reactive oxygen species (ROS), which function as signaling molecules and can lead to oxidative stress when generated in excess. Moreover, mitochondrial dysfunction is one of the leading cause of neuroinflammation. In this regard, we have rationally designed a triazine derived mitochondriotropic antioxidants (Mito-TBA), based on gallic acid and triphenylphosphonium (TPP) cation to specifically target mitochondria to mitigate neuroinflammation. *In vitro* Mito-TBA-3 inhibits mitoautophagy, offers neuroprotection by inhibiting the LPS induced TLR-4 activation and activating the Nrf-2/ARE pathway in PC-12 derived neurons. *In vivo* Mito-TBA-3 rescue memory deficit, reversed depression like behavior, inhibited neuroinflammation, and decreased proinflammatory cytokines in LPS induced neuroinflammation rat model. Overall, based on biophysical, *in vitro* and *in vivo* analysis, Mito-TBA-3 offers valuable insights as a potent therapeutic lead molecule to combat neurodegeneration even outperforming a well-known non-steroidal anti-inflammatory drug (Aspirin), it also has the potential to use as a promising therapeutic candidate for other mitochondrial oxidative stress related disorders.

1. Introduction

Neurodegenerative disease (NDD), intracranial tumours, craniocerebral trauma, cerebrovascular disease, spinal cord disease, and other neurological disorders are among the leading causes of disability, death, and morbidity worldwide [1,2]. In the past few years population expansion, ageing, urbanization, and longer life expectancy had a significant impact on the epidemiological patterns, which has led to concerns about a surge in neurological disorder and its associated burden [3,4]. Neurological disorders itself caused around 10 million fatalities in the year 2019 alone [5]. Pathological disorders, including Alzheimer's disease (AD), Parkinson's disease (PD) and stroke, are the most common forms of neurodegeneration, which progressively damages the structural or functional activity of neuronal cells [6,7]. Synaptic dysfunction, cell death in the nervous system, and the acceleration of neurodegenerative disease progression are all results of an excessive consumption and release of ROS and proinflammatory cytokines induced by various pathological factors in neuroinflammation [8–11]. In response to excessive ROS cells have developed an antioxidant defense systems to mitigate the detrimental impact of excessive ROS generation [12,13]. These methods involve enzymes like superoxide dismutase (SOD) and catalase (CAT), which aid in the neutralization of ROS and hinder their buildup. If the formation of ROS surpasses the capacity of the antioxidant defense system, it results in oxidative stress, which can cause cellular malfunction and contribute to the development of many disorders ranging from cardiovascular to neurological [14,15]. Hence, it is important to uphold an equilibrium between the generation of ROS and the protection provided by antioxidant defense mechanisms in order to preserve cellular well-being and avert oxidative damage.

Despite comprising only 2 % of the body weight, the brain receives 15 % of the cardiac output and 20 % of the total oxygen consumed by the body [16,17]. A major portion of this energy demand comes from neurons, as they need to maintain a homeostasis condition across the plasma membrane to generate an action potential. Neurons are heavily

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ACS APPLIED MATERIALS & INTERFACES

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Research Article

Generation of Functional Neurons from Mesenchymal Stem Cells Using Neural Differentiator and Engineered Peptide Hydrogel: Potential Therapeutic Lead for Traumatic Brain Injury

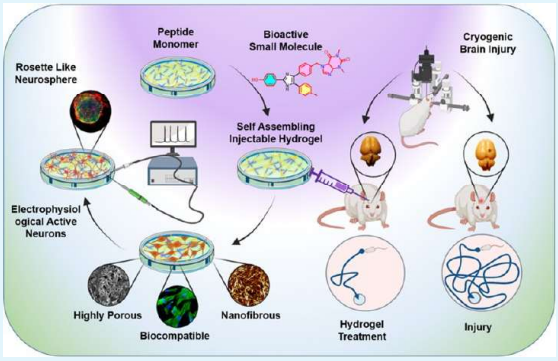
Aniket Jana,[#] Shubham Garg,[#] Satyajit Ghosh, Juhee Khan, Rajsekhar Roy, Nabanita Mukherjee, Moumita Jash, Varsha Gupta, Prasunpriya Nayak, and Surajit Ghosh*

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ABSTRACT: Traumatic brain injuries (TBIs) cause multifaceted disruption in the neural network, initiate huge inflammation processes, and form glial scars that result in severe damage to the brain. Thus, the treatment of TBI is a challenging task. To address this challenge, a newer and innovative approach is extremely important to develop a successful therapeutic strategy. Toward this aim, we hereby report an extremely effective therapeutic strategy. This interesting approach showcased the development and validation of a combination therapy comprising a neuro-regenerative protective peptide hydrogel (SLNAP) and a potent neuro-regenerative chemical modulator (NCM). It is noteworthy to mention that this hydrogel formulation has injectable nature, which allows it to be applied at focal injury site of brain. Remarkably, our results reveal excellent transdifferentiation of human umbilical cord-derived mesenchymal stem cells (hMSCs) into functional neuron upon treatment with this combination therapeutic formulation. The functionality of regenerated neurons was thoroughly checked through observation of active signals generated from those neurons in electrophysiology recording using patch clamp. Further, we also observed that this strategy not only successfully converts hMSCs into neuron but also spontaneously formed neural stem cells (NSCs) like neurosphere. This work also showcased that this multidomain self-assembling peptide hydrogel emerges as an attractive soft-biomaterial due to its capability of slow and sustained release of the drug at the injury site upon topical application. This resulted in significant regeneration of functional neuron at the injury site. Fascinatingly, we found that this combination therapeutic strategy is highly effective in *in vivo* brain injury model establishing that this could be a potential and highly effective therapeutic strategy for TBI.



KEYWORDS: *traumatic brain injury, regenerative medicine, peptide hydrogel, mesenchymal stem cell, transdifferentiation, electrophysiology*

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INTRODUCTION

Traumatic brain injury (TBI) causes severe brain tissue damage, which enforce associated physical damages that triggered long-term secondary cascade of reactions like neuroinflammation and neurodegeneration.[1] Due to limited regenerative potential of mature neurons, the number of neurons at the lesion site is significantly reduced and results in motor and cognitive dysfunction.[2] Due to the complex pathophysiology of TBI as well as the unavailability of effective therapeutics, its treatment is a humongous task and currently available therapeutics involve intravenous or oral administration of growth factors. ROS scavengers can only delay the disease progression to a certain extent.[3] It has been shown that one of the interesting approaches to treat is the transplantation of stem cells, as they have lower immunogenic risk and can be successfully differentiated into nerve cells, which can facilitate the repair of neural damage. However, successful transplantation of stem cells is a very challenging task due to the complexity of the CNS and the hostile environment around the lesion site. Further challenges involved in TBI treatment are the crossing of the blood–brain barrier (BBB) and poor bioavailability of therapeutics.[4,5] To overcome this problem, intracranial drug delivery at the injury site is an alternative approach; however, it requires an extracellular matrix mimicking three-dimensional scaffold. This biomaterial scaffold is crucial for cell adhesion, directed migration, and differentiation of stem cell into functional neuron. In addition, in particular to the effective trans-

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Ratiometric Fluorescent Probe Promotes Trans-differentiation of Human Mesenchymal Stem Cells to Neurons

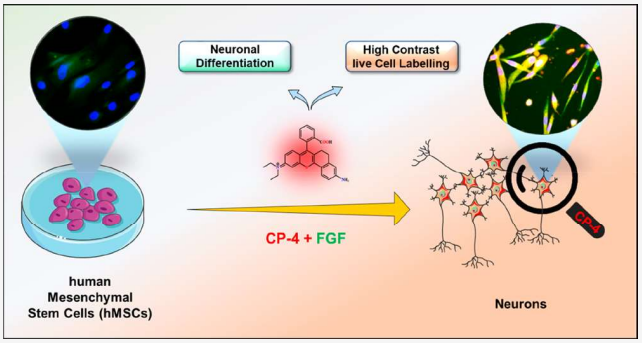
Varsha Gupta,[#] Prabir Kumar Gharai,[#] Chirantan Kar,[#] Shubham Garg, and Surajit Ghosh*

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ABSTRACT: Development of multifunctional theranostics is challenging and crucial for deciphering complex biological phenomena and subsequently treating critical disease. In particular, development of theranostics for traumatic brain injury (TBI) and understanding its repair mechanism are challenging and highly complex areas of research. Recently, there have been interesting pieces of research work demonstrated that a small molecule-based neuroregenerative approach using stem cells has potential for future therapeutic lead development for TBI. However, these works demonstrated the application of a mixture of multiple molecules as a "chemical cocktail", which may have serious toxic effects in the differentiated cells. Therefore, development of a single-molecule-based potential differentiating agent for human mesenchymal stem cells (hMSCs) into functional neurons is vital for the upcoming neuro-regenerative therapeutics. This lead could be further extraploted for the design of theranostics for TBI. In this study, we have developed a multifunctional single-molecule-based fluorescent probe, which can image the transdifferentiated neurons as well as promote the differentiation process. We demonstrated a promising class of fluorescent probes (CP-4) that can be employed to convert hMSCs into neurons in the presence of fibroblast growth factor (FGF). This fluorescent probe was used in cellular imaging as its fluorescence intensity remained unaltered for up to 7 days of trans-differentiation. We envision that this imaging probe can have an important application in the study of neuropathological and neurodegenerative studies.

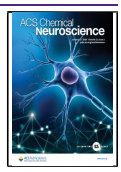


KEYWORDS: *Traumatic brain injury, Theranostics, Fluorescent probe, hMSC, Trans-differentiation, Neuron*

Neurological injuries, the functional loss of neurons due to the death of nerve cells over time, and cognitive deficit are all symptoms of neurodegenerative diseases like Alzheimer's disease (AD),[1] Parkinson's disease (PD),[2] Huntington's disease (HD),[3] spinal cord injury (SCI),[4,5] stroke,[6] and amyotrophic lateral sclerosis (ALS).[7] Neurodegenerative processes start years before any clinical symptoms appear and progress slowly and irreversibly over time.[8,9] Only a limited amount of progress has been made in this area, despite significant research and ongoing clinical efforts, such as diagnosable treatments and precautions to enhance patients' quality of life. A timely diagnosis is crucial for the implementation of therapeutic modalities in clinical testing before the onset of moderate dementia, to assess and apply counter measures to preserve cognitive functions as much as possible, or to slow the progression of these disorders.[10] Neuroimaging techniques such as radio ligand-based positron emission tomography (PET),[11,12] 3D single-photon emission computed tomography (SPECT),[13] and magnetic resonance imaging (MRI)[14] are the principal advancements in medical technologies for the timely examination of neurodegenerative disease pathology. Although PET and SPECT are the most frequently used imaging approaches for neurodegenerative diseases,[15] their uses are limited due to their high cost, dangerous radio-labeled compounds, the necessity of using advanced instruments, and the use of multifaceted data management and analysis protocols. The MRI technique has a narrow spatial resolution and is inherently insensitive to distinguishing morphological variations between disease biomarkers and the surrounding tissue. Fluorescence diagnosis techniques have recently become an enticing and promising alternative for diagnosing and investigating the progression of neuronal disease due to their being rapid, sensitive, simple, low-cost, attractive, real-time, and high-resolution in nature.

MSCs are versatile pluripotent stem cells that have recently been investigated as a preventive and curative measure for a wide range of neurodegenerative diseases.[16−18] Utilizing MSCs[19,20] over other cells has a number of benefits, including

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Discovery of Carbazole and Theophylline-Based Amyloid Inhibitor for the Promotion of Neuroprotection

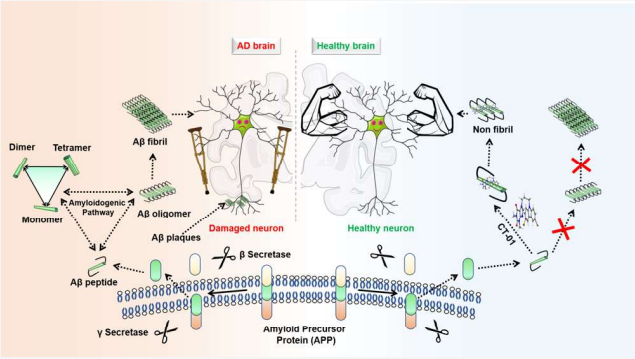
Prabir Kumar Gharai, Juhee Khan, Rathnam Mallesh, Shubham Garg, Sanju Gupta, Parasuraman Jaisankar, and Surajit Ghosh*

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ABSTRACT: The amyloid-beta 42 ($A\beta_{42}$) peptide assembles into neurotoxic soluble oligomers and extracellular fibrillary aggregates during the progression of Alzheimer's disease (AD), which ultimately leads to amyloid plaque in the brain, causing major disruption of the neural circuit and leading to the severe loss of memory. Thus, perturbation or inhibition of this process through the development of advanced inhibitors is crucial for the treatment of AD. Here, we adopted an advanced strategy that showcases the design of a carbazole-based chemical inhibitor targeting the $A\beta$ peptide. The new inhibitors are designed in such a way that they can bind selectively with the $A\beta_{42}$ peptide and inhibit the assembly process and disease progression. Initial assessments using the thioflavin-T assay and molecular docking experiments help screen the carbazole and theophylline-based CT-01 as effective lead molecules, which bind at the N-terminal hydrophobic region of the $A\beta_{42}$ peptide and inhibit the formation of soluble oligomers and extracellular fibrillary aggregates. Further, FT-IR spectroscopy, CD, TEM, dot blot, and ITC experiments suggest the inhibition potency of CT-01. Finally, the neuroprotection and apoptosis assay confirm that CT-01 reduces amyloid-mediated toxicity in neurons. The serum-stable CT-01 can also protect the NGF-deprived neurons and has the ability to cross the blood–brain barrier.



KEYWORDS: *Alzheimer's disease, amyloid-beta peptide, toxicity, carbazole, theophylline*

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive, incurable, age-related neurological disorder that affects millions of people globally.[1] Clinical symptoms of AD involve memory loss, cognitive dysfunction, and behavioral changes that impair routine activities of patients.[2,3] Histopathologically, AD is characterized by the extracellular deposition of amyloid fibrils and intraneuronal fibrillary tangles.[4−6] It has been well documented that hypoxic or oxidative stress conditions induce β-secretase by increasing BACE1 gene expression, resulting in excessive $A\beta$ production.[7] During the progression of AD, the main component of neurotic plaques, $A\beta$ is generated from the amyloid precursor protein as heterogenic in size through sequential proteolytic cleavage by β- and γ-secretases. Pathologically, $A\beta_{40}$ and $A\beta_{42}$ are considered as the toxic forms, and they can be found in various forms, such as soluble oligomers, protofibrils, and extracellular fibrillary aggregates. Six acidic residues (D1, E3, D7, E11, E22, and D23) and three basic residues (R5, K16, and K28) contribute to the production of both amyloids, $A\beta_{40}$ and $A\beta_{42}$, with a net charge of −3. These residues provide a hydrophobic domain that facilitates the attachment to the membranes and proteins, promoting oligomerization.[8] Various pathogenic pathways of the $A\beta$ oligomer and fibrils can directly harm neurons,[9] generate oxidative stress,[10] obliterate electrochemical signaling,[11] accumulate in the mitochondria,[12] and damage the respiratory chain. The prevention of $A\beta$ peptide aggregation is one of the potential strategies toward the development of AD therapeutics. Previously, attempts have been made to suppress the development of $A\beta$ aggregation in AD[13,14]; however, only a few inhibitors have entered clinical trials due to severe side effects. In this report, we aim to develop advanced therapeutics for AD by targeting the hydrophobic stretch of the $A\beta_{42}$ peptide using a carbazole and theophylline scaffold. The rationale for choosing these two moieties are as follows: (i) carbazole-based derivatives selectively bind to $A\beta$ peptides, inhibiting $A\beta$ aggregation/fibrillogenesis,[15] improving tau phosphorylation, and reducing neuroinflammation,[15] as their π-conjugated aromatic rings interact with the aromatic

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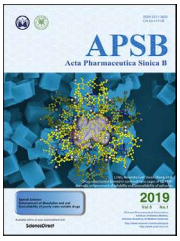
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ORIGINAL ARTICLE

Discovery of powerful multifaceted antioxidant for combating oxidative stress associated with neurodegenerative disorders

Juhee khan[a,b], Prabir kumar Gharai[a,b], Shubham Garg[b], Sanju Gupta[b], Mohammad Umar Arshi[b], Rathnam Mallesh[a,b,c], Surajit Ghosh[a,b,c,*]

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KEY WORDS

Neuroprotection;
Oxidative stress;
Polyphenol;
Nrf2/ARE pathway;
Ischemic stroke;
tBCCAO;
Antioxidant;
Hydrogen peroxide

Abstract Amidst the tangled web of neurons, antioxidants stand as silent sentinels, shielding the delicate threads from the raging storm of oxidative stress in the realm of neurological affliction. Herein, we showcased an innovative design strategy to develop a novel powerful antioxidant small molecule (AOX), designed with the synergistic integration of EGCG (epigallocatechin gallate), gallic acid, and coupled with the metal-chelating capabilities of 8-hydroxy quinoline functional moieties that exhibit multifunctional activity in combating oxidative stress *via* activating the anti-oxidative, anti-apoptotic and anti-inflammatory activity, showcasing the potential for a transformative impact in neuroprotection from oxidative insults. Our work addresses oxidative stress in neuronal systems by providing a thorough examination of oxidative stress caused by hydrogen peroxide in PC12 cell line-derived neurons by shedding light on the antioxidative mechanisms orchestrated by our novel small molecule. Particularly our designed molecule (AOX) provides neuroprotection by mitigating mitochondrial impairment and activating the Nrf2/ARE (nuclear factor erythroid 2-related factor 2/antioxidant response element) pathway and it also demonstrates remarkable resilience against neuroinflammation, as evidenced by minimal alterations in neuroinflammatory markers such as GFAP, IBA1, and S100β in a transient bilateral common carotid artery occlusion (tBCCAO) ischemic stroke model.

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Discovery of imidazole-based GSK-3β inhibitors for transdifferentiation of human mesenchymal stem cells to neurons: A potential single-molecule neurotherapeutic foresight

Varsha Gupta[1], Tanushree Mahata[1], Rajsekhar Roy[2], Prabir Kumar Gharai[1], Aniket Jana[3], Shubham Garg[2] and Surajit Ghosh[1,2,3]*

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The transdifferentiation of human mesenchymal stem cells (hMSC) to functional neurons is crucial for the development of future neuro-regenerative therapeutics. Currently, transdifferentiation of hMSCs to neurons requires a "*chemical cocktail*" along with neural growth factors. The role of the individual molecules present in a "chemical cocktail" is poorly understood and may cause unwanted toxicity or adverse effects. Toward, this goal, we have showcased the discovery of an imidazole-based "single-molecule" transdifferentiation initiator SG-145C. This discovery was achieved *via* screening of a small molecule library through extensive *in silico* studies to shortlist the best-fitting molecules. This discovery evolved through a careful selection to target Glycogen synthase kinase-3β (GSK-3β), which is one of the important proteins responsible for neurogenesis. Rigorous computational experiments, as well as extensive biological assays, confirmed that SG-145C has significant potential to transdifferentiate hMSCs to neurons. Interestingly, our results suggest that SG-145C can inhibit the proteasomal degradation of phosphorylated β-catenin, in turn promoting transdifferentiation of hMSCs into neurons *via* the Wnt pathway.

KEYWORDS

hMSC, transdifferentiation, GSK-3β, β-catenin, imidazole, β-III tubulin, neurodegeneration

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An overview of key potential therapeutic strategies for combat in the COVID-19 battle

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The sudden ravaging outbreak of a novel coronavirus, or SARS-CoV-2, in terms of virulence, severity, and casualties has already overtaken previous versions of coronaviruses, like SARS CoV and MERS CoV. Originating from its epicenter in Wuhan, China, this mutated version of the influenza virus with its associated pandemic effects has engulfed the whole world with awful speed. In the midst of this bewildering situation, medical and scientific communities are on their toes to produce the potential vaccine-mediated eradication of this virus. Though the chances are really high, to date no such panacea has been reported. The time requirements for the onerous procedures of human trials for the successful clinical translation of any vaccine or potential therapeutics are also a major concern. In order to build some resistance against this massive pandemic, the repurposing of some earlier antiviral drugs has been done, along with the refurbishment of some immune-responsive alternative avenues, like monoclonal antibody mediated neutralization, interferon treatment, and plasma therapy. New drugs developed from the RBD domain of the virus spike protein and drugs targeting viral proteases are also undergoing further research and have shown potential from preliminary results. The sole purpose of this review article is to provide a brief collective overview of the recent status of therapeutics advances and approaches, and their current state of implementation for the management of COVID-19.

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Introduction

As COVID-19 continues to spread around the globe, researchers and drug manufacturers are moving towards the development of potential therapeutics into clinical trials at a dizzying pace. The situation has been declared an emergency by the World Health Organization (WHO) and governments across the world have given urgent consideration to controlling the transmission of this disease. Worldwide the generally accepted plan to combat this pandemic has already been taken by imposing a "lockdown" aiming to prevent the spread of virus, as SARS-CoV-2 is hugely a human-to-human contagious disease, which has also been catastrophic to the medical infrastructure of all countries. Along with governments, the people who are working as the frontline warriors in the campaign against the novel coronavirus are scientists and doctors. Researchers and medical experts are working very hard to find a promising outcome to treat this deadly disease. As we all know, viruses are non-living infectious species, which require a host cell to divide and sustain themselves. Here SARS-CoV-2, the virus causing COVID-19, is a single-stranded RNA virus, which utilizes its spike-like receptor binding domain to interact with the host cell.[1] In the lungs, the virus targets cells expressing angiotensin-converting enzyme 2 (ACE2), which are situated in the lining of the lungs (called pneumocytes), and cause respiratory disorder.[2] This results in a reduction in oxygen levels in the blood, which can finally lead to a fatal condition. A recent report reveals that the virus can interfere with the iron-containing compound of blood.[3] Another study also proposes that patients with blood group A are more susceptible to SARS-CoV-2 in comparison to others.[4] In addition, there is prevailing evidence that patients living with heart disease and diabetes are more vulnerable to this disease due to over-expression of a protein called ACE2, which SARS-CoV-2 can bind to and later use the host cell machinery for producing its duplicate copies. Hence, this further increases the rate of infection for these patients.[5] Currently, the most generally adopted approach to treat COVID-19 infected people is to ease the patient's symptoms (which feature pneumonia), while the campaign to develop a complete cure for the disease is still a major challenge. SARS-CoV-2, being an RNA virus, can be

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An NIR-emissive AIEgen with dual sensing ability: An azine-based chemodosimeter for discriminative ppb-level detection of hydrazine and bisulfite ions

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Sensing in live cells

ABSTRACT

A new near-infrared (NIR) emissive aggregation-induced emission (AIE) active unsymmetrical azine (benzophenone-azine-phenyl-cyanovinyl-pyridinium chloride, **BAPCP**) with dual sensing ability is developed for discriminative detection of hydrazine (N_2H_4) and bisulfite (HSO_3^-) ions. The azine-based chemodosimeter, **BAPCP** was synthesized in a sustainable way adopting a solvent-free one-pot tandem mechanochemical route. The probe is nonfluorescent in organic solutions and displayed strong AIE at higher water fractions (upto 99% of H_2O) with an emission maximum at 718 nm and a large Stokes shift of 333 nm. **BAPCP** could detect N_2H_4 by a ratiometric response at 525 nm by cleavage of the cyano-pyridinium ethylene group and showed a turn-off response against HSO_3^- by its nucleophilic addition across the olefinic bond of **BAPCP**. The probe showed excellent selectivity and low limit of detection (LOD) of 4×10^{-8} M (1.2 ppb) and 2.5×10^{-8} M (2 ppb) for N_2H_4 and HSO_3^-, respectively. The practical utility of the probe was demonstrated by solid-phase detection of these analytes on silica-coated TLC plates, followed by ImageJ analysis for on-site quantitation purposes. **BAPCP** is also capable of the detection of intracellular N_2H_4 and HSO_3^- in live cells.

1. Introduction

Hydrazine (N_2H_4) is a commercially used common reducing agent, which finds its application as a blowing agent, fuel, antioxidant, anti-corrosion agent as well as in the pharmaceutical and pesticide industries [1–3]. Its excessive use elevates its concentration in water bodies and soil beyond its threshold level, and being volatile in nature, it easily gets transported by oral, dermal, or inhalation into humans, thereby, causing serious damage to the lungs, liver, kidneys, central nervous system, and respiratory system [4]. The threshold limit value (TLV) of this potential carcinogen has been set as 10 ppb in drinking water by the United States Environmental Protection Agency (USEPA) [5]. Its profound toxicity even at a low level makes it imperative to develop sensitive detection tools for N_2H_4. Standard analytical tools like voltammetry [6], electrochemistry [7], surface-enhanced Raman spectroscopy [8], and high-performance liquid chromatography (HPLC) [9] are available for the estimation of hydrazine level. Apart from these techniques, which often need expensive instrumentation, tedious sample preparation, and rigorous operational skills, a more common approach is the use of reaction-based molecular probes (i.e., chemodosimeters) for user-friendly and on-site measurements of hydrazine [10]. Chemodosimeters are not only sensitive but also highly selective due to cite-specific reactions like hydrazine-driven deprotection of esters [11,12], phthalimide removal [13], cleavage of cyano-ethylene moiety [14], etc.

On the other hand, sulfites (SO_3^{2-}) and bisulfites (HSO_3^-), the hydrated sulfur di-oxide (SO_2) derivatives, are extensively used in the preservation of foods, pharmaceuticals, and beverages [15], antioxidants, and color stabilizers [16]. However, excessive SO_3^{2-}/HSO_3^- intake causes allergic reactions, skin or eye irritation, and even tissue damage [17]. Because of the harmful effects several conventional analytical techniques like chromatography [18], electrochemical analysis [19], surface-enhanced Raman spectroscopy [20], and capillary electrophoresis [21] have been developed for the estimation of these anions. However, small-molecule fluorescent probes are more convenient for quick and sensitive detection of HSO_3^- (or SO_3^{2-}) [22–26]. Commonly,

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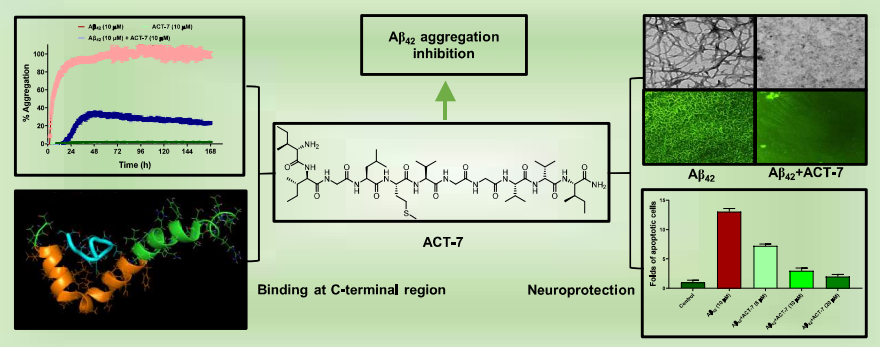
Hydrophobic C-Terminal Peptide Analog $A\beta_{31-41}$ Protects the Neurons from $A\beta$-Induced Toxicity

Rathnam Mallesh, Juhee Khan, Prabir Kumar Gharai, Mohammad Umar Arshi, Shubham Garg, Sanju Gupta, and Surajit Ghosh*

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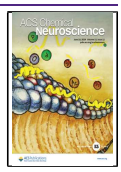
ABSTRACT: Spontaneous aggregation of amyloid beta ($A\beta$) leads to the formation of neurotoxic senile plaque considered as the most crucial event in Alzheimer's disease (AD) progression. Inhibition or disruption of this deadly aggregate formation is one of the most efficient strategies for the development of potential therapeutics, and extensive research is in progress by various research groups. In this direction, the development of a peptide analogous to that of the native $A\beta$ peptide is an attractive strategy. Based on this rationale, β-sheet breakers were developed from the $A\beta$ central hydrophobic core. These peptide derivatives will bind to the full length of the parent $A\beta$ and interfere in self-recognition, thereby preventing the folding of the $A\beta$ peptide into cross β-sheet neurotoxic aggregates. However, this approach is effective in the inhibition of fibrillar aggregation, but this strategy is ineffective in the $A\beta$ neurotoxic oligomer formation. Therefore, an alternative and efficient approach is to use the $A\beta$ peptide analogous to the C-terminal region, which arbitrates fibrillation and oligomerization. Herein, we developed the $A\beta$ C-terminal fragment (ACT-1 to ACT-7) for inhibition of oligomerization as well as fibrillar aggregation. Screening of these seven peptides resulted in an efficient anti-$A\beta$ peptide aggregative agent (ACT-7), which was evaluated by the ThT assay peptide. The ThT assay reveals complete inhibition and showed significant neuroprotection of PC-12-derived neurons from $A\beta$-induced toxicity and reduced cell apoptosis. Further, analysis using CD and FTIR spectroscopy reveals that the ACT-7 peptide efficiently inhibits the formation of the β-sheet secondary structure content. HR-TEM microscopic analysis confirmed the inhibition of formation. Therefore, the inhibition of β-sheet $A\beta$ fibrillary aggregation by the protease-stable ACT-7 peptide may provide a beneficial effect on AD treatment to control the $A\beta$ aggregates. Finally, we anticipate that our newly designed ACT peptides may also assist as a template molecular scaffold for designing potential anti-AD therapeutics.

KEYWORDS: *amyloid beta aggregates, oligomer inhibition, fibril inhibition, neuroprotection, cell apoptosis, Alzheimer's disease*

1. INTRODUCTION

Considerable evidence from genetic and biological studies confirms that the irregular folding of the amyloid beta peptide ($A\beta$) into neurotoxic aggregates is a crucial event in the progression and development of Alzheimer's disease (AD) in the brain.[1,2] The $A\beta$ peptide is formed from the sequential hydrolysis of amyloid precursor protein (APP) by the action of β- and γ-secretase enzymes with different structural variants ($A\beta_{40}$, $A\beta_{42}$, etc.) in the brain.[3,4] In normal conditions, the $A\beta$ peptide exists as an unordered random coil, but in the abnormal condition, the $A\beta$ peptide turns the direction toward a β-sheet pattern and then ultimately leads to depositions as plaques around the neurons.[5−7] All forms of $A\beta$ share a common β-sheet motif in their structure, and the majority of the $A\beta$ aggregates are assumed to be toxic to neurons.

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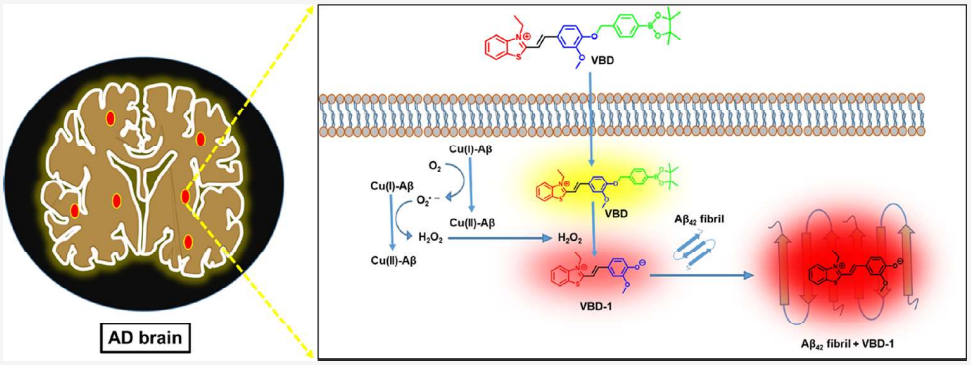
Vanillin Benzothiazole Derivative Reduces Cellular Reactive Oxygen Species and Detects Amyloid Fibrillar Aggregates in Alzheimer's Disease Brain

Prabir Kumar Gharai, Juhee Khan, Rathnam Mallesh, Shubham Garg, Abhijit Saha, Subhajit Ghosh, and Surajit Ghosh*

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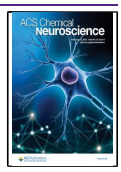
ABSTRACT: The misfolding of amyloid beta (Aβ) peptides into Aβ fibrillary aggregates is a major hallmark of Alzheimer's disease (AD), which responsible for the excess production of hydrogen peroxide (H_2O_2), a prominent reactive oxygen species (ROS) from the molecular oxygen (O_2) by the reduction of the Aβ-Cu(I) complex. The excessive production of H_2O_2 causes oxidative stress and inflammation in the AD brain. Here, we have designed and developed a dual functionalized molecule VBD by using π-conjugation (C═C) in the backbone structure. In the presence of H_2O_2, the VBD can turn into fluorescent probe VBD-1 by cleaving of the selective boronate ester group. The fluorescent probe VBD-1 can undergo intramolecular charge transfer transition (ICT) by a π-conjugative system, and as a result, its emission increases from the yellow (532 nm) to red (590 nm) region. The fluorescence intensity of VBD-1 increases by 3.5-fold upon binding with Aβ fibrillary aggregates with a high affinity (K_d = 143 ± 12 nM). Finally, the VBD reduces the cellular toxic H_2O_2 as proven by the CCA assay and DCFDA assay and the binding affinity of VBD-1 was confirmed by using in vitro histological staining in 8- and 18-month-old triple transgenic AD (3xTg-AD) mice brain slices.

KEYWORDS: *fluorescent probe, H_2O_2, amyloid beta fibril, Alzheimer's disease brain*

1. INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease and the leading cause of dementia. More than 35 million people around the world have been identified with the increasing tendency of AD. There are several symptoms like irreversible memory loss, disability in cognitive function, mood swing, abnormal behaviour, language disorder, and causes health risk in the aging population.[1−3] However, there is a lack of availability of promising therapies. Amyloid beta (Aβ) is produced from a transmembrane protein called amyloid precursor protein (APP)[4] by sequential hydrolysis by β- and γ-secretase, which is generally found in the membranes of cells and organelles such as mitochondria. These Aβ peptides are self-assembled into fibrillar aggregates, which induce neurotoxicity in AD brain. The genesis of Aβ peptides is governed by enzymatic mechanisms that convert APP to Aβ. There are two important membrane-bound endoprotease activities: β- and γ-secretase break APP in a sequential manner. A large secreted derivative, sAPPβ, is produced after the first cleaving of APP by β-secretase. Next, a large 99 amino acid-based fragment that is still membrane-bound is quickly broken down by γ-secretase to produce Aβ. There are so many fragments formed, but 40 amino acid-based $A\beta_{40}$ is the most abundant followed by $A\beta_{42}$. But, the important point is toxicity generation. The two extra amino acids make $A\beta_{42}$ more prone to fibril formation through dimer, tetramer, and oligomer formation, resulting in higher

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Review

Venom: A Promising Avenue for Antimicrobial Therapeutics

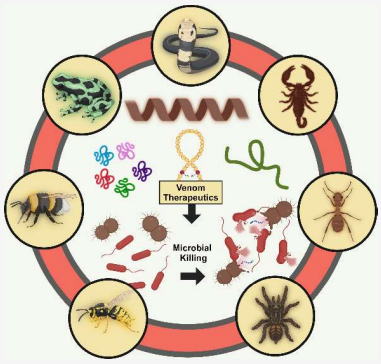
Ramkamal Samat, Samya Sen, Moumita Jash, Satyajit Ghosh, Shubham Garg, Jayita Sarkar, and Surajit Ghosh*

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ABSTRACT: Venom in medicine is well documented in the chronicles of ancient Greece and the Roman Empire and persisted into the Renaissance and even into the modern era. Venoms were not always associated with detrimental consequences. Since ancient times, the curative capacity of venom has been recognized, portraying venom as a metaphor for pharmacy and medicine. Venom proteins and peptides' antimicrobial potential has not undergone systematic exploration despite the huge literature on natural antimicrobials. In light of the escalating challenge of antimicrobial resistance and the diminishing effectiveness of antibiotics, there is a pressing need for innovative antimicrobials capable of effectively addressing illnesses caused by multidrug-resistant microorganisms. This review adds to our understanding of the effectiveness of different venom components against a host of pathogenic microorganisms. The aim is to illuminate the various antimicrobials present in venom and venom peptides, thereby emphasizing the unexplored medicinal potential for antimicrobial properties. We have presented a concise summary of the molecular examination of the venom peptides' functioning processes, as well as the current clinical and preclinical progress of venom antimicrobial peptides.



KEYWORDS: *infectious disease, antibiotics, antimicrobial peptides, antimicrobial resistance*

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1. INTRODUCTION

The prevalence of microorganisms (like bacteria, fungi, and protozoa) becoming resistant to antimicrobials has been increasing significantly. This issue has increased due to the overuse and misuse of antimicrobials in agriculture and veterinary settings. In 2017, the World Health Organization (WHO) published a list of high-priority multidrug-resistant (MDR) bacterial strains.[1] A dearth of new antibiotics, however, means that drug-resistant illnesses remain a serious concern.[1] According to the United Nations Interagency Coordination Group (IACG) on antimicrobial resistance (AMR), at least 700,000 people worldwide are dying due to drug-resistant illnesses per year.[2] By 2050, AMR is projected to kill ten million people annually, with an estimated death toll of 4.95 million deaths in 2019.[3] Since multidrug-resistant pathogens threaten long-term care residents and critical care patients, effective infection control is essential.[4] Given this dire situation, natural agents that are less toxic, more effective, and employ a multimodal mechanism of action. The natural world contains many strong therapeutic substances, primarily obtained from prokaryotes like bacteria or eukaryotes like fungi or plants.[5] Antimicrobial amphipathic peptides are found in many species in the Monera (eubacteria), Protista (bacteria and algae), Fungi (yeasts), Plantae (plants), and Animalia kingdoms.[6] Poisonous animal species like snakes, frogs, scorpions, etc., are also being extensively studied for an invaluable natural repository of antimicrobial substances, namely, venom.[7] Their various antimicrobial components have motivated researchers worldwide to study the curative properties of animal venoms. Cancer, renal failure, heart disease, diabetes, and HIV infection have immunocompromised a large segment of the population, making them more susceptible to infections. Thus, an urgent need for newer and more effective therapeutics has arisen for managing MDR infections, prompting the discovery and application of antimicrobial agents, like venom-derived antimicrobial peptides (AMPs) (Table S1). In this review, we will attempt to explore the details of bioactive and antimicrobial components of snake, frog, spider, scorpion, wasp, ant, and bee venom (Figure 1). The review will provide a succinct overview of the molecular analysis of the bioactive venom component's functional mechanisms, their modification/design, and development using cutting-edge computational methods, and their probable future applications, along with the latest advancements in the clinical and preclinical development of venom antimicrobial peptides.

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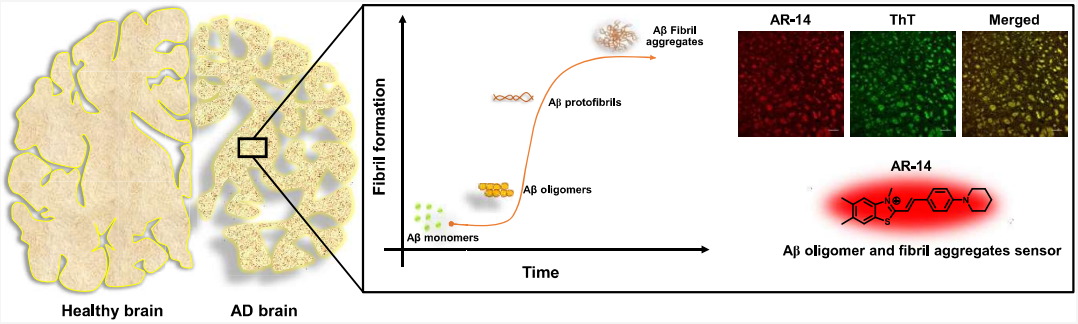
High-Affinity Fluorescent Probes for the Detection of Soluble and Insoluble Aβ Deposits in Alzheimer's Disease

Rathnam Mallesh, Juhee Khan, Prabir Kumar Gharai, Subhajit Ghosh, Shubham Garg, Mohammad Umar Arshi, and Surajit Ghosh*

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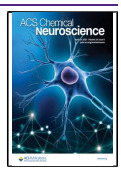
ABSTRACT: The overproduction and deposition of the amyloid-β (Aβ) aggregates are accountable for the genesis and development of the neurologic disorder Alzheimer's disease (AD). Effective medications and detection agents for AD are still deficient. General challenges for the diagnosis of Aβ aggregates in the AD brain are (i) crossing the blood−brain barrier (BBB) and (ii) selectivity to Aβ species with (iii) emission maxima in the 500−750 nm region. Thioflavin-T (ThT) is the most used fluorescent probe for imaging Aβ fibril aggregates. However, because of the poor BBB crossing (log P = −0.14) and short emission wavelength (482 nm) after binding with Aβ fibrils, ThT can be limited to *in vitro* use only. Herein, we have developed Aβ deposit-recognizing fluorescent probes (ARs) with a D-π-A architecture and a longer emission wavelength after binding with Aβ species. Among the newly designed probes, AR-14 showed an admirable fluorescence emission (>600 nm) change after binding with soluble Aβ oligomers (2.3-fold) and insoluble Aβ fibril aggregates (4.5-fold) with high affinities K_d = 24.25 ± 4.10 nM; K_a = (4.123 ± 0.69) × 10^7 M^{-1} for fibrils; K_d = 32.58 ± 4.89 nM; and K_a = (3.069 ± 0.46) × 10^7 M^{-1} for oligomers with high quantum yield, molecular weight of <500 Da, reasonable log P = 1.77, stability in serum, and nontoxicity, and it can cross the BBB efficiently. The binding affinity of AR-14 toward Aβ species is proved by fluorescence binding studies and fluorescent staining of 18-month-old triple-transgenic (3xTg) mouse brain sections. In summary, the fluorescent probe AR-14 is efficient and has an admirable quality for the detection of soluble and insoluble Aβ deposits *in vitro* and *in vivo*.

KEYWORDS: *amyloid-β deposits, fluorescent probe, Alzheimer's disease, amyloid inhibitor*

1. INTRODUCTION

Alzheimer's disease (AD) is a diverse widespread neurological disorder. The pathological features associated with AD are progressive loss of essential neuronal functions including cognitive decline, deficits in language, and dementia in older people.[1] Currently, there are no proper diagnostic agents and medications available to identify and prevent or cure the disease. The available medications are still limited in their ability to reduce the symptoms. This gap shows the great tasks for the researchers to develop specific diagnostic tools and effective therapeutic agents.[2] The amyloid cascade hypothesis describes that overproduction and deposition of insoluble amyloid-β plagues (Aβ) are accountable for the progression of AD in the brain.[3−5] However, current studies show that the deposition of soluble Aβ oligomers is more neurotoxic than the insoluble Aβ fibril deposits and induces neurotoxicity[2,3] and neuroinflammation.[6] These results directly indicate that soluble Aβ oligomer deposits play a crucial role in the early stage of AD progression.[6−8] Therefore, the targeting and detection of neurotoxic soluble Aβ deposits in the AD brain are crucial in the early diagnosis and monitoring of the disease progression.[9,10] Currently, there is a requirement for fluorescent probes, which can selectively bind to the Aβ

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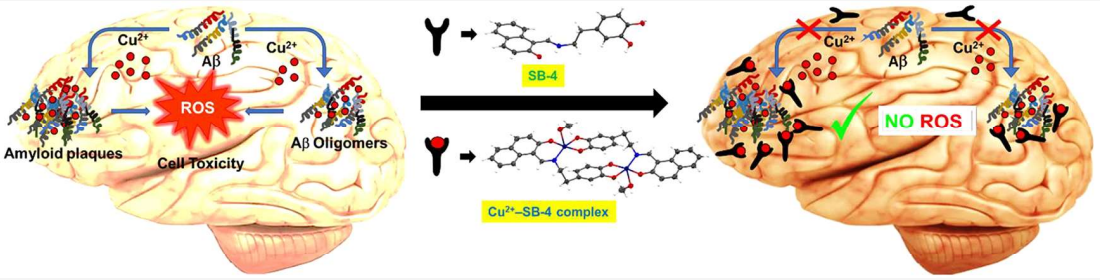
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Power of Dopamine: Multifunctional Compound Assisted Conversion of the Most Risk Factor into Therapeutics of Alzheimer's Disease

Prabir Kumar Gharai, Juhee Khan, Krishnangsu Pradhan, Rathnam Mallesh, Shubham Garg, Mohammad Umar Arshi, Surajit Barman, and Surajit Ghosh*

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ABSTRACT: In Alzheimer's disease (AD), reactive oxygen species (ROS) plays a crucial role, which is produced from molecular oxygen with extracellular deposited amyloid-β (Aβ) aggregates through the reduction of a Cu^{2+} ion. In the presence of a small amount of redox-active Cu^{2+} ion, ROS is produced by the Aβ-Cu^{2+} complex as Aβ peptide alone is unable to generate excess ROS. Therefore, Cu^{2+} ion chelators are considered promising therapeutics against AD. Here, we have designed and synthesized a series of Schiff base derivatives (SB) based on 2-hydroxy aromatic aldehyde derivatives and dopamine. These SB compounds contain one copper chelating core, which captures the Cu^{2+} ions from the Aβ-Cu^{2+} complex. Thereby, it inhibits copper-induced amyloid aggregation as well as amyloid self-aggregation. It also inhibits copper-catalyzed ROS production through sequestering of Cu^{2+} ions. The uniqueness of our designed ligands has the dual property of dopamine, which not only acts as a ROS scavenger but also chelates the copper ion. The crystallographic analysis proves the power of the dopamine unit. Therefore, dual exploration of dopamine core can be considered as potential therapeutics for future AD treatment.

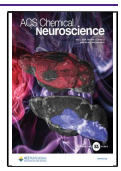
KEYWORDS: *Alzheimer's disease, multifunctional compound, dopamine, copper chelator, amyloid aggregation inhibitor, antioxidant, mitochondrial damage, crystallography*

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1. INTRODUCTION

Alzheimer's disease (AD) is a chronic neurological disorder and is the most common form of dementia.[1] In the early stage of this disease, memory loss is very severe, but with time the patient suffers from multiple neurological disorders that severely affect routine life. Extracellular amyloid plaques[2,3] and intracellular neurofibrillary tangles[4] are considered signature components of AD. The amyloid-β peptide (Aβ), which is produced by the successive proteolysis of the transmembrane protein called amyloid precursor protein (APP) by β- and γ-secretases, is universally believed to play a direct role in the pathogenesis of AD.[5,6] Amyloid plaques are composed of aggregated Aβ peptide with 40−42 amino acids in AD brains.[7] $A\beta_{40}$ is the most abundant in AD patients' brains, whereas $A\beta_{42}$ is more prone to aggregation. Due to the complex nature of the disease, the mechanisms of this pathogenesis were poorly understood. During the progression of the disease, polymerization of Aβ into different toxic Aβ species such as amyloid oligomers as well as fibrils is a critical step. Therefore, the prevention of Aβ polymerization has been recognized as the therapeutic strategy for curing or preventing the disease progression.[8] In the AD brain, the concentration of Cu^{2+} is very high, nearly 0.4 mM.[9] Recently, some transition metals like copper and zinc have been noticed to directly interact with amyloid plaques.[10,11] These metal ions are expected to be involved in the assembly and neurotoxicity of Aβ species. Cu^{2+} accelerates the polymerization of Aβ species into different toxic species and also changes their conformational transformation. In AD, the main reason for neuronal

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Brain-on-a-Chip

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Unravelling the microenvironment of the human brain to investigate the different pathophysiological conditions is a pivotal task. The demarcation of the central nervous system from the peripheral nervous system makes the human brain developmental process a more protracted one. Increasing number of various neurodegenerative diseases causes a major challenge to the management of global public health. Although traditional in vivo animal models and in vitro cell culture models facilitate the evaluation of the structural and functional aspects of the brain, they have certain limitations too. This calls for an innovative approach in the shape of Microfluidics Technology that can offer a single platform to culture neurons in a quasi-physiological confined microenvironment, to reconstitute neural framework under spatiotemporal regulation and external stimulation for modelling of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and more followed by high-throughput analysis of effective drug delivery systems. In this chapter, we have highlighted the various structural and functional aspects of the human brain by the reconstitution of the central nervous system and the peripheral nervous system on chip. We have thereby highlighted the neurological disorders that are associated with the malfunctioning of both the nervous systems succeeded by high-throughput analysis of drugs on chip that have been screened till date. Therefore, these lab-on-chip technologies hold promises for mimicking the complex brain microenvironment imparting novel platform for disease modelling and drug screening.

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Chapter 2

Facile Method of Tubulin Purification from Goat Brain for Reconstitution of Microtubule-Associated Intracellular Function

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Tubulin/microtubule plays crucial role in eukaryotic cell division. Polymerization of $\alpha\beta$-tubulin heterodimers forms the microtubules, which is essential for the segregation of chromosomes during cell division and organelle positioning. Our method of tubulin purification from the goat brain includes isolation of goat brain, multiple cycles of polymerization (warming at 37 °C)-depolymerization (cooling at 4 °C) followed by centrifugation process. The purified tubulin from goat brain is highly functional and successfully used in different applications including reconstitution of cell like environments and understanding molecular mechanisms. Toward the end of the chapter, we have discussed, how this purified tubulin can be used for reconstitution of intracellular microtubule-associated events or function. To enable our reconstitution approach, we have developed various micropatterned-based platform and their fabrication methodology with single ligand and dual-ligand functionalizations, which are also demonstrated. These chemically functionalized micropatterned platforms are extremely useful for immobilization of tubulin/microtubule onto the localized defined area, which will be helpful in mimicking cellular phenomena like kinesin-driven transport, microtubule dynamics, etc.

Keywords Tubulin, Microtubule, Kinesin, Dynamics instability, Surface functionalization, Biotin, Tris-NTA, 2D-micropatterned surface

Microtubules are highly
dimer of α- and β- tubulin and plays diverse role in the cell, starting from providing an organizational framework, structural support, organization of mitotic spindle, providing track for motor protein kinesins and dynein. The α, β-tubulin heterodimer contains binding sites for one GTP molecule [1, 2]. The nucleotide on the β-tubulin is exchangeable and remains in either GTP or GDP bound state. $\alpha\beta$-tubulin heterodimers are stacked head to tail, which form a protofilament; this type of 13 protofilaments folded into tube to

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CHAPTER

Organ-on-a-chip for multianalyte monitoring

15

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Introduction

Enhancing the accuracy of preclinical predictions is essential in pharmaceutical research and development since current drug development methods are insufficient for creating novel medications to treat a wide range of human ailments. Despite being the industry standard for preclinical drug testing, animal models frequently fail to accurately anticipate a human response, which drives the demand for more sophisticated model platforms. The ethical issues, high cost, and accurate extrapolation of results from animal experiments to people are critical barriers to using animal models as a fundamental standard [1]. Therefore in vitro and in vivo experimental models are required to study human drug responses on alternative platforms [2].

Over the past 20 years, microphysiological systems (MPS, often referred to as "OoC," "body-on-a-chip," or "human-on-a-chip") have become popular systems to examine how the body reacts to drugs or other compounds. These methods are especially effective at anticipating human reactions before clinical drug testing or as an addition to clinical trials to investigate underlying causes. The benefits of MPS include a recapitulation of tissue architecture, drug and signaling component diffusion kinetics, and physiological flow conditions. Microfluidics is applied in micropropulsion, microthermal technologies, DNA chips, and lab-on-a-chip (LOC) technologies. It allows the handling of pico- to micro-liters of fluid volume, which reduces the necessary volume of sample, consumption of reagents, time of analysis, and costs. Nuclear magnetic resonance, high-performance liquid chromatography, spectroscopy, mass spectrometry, and gas chromatography are traditional techniques for identifying and separating cell metabolites. In addition, there are UV-vis spectrophotometers or a fluorescence microscope/spectrophotometer for colorimetric and fluorimetric assays, respectively. The target molecule can be separated using these well-proven techniques, which have good sensitivity and repeatability. However, these techniques rely on relatively expensive equipment, are labor-intense, economically resource-intensive, and generally inapplicable to continuous monitoring systems [3,4].

A microfluidic chip created to replicate the characteristics of an organ-specific microenvironment is necessary to create MPS models. The spatial arrangements of cells, the existence of biomechanical stresses, and tissue characterization methods are some of the crucial elements that must be considered. Microfabricated cell culture systems called organ-on-a-chip (OoC) can simulate the structure and function of human organs more straightforwardly [5]. Human organ models, including

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Certificate of Participation

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This letter certifies your participation as a(n) Attendee at the Gordon Research Conference on Barriers of the CNS held 06/09/2024 - 06/14/2024 at Colby-Sawyer College in New London, New Hampshire, United States.

Poster Entitled: Bioactive Polydopamine Based Nanocomposite as Antioxidant for the Combinational Therapy in Traumatic Brain Injury



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