

Scaffold Hopping in Drug Discovery
Making Grignard reaction safer and cleaner
A cross-sectional study on pain perception
CRIPS Digest

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EDITORIAL**Review Articles**

Scaffold Hopping in Drug Discovery: Innovation in Pharma Industries and Academia

Making Grignard reaction safer and cleaner in continuous flow synthesis and applying for synthesis of active pharmaceutical ingredients (APIs) and key starting materials (KSMs)

A cross-sectional study on pain perception toward needle-free injections in Gujarat state, India

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The cover page contains a figure from the article of Prof. Sankar K. Guchhait

EDITORIAL

93 Needle-free injection technology has received a lot of interest in recent years as a safer and more convenient alternative to traditional hypodermic needle injections. Rajesh & coworkers discussed the future of injectable therapy and the role that needle-free injection technology will play in advancing medical treatment and increasing patient outcomes. Needle-free injection technology has the potential to transform the way we receive injections, making medical care safer, more convenient, and less unpleasant. As technology advances, we expect it will play an important role in improving the delivery of injectable medicines. This technology may transform the delivery of many biologics, and poorly bioavailable drugs and more importantly the delivery of vaccines and anti-diabetic drugs like Insulin. There is a great scope for all academic and industry partners to contribute to the advancement of this technology to improve the delivery and therapeutic performance of a wide range of therapeutic agents with much-improved patient compliance. The Needle-free injection technology may avoid issues associated with injectables such as anxiety or discomfort associated with old injection procedures.

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100 Drug development is a multi-step process that necessitates innovative methods and cutting-edge technologies. Scaffold hopping has emerged as a viable strategy in drug development in recent years, providing new chances for pharmaceutical companies and university researchers to identify new, effective, and affordable therapies for a wide range of ailments. Scaffold hopping allows scientists to swiftly explore new chemical space and develop novel compounds with similar or enhanced activity by exploiting current molecules as a starting point. This method is very beneficial for discovering drugs for conditions when present therapies are either unavailable or inefficient. Scaffold hopping is positioned to play a crucial role in developing affordable medicines and may contribute immensely to improving patient outcomes because of its capacity to minimize time and cost as well as its potential to uncover new classes of medications.

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The Grignard reaction is a powerful and versatile organic chemistry synthetic technique for forming new carbon-carbon bonds between reactive organometallic reagents and organic substrates. In recent years, the Grignard reaction has received attention as a safer and cleaner alternative in the continuous flow synthesis of active pharmaceutical ingredients (APIs).

In continuous flow synthesis, the Grignard reaction is a potential technique for the synthesis of active medicinal components, giving better safety, decreased waste, and improved process control. As technology advances, we expect it will play an important role in improving the impurity profiles of the wide range of APIs. We must continue to invest in new and creative technologies that enhance healthcare outcomes and make medical treatment available to everyone.

Dr Chandraiah Godugu

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Scaffold Hopping in Drug Discovery: Innovation in Pharma Industries and Academia

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The medicinal chemistry strategy plays crucial roles in the drug discovery process starting from selection and synthesis of new compounds to the study of structural modulation for required preclinical profile of compounds. Modern medicinal chemists are currently implementing scaffold hopping strategy to expand the existing drug and chemical space, introducing new molecules with chemically different core structures, and yet binding to the same biological target. Scaffold hopping is important not only in the early stages of drug discovery where a novel active compound must be identified, but also in lead optimization where a variety of chemical properties, physicochemical properties, PK-PD, and toxicity problems of a bioactive molecule or natural product can be resolved via identification of a novel core structure. Scaffold replacements afford to discover the architecture of the therapeutic-valued molecules within patent space of interest. This review presents a brief account of scaffold hopping, creating new chemical space, applications to various stages of Pharmaceutical science research, and successful discovery of drugs.



Scaffold hopping as a concept and patentability

In the year 1999, Gisbert Schneider coined the term Scaffold hopping.¹ It is a process that identifies isofunctional structures with different molecular backbones. This can be achieved by modifying either the central core structure or side chains of the known active compound, which leads to a novel molecular structure that has 3D structure and biological properties similar to the parent compound.² The new molecules developed through this strategy successfully dodge the patent space of the original drug and become patentable. Patentability-claim of generated new chemical entities is always a

Keywords: Scaffold hopping; Drug discovery; chemical space; Patentability; PK-PD

challenging issue. The scaffold hopping strategy overcomes the issue of patentability and creates the new chemical space outside of existing patented chemical island.³

Balancing novelty with drug-like properties in chemical space.

Scaffold hopping is a useful strategy in drug design to 'jump' in different areas of chemical space. The key feature of scaffold hopped analogues is that they have structurally distinct templates with similar biological activity. This approach is employed by medicinal chemists in order to get drug-like qualities, avoid unfavourable ADME-tox features, find readily synthesizable molecules that resemble complex natural products, and to secure intellectual property right. Thus, this is an attractive strategy to achieve

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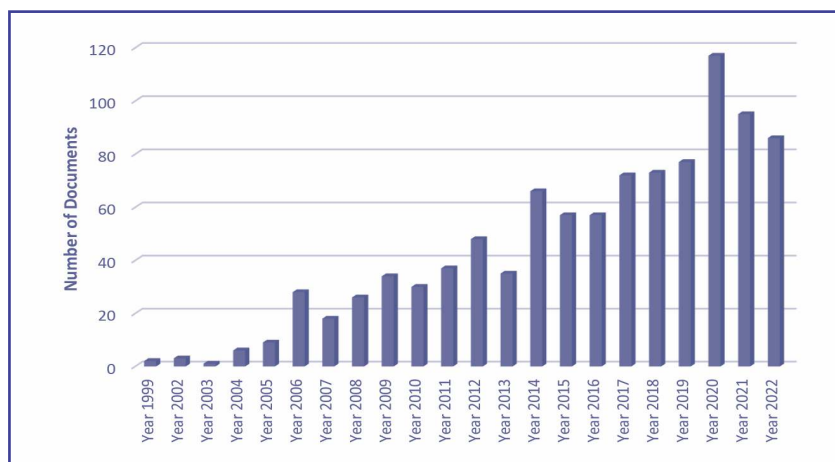


Figure 1. "Documents" (article, review, conference paper, bookchapter, patent, letter, Editorial) published; data collected from Reaxys.

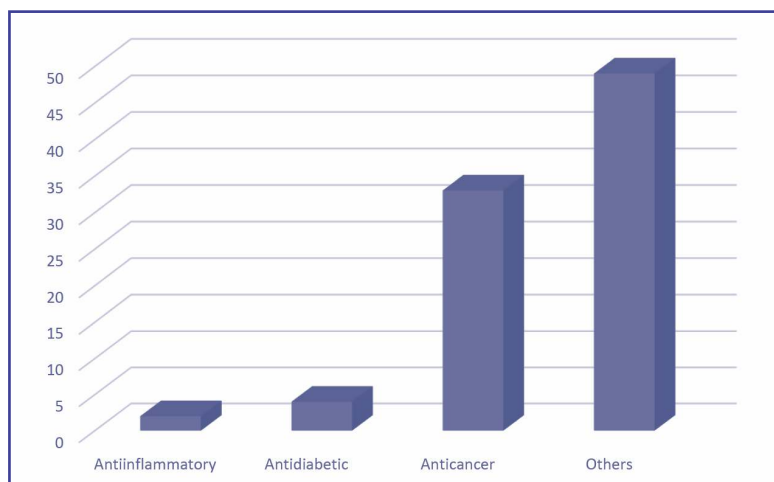


Figure 2. Scaffold hopping strategy in various therapeutic research from 2019-2022 (Number of Publications and patents); data collected from Reaxys.

novelty within confined drug-relevant chemical space.⁴

Scaffold hopping - a highly practiced research area

The number of documents generated from research on scaffold hopping, as presented in the Figure 1, clearly indicates that year-wise documents publication is highly increasing and scaffold hopping is a highly practiced research area in the pharma sector and academia.

Scaffold hopping - therapeutic research area

Scaffold hopping as an important strategy is frequently considered in various therapeutic research. Among various diseases, the anticancer drug discovery uses more frequently the scaffold hopping strategy (Figure 2).

Scaffold hopping in Pharmaceutical industry

Various drugs have been successfully discovered by using the scaffold hopping strategy.

Kinase Inhibitor

Heteroatom replacement, a strategy of scaffold hopping, was investigated by Wyeth Pharmaceutical company. The research focused on the AstraZeneca drug gefitinib that was introduced to market in 2003, which led to the successful development of bosutinib drug, approved by USFDA in 2012. 'Nitrogen' atom in the quinazoline ring is replaced with 'Carbon' to form quinoline ring system (Figure 3). To retain the pharmacophoric importance of quinazoline ring 'N' atom, a cyano group is introduced at relevant position of quinoline ring.³

PDE 5 Inhibitor

In 1994, Pfizer filed a patent covering the use of sildenafil drug that was approved by the USFDA in the year 1998 and Bayer Pharmaceuticals introduced in 2003 the vardenafil drug (Figure 4). Both drugs are phosphodiesterase 5 (PDE5) enzyme inhibitors. The major structural variation between these drugs is the swap of a carbon atom with a nitrogen atom in the 5-6 fused ring.⁵

Cyclooxygenase (COX 2) inhibitor

The cyclooxygenase 2 (COX-2) inhibitor drugs rofecoxib (VioxxTM) and valdecoxib (BextraTM) were discovered by use of scaffold hopping strategy on the structure of celecoxib drug, a COX-2 inhibitor. They differ by only the five-member hetero ring connecting two phenyl rings (Figure 5). Rofecoxib was introduced to market by Merck in 1999 and the valdecoxib drug was developed by Pharmacia/Pfizer and approved by USFDA in 2001.⁶

Classification of Scaffold hopping

In the scaffold hopping strategy, various types of structural modulation of drugs/ bioactive agents while retaining the key pharmacophoric features are practiced by the pharma sector and academia.⁷

Heterocycle replacement (1° hop)

1° hop refers to minor alterations, such as switching or substituting heteroatoms and carbon in a backbone ring. (Figure 6). Replacing the heteroatoms in a heterocycle that functions as the core of the

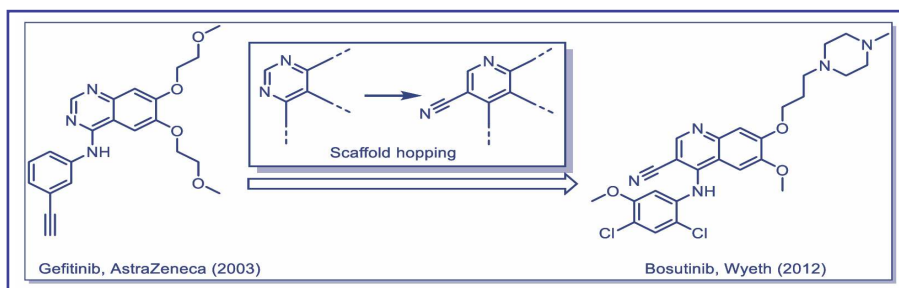


Figure 3. Scaffold hopping strategy in kinase inhibitor.

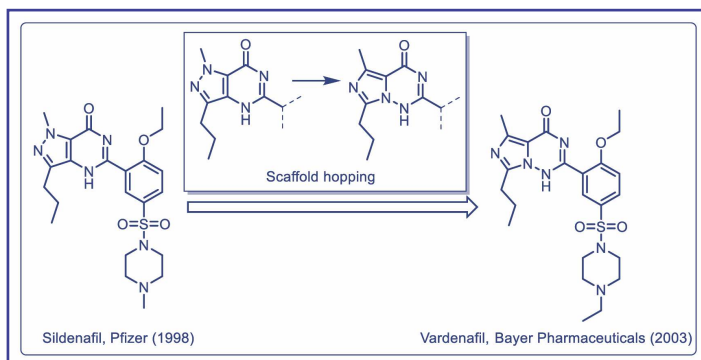


Figure 4. Scaffold hopping strategy in PDE 5 inhibitor.

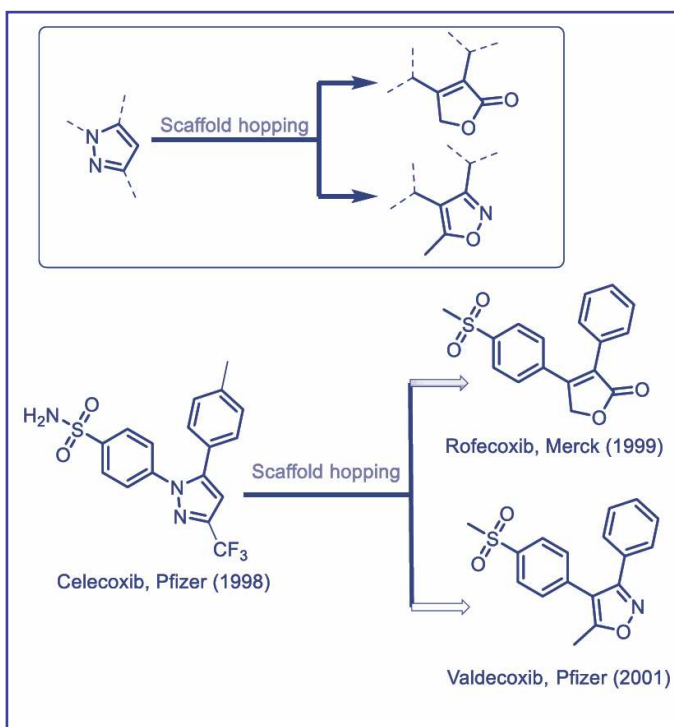


Figure 5. Example of scaffold hopping strategy in cyclooxygenase (COX 2) inhibitors.

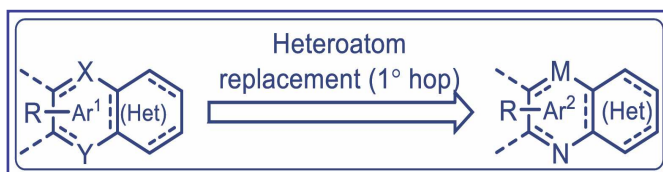


Figure 6. Heterocycle replacement (1° hop).

drug molecule while retaining linked functional motifs and pharmacophores results in novel scaffold. Pizotifen, a more effective medication for the treatment of migraines, is created when one of the phenyl rings in the antihistaminic drug cyproheptadine is switched out for thiophene. The solubility of the molecule is increased when one of the phenyl

rings in cyproheptadine is replaced with a pyrimidine ring to create azatadine.⁸

Ring opening and closure (2° hop)

Most drug-like molecules possess at least one ring system. The ring opening and ring closure are two scaffold hopping 2° hop strategies to create novel scaffolds (Figure 7). 2° hop is a useful strategy for improving the drug-like properties, because molecular flexibility or rigidity aspect contributes greatly to the entropic component of the binding free energy, membrane

penetration, absorption, and to manipulate the flexibility of a molecule by controlling total number of free rotatable bonds. Morphine, an opioid receptor agonist is a rigid 'T' shaped molecule, breaking six ring bonds and opening three fused rings, the new drug tramadol is more flexible, resulting in reduced potency and reduced side effects.⁹

Pseudopeptides and peptidomimetics (3° hop)

Replacement of a peptide backbone with a non-peptidic moiety is included into the category of scaffold hopping 3° hop. Due to their poor metabolic stability and limited bioavailability, peptides' clinical usage is significantly hampered. Using active peptide conformations as templates, small molecules that mirror the structural characteristics of peptides have shown encouraging outcomes (Figure 8). The major goal of peptide-based drug discovery is to reduce the peptide character for enhancing the molecular resistance to proteolysis, while retaining the key chemical features for molecular recognition. Replacing the central residues of angiotensin II (Ang II) containing Tyr at position 4 and Ile at position 5 with a benzodiazepine-based mimetic, a well-known b-turn scaffold exhibited high binding affinities against both angiotensin II receptor type 1 (AT1) and angiotensin II receptor type 2 AT2 receptors with K_i values of 14.9 nM and 1.8 nM.¹⁰

Topology or shape-based scaffold hopping (4° hop)

A complete new chemical backbone that only retains molecular interactions of the ligand with the target is characterized as a 4° hop scaffold hopping (Figure

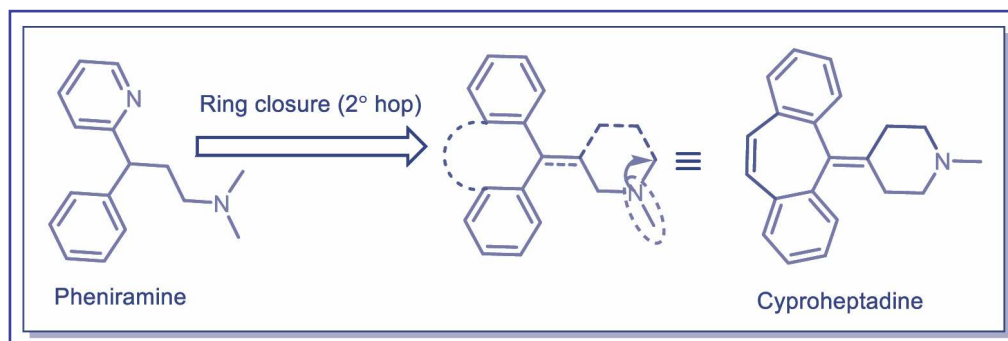


Figure 7. Ring closure (2° hop).

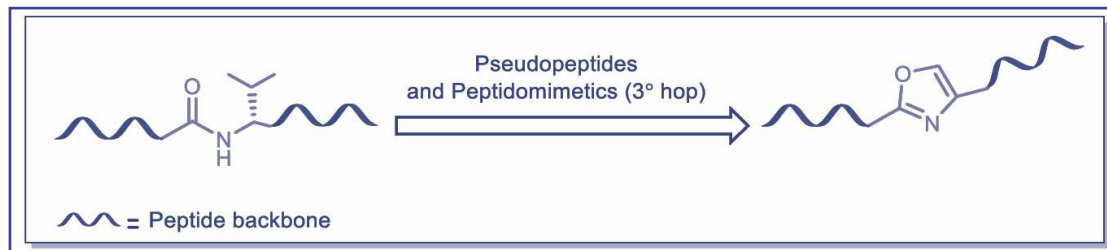


Figure 8. Pseudopeptides and peptidomimetics (3° hop).

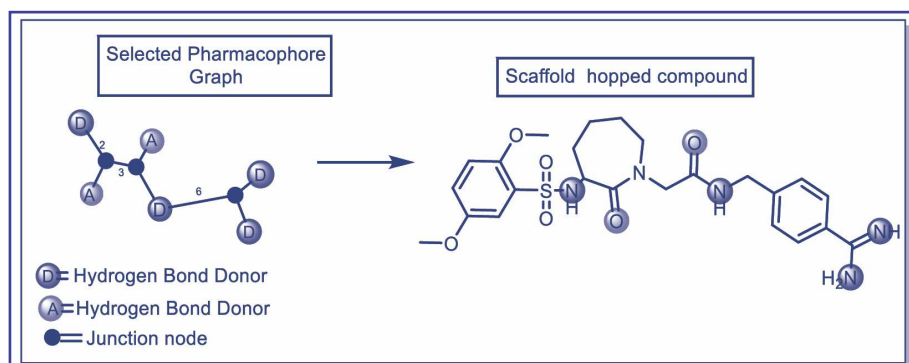


Figure 9. Topology or shape-based scaffold hopping (4° hop).

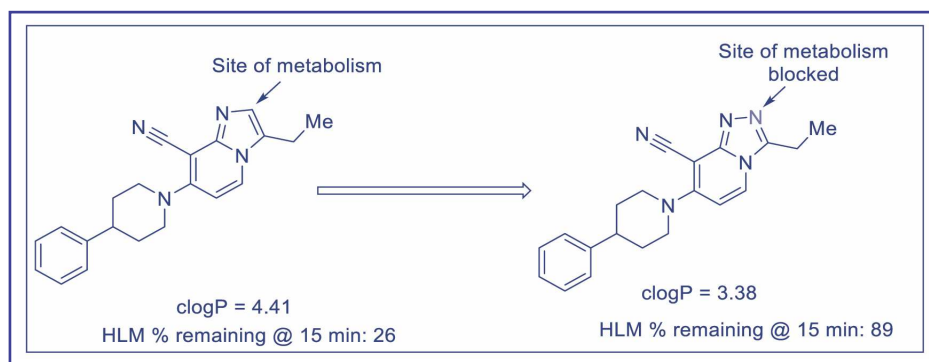


Figure 10. Improvement in metabolic stability using scaffold hopping strategy.

9). There are many examples of topology or shape-based scaffold hopping in this category. Instead of scaffold hopping, the procedure may be referred to as structure-based virtual screening when the new chemotype differs greatly from its original template.^{11,12}

Scaffold hopping in changing PK-PD profile

Metabolic stability

Cid et al. reported¹³ an interesting application of scaffold hopping by switching from imidazopyridine to 1,2,4-triazolopyridine scaffold (Figure 10). It afforded

significant improvement in metabolic stability with decrease in clog P property of the molecule. The metabolism site in the imidazopyridine ring is

blocked by incorporation of an additional nitrogen atom in the ring as 1,2,4-triazolopyridine motif. In addition, lipophilicity of the molecule is reduced. The scaffold hopped 1,2,4-triazolopyridine-based analog showed improved metabolic stability in human liver microsomes.

Yeung et al.¹⁴ described, when a phenyl motif is changed to a pyridyl or pyrimidyl ring by scaffold

hopping strategy, it imparts metabolic stability (Figure 11). The metabolic stability is typically increased by adding nitrogen atoms to aromatic systems to enhance half-life.

Pharmacodynamic, physicochemical and pharmacokinetic properties

Liu et al. investigated¹⁵ the scaffold hopping strategy for improvement in physicochemical and pharmacokinetic profile,

while retaining the *in vitro* activity. The scaffold hopping of imidazo[1,2-a]pyrazines with pyrazolo[1,5-a] pyrimidines increases polarity at relevant site of molecule and improves physicochemical and pharmacokinetic profiles, while retaining the molecule's pharmacodynamic potency (Figure 12).

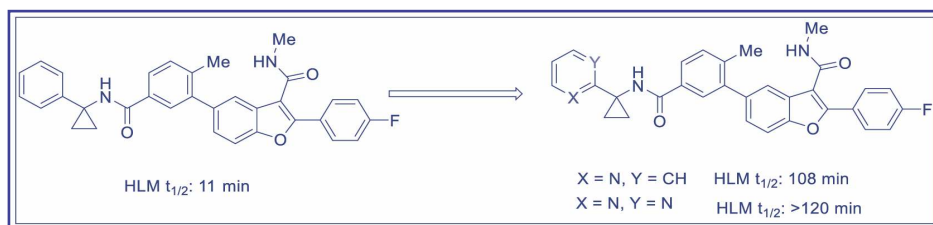


Figure 11. Metabolic stability using scaffold hopping.

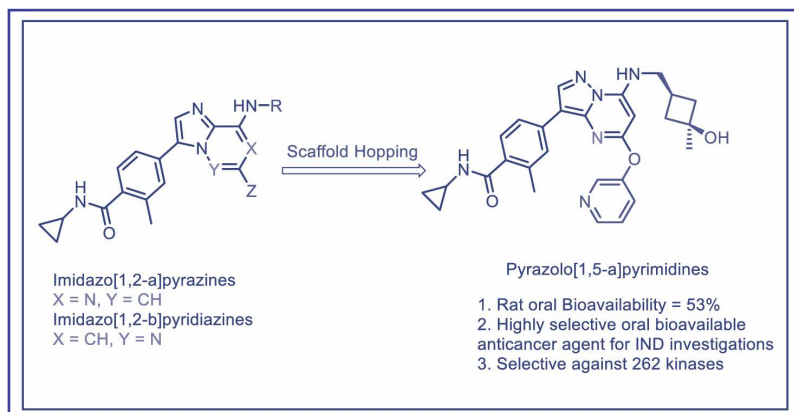


Figure 12. Scaffold hopping strategy for improvement in *in vitro* activity, physicochemical and pharmacokinetic profile, while retaining *in vitro* activity.

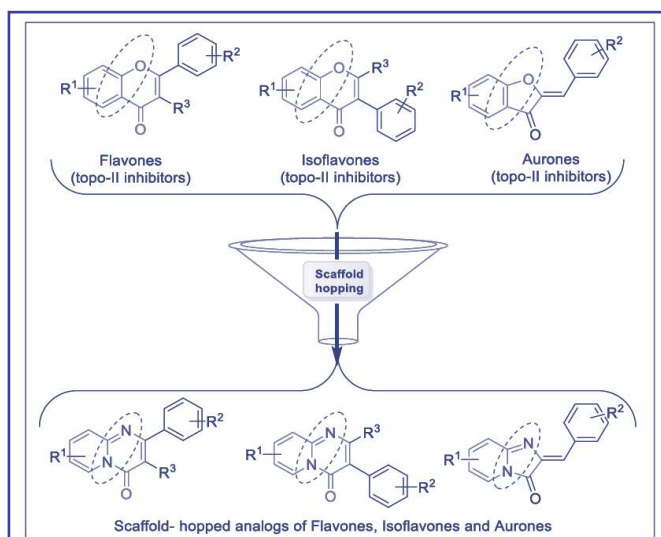


Figure 13. New scaffold hopped analogs of flavones, isoflavones, and aurones; improvement in activities and reduction in toxicity.

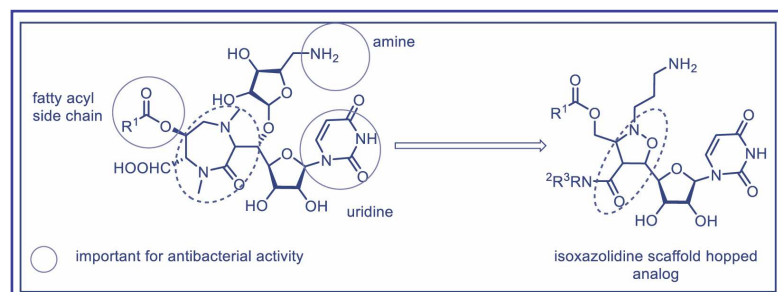


Figure 14. Scaffold hopping strategy in caprazamycin antibiotic.

Scaffold hopping of Natural products

Our group reported¹⁶ for the first-time the scaffold hopping strategy on bioactive natural product flavonoids. Compared to parent flavones, isoflavones and aurones with equivalent functional motifs, the scaffold hopped analogs of these flavonoids displayed very high hTopoII-inhibitory activities, cytotoxic activities, and less toxicity to normal cells. The study showed the importance of a natural product-based scaffold hopping technique in the drug discovery (Figure 13).

Our group¹⁷ also considered an iterative scaffold hopping strategy on scaffold hopping analogs of aurones by incorporation of the structural skeletons frequently present in known anticancer agents. They were synthesized via a new method of organocatalyzed umpolung chemistry. These iterative scaffold hopped analogs were found to possess important anticancer activities and appropriate physicochemical properties.

Yamaguchi et al. reported¹⁸ scaffold hopping of glycyuridine antibiotic caprazamycins, those are excellent antimycobacterial agents effective against both drug-susceptible and multi-drug-resistant *Mycobacterium tuberculosis* strains (Figure 14). They replaced the structurally complex diazepanone moiety of caprazamycin with the isoxazolidine scaffold (Figure 14). The isoxazolidine-containing uridine derivatives exhibited good activity against *H. influenzae* ATCC 10211 (MIC 0.25–0.5 $\mu\text{g mL}^{-1}$) and significant activity against vancomycin-resistant *E. faecalis* SR7914 (MIC 4–8 $\mu\text{g mL}^{-1}$).

Conclusions

Scaffold hopping approach is an emerging strategy in the drug discovery process and it enables medicinal chemists to explore varied array of biologically-important new patentable molecules. The strategy has found promising applications in the research area from pharmacoinformatics to pre-clinical investigations. It provides improvement in pharmacodynamic potency, physicochemical properties, and pharmacokinetic profile, as well as reduction in toxicity of molecules. Scaffold hopping is highly practiced in pharmaceutical industries sector and academia. Many drugs and clinical trial

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agents were successfully discovered by the scaffold hopping strategy. This medicinal chemistry strategy in combination with other design processes has potential in applications to various stages of pharmaceutical science research from computer-aided design to preclinical profile improvement.

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Making Grignard reaction safer and cleaner in continuous flow synthesis and applying for synthesis of active pharmaceutical ingredients (APIs) and key starting materials (KSMs)

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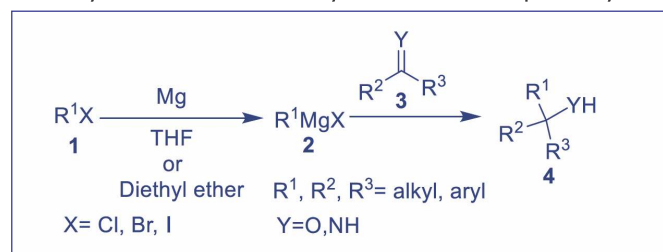
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Grignard reaction was discovered by Victor Grignard. Victor Grignard was awarded Nobel prize in the year 1912. This is an efficient reaction for C-C, C-hetero atom bond formation, for synthesis of several organic building blocks and active pharmaceutical ingredients. The drawback of this reaction is sudden spike in reaction temperature of environment friendly some substrates and slow addition to the substrates. This makes the process time consuming and formation of byproducts. The starting material is converted to product in a few minutes, in continuous flow process. The hazardous, unstable intermediates are well managed in a flow reactor. The safe operation and product with improved purity are the advantages of adopting continuous process in active pharmaceutical ingredients synthesis. This review article highlights recent reported literatures related to generation and applications of Grignard reagents for synthesis of precursors, active pharmaceutical ingredients, using flow reactors.

Introduction

Grignard reagents are the highly predominant reagents used in organic synthesis, for C-C, C-hetero atom bond formation (Scheme 1) and for the synthesis of active pharmaceutical ingredients (API). These are inexpensive and robust organometallic compounds.¹ Victor Grignard discovered the Grignard reaction and he was awarded Nobel Prize in Chemistry in the year 1912. Grignard reagent is denoted as RMgX , the R is an alkyl or aryl functional group, and X is generally a halogen group such as Cl or Br. It is synthesized by reaction of alkyl or aryl halide with Mg metal in tetrahydrofuran or diethyl ether.² The polarity and



Scheme 1. Generation of Grignard reagent and its reactivity with electrophiles.

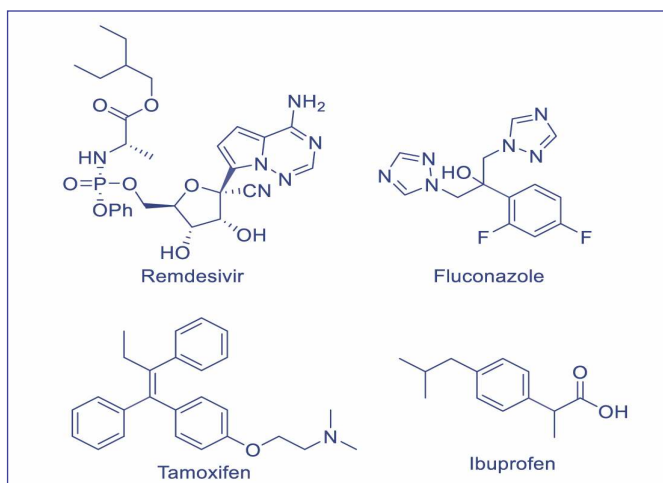


Figure 1. Familiar drug molecules synthesized using Grignard reagents.

electronegativity difference of the $\text{C}(\text{d}^-)\text{-Mg}(\text{d}^+)$ in the Grignard reagent implies that it reacts as a nucleophile, and facilitates the addition to an electrophilic substrate such as carbonyl, imine, cyano compounds etc.²

The Figure 1 describes the structures of a few

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essential, highly prescribed drug molecules such as remdesir,³ fluconazole,⁴ tamoxifen,⁵ and ibuprofen,⁶ synthesized using Grignard reagents. Grignard reagents are stored at room temperature. But these are extremely moisture, air sensitive, and should be stored in air tight closed container, as they lose reactivity over time, leading to lower yield. The other drawback is that it takes more time for initiation of the reaction for some substrates and once initiated, the reaction generates a lot of heat quickly, which must be dissipated. In order to control the sudden spike of temperature, the reagent is added slowly in drop-wise manner. This increases the reaction time, and promotes formation of byproducts. In continuous flow synthesis, the entire starting material is converted to product in a matter of minutes. The product purity is improved, and produced amount of product could be tailored to requirements.⁷

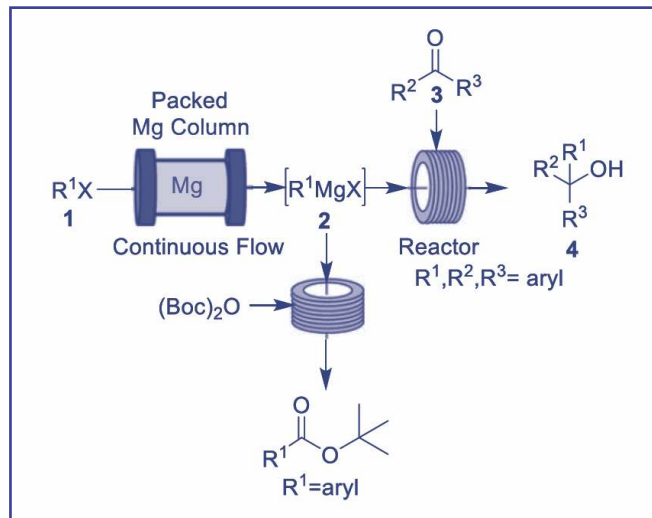
In Flow chemistry, channels or tubing or microreactors are used to carry out a reaction in a continuous stream rather than using a traditional reaction vessel. The solvents, solution of reagents is flown through the reactor using HPLC or peristaltic or other suitable pumps. The solution is pumped with precise flow rate in a controlled manner.⁸ The process is faster, as well as safer to handle hazardous reagents and unstable intermediates.⁸ Hence, flow reactor has got significant attention of academic and industrial synthetic chemists. This review article describes the generation of Grignard reagents in flow reactor, their applications for synthesis of different organic building blocks and API.

Grignard reagent generation in flow reactor

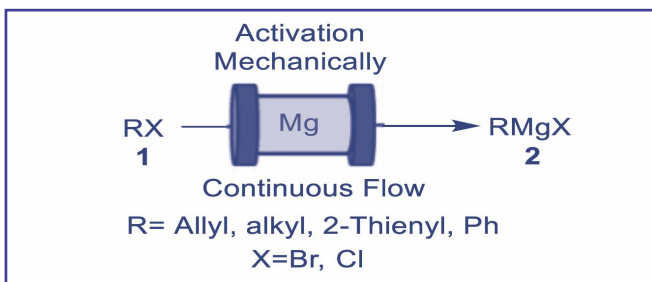
Alcazar and coworkers prepared Grignard reagent in a packed magnesium column in a flow reactor (Scheme 2).⁹ Magnesium metal of 20-230 mesh size was ideal for packing in a column, as it did not generate excess pressure during the reaction. The unwanted oxide layer on Mg surface was removed by passing diisobutylaluminium hydride solution through the column. The Mg metal was activated by flowing a solution of trimethyl silyl chloride in THF and followed by 1-bromo-2-chloroethane in THF. The generated Grignard reagent reacts with electrophiles such as keto compounds, di-tert-butyl dicarbonate [(Boc)₂O] to get alcohols, esters, respectively.

Menges-Flanagan used a jogging motor to activate Mg turning by causing abrasion on its surface, and

Grignard reagent generation in flow reactor

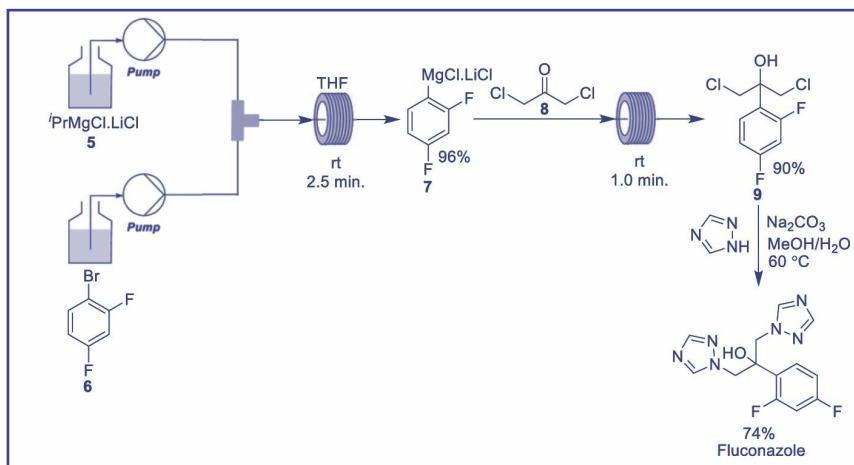


Scheme 2. Generation of Grignard reagent in packed Mg column in flow reactor.

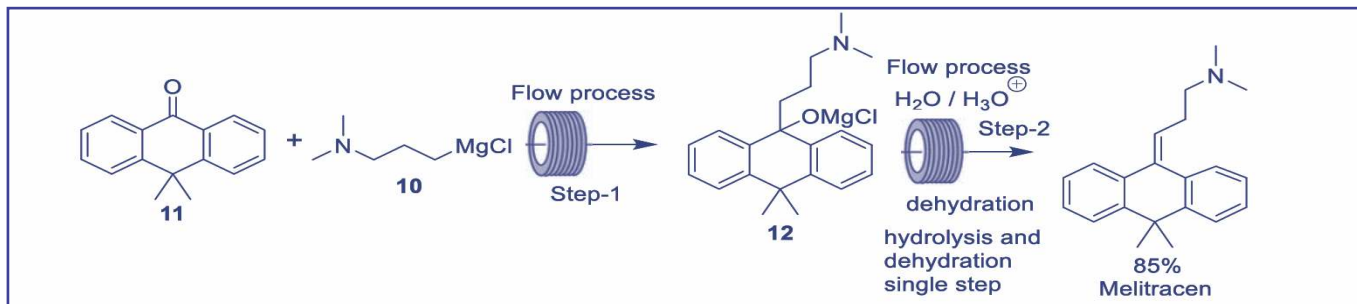


Scheme 3. Activation of Mg mechanically and preparation of Grignard reagent in flow reactor.

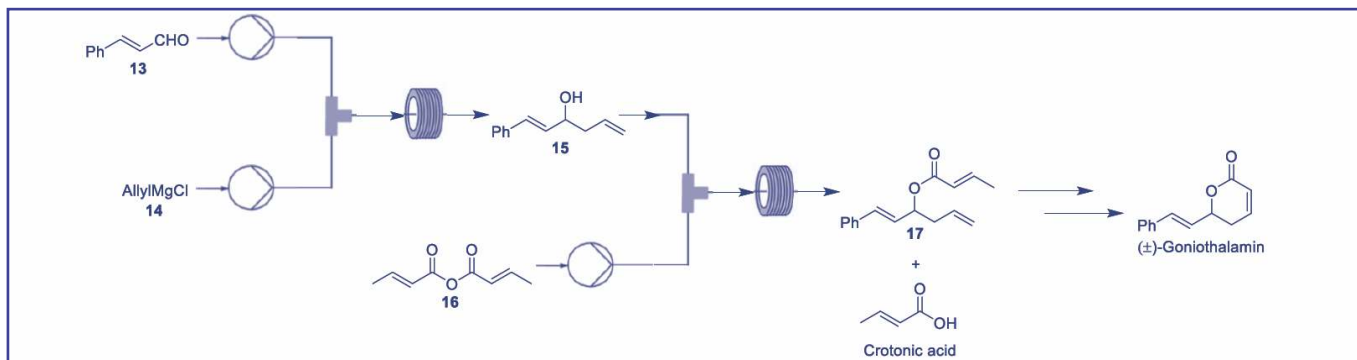
generated Grignard reagent on continuous flow mode (Scheme 3).¹⁰ Excess of Mg (5 to 25 molar) was used for 100% conversion and to prevent formation of unwanted side products. The monitoring of progress of the reaction was performed using inline IR technique. The condition was suitable for preparation of $PhMgBr$, $EtMgBr$ and $AllylMgCl$ on both



Scheme 4. A standard schematic diagram of continuous flow process for Fluconazole.



Scheme 5. A continuous flow process for synthesis of Melitracen.



Scheme 6. Synthesis of key starting material (KSM) for (±)-Goniathalamin.

the laboratory, as well as pilot scales.

Synthesis of Fluconazole

Fluconazole is an antifungal drug prescribed for treatment of localized and disseminated mycoses.¹¹ The *i*PrMgCl·LiCl (turbo Grignard reagent) which promotes the halogen magnesium insertion was used for conversion of bromo compound **6** to Grignard reagent **7** (Scheme 4).¹² Two separate pumps were used for *i*PrMgCl, bromo compound **6** and mixing of both reagents happens at T-joint and passed through the reactor at rt. There was formation of 96% Grignard reagent **7** and the residence time was only 2.5 min. The obtained compound **7** was reacted with 1,3-dichloro acetone in flow reactor with residence time of 1.0 min. to get 90% of alcohol compound **9**. Finally, treatment of compound **9** with 1,2,4-triazoles provides desired Fluconazole with 74% yield.

Synthesis of Melitracen

Melitracen is a drug used for the treatment of anxiety and depression.¹³ Pedersen reported a continuous flow process for synthesis of Melitracen.¹⁴ In flow process both hydrolysis and dehydration were performed in single step (Scheme 5). The phase separation step was also eliminated and only tetrahydrofuran (THF) was used as a solvent in comparison to the batch process, which requires toluene-THF solvent mixture. The both Grignard reaction (step 1), hydrolysis and dehydration (step

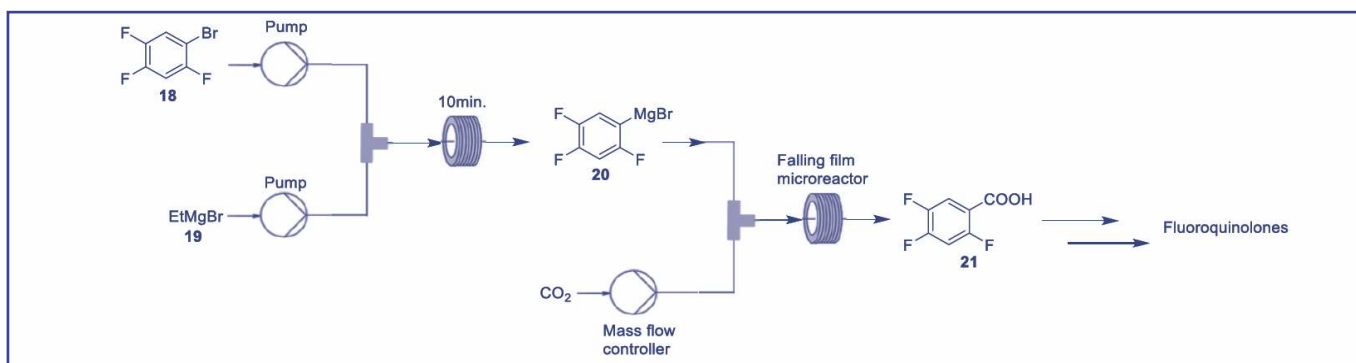
2) were performed at ambient temperature, whereas the batch process reaction happens at 50 °C.

Goniathalamin Precursor synthesis

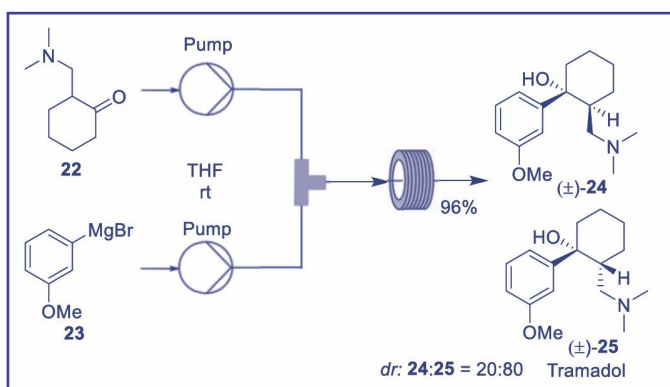
Goniathalamin is obtained from Goniothalamus species, which shows anticancer activity against breast cancer.¹⁵ A continuous flow gram scale process reported for synthesis of (±)-goniathalamin, by Pilli and Ley (Scheme 6).¹⁶ The continuous flow 1,2-addition reaction of allylmagnesium chloride with aldehyde **13**, followed by an acylation using compound 16 provided compound 17, the KSM for goniathalamin, with an output of 7 g·h⁻¹.

Preparation of KSM for Fluoroquinolones

Fluoroquinolones are considered as broad spectrum antibiotics, used for treatment of Gram-negative, as well as Gram-positive bacilli infections.¹⁷ Ciprofloxacin is the first generation fluoroquinolone and the next generation fluoroquinolones are levofloxacin, sparfloxacin, moxifloxacin, gatifloxacin etc. Many fluoroquinolones could be synthesized from the KSM 2,4,5-trifluorobenzoic acid. Deng synthesized 2,4,5-trifluorobenzoic by a continuous flow method (Scheme 7).¹⁸ The aryl-Grignard reagent **20** is unstable in nature. The Grignard reagent **20** is generated from bromo compound **18** using EtMgBr. The generated Grignard reagent **20** reacts with CO₂ (delivered through a mass flow-controller). The falling film microreactor helps in



Scheme 7. Synthesis of precursor for Fluoroquinolones.



Scheme 8. Synthesis of tramadol in continuous flow process.

thorough gas-liquid mixing, and facilitates biphasic reaction of compound **20** with CO₂ at atmospheric pressure efficiently. The increase in reaction temperature to 50°C, improved the yield to 93%. The batch process of the same reaction occurs at low-temperature and slow controlled addition is required, which makes the process expensive and inefficient.

Synthesis of Tramadol

Tramadol is an analgesic drug used for management of moderate to severe pain.¹⁹ Tramadol was synthesized with improved yield (96%) in a continuous flow reactor (scheme 8),²⁰ in comparison to traditional batch process.²¹ The reaction between (3-methoxyphenyl)magnesium bromide **23** and (±)-2-((dimethylamino) methyl)-cyclohexanone **22** generated tramadol with good diastereoselectivity (dr: 80:20).

Conclusion

Grignard reagents generation in a safer way and with good yield in continuous flow process have been reported in recent literatures. Several APIs and KSMs were synthesized using Grignard reagents in continuous flow synthesis ally magnesium chloride.

This technology will help in manufacturing of API, KSM with improved purity and safe operation of hazardous chemicals.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgements

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A cross-sectional study on pain perception toward needle-free injections in Gujarat state, India

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Aim: Main purpose of study was to understand awareness and preference related to needle free injection in Gujarat. Hypodermic needle related different dimensions were evaluated like fear, pain, inconvenience, side effects and reasons behind side effects and how they create avoidance of therapies by people. Study focuses to describe whether people are aware about needle free injection (NFI) and how much they are willing to use such device as alternative device to needle based injection. This conclusive cross-sectional study uses simple non convenient random sampling techniques were 390 respondents from 12 year to 55-year age group were selected who have once been administrated with needle injection in their life span, hence their perception can be known. An online structured surveys form was created. The data were analysed for descriptive analysis (mean, standard deviation), factor analysis (dimension reduction), and model building and structural equation modelling using the analysis of moment software. The association between gender and injection fear was evaluated using chi-square test ($p < 0.05$). Key findings reveal that factors such as fear of injection, injury risk, awareness of needle free injection, and preference have a significant impact on people's perception of needle free injection. Factors related with needle pain perception raise awareness questions among people, thinking about an alternative. Pain (66.8%) is the biggest reason for negative perception of needle injection. NFI is unfamiliar to 48% of respondents. A total of 81.1% of people prefer NFI as an alternative to needle-based injection. There is high preference and moderate awareness of needle free injection among people of Gujarat. The NFI survey may help doctors, nurses, and other medical professionals to comprehend the rationale and necessity for incorporating needle-free injection into routine practices and large vaccination programme. Additionally, pharmaceutical companies will know about NFI market's potential for growth and patient centric approach.

Introduction

A. Background to study

Needle procedures are regularly performed on both healthy and chronically ill people throughout their lives. Some medical equipment is used on a regular basis on a worldwide scale since it is so important in nursing and medicine. One such device is a hypodermic needle or injection. Approximately 16 billion injections per year are administrated worldwide.¹ It is used for drug delivery into dermal, vascular, intramuscular, subcutaneous, and other various tissues. Injections are widely used in health care settings such as hospitals, and community centres serving as both home and health clinics for individuals with disabilities. Intramuscular and intradermal routes are frequently used in clinical practices.²

There are various psychological aspects related to needle insertion by which the patient is affected.

KEY WORDS: Injection, Needle fear, Needle-free injection, Painless, Perception.

One such emotion seen in individuals who may fear pain, discomfort, or fainting is a fear of needles or worry, and other side effects such as redness, swelling, observing needle size, lumps, and other issues.³

The related fear and avoidance may have a negative impact on critical aspects of patients' lives, including as their ability to choose a career, and get necessary medical treatments, such as self-injected insulin for diabetics.⁴ There are various drawbacks related to needles shown in Figure 1.

The limitation of needle-based injection is that psychological fear related to resistance to self-injection.⁵ The need for novel drug delivery technology is growing all the time. Needle Free Injection Technology has attracted a lot of interest as a way to solve several challenges associated with needle-based injections.⁶

- Injections without needles are painless.
- They do not cause any side effects.
- They are easy & fast to use.

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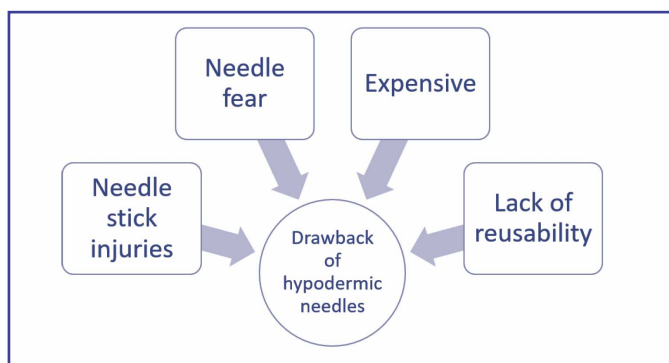


Figure 1. Drawback Related to Needles.

- Safe alternative for needle injections.
- Improve bio-availability over alternative non-invasive drug delivery systems by providing fast delivery and reprehensibility comparable to needles and syringes.
- Devices are available in reusable forms.

This method was first time documented in the 19th century in France, by H Galante produced an instrument called "aqua puncture". It was commercialized in the 1960s in the United States. Patients dislike needles, also healthcare professionals are concerned regarding accidental needle stick injuries, and pharmacy firms are looking for various novel ways to distribute their medicines.⁷ PowderJect Pharmaceuticals was the first to design needle-free injections for powder medications. In addition, to avoid the need of needles, the method allowed a drug to be administered in solid dosage form, which improved the product's stability along with allowing it for controlled release.⁸

This technique works by forcing fluids via a tiny aperture against the skin at a high speed. This results in an ultra-fine jet of high-pressure fluid that penetrates the skin without any need of a needle.⁹ These technologies were created to inject liquid formulations as well as solid dosage forms and vaccinations.

Needle-free medicine delivery is beneficial for a variety of reasons, including improved safety, increased compliance, reduced pain at the injection site, and easy and faster delivery of medicine.¹⁰ There are several different kinds of NFIs include Ojector, cool click, Vita jet and others are available in market.¹¹ Manufacturers are attempting to create technology that can deliver more types of drugs in addition to ones that are safer and simpler to use.

The market's primary growth drivers include the rising prevalence of chronic diseases, an increase in the frequency of communicable diseases brought on by needle stick injuries, the increased demand of self-injection devices and bio-similar as well as the

benefits of novel drug delivery technology.¹²

The most difficult aspects of patient care have been identified as fear and discomfort and they can be barrier to successful treatment.¹³ Needle Free Injection Technology (NFIT) has attracted a lot of interest as a way to solve several problems associated with needle-based injections.¹⁴ World health organization and the Centre for Disease Control (CDC) are among the organization that promotes NFI.¹⁵ Needle-free devices offer a number of benefits, including increased patient compliance, reduced injection site pain, and easier and faster drug delivery. Not only in the pharmaceutical industry, but this technology is also helpful in mass immunization programs, reducing the likelihood of needle stick injuries and other issues brought on by repeated use of a single needle.¹⁶ A survey to analyse the perception of injections among health-care seekers is required to appropriately address this problem.

We conducted a survey understanding people perception of needle fear, various reasons for needle fear, major side effects experienced, and avoidance of treatment due to same. Apart from this, survey includes respondents' awareness towards Needle free injection and awareness on parameter like painless delivery, ease and use convenience and market availability. An educational video regarding technique and knowledge was kept in Google form circulated for collecting responses to make the unaware respondent aware about NFI technology. Further phase of study was asked regarding their preference for NFI over needle injection, perception of usage in children for various therapies and as overall usage alternative to needle.

Gap analysis

The related fear has a negative impact on critical aspects of patients' lives such as:

- Avoidance of necessary medical treatments
- Delay in therapy initiation
- Vaccination avoidance (studies show that 50% of adults faced fear while vaccinating and 11% of Indians avoided vaccine due to needle fear in 2021.¹⁷
- Resistance towards self-injection.

Therefore, there is must need for some alternative to needles.

B. Why there is a need for study?

- Patients have a fear of needles.
- Patient suffers from different complications related to injections.
- Because needles are used so frequently in

professional settings, it's necessary to know who's at risk for needle phobia and what the consequences are.

- To understand patient awareness about alternatives to injection or what can be the scope of needle-free devices in the future.

C. Aim of Study

To study pain perception of people towards needle-free injections in Gujarat state.

D. Objectives

1. To understand physiological and behavioural variables in patient mind for fear, pain or side effects due to injection administration.
2. To understand Awareness of needle free injection among different age population.
3. To understand the willingness of people to prefer needle-free injection as an alternative to needle injections.
4. To check the future scope of needle-free injection in terms of factors like cost effectiveness, and self-administration.
5. To understand significant variables that has perception of people towards needle free injections.

Literature review

Both the terms "needle fear" and "needle phobia" refers to anxiety which is related to the use of needles and scenarios involving injections use in practices. That needle, the phobia is defined as more psychiatric disorder than simple generalized fear in the Diagnostic and Statistical Manual of Mental illnesses. The research by McLenon and Rogers, 2019¹⁸ yielded that needle fear was common to those who were undergoing blood donation, puncture in vein and patients with long term diseases need alternate injection. Hence, research gives direction towards gap analysis that why there is requirement of needle free injection for self-administration to chronic therapy patients along with fear being most common cause.¹⁸

According to a study, there was a considerable difference in correlation between injection and associated fear to get any injection treatment while being a patient. The authors recommended that anxiety and fear might influence health promotion behaviours to improve health and access to medical care, and also could affect procedures for detecting diseases and blood donations, by either reducing care seeking for people or by causing avoidance to care. Hence, a significant issue for services in clinical or preventive practice is due to needle anxiety or blood-related injury-injection phobia.¹⁹

According to an evaluation of ten key regions by WHO, each person in those areas received an average of 2 to 11 needle injections annually. Injections are among the most frequently performed medical treatments and are essential for both prevention and treatment. Even so, the fear of needle lead to delay in avoidance of preventive actions like vaccination, blood donation, and vein puncture while doing a normal clinical assessment and prescribing the appropriate care for various acute and chronic illness situations. From article John Yelland shows that most nations around world have seen improvements in injection procedures, still further efforts are needed to eliminate unsafe practices in healthcare settings. Needle free can be one such approach to eliminate unsafe practices.²⁰

A research study that tries to understand the relationship between needle avoidance, vaccination intention (VI), and vaccination fear (VF), as well as reasons for avoiding vaccination (RAV). This relationship was found positive for these factors RAV and VI, but negative for VI with needle phobia. The findings are addressed and steps to decrease Vaccination fear (VF) and increase vaccination intention (VI) are advised. Malas and Tolsá 2022 study concluded that needle phobia is one of the major reasons behind vaccination administration.²¹

A short study on paediatric patients' pain tolerance to various dental operations under anaesthesia was compared using a vibrating needle and a traditional syringe. A statistically significant difference in visual analogue scale (VAS) and face pain rating scale (FRS) was discovered between the two procedures, but when physiological indicators like heart rate, blood pressure, and temperature at different intervals were examined. Findings demonstrate, Vibraject because less discomfort and pain while administrating local anaesthetic injection as compare to traditional injection. Reference study of Chaudhry, 2015 helps author to give more insights about how NFI device and traditional injection have significant impact on pain perception. As well as such studies motive author to understand more from society on needle free device awareness, usage, and preference to alternatives.²²

McMurtry in his paper present an overview of discomfort and apprehension in relation to needle procedures. Few people consider this pain as minor pain but for others, these needles are not just a poke. While compared to adults, children have more anxiety about needle pain and desire to get therapies at lower pain intensity levels. Needle procedures like vaccine injection are common across the life, commonly in childhood. Across the lifespan,

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people report an absolute increase of about 10% in willingness to receive a vaccination if treatment is painless. The emotional after effects of the experience can remain long after the sharp pain has subsided. Unrelieved discomfort might eventually lead to fear, which can lead to suffering during subsequent treatments.²³

Liquid jet injector administers vaccine to dermal, subcutaneous, and muscle areas. The process of ballistic inoculation involves injecting vaccinations into skins outermost layers as in powder form. It has become common practice to use powdered lidocaine to deliver quick local analgesia to the back of the hand. Literature views about needle free injection mechanism, types and advantages are explained in article.²⁴

Methodology

A Conclusive, descriptive cross sectional research design study was conducted from 3rd December, 2021 to 9th December, 2021 with age group of 12 to 55 years old from state of Gujarat, India. By keeping in mind, the people of Gujarat who have once been administrated any medication with hypodermic needle in their life time are selected as relevant respondents for research study. Collectively 390 responses were collected by adopting non-convenient random sampling technique. An online Google based research questionnaire was circulated by various social media applications to people living in different districts of Gujarat. A total of 401 responses were collected, but out of that 390 have taken hypodermic needle at least once in their life time hence were sorted for data analysis. Remaining 11 responses were not taken in data analysis. A total of 390 data was collected for doing analysis. Data was analysed using SPSS V 23. Continuous variables were summarised using mean and standard deviation whereas categorial variables were done using frequencies and percentages. Prior to the survey, all respondents were informed that the information will be kept confidential. Prior to study, each responded gave their consent also. Referencing Hair²⁵ discussion with a view to the application of confirmatory factor analysis (CFA) and SEM structural equation modelling. The authors conclude that this sample is sizeable. This sample is considerable as per the authors' opinion by refereeing the discussion presented by Hair, 2019 with a view to the application of CFA and SEM.

Data Analysis Plan:

1. Data preparation: Data entry into Excel, SPSS V23 data files preparation
2. Data Cleaning: Fill-up missing data points, removal of incomplete cases
3. Model building

4. Descriptive statistics with necessary graphs and statistics for gender, age, income level, healthcare professional and area are indicated by frequency (number of respondents) and percentage out of 100 in table 1. (Q 1 to Q 5 described under questionnaire design).
5. Exploratory factor analysis (EFA) -varimax rotation (Q6 to Q20 described under questionnaire design).
6. Confirmatory factor Analysis (CFA) -significant value 0.05 and confidence interval level 95%, Hair and babin, 2019 method taken maximum likelihood estimation (MLE) (for Q6 to Q20 described under questionnaire design). This study uses Principal Component Analysis (PCA) in SPSS V20 utilising varimax rotation to determine the underlying element and perception of needle-free injection. Four significant factors were as a result extracted. These four elements were determined to be needle injection awareness; needle injection preference, needle injection fear, and needle injection harm risk. In order to confirm the underlying findings, confirmatory factor analysis was carried out. CFA was employed to the measure construct of validity. Confirmatory Factor Analysis (CFA) is one of the most widely used techniques for evaluating the construct validity of an instrument, Hair et al, 2019. In comparison to Exploratory Factor Analysis (EFA), this method also provides a more accurate interpretation of dimensional data, Diana.²⁶ SEM technique was used in this work in examine the hypotheses. To determine whether the gathered data fit the suggested model, model fit analysis must be performed prior to testing the association, Hair, 2019. Maximum likelihood estimation is used for SEM-AMOS estimation.²⁷
7. Reliability and validity check

I. Study design: The major study is conclusive cross sectional in nature. It is primary research which includes 20 close ended questionnaires. Data was collected from people with age group between 12 years to 55 years old.

II. Sample size and sample technique: The data was obtained from 390 respondents. The method used for collection of samples was convenient non-random sampling through an online survey. By refereeing the discussion presented by Hair and Babin, 2019²⁵ with a view to the application of SEM and CFA. Considering the summary of discussion (as provided below points) on the ideal sample size suitable for CFA and Communalities, the present research sample can be considered as adequate:

III. Statistical test and Software used for statistical analysis and visualization: Tools like Power BI, IBM SPSS V23, IBM AMOS V23 and Excel were used to perform statistical tests. The graphical dashboard in Figure 3 is created through Power BI. Factor analysis done for dimension reduction and chi-square analysis was performed through IBM SPSS tool. IBM AMOS software is used to study Structural equation modelling (SEM) and for model building. In excel various charts and numerical value such as percentages to each study was carried out, bar graph in Figure 4 was prepared using Excel 2019 version.

IV. Procedure: Firstly, a focus group study was conducted with 7 questions asked out of which 3 were open ended questions and other 4 questions were "yes" and "no" answered based questions asked to different age group. Total of 17 participants were asked about needle related fear and pain, avoiding treatment due to fear and pain while administration, awareness of needle free injection and preference to needle-

needle, only those were selected as sample for target population for research. Finally, 390 responses were sorted for data analysis. Total of 20 questions were asked to respondents. I have mentioned that point in my questionnaire that I won't share this data or information provided by the respondents with any third party, this is solely for research purpose and in the possession of NIPER-A. I have kept the questions by carrying pure research intention in mind and this is not indulging any hurtful sentiments and beliefs of respondents from any group of people.

First five questions (Q.1 to Q.5) were on age, gender, income level, healthcare professional, belonging to urban or rural were asked to respondents. Next seven questions concerned or reaction to perception of needle-based injection about fear of injection before taking injection, pain perception while administration and side effects occur post injection effects were asked. Major parts of questionnaire were adopted from Siddiqui paper.²⁶ The researcher has developed the questionnaire by referring the literature on internet.^{27,28} The data for first 6

questions has a 5 point scale with following options: 1 highly disagree, 2 for disagree, 3 for neutral, 4 for agree and 5 for strongly agree.

Q.6 Do you experience any fear/anxiety for needle before taking injection?

Q.7 Do you experience any type of pain while taking needle injection?

Q.8 Do you feel inconvenience while taking injection? (Place of administration, etc)

Q.9 Do you think that needles present in injections can be risky to cause injury?

Q.10 Do you experience any side effect after taking injection?

Q. 11 What are the reasons for needle fear? (Multiple selection option was asked for 7th question: Pain, watching needle, Nurse discussing with other about injection, seeing size of needle, observing other people getting vaccinated, previous bad experience).

Next four questions were asked about the Awareness of people towards Needle free injection. The Likert scale point for this awareness question were as 1- Not Aware at all, 2- Unaware, 3- Neutral, 4- Aware, 5- Extremely Aware.

Q.12 Needle free injection does not cause any pain.

Q.13 Needle free injections do not cause any side effects.

Q.14 Needle free injections are easy & fast in use

Size of sample minimum	No. of observed variable per factor	Communalities
100	3 and more	0.6 or more
150	3 and more	Modest level -0.5
300	<3 (few out of 7)	Lower level <0.45
500	<3 or equal to 3	Few with lower level

less injection as an alternative option of needle injections. Once the review obtained, 20 final questionnaires with different scales were prepared by doing literature review and circulated via Google form (online mode). The questions were asked regarding their perception to pain and other problem faced while administration of needle-based injection, awareness of needle free injection NFI and preference to NFI along with demographic questions like age, gender, healthcare professional, income level.

V. Questionnaire design: Close-ended questions were asked to respondents on the survey to look at their demographic profiles and Likert scale was used to record their comments on the factors that affect needle injection fear. The Likert scale has a maximum of five points, from one for strongly disagreeing to five for strongly agreeing and some awareness towards NFI were recorded on 5 scale where 1 being not aware at all to 5 being highly aware. A question asking "Have you ever taken injection before?" and 97.2% respondents answered "yes"- that they have experienced administration of hypodermic

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and safe alternative for needle injections.

Q.15 Are you aware about various needle free injections available in market? (E.g., Pharma JET, Cross jet, etc)

To get the insights on willingness of people to prefer needle free injection as an alternative over needle injections & its scope in market following next five questions were asked as follow. Five pointer Likert scale of 1 to 5 as above.

Q.16 Would you prefer Needle free injections if they are available for all range of disease therapies?

Q.17 Would you prefer needle free injections if they are cost effective than needle injections?

Q.18 Would you feel free to recommend needle free injections for children's therapies?

Q.19 Do you feel needle free injections are user friendly for self-administration (e.g. - Insulin)?

Q.20 Would you prefer needle free injections as an alternative over needle injections?

Research hypothesis

Null hypothesis: There is no association between fear of needle, injury risk, awareness to NFI, preference to NFI perception towards NFI among people and

Alternative hypothesis: There is association between fear, risk to injury, awareness to NFI,

Research hypothesis

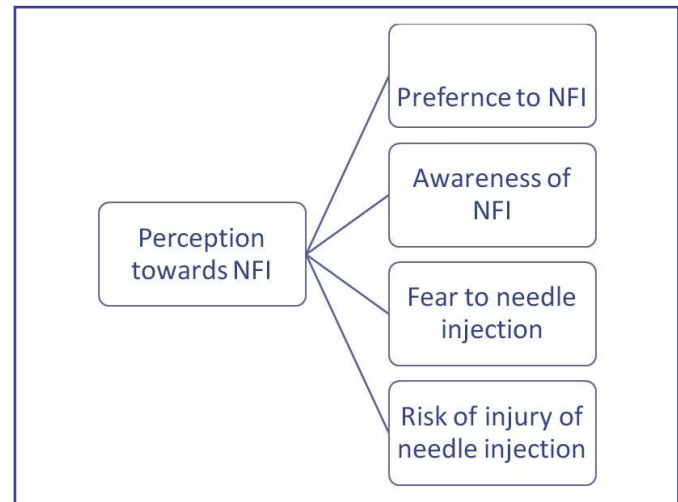


Figure 2. Hypothesized model.

preference to NFI and perception towards NFI.

Major objective of this paper is to check the usefulness of the CFA in order to test the fitting of a predesigned theoretical model. This is a study to understand how different estimation methods of CFA should be considered to fit the theoretical model on the data.

RESULTS & DISCUSSION

The numbers of individuals recruited for research study are 390 living in Gujarat state.

Table 1. Demographic Profile

VARIABLE	CATOGORY	FREQUENCY	PERCENTAGE
Gender	Male	196	50.3%
	Female	194	49.7%
Healthcare professional	Yes	185	47.4%
	Not healthcare professional	205	52.6%
Income level	No income yet	147	37.7%
	< 50,000 INR	66	16.9%
	50,000- 2,50,000 INR	49	12.6%
	2,50,000-5,00,000 INR	58	14.9%
	5,00,000-10,00,000 INR	47	12.1%
	>10,00,000 INR	23	5.9 %
Area	Urban	294	75.4%
	Rural	96	24.6%
Do you experience fear/anxiety before taking injection?	Agree	176	45.1%
	Neutral	107	27.4%
	Not agree	107	27.4%
Would you prefer needle free injection as alternative to needle injection?	Agree	319	81.8%
	Neutral	34	8.7%
	Disagree	37	9.5%

a . D E M O G R A P H I C PROFILES:

Table 1 displays some demographic data for the population, including gender, age, region, healthcare professional and income level and preference to needle free injection over needle injection administration choice.

From 390 respondent people from 12 year to 55-year age group all have participated in study. The survey was conducted to understand people perception of fear of injection which shows, 45.1% feels they have anxiety before taking injection. And at last, when preference was taken for Needle free injection over needle injection 81.1%

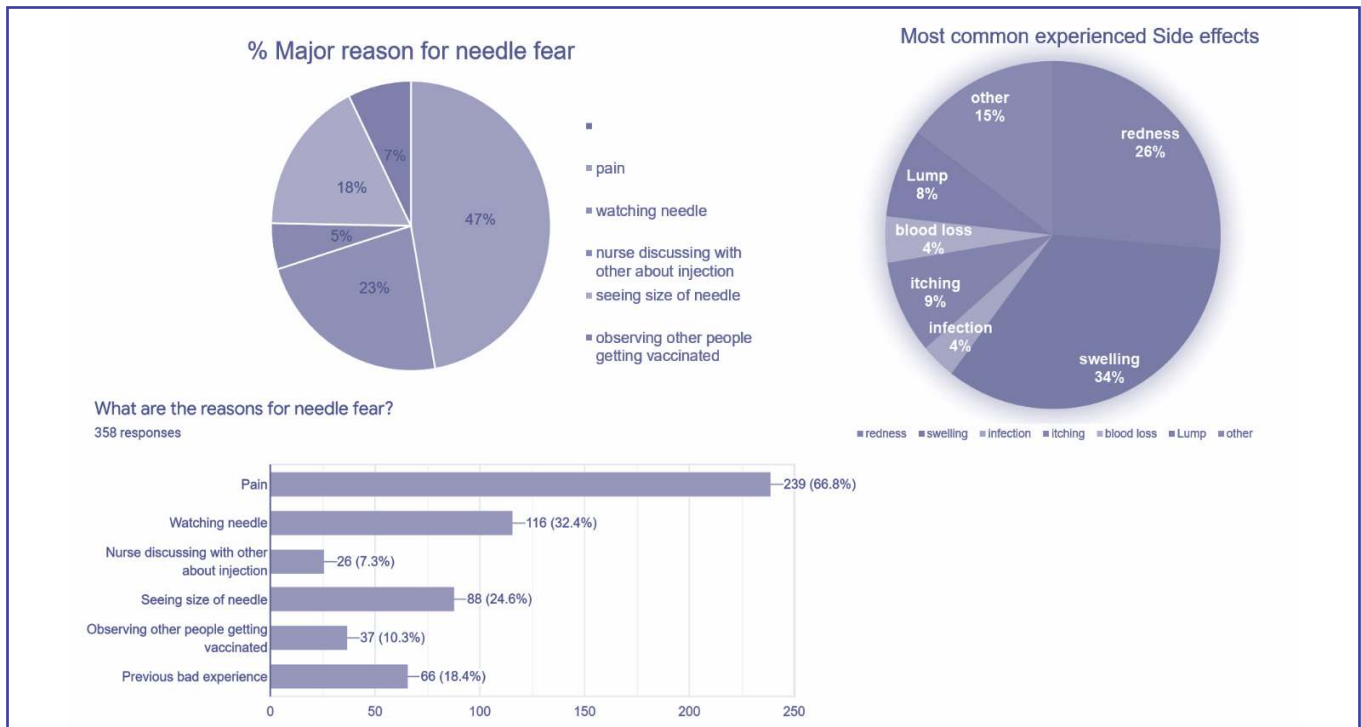


Figure 3. Needle fear related reason analysis.

want to have NFI in their treatment way option. People's perceptions toward pain, fear, and other aspects of administering injections with needles: figure 3 indicates various reason of needle fear as shown pain, watching needle followed by size of needle are major reason why people fear of injection and which ultimately affect therapy or healthcare of patient. When asked for most frequent side effects, pain (47%) being major one, followed by nurse discussing with other about injection (23%). Graphical represented dashboard was created using power BI represent that there are various problems like pain, seeing size of needle, and other responsible for fear to injection.

Figure 4 shows that from total number of those respondents who do not belong to healthcare profession (205), out of them only 32% know about NFI, while rest have no clue to this device. From them 77% wants to prefer NFI as they find it effective alternative to needle-based injections. Similarly, total number of healthcare professional participated in study (185) out of which only 38% are aware about NFI, hence, there is high requirement that a proper awareness of NFI among healthcare professional is also required. Those having healthcare professionals background from them 87% wants to prefer NFI as alternative to injections.

Association between gender and avoidance of treatment (Pearson Chi-square test):

Hypothesis

Ho: There is no association between being gender and avoidance to treatment.

H1: There is an association between gender and avoidance to treatment.

Statisticians determine the probability of an event occurring (Probability value) by chi-square on basis of degree of freedom. The number of subjects that can vary independently, minus one, is the degree of freedom (n-1). Because we have two phenotypic classes, then degree of freedom is 1. The calculated chi-square value from our findings can be compared to the numbers in the table that correspond to the particular degree of freedom we observe. This will inform us whether the differences (between what we predicted and also what we actually witnessed) are caused by chance or whether our hypothesis or assumption can be confirmed. If calculated value of chi-square is higher than critical or tabulated values, then you reject your null hypothesis. Here, calculated value is 5.191 which is greater than tabulated value which is 3.841. Hence, Pearson chi-square t-value was found significant indicates there is association between gender and avoidance of treatment as shown in Table 2. Our null hypothesis is rejected. Females tend to avoid treatment more compare to males.

Perception and Preference Analysis: Figure 4 indicates graphical analysis of few important questions asked during survey. Here, 5 major criteria

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were kept in mind while questionnaire designing and few questions related to each criterion were asked as indicated in Table 3.

b. Data analysis:

As data is moderately normally distributed and the measurement type of all the chosen variables are ordinal (scale- notation in SPSS data- file), maximum likelihood estimation can be an appropriate method. With the help of SPSS V23 software, most of underlying variables were extracted using factor analysis, which was utilised to reduce large number of variables. Results from the remaining factors generated a reliable model, which was further taken into consideration for validity and dependability. To determine if the various data components are correlated with one another, the greatest likelihood estimation. In this type of analysis different variables are grouped together. In Table 4, all the variables above 0.5 are grouped together. Before factorization, the sample adequacy for performing factor analysis on data is evaluated using the Bartlett test and KMO (Kaiser-Mayer-Olin), as indicated in Table 3. The sample is sufficient for factor analysis based on the KMO's p-value of 0.843 (>0.7).

Bartlett's test of sphericity p-value is 0.000 which indicates that factors can be performed from sample data. 48% of data is normal; however, considering the importance of valuable data, the other non-normal data along with normal (total sample) is taken for factor analysis study.

The data having commonality of higher than 0.5, that will explain more than half of the variance.

The analyses were performed utilizing IBM SPSS V 23 software. In order to check for connections across latent factors and to identify which factors are impacting which variables in the predicted model, the factored data was then used to conduct confirmatory factor analysis in IBM AMOS V 23 software and structural equation modelling. To do this, the reliability of Cronbach's alpha²⁵ (Table-5) was examined. The alpha values are displayed above, and we may consider them satisfactory values, indicating that the factor analysis-based variable grouping is closely related to one another, and thus a good indication to conduct Confirmatory Factor Analysis (CFA)²⁵ and Structural Equation Modelling (SEM)²⁵ on determined data set. The proposed model was created using AMOS graphics to conduct the

CFA and show that it fits the data perfectly.

In order to test our research hypothesis, all endogenous and exogenous variables were properly entered into the hypothesized model created in AMOS graphics, along with error factors, even before variables were connected with arrows to test our research theory. When the analysis is finished, the factor loading number in arrows is checked and the following statistics were examined for model fit: TLI (Tucker Lewis Index), CFI (Comparative Fit Index) and RMSEA Root Mean Square Error, must be near to ideal.²⁵

Regression Weights/ Factor Loading (Group number 1- Default Model)

The above results were acquired by a study

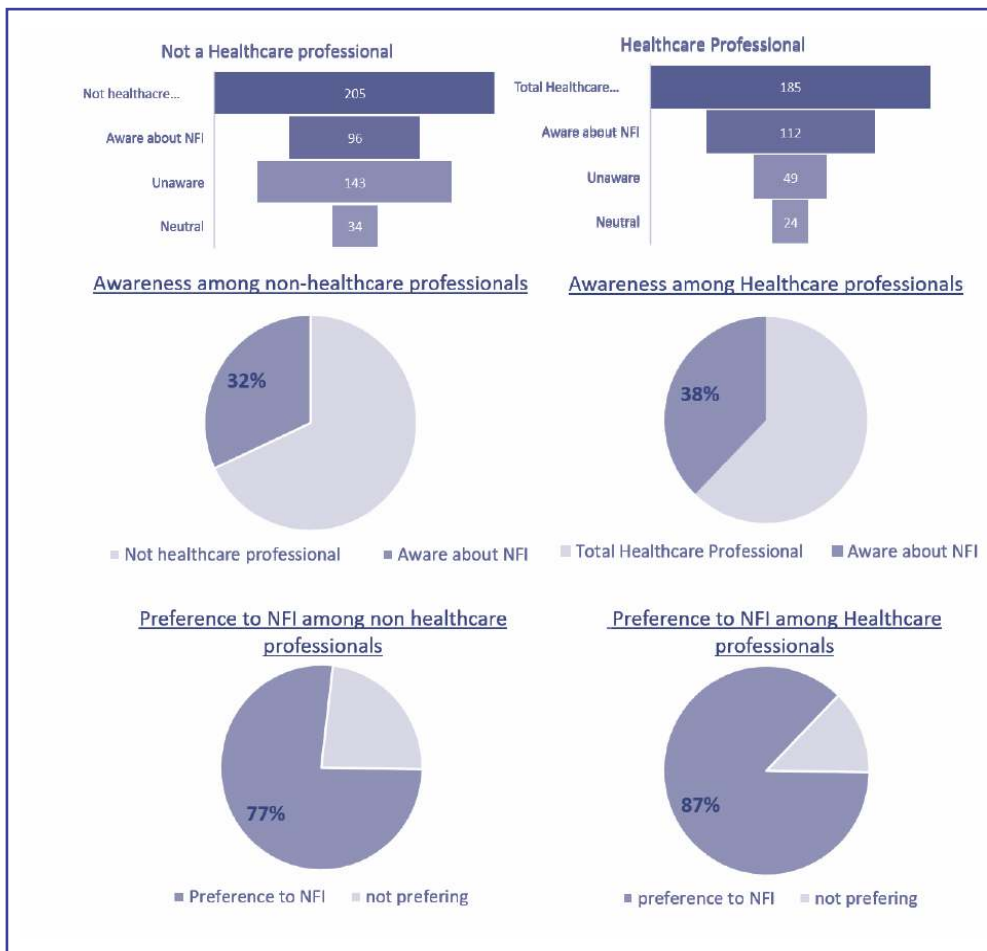


Figure 4. Profession-wise perception study.

Table 2. Pearson Chi-square test.

			A2		Total
			No	Yes	
G	Female	Count	129	65	194
		Expected Count	139.8	54.2	194.0
	Male	Count	152	44	196
		Expected Count	141.2	54.8	196.0
Total		Count	281	109	390
		Expected Count	281.0	109.0	390.0

P value	0.014984128
Alpha	0.05
Degree of freedom	1
Calculated chi-square	5.191833307
Tabulated chi-square	3.841458821

performed in AMOS (Analysis of moment structure), as shown in the table, there are variables that have a significant impact on each other (95% confidence), as the Probability value (P- value) (significance) is 0.001(in the Table 7 output it is represented as *).²⁵ Originally for significance, the value of P should be < 0.05. Fear related factors such as side effects, pain, fear, and inconvenience during administration and risk to injury, similarly awareness towards NFI and preference of needle free injection shows significant impact on needle free injection perception.

Goodness of Fit

Goodness of Fit basically, it gives an indication of the fitting of the theoretical model. Higher the value of GFI (goodness of fit index) indicates the better fit of the theoretical model to the sample data. The static values of different model fit parameters obtained are Root means square error (RMSEA) 0.065

Table 3. Analysis.

Major Criteria	Perception	Strongly agree & agree out of total 390 samples (100%)
1. Before Taking Needle Injection	Fear before taking injection	45.1%
	Injury Risk Before taking injection	42.0%
2. While Taking Needle Injection	Inconvenience while administration	46.4%
	Pain felt while administration	61.8%
3. After Taking Injection	Side Effects Experienced after injection administration	28.4
4. Awareness to Needle free Injection (NFI)	Awareness to NFI	48% (not aware at all)
1. Preference to NFI	Preference to children’s therapies	84.6%
	Preference as alternative to Needle injection	81.8%

near to 0.06 said to be in the non-error range, Comparative fit Index (CFI) 0.938, TFI (Tucker Lewis Index) 0.926 and the CFI (Comparative Fit Index) is 0.938 signifies that the model has satisfactory results and is considered to be fit model. The probability level was significant (P= 0.001) at that level. The components having Cronhbach's alpha value which is greater than >0.7 and is regarded as "acceptable". Figure 5 shows the results of SEM (Structural Equation Modelling) in addition to this CFA (Confirmatory Factor Analysis).

Factor Analysis).

AMOS was used to generate the mentioned in Table-8 results. It is evident that there are variables that have a significant relationship with each other because the P (significance) value is 0.001, showing 95 percent confidence.

The findings suggest that the Perception of people towards needle free injection has been impacted by Fear of needle injection, injuries related to them, which leads to awareness of NFI and preference of NFI.

Generalisability (external validity)

Convergence validity is the level of assurance in a feature that is well measured by its indicators, whereas discriminant validity is the level of assurance in measuring various qualities that are unrelated to one another. The degree of common variance shared among the implicit variables of the model is assessed using the Fornell-Larcker (1981) criterion in CFA. With Average Variance Extracted [AVE] and Combined Reliability, the measurement model's convergent validity can be assessed (CR-Composite Reliability [CR]). The acceptable range for both the CR and AVE is 0.70 and above, but 0.50 and above is sufficient. For the purpose of determining the discriminant validity, AVE> MSV (Maximum Shared Variance) criteria were used. The analyses in the table offer speculative conclusions about generalizability.

Reliability: the CR for Injury is slightly less than 0.70.

Convergent Validity: the AVE for Injury is equal to 0.50.

Discriminant Validity: the AVE for Injury is more than MSV.

Here, everything is in range, but reliability of injury to risk factor is slightly away from

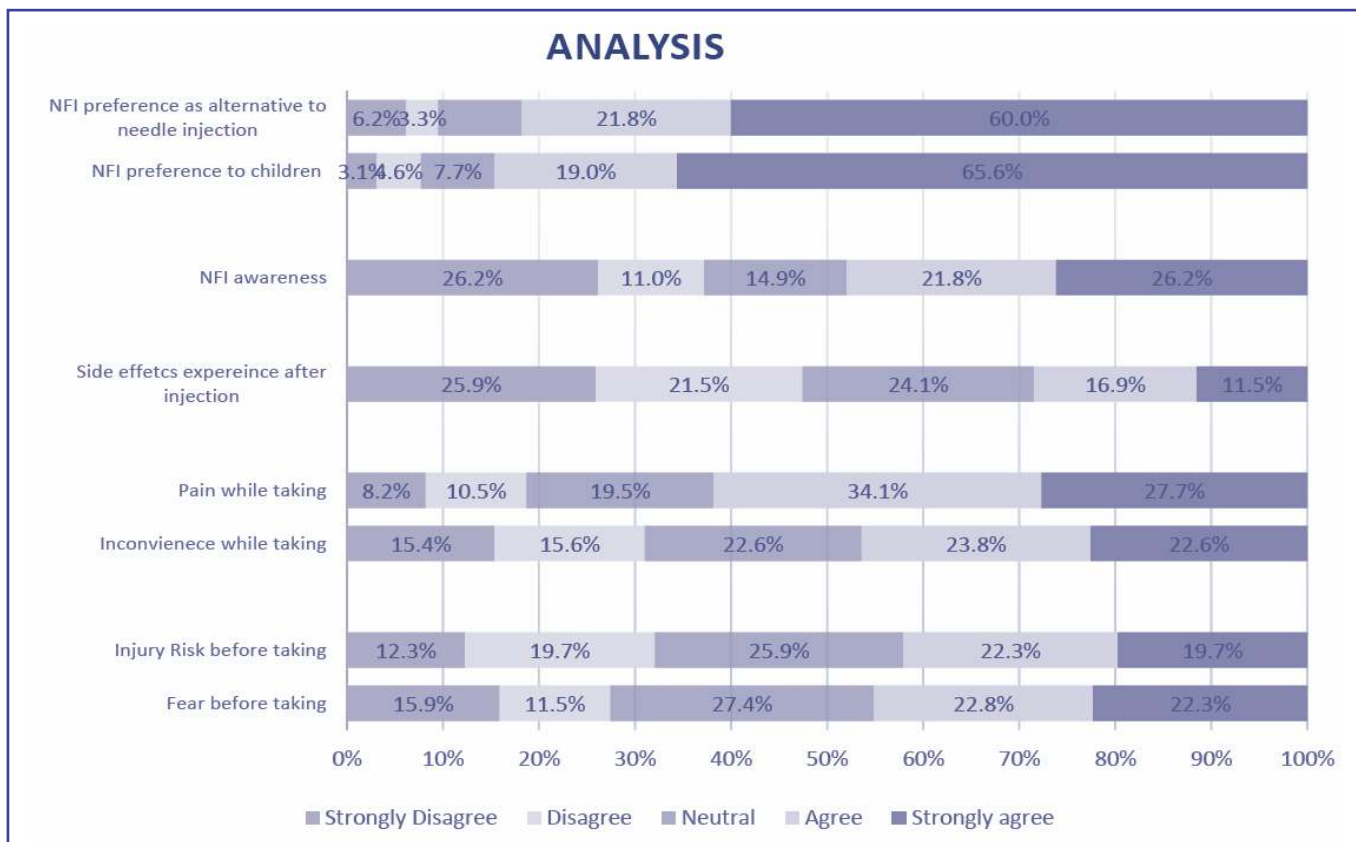


Figure 5. Various Perception and Preference Analysis.

Table 4. KMO and Bartlett's test.

KMO measure of sampling adequacy		0.843
Bartlett's Test Sphericity	Approx. chi-square	3081.929
	Degree of freedom	136
	Significance	.000

what need to be, thus that factor can be a concern during generalisability.

Conclusion

The study's main objective was to understand the important factors that influence how people perceive needle free injection (NFI). Such an NFI survey may assist healthcare practitioners, nurses and other professionals incorporate needle-free injection into routine procedures and large vaccination programs. Physical and psychological factors such as pain (61.8%), inconvenience (46.4%), fear (45.1%), needle injury (42%) and various side effects (28.4%) such as redness, swelling, itching and lump together significantly contribute to the perception of alternative options for needles, such as needle free injections, to prevent such issues. In addition, only a small percentage of healthcare professionals and even fewer people with no medical background are aware of this

new pain-free method of administration, despite a high level of desire for NFI, according to the study. Survey provides insights that whether there is need of needle free injection to overcome this perception of pain with needles or not. Preference to

Table 5. Rotated component matrix.

	Components			
	1	2	3	4
Preference to NFI				
P1	0.752			
P2	0.751			
P3	0.784			
P4	0.790			
P5	0.828			
P6	0.608			
Awareness to NFI				
A1		0.867		
A2		0.899		
A3		0.891		
A4		0.860		
Fear of needle				
F1			0.894	
F2			0.824	
F3			0.710	
F4			0.691	
Risk of injury of needle				
R1				0.767
R2				0.469
R3				0.797

Table 6. Descriptive statistics.

	N	MEAN	SD	CronhBach's alpha
PREFERENCE TO NFI				0.850
P1	390	3.241	1.3485	
P2	390	3.226	1.3663	
P3	390	3.626	1.2225	
P4	390	2.697	1.4767	
P5	390	3.174	1.2948	
P6	390	3.874	1.3171	
AWARENESS TO NFI				0.910
A1	390	2.667	1.3325	
A2	390	3.108	1.5541	
A3	390	3.026	1.5919	
A4	390	3.177	1.5585	
FEAR OF NEEDLE				0.831
F1	390	2.810	1.5888	
F2	390	4.267	1.0517	
F3	390	4.254	1.0706	
F4	390	4.395	1.0206	
RISK TO INJURY OF NEEDLE				0.639
R1	390	4.300	.9619	
R2	390	4.497	.9693	
R3	390	4.262	1.1443	

NFI being another significant factor creating perception towards needle free injection such preference for all range of disease therapies, for children and self-administration are high. An overall conclusion, there is high preference and moderate awareness of needle free injection in routine healthcare practices. Study contributes to a better understanding for healthcare professional about willingness of people (81.1%) to accept NFI as alternative to hypodermic needle and understand future market of needle free injection. Children typically have more injection anxiety, thus the study also examined how willing people want to use this novel technique on them. The majority of adults (84.4%) said they would be willing to use NFI on children. In this way, individuals who avoid therapy or don't comply with it because they find it uncomfortable or difficult to get injections with needles can also receive treatment. Thus, these four physiological and behavioural factors-fear of needles, injury from needle use, awareness and preference for NFI-significantly influence perceptions of NFI. As a result, this research has important clinical significance for healthcare settings and pharmaceutical firms developing patient-centric approaches.

settings and

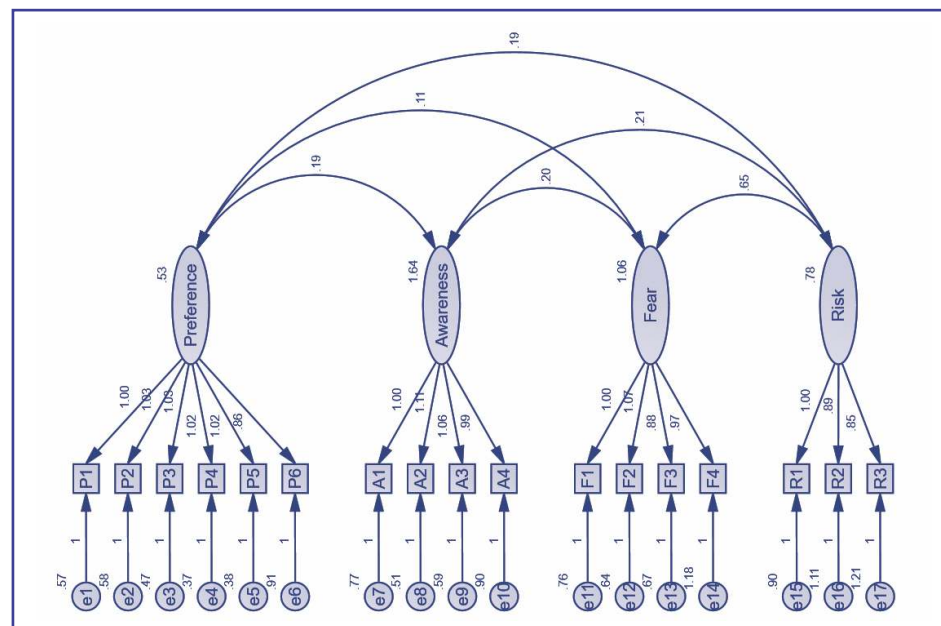


Figure 6. Confirmatory Factor Analysis Using Amos.

Limitation and future scope

Study is conducted for age group above 12 only. Children from age group 5-12 experience high pain and have side effects also such study has to be done from their guardian. Apart from this as per responses receive not only children but adults are also having very painful perception related needle. Swelling, blood loss, lump, redness and other are major concern of people from long ago, yet now various devices are available but due to lack of awareness in Indian

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Table 7. Regression Weights/Factor Loading.

Variables		Factors	Estimate	S.E. (Standard Error)	C.R. (critical ratio)	P (Probability value)	Label
X7	<---	Preference	1.000				
X8	<---	Preference	1.029	.080	12.840	***	par_1
X9	<---	Preference	1.019	.080	12.750	***	par_2
Y1	<---	Preference	1.022	.078	13.130	***	par_3
Y2	<---	Preference	1.009	.075	13.395	***	par_4
Y3	<---	Preference	.857	.087	9.806	***	par_5
X3	<---	Awareness	1.000				
X4	<---	Awareness	1.111	.052	21.267	***	par_6
X5	<---	Awareness	1.057	.052	20.331	***	par_7
X6	<---	Awareness	.996	.055	18.259	***	par_8
V4	<---	Fear	1.000				
V2	<---	Fear	1.142	.084	13.542	***	par_9
V3	<---	Fear	1.220	.085	14.395	***	par_10
X2	<---	Fear	1.121	.090	12.432	***	par_11
V6	<---	Injury Risk	1.000				
V7	<---	Injury Risk	.881	.107	8.260	***	par_12
V8	<---	Injury Risk	.876	.098	8.960	***	par_13

Table 8. Estimates of Different Factors.

			Estimate	S.E. (Standard Error)	C. R. (critical ratio)	P (Probability value)	Label
Preference	<-->	Awareness	.190	.055	3.440	.000	
Preference	<-->	Fear	.114	.045	2.505	.012	
Preference	<-->	Risk	.188	.047	4.050	.000	
Awareness	<-->	Fear	.195	.077	2.528	.011	
Awareness	<-->	Risk	.214	.076	2.825	.005	
Fear	<-->	Risk	.655	.083	7.877	.000	

Table 9. Reliability and Generalisability.

Factor estimate	CR	AVE	MSV
Awareness	0.8	0.6	0.5
Preference	0.8	0.5	0.1
Fear	0.9	0.7	0.0
Injury risk	0.68	0.5	0.4
Normal criteria	0.700	0.500	<AVE

Note: CR= composite reliability, AVE is average variance extracted and MSV is Maximum shared squared variance.

demographic it has delay the use of NFI in routine in the hospital and self-administration also. A proper awareness program has to be done at Hospital and PHC to make people and healthcare professional aware about it, as it has direct impact on adherence of treatment of patient, mass immunization like program and ultimately health of patient. The cost of NFI is high hence some research needs to be done in that field again.

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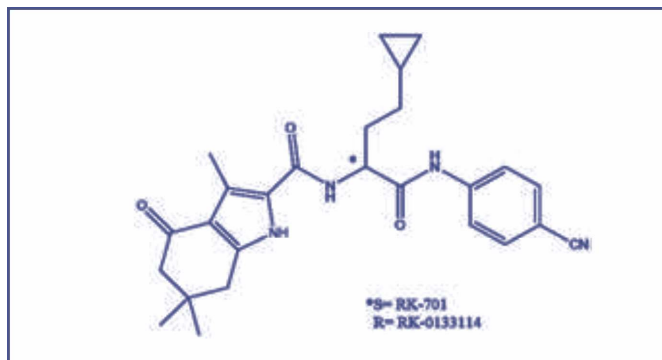
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CRIPS Digest

A specific G9a inhibitor unveils BGLT3 lncRNA as a universal mediator of chemically induced fetal globin gene expression

Mutations in the β -globin gene results in the heritable illness known as sickle cell disease (SCD). Fetal γ -globin induction is a recognised therapeutic approach. Epigenetic modulators, such as G9a inhibitors, have recently been suggested as potential therapeutics. The molecular processes via which these little molecules revive γ -globin are still unknown. In this research, it is reported that RK701 is a highly selective and non-genotoxic G9a inhibitor.



In this study an enantiomer is also reported along with the molecule RK-701 which is RK-0133114 and it shows 100-fold weaker inhibition as compared to RK-701. The Caco-2 permeability assay and the parallel artificial membrane permeability assay (PAMPA) both showed that RK-701 had reasonable cell permeability. HUDEP-2 cells and primary human CD34+ hematopoietic cells were taken for induction of the fetal globin by treating with RK-701 and the activity was observed as enhanced percentage of cells producing HbF in a concentration-dependent manner and raised the RNA level of γ -globin but not β -globin (observed in case of HUDEP-2 cells).

Treatment with RK-701 causes the expression of foetal globin in mouse and human erythroid cells. It was discovered that BGLT3 long non-coding(lnc) RNA located inside g-globin gene locus is upregulated selectively by RK-70 for the induction of γ -globin. By preventing the binding of two important γ -globin repressors in conjunction with G9a to the BGLT3 gene locus via CHD4, a member of the NuRD complex, RK-701 specifically upregulates BGLT3. Surprisingly, BGLT3 is required for the induction of γ -globin by inducers such as hydroxyurea, RK-701, and other inducers. The fact that BGLT3 is involved in γ -globin

induction everywhere suggests that treating Sickle Cell Disease (SCD) should be a priority (Nature Communications (2023), 14:1-8).

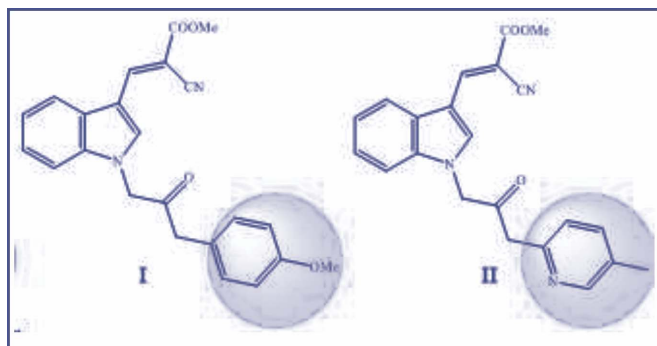
Indole Derivatives as New Structural Class of Potent and Antiproliferative Inhibitors of Monocarboxylate Transporter 1(MCT1; SLC16A1)

Cancer is one of the leading causes of death as multiple drug resistance (MDR) is developed by continuous adaptations in cancer cells for their survival; hence, cells keep renovating their metabolic pathway and mechanism of various transporter through mutations thereby most first-line and second-line treatment becomes ineffective. Thus, researchers are inclined more towards a novel target to win the battle against cancerous cells.

Monocarboxylate 1 (MCT1) is a novel drug target significant against cancer cells, inhibition of this target prevents the supply of lactate and pyruvate uptake by anaerobic and aerobic cells which ultimately results in the scarcity of glucose and causes apoptosis. There are limited MCT-1 inhibitors reported based on indole or indole-like scaffolds which include syrosingopine, lonidamine, and many more. Puri et al., synthesized a 16-indole-based active compound which has a significant IC_{50} value than the previously reported compounds.

All 16 indole-based compound shows inhibition of MCT-1 transporter in the sub-micromolar range, compound I with IC_{50} value of 81.0 nM was the most active amongst the series and compound II with IC_{50} value of 82.0 nM second most active as well as compared to the previously reported compound which has IC_{50} value 87.0 nM.

The antiproliferative activity of all 16 compounds was tested against MCT1 Expressing Cancer Cell



Lines and Cancer Cell viability was checked by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Also, the Cell Cycle Distribution Analysis, Apoptotic assay Inhibitory Activity against Multidrug Transporters ABCB, ABCC1, and ABCG2 of compound I and it was found that all compounds exhibit cytotoxic activity.

Computational analysis showed the most potent lead compound I exhibited very low free binding energy of -9.5 kcal mol⁻¹ and made hydrogen bonds with Arg313, Ser154, and Lys38, along with one carbon-hydrogen bond with Ser371. Also, it exhibits two electrostatic π -cation interactions with Lys38 and hydrophobic interactions, with Leu66, Pro406, Pro37, and Val282.

The physicochemical properties of all compounds are determined by SwissADME software from which it is concluded that chiefly drug likeliness of all compounds according to Lipinski's rule of five. All compounds are superior to previously discovered compounds and possess the potential to serve as novel anticancer agents which can inhibit multiple cancer cell metabolism pathways. (J. Med. Chem. (2023), 66, 657-676)

The role of Pyrazolo[3,4-d]pyridazinone Derivatives as a selective DDR1 inhibitor for inflammatory bowel syndrome therapy

Discovery of pyrazolo [3, 4-d] pyridazinone derivatives as selective DDR1 inhibitors via deep learning based design, synthesis, and biological evaluation.

Discoidin domain receptor 1 (DDR1) is a crucial member of the class of transmembrane receptor tyrosine kinases (RTKs) which exhibits a crucial role in several cellular processes includes cellular proliferation, migration, proliferation and invasion. The activation of DDR1 is associated with the generation of various kind of cytokines like IL and TNF and generates significant impact upon inflammatory disorders. A few inhibitors of Discoidin domain receptor 1 (DDR1) have been reported but most of those have specific disadvantages like poor target specificity. Therefore, a three-step scaffold-based molecular design process is used to create a highly selective antagonist of the Discoidin domain receptor 1 (DDR1). This three-step process is based upon the matched molecular pairs (MMP) algorithm to create a large library of fragments with different combinations of functional groups. Formation of scaffold-based virtual molecular library was carried out using chEMBL database, kinase activity virtual profiling was carried out by using KinomeX tool and molecular docking screening was carried out through the Glide program within the Schrodinger software.

A new series of new pyrazolo[3,4-d]pyridazinone derivatives were generated using a machine learning based activity score and through the process of virtual screening. Kinase activity virtual profiling was carried out using KinomeX tool. A total of 16 different types of kinases have been generated by this tool. In comparison to other kinases, the produced compounds displayed a greater than expected active probability towards DDR1. Tree maps (TMAPs) were plotted to generate a 2D layout of a minimum spanning tree that clusters molecules according to resemblance. This allowed us to see how structure similarity and docking scores relate to one another. Molecular docking result showed the pyridazine moiety of the lead compound (DC1) forms three hydrogen bonds with the residue Met704 and Asp702 in the hinge region of DDR1 receptor. In the c-Helix and the DFG motif, respectively, Glu672 and Asp784 of the linker amide created two hydrogen bonds. The N-Phenyl substituted portion of the lead molecule reached the solvent exposed area of DDR1. The modified benzofuran ring fills the hydrophobic pocket. The phenyl ring is modified to show prominent interactions with the allosteric site of the DDR1 receptor. On the basis of the docking score top 2 compounds were synthesized and bioactivity experiments were carried out.

With IC₅₀ values of 1.2 nM and 1.9 nM, respectively, the top 2 compounds showed strong inhibitory action against DDR1. A scaffold-based molecular design workflow was designed for finding potential promising drug candidates for DDR1 inhibition. Through this approach two compounds were discovered that exhibited potent inhibitory activity against DDR1 and inhibits the expression of pro-inflammatory cytokines and DDR1 autophosphorylation in cells. (J. Med. Chem. (2021), 65, 103-119)

Discovery, drug action and future aspects of MK-8189, a Potential and Selective inhibitor of PDE10A against Schizophrenia

MK-8189 is a highly potent selective PDE10A inhibitor which is under phase 2b clinical studies (NCT04624243). It selectively inhibits PDE10A one of the phosphodiesterase enzymes which hydrolyses secondary messengers cAMP and cGMP involved in the neuronal signaling in striatum. Excessive expression of PDE10A results in abnormal striatal output which is strongly associated with schizophrenia pathophysiology. Thus, inhibition of striatal PDE10A can improve neuronal signaling, cure positive symptoms and improve cognition.

Fragment screening identified 4,6-dichloro-2-cyclopropyl-5-methylpyrimidine molecule as potential inhibitor of PDE10A having good efficiency of ligand

binding (LBE=0.57). Structure of PDE10A bound to inhibitor obtained by X-ray crystallography and rational design aided with parallel library synthesis resulted in molecules with PDE10A inhibitory activities at picomolar concentrations but suffered from weak pharmacokinetic profile including poor oral bioavailability, high unbound clearance, poor aqueous solubility, off target ion channel activity against hERG, reversible inhibition of CYP2C9 and CYP3A4 and activation of CYP3A4PXR. Rigorous derivatization and optimization at both the side chains as well as core portion resulted in MK-8189, which has pyrimidine core, 2(5-methylpyridin-2-yl)-cyclopropylmethoxy as side chain 1 and (5-methyl-1,3,4-thiadiazol-2-yl) methyl amino as side chain 2. It has 8 times better PDE10A inhibitory potency, moderately improved solubility at pH 7, reduced CP3A4 PXR activation, CYP2C9 and CYP3A4 inhibition and rat unbound clearance.

X-ray crystallography structure of catalytic domain (residues 439 to 779) of PDE10A in complex with MK-8189 having resolution of 2.1 Å (PDBID: 8DI4) was used to study molecular interactions. MK-8189 interacts via multiple interactions with the crucial residues at the active site of the target enzyme PDE10A. The 5-methylpyridine ring of chain A forms hydrogen bond interaction with the Tyr683 in selectivity domain. The 2-methylpyrimidine core forms π - π stacking interactions with benzyl side chain of Phe719, whereas, N1 nitrogen forms hydrogen bond with Gln716 and N3 nitrogen along with 4-amino linker forms water mediated hydrogen bonds

with sidechain residues respectively Ser667 and Tyr514. The pyrimidine core's N3 nitrogen also forms auxiliary interactions with the binding site of PDE10A.

The small size (MW=382) and significant lipid solubility (LogD=2.1) of MK-8189 results in better efficiency of ligand binding (LBE = 0.54) and ligand lipophilicity efficiency (LLE=7.8). The pharmaceutical profile is sufficiently high throughput solubility at pH 7 (167 mM) crystalline free base solubility in simulated intestinal fluid (0.17 mg/mL) and in acidic simulated gastric fluid (5mg/mL) is excellent as well. MK-8189 has significant plasma clearance and smaller volume of distribution leading to a half-life of 4.8 h in rats and 4.2 h in rhesus monkeys. The oral bioavailability in rats was 46% and in monkeys 41%. It showed significant plasma protein binding in rat, monkey as well as human plasma with average unbound fraction in plasma of 8.2% in rat, 8.7% in monkey and 4.0% in human plasma. At the same time, MK-8189 does not potentially inhibit the CYP3A4 and CYP2C9 and in the PXR assay it was not active ($EC_{50} > 30$ mM). MK-8189 has excellent off target profile. It exhibits excellent profile against ion channels (Iks, Cav1.2 and Nav 1.5 >30 mM and functional hERG Ikr $IC_{50} = 33$ mM). MK-8189 has high passive permeability (35.4 to 42.6 X 10⁻⁶ cm/s) and is not substrate for human and monkey P-gp (B-A/A-B ratio <2). The physicochemical and pharmacological profile, pharmacokinetics, high selectivity and interaction with PDE10A makes the MK8189 a potential therapeutic for schizophrenia acting as highly selective PDE10A inhibitor (Layton et al. J. Med. Chem. (2023)).

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