

Synthesis, characterization and analysis of physico-chemical properties of 1H-indol-5-yl-[4-(2-phenoxyethyl)piperazin-1-yl]methanone

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UNIVERSITY OF ZAGREB
FACULTY OF CHEMICAL ENGINEERING AND TECHNOLOGY
UNIVERSITY UNDERGRADUATE STUDY

Ian Horvat

UNDERGRADUATE THESIS

Zagreb, September 2022.

SVEUČILIŠTE U ZAGREBU
FAKULTET KEMIJSKOG INŽENJERSTVA I TEHNOLOGIJE
SVEUČILIŠNI PREDDIPLOMSKI STUDIJ

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SYNTHESIS, CHARACTERIZATION AND ANALYSIS OF PHYSICO-
CHEMICAL PROPERTIES OF 1H-INDOL-5-YL(4-(2-
PHENOXYETHYL)PIPERAZINE-1-YL)METHANONE

UNDERGRADUATE THESIS

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Zagreb, September 2022.

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SINTEZA, KARAKTERIZACIJA I ANALIZA FIZIKALNO-KEMIJSKIH
SVOJSTAVA 1H-INDOL-5-IL-[4-(2-FENOKSIETIL)PIPERAZIN-1-
IL]METANONA

ZAVRŠNI RAD

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Abstract

Synthesis, characterization and analysis of physico-chemical properties of 1H-indol-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone

With the aim to prepare 1H-indol-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone three synthetic steps were performed. First step in synthesis included making a good leaving group from commercially available alcohol by nucleophilic substitution with mesyl-chloride. Obtained sulfonate ester was further substituted with piperazine using triethylamine as base. In the final step of synthesis, obtained intermediate reacted with indole-5-carboxylic acid accordingly to the Steglich mechanism of amidation using DMAP as the catalyst. During the isolation of compounds, based on the prediction of pKa values, yields of intermediate products as well as the final compound were optimized. In addition, alternative synthesis was proposed with the aim to increase overall yield due to possible application for the scale-up synthesis. Furthermore, analysis of the physico-chemical and ADME (absorption, distribution, metabolism and excretion) properties of target molecule were performed using ACDLabs/Percepta software, with aim of profiling the compound as a potential drug according to Lipinski's Ro5 rules. Performed synthetic route was not described in the literature, nor is the final compound. Synthesis of analogues showing activity in *in vitro* assay on Kv1.5 potassium ion channel was described in the literature, which is validated biological target in the treatment of arrhythmia. With regards to this result, 1H-indol-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone is potentially interesting compound for further *in vitro* profiling of Kv1.5 inhibition.

Keywords: piperazine, nucleophilic substitution, Steglich amidation, ADME prediction, Kv1.5 inhibition

Sažetak

Sinteza, karakterizacija i analiza fizikalno-kemijskih svojstava 1H-indol-5-il-(4-(2-fenoksietil)piperazin-1-il) metanona

U cilju pripreme 1H-indol-5-il-[4-(2-fenoksietil) piperazin-1-il] metanona provedena je sinteza u tri stupnja. Prvi korak u sintezi je prevođenje komercijalno dostupnog alkohola u dobru izlaznu skupinu reakcijom nukleofilne supstitucije sa mesil-kloridom, potom slijedi supstitucija dobivenog sulfonatnog estera sa piperazinom uz prisustvo trietilamina kao baze. Dobiveni produkt u 3. sintetskom stupnju reagira sa indol-5-karboksilnom kiselinom prema Steglich mehanizmu amidacije uz DMAP kao katalizator. Prilikom izolacije spojeva, na osnovu predikcije pKa vrijednosti optimirana su iskorištenja međuprodukata kao i finalnog spoja, te predložena alternativna sinteza u svrhu većih ukupnih iskorištenja, te primjene u sintezi na uvećanoj skali. Nadalje, provedena je analiza fizikalno-kemijskih i ADME (apsorpcija, distribucija, metabolizam i izlučivanje) svojstava dobivene ciljne molekule primjenom ACDLabs/Percepta Software-a, a u smislu profiliranja spoja kao potencijalnog lijeka prema Ro5 pravilima Lipinskog. Provedeni sintetski put nije literaturno opisan kao niti finalni spoj, no literaturno je opisana sinteza analoga koji pokazuju aktivnost prilikom *in vitro* testiranja na Kv1.5 kalijev ionski kanal, koji je validirana biološka meta u tretmanu aritmije. U tom smislu 1H-indol-5-il-(4-(2-fenoksietil)piperazin-1-il)metanon je potencijalno zanimljiv spoj u daljnjem *in vitro* profiliranju inhibicije Kv1.5.

Ključne riječi: piperazin, nukleofilna supstitucija, Steglich amidacija, ADME predikcija, inhibicija Kv1.5

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1. INTRODUCTION

Stroke is the leading cause of death and disability worldwide [1]. Atrial fibrillation (AF) is the most common cardiac arrhythmia, afflicting 13% of men and 11% of women over 85 years of age and confers a substantial risk of stroke [2]. In patients with AF, the upper and the lower chambers are not coordinated, causing the heart to beat too slowly, too quickly or irregularly. The normal beating in the upper chambers of the heart (the two atria) is irregular and blood doesn't flow as well as it should from the atria to the lower chambers of the heart (the two ventricles). AF may happen in brief episodes, or it may be a permanent condition [3]. Antiarrhythmic drugs have been used for nearly 100 years in the treatment of AF in the clinical trials till now, but they are limited in clinical use because of the production of negative feedback on the ventricular repolarizations and can be potentially life threatening [4]. For example, amiodarone, the available agent that is most effective, causes serious adverse effects [5]. Therefore, novel atrial-selective drugs with improved safety and efficacy are urgently required [6].

K^+ channels play a critical role in cardiac electrophysiology, and their dysfunction is linked with many cardiovascular diseases [7]. Numerous K^+ channel types are expressed in the sarcolemmal membrane of the heart, where they exert precise control over action potential (AP) duration (APD) [8]. The upstroke of the AP is caused by inward sodium current which is followed by a partial early repolarization caused by outward potassium flux through rapidly activating and inactivating K^+ channels. The extent of this early repolarization affects the time course of the other voltage-gated currents and, therefore, indirectly controls APD [9]. Each K^+ channel type has distinct kinetics and regulation, allowing control over particular phases of the AP. Thus, modulation of these channels can profoundly affect physiological processes including neuronal integration, vesicle secretion, and muscle contraction [10]. Recently, voltage-gated potassium channel 1.5 ($Kv1.5$) has emerged as a putative atrial-selective target, as it arbitrates the ultra-rapid delayed rectifier current (I_{Kur}) and works atrial-specifically [11]. Therefore, selective $Kv1.5$ blockers are thought to represent a safer pharmacologic intervention strategy for AF drug administration and the prevention of recurrence of AF when compared with existing antiarrhythmic agents. In addition to antiarrhythmic activity, $Kv1.5$ blockers may also improve atrial contractility and reduce thromboembolic risk [12]. Blockade of the transient outward current (I_{To}) and I_{Kur} prolongs APD exclusively in the atria and might thereby enhance atrial contractility without adverse effects on the ventricle.

Herein we present design, synthesis and evaluation of physico-chemical properties of 1H-indol-5-yl(4-(2-phenoxyethyl)piperazin-1-yl)methanone. With the aim to discover novel Kv1.5 potassium channel inhibitors in design of the target molecule we considered key common segment as alkoxy chain including two carbon present in the drugs now on the market (amiodarone, dronedarone and vernakalant). Furthermore, saturated 6-membered N containing ring such as piperazine was introduced based on the improved *in vitro* potency by whole cell patch clamp technique described in the literature [9] as well as indole scaffold. Structures of intermediates as well as final compound were determined by spectroscopic techniques (1D and 2D NMR and MS).

2. GENERAL

2.1. Ion channels

Ion channels are membrane-spanning or transmembrane proteins with three essential functional properties: a central tunnel or pore in the cell membrane through which ions flow down their electrochemical gradient; a selectivity filter that dictates which ions are allowed to cross the pore; and a gating structure that controls switching between open and closed conformations and thus determines whether permeation occurs. These proteins are more efficient than enzymes since a single conformational change allows permeation of up to 10^8 ions/s. Ion channels may be gated by electrical, chemical or mechanical forces which are detected by a sensor that is linked to the access gate. The behaviour of ion channels can be modified by intracellular processes, toxins or drugs. The latter makes them potential molecular targets for therapeutic drugs [10].

2.2. Voltage-gated ion channels

The gating mechanism of ions can be of many types, such as voltage, stretch, or temperature. Voltage-gated ion channels (VGICs) (Fig.1) form one of the largest groups. These ion channels are involved in a great variety of cellular functions, such as generation of action potentials (AP) in excitable cells or activation in numerous non-excitabile cell types, such as lymphocytes and tumour cells. VGICs typically consist of four subunits (potassium channels) or four domains (calcium and sodium channels), each of which is made up of six transmembrane helices. The first four helices together (S1–S4) are called the voltage-sensing domain (VSD), while the rest (S5–S6) build up the pore [13]. The voltage sensing response mostly comes from the movement of S4, which has net positive electric charge, originating from positively charged amino acids (arginines and lysines). In response to a membrane potential change, these proteins open their ion-selective pores through which ions move passively across the membrane, driven by the electro-chemical potential difference [14,15].

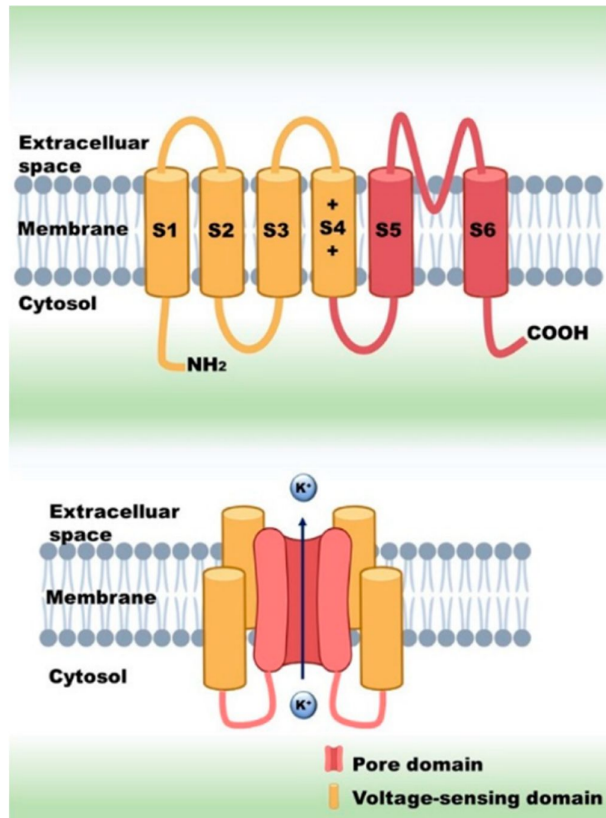


Fig. 1. Structure of voltage-gated potassium channels [21]

Among VGICs, the Kv form a large family with some 40 members [16]. They are highly selective for potassium ions over other cations and expressed in almost all cell types, including muscle cells, neurons and immune cells, playing active roles in a variety of cellular functions. Kv channels provide the outward cation currents required to terminate the AP in excitable cells and allow the membrane potential to return to a negative resting potential following an AP. Several Kv channels contribute to shaping of the AP in the heart. The currents produced by these channels in cardiomyocytes include: the transient outward potassium current (I_{TO}) and the delayed rectifier potassium channel currents (I_K), which are named based on the speed at which they activate: slowly activating (I_{Ks}), rapidly activating (I_{Kr}) and ultra-rapidly activating (I_{Kur}) [11].

2.3 Kv1.5 potassium channel

I_{Kur} is generated by the potassium current through the Kv1.5 channel, which is an outwardly rectifying and highly selective K^+ channel with a rapid and sigmoidal time course of activation [17]. I_{Kur} is present in human atrial myocytes but not in the human ventricle [18].

Many studies have concluded that inhibition of I_{Kur} could prolong the APD of AF patients [19], and by this, it can terminate the fibrillation, indicating that Kv1.5 is a potential target for AF therapy [20].

Kv1.5 potassium channel, which mediates the I_{Kur} current in human cells, has a significant role in AF in the repolarization of the atrial action potential [21]. Kv1.5 potassium channel is recognized as an ideal target because it is only recorded in the human atrial myocytes while is absent in the ventricular myocytes [13]. Its mutations have been linked to hereditary forms of AF, therefore it is of great importance to find the solution for this problem. The development of I_{Kur} blockers in order to treat AF resulted in small molecule Kv1.5 inhibitors. The selectivity of the blocker for the target channel plays an important role in the potential therapeutic application of the drug candidate: the higher the selectivity, the lower the risk of side effects [22]. Recent studies have demonstrated that, in general, atrial contractility and blood flow velocity can be significantly enhanced by direct positive inotropic stimulation in patients undergoing cardioversion of atrial flutter or AF [23]. However, the pharmacological compounds used in these studies are of questionable benefit in the clinical setting of AF because of their potential proarrhythmic activity. The present study shows that I_{TO}/I_{Kur} blockade by AVE-0118 blocker (provided that its efficiency can be proved in humans) might be a realistic therapeutic approach to increase atrial contractility without the cost of proarrhythmic side effects [13]. Although we could recently report a pronounced increase in active force development in atrial muscle bundles of patients with chronic AF in the presence of AVE-0118 [24]. However, it remains to be determined whether I_{TO}/I_{Kur} blockade effectively and safely restores atrial contraction in patients after cardioversion of AF.

Src is a non-receptor tyrosine kinase protein that in humans is encoded by the SRC gene and contains a number of domains, which Src homology 3 (SH3) is one of [19]. The human Kv1.5 potassium channel (hKv1.5) contains proline-rich sequences identical to those that bind to SH3 domains. Direct association of the Src tyrosine-kinase with cloned hKv1.5 and native hKv1.5 in human myocardium was observed. This interaction was mediated by the proline-rich motif of hKv1.5 and the SH3 domain of Src. Furthermore, hKv1.5 was tyrosine phosphorylated, and the channel current was suppressed. These results provide direct biochemical evidence for a signaling complex composed of a potassium channel and a protein tyrosine kinase. Potential consequences of a closely associated channel-kinase signaling complex include increased specificity of signaling pathways, faster coupling, and a higher probability of channel phosphorylation after kinase activation [25].

The most promising strategy to treat AF that avoids ventricular proarrhythmic side effects is the development of drugs known as “atrial selective drugs”. This concept would exploit distinct differences in expression patterns of individual ion channels and their different contribution to refractoriness between atrial and ventricular myocytes. Inhibition of the ion flow through Kv1.5, i.e., blocking I_{Kur} , eliminates a component of the repolarizing current during atrial AP, thus prolonging the APD. Almost all of the known Kv1.5 blockers are exclusively small molecules. Pharmaceutical companies have made great efforts to develop selective I_{Kur} blockers as new pharmacological agents against AF. As a result, many new I_{Kur} blockers have been developed and tested since the beginning of this century [22].

2.4. Class III antiarrhythmic drugs

Class 3 antiarrhythmics are drugs that block cardiac tissue K channels. The medications in this class include amiodarone, dronedarone, sotalol, ibutilide, dofetilide, and bretylium. The main mechanism of action includes blocking the cardiac K channels to prolong repolarization. However, some medications in this class also exert effects on Na and Ca channels, and adrenergic receptors. Indications vary among the medications, but include both atrial and ventricular arrhythmias. Because these medications prolong the QT interval, torsades de pointes are potential complication of therapy.

Considering the alkoxyl chain including two carbons is a common segment in the drugs such as amiodarone, dronedarone and vernakalant, in structure-based design we introduced this fragment into the structure of final molecule (Fig. 2), as well as piperazine ring present in the hit molecule **1** [9]. New structural modification includes thiophene replacement with fused heterocyclic ring indole. It appears that fused heterocyclic ring furane is tolerated in the structure of amiodarone and dronedarone. Our aim was to explore indole as furane replacement with possibility of an additional hydrogen bonding.

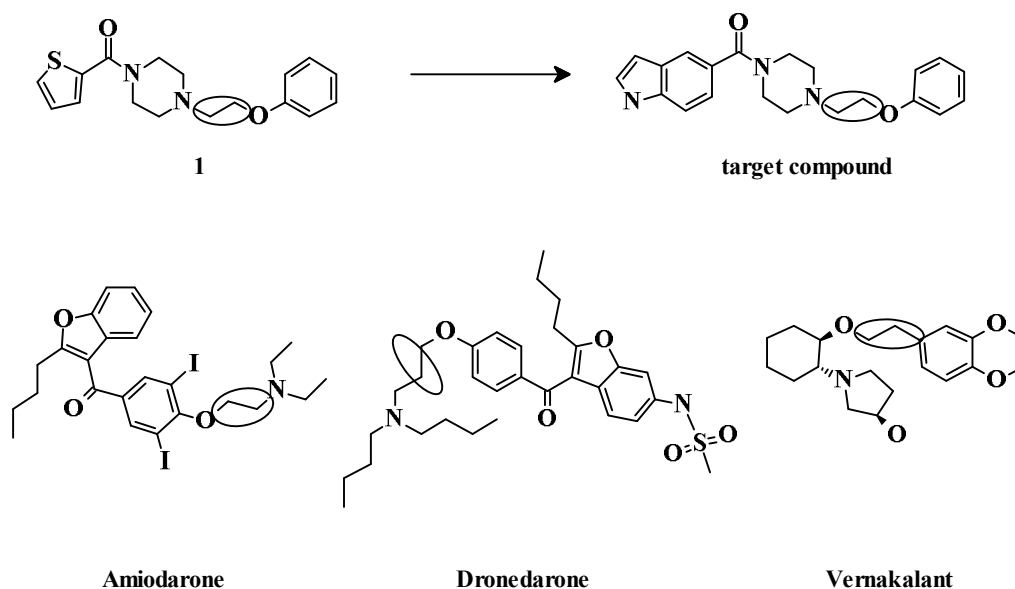


Fig. 2. Design of target compound with comparison of hit 1 and drugs on the market.

2.4.1. Piperazine moiety in drug design and discovery

Piperazine moiety is well studied heterocycle in drug design that has found gainful application as scaffold and terminal element for enhancing the aqueous solubility of a molecule due to two nitrogen atoms that benefit bioavailability, also with a high number of positive hits encountered in biological applications. Piperazine template deserves the molecular backbone as it possesses versatile binding properties and behaves as a potent and selective ligand for a range of different biological targets in medicinal chemistry. It can also be used as a replacement for other nucleophiles in order to positively influence the pharmacological profiles of the targeted molecule. In medicinal chemistry the main function of this framework is to provide a way to build a collection based on one primary design and screen it against a variety of different receptors. The piperazine scaffold has been regarded as a core and is frequently found in naturally active substances across a variety of different therapeutic drugs. Piperazine and its derivatives offer a wide range of biological activities including antimicrobial, antituberculosis, anticancer, antiviral and antimalarial activity [26].

Maintaining balance between pharmacodynamic and pharmacokinetic profiles of drug-like molecules is an important factor in designing and developing new drugs. Thus, one of the goals in drug discovery process is to design molecules with high affinity for its targets and appropriate physico-chemical properties. For this purpose, the characteristics of the piperazine template

make this molecular subunit useful and well-positioned system in the design of drugs. Our final molecule contains piperazine ring.

Furthermore, the optimization of drug candidates that incorporate these heterocycles in an effort to refine potency, selectivity and developability properties has also stimulated the design and evaluation of a wide range of bioisosters that can offer advantage (Fig. 3).

More than 100 FDA-approved drugs contain the piperazine moiety [27]. Piperazine-based analogues may advantageously alter important pharmacokinetic properties when grafted onto molecular scaffolds [28]. In 2018, chemists showed that replacing a piperazine ring in the drug Olaparib with the spirodiamine analogue beneficially affected activity and reduced cytotoxicity of the parent compound [29]. We have designed compound containing piperazine ring which, depending on *in vitro* biological results, could be further expanded to library compounds with bioisosteric replacement.

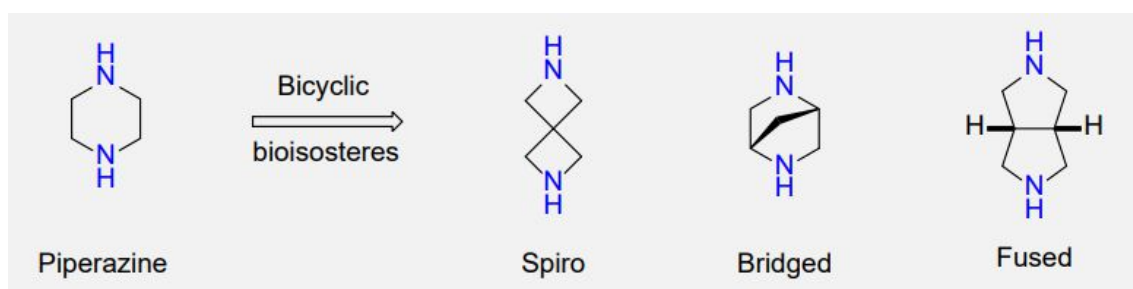


Fig. 3. Piperazine and bioisosteric replacement.

2.4.2. Indoles

Heterocycles are carbocyclic compounds, in which one or more carbon atoms of the ring are replaced by hetero atoms such as nitrogen, sulfur and oxygen. The study of the heterocycles occupies a prominent place in chemistry due to their wide range of applications in the fields of medicinal chemistry, material chemistry, photochemistry etc. [30]. In 2010, US retail sales reported that 80% of the drug molecules are containing heterocycles [31]. Functionalized nitrogen and sulfur containing heterocycles achieved a historical development in organic synthesis due to their high stability.

Indole (Fig. 4) is a planar bicyclic molecule in which the benzene ring is fused through 2 and 3 positions of N-containing pyrrole ring. Indole, having 10 π -electrons (8 from double bonds and 2 from lone pair of electrons on nitrogen) is said to be aromatic in nature according to Huckel's rule. Due to delocalization of excessive π -electrons, indole readily undergoes electrophilic

substitution reactions similar to benzene ring and are very reactive with strong acids due to weak basicity like pyrrole. Based on the molecular orbital calculations, 3-position of indole has the highest electron density and it is the most reactive position for electrophilic substitution reactions. Since the N-H bond in indole is slightly acidic, it undergoes N-substitution reactions under basic conditions. [32]

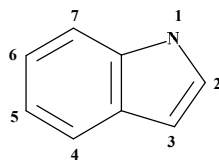


Fig. 4. Indole.

Indole is an important heterocyclic system because it is built into proteins in the form of amino acid tryptophan, also it is the basis of drugs like indomethacin and provides the skeleton of indole alkaloid-biologically active compounds from plants including strychnine and LSD. The incorporation of indole nucleus, a biologically accepted pharmacophore in medicinal compounds, has made it versatile heterocyclic possessing wide spectrum of biological activities specifically due to varying of the substituents at various positions of indole ring. We have designed target molecule with the aim of explore 5-position of indole ring in analogy to the 5-position of benzofuran ring in dronedarone (Fig. 2).

2.4.3. Lipinski's rule of five

Lipinski's rule of five (RO5) is a 'rule of thumb' to evaluate 'druglikeness' to determine if a chemical compound with a certain biological activity has chemical and physical properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most orally administered drugs are relatively small and moderately lipophilic molecules. [33]

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including absorption, distribution, metabolism and excretion (ADME). However the rule does not predict if a compound is pharmacologically active. Ro5 is important to keep in mind during drug discovery when a pharmacologically active lead structure is optimized stepwise to increase the activity and selectivity of the compound as well as to ensure drug-like physicochemical properties are maintained as described by Lipinski's rule. Candidate drugs that

conform to the RO5 (Fig. 5) tend to have lower attrition rates during clinical trials and hence have increased chance of reaching the market.

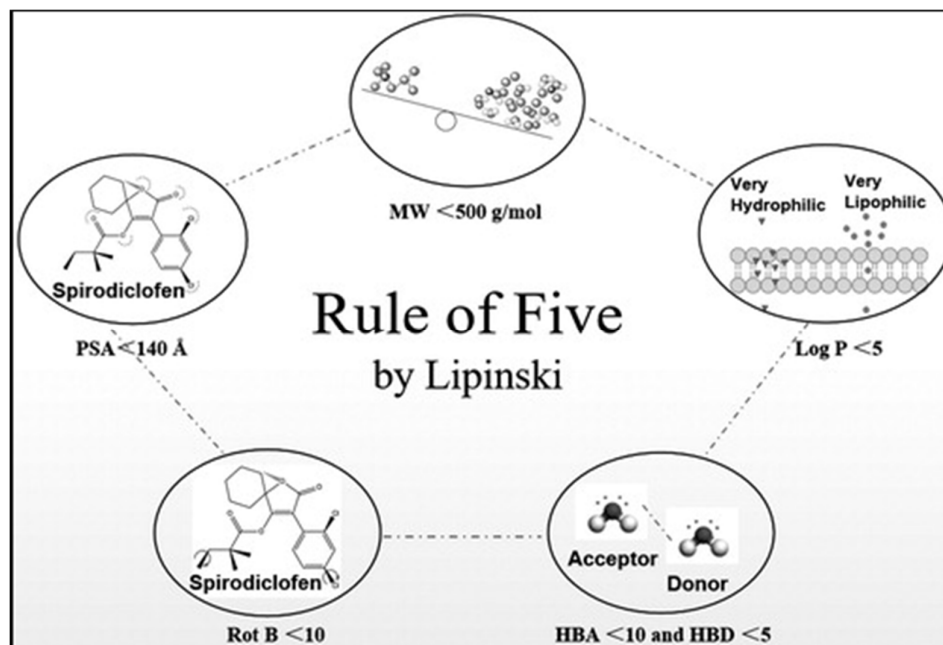


Fig. 5. Lipinski Ro5

About 50% of orally administered new chemical entities actually obey it. Studies have demonstrated that some natural products break the chemical rules used in Lipinski filter such as macrolides and peptides [34].

Prediction of drug-like properties of targeted molecule 1H-indol-5-yl(4-(2-phenoxyethyl)piperazin-1-yl)methanone according to the Ro5 will be further discussed.

3. RESULTS AND DISCUSSION

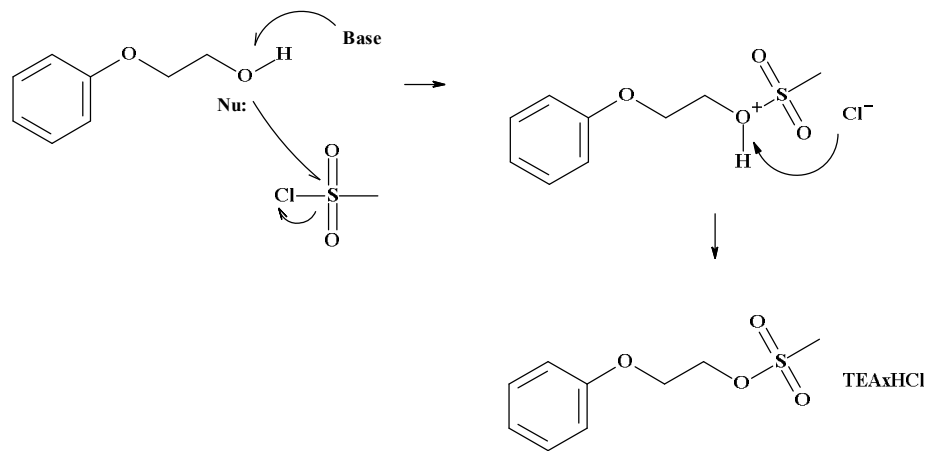
3.1. Introduction

With the aim to discover novel Kv1.5 potassium channel inhibitors, design, synthesis and evaluation of physico-chemical properties of 1H-indol-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone were performed. In the rational design target molecule contains key common segment as alkoxy chain including two carbons present in the drugs on the market (amiodarone, dronedarone and vernakalant). Furthermore, saturated 6-membered N containing ring such as piperazine was introduced based on the improved *in vitro* potency by whole cell patch clamp technique described in the literature [9] as well as indole scaffold substituted at 5-position in analogy to the 5-position of benzofuran ring in dronedarone.

Structures of intermediates as well as final compound were determined by spectroscopic techniques (1D and 2D NMR and MS), pKa values and drug-like properties were calculated by ACD Percepta software.

3.1.1. Synthesis of 1H-indol-5-yl(4-(2-phenoxyethyl)piperazin-1-yl)methanone

Target compound was synthesised in three steps of which the first synthetic step involved converting of primary alcohol 2-phenoxyethanol with mesyl-chloride using triethylamine (TEA) as a base, into a suitable sulfonate such as mesylate (OMs) as a good leaving group by S_N2 mechanism. Alcohols are poor substrates for substitution reactions since hydroxyl group is strong base. [35]



Scheme 1. Synthesis of intermediate and proposed mechanism of mesylation.

Reaction was performed in dry toluene with TEA as base. Reaction mixture and formation of the desired product were determined by UPLC-UV (TIC) chromatography (Fig. 6) at basic pH. Desired product was observed at $R_t= 0.94$ min, while toluene showed $R_t=1.15$ min. Desired product did not ionize by MS spectra, therefore UPLC-UV (TIC) spectra of starting alcohol was recorded under same method. As shown at (Fig. 7) $R_t= 0.77$ min was observed starting alcohol. At Fig 6. starting material has remained in traces, therefore reaction was stopped.

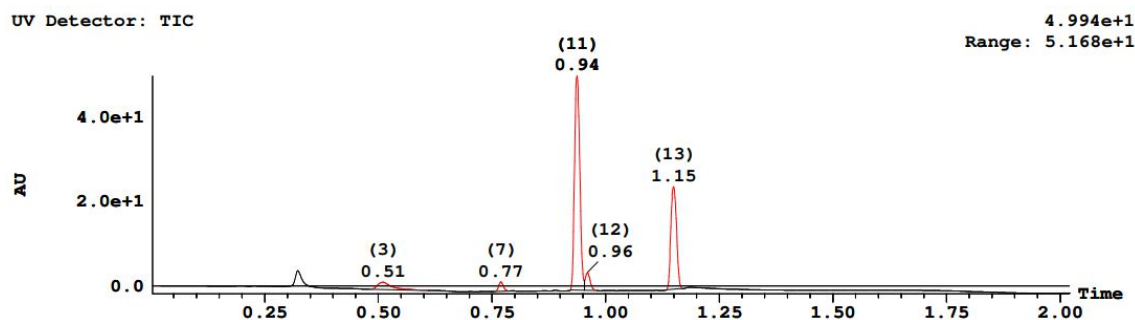


Fig. 6. UPLC-UV of reaction mixture of mesylation step.

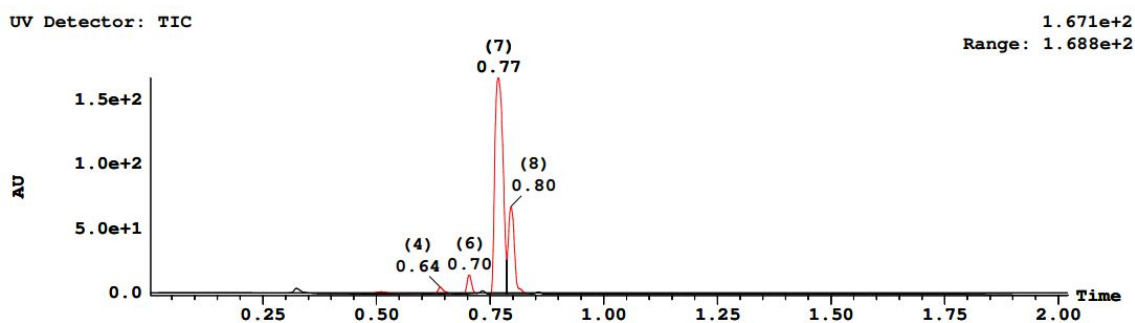


Fig. 7. UPLC-UV of starting alcohol.

After work-up and evaporation in vacuo, desired product was obtained in the high yield of 97% and used in the next step without additional purification. Purity of final 2-phenoxyethyl methanesulfonate was determined by UPLC-UV at 254 nm (Fig. 8) and structure confirmed by ^1H and COSY NMR.

NMR spectra (Fig. 9 & 10) showed correlation peaks of methylene protons in the linker, as well singlet of 3H at 3.09 ppm.

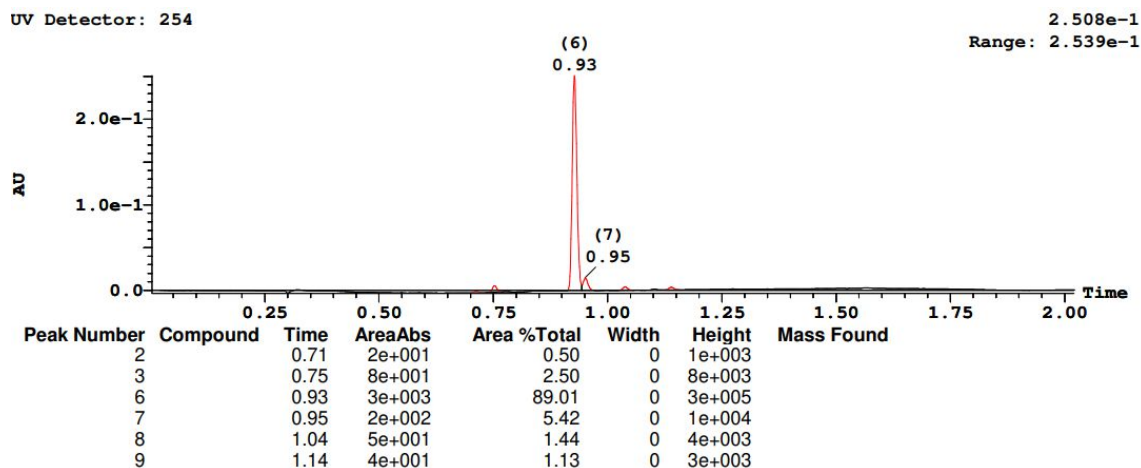


Fig. 8. UPLC-UV purity of 2-phenoxyethyl methanesulfonate.

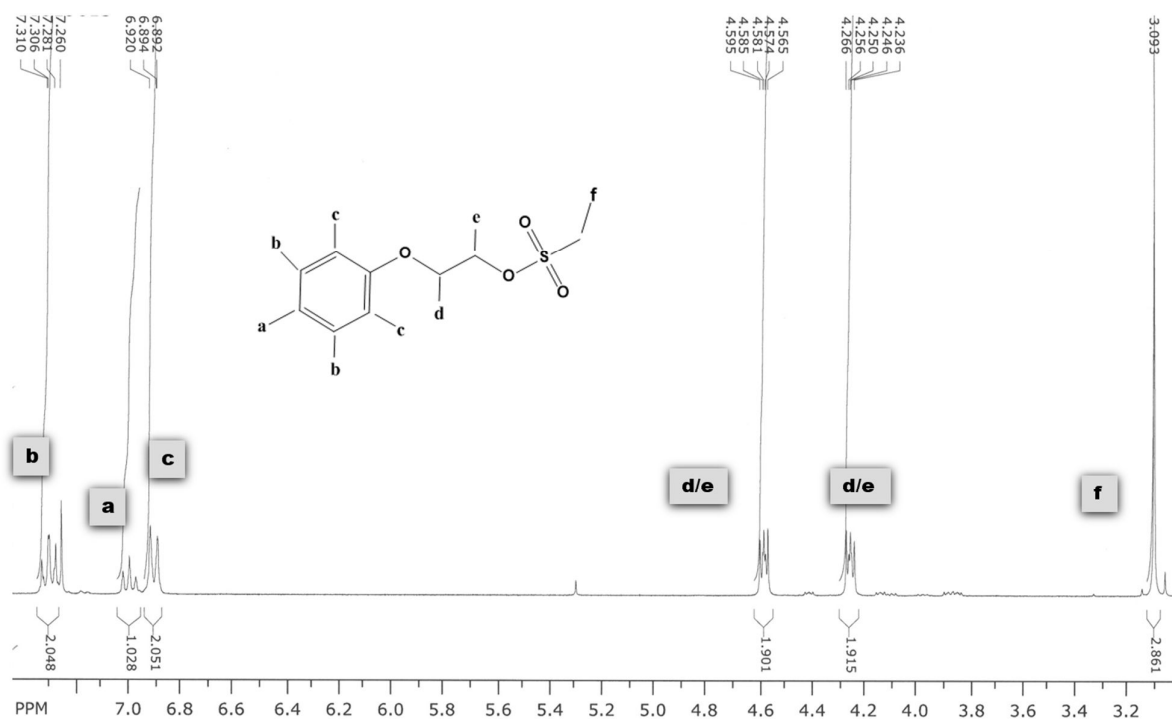


Fig. 9. ¹H NMR spectra of 2-phenoxyethyl methanesulfonate

Protons Hd and He form 2 similar triplets at 4.25 and 4.58 ppm with coupling constants of 9 Hz, while the phenyl part is visible in characteristic lower field with 2 triplets (Ha, 2Hb) with coupling constants of 7.5 Hz and 1 doublet (2Hc) with coupling constant of 8.1 Hz. Three mesyl hydrogens (Hf) make a singlet at characteristic low chemical shift 3.09 ppm.

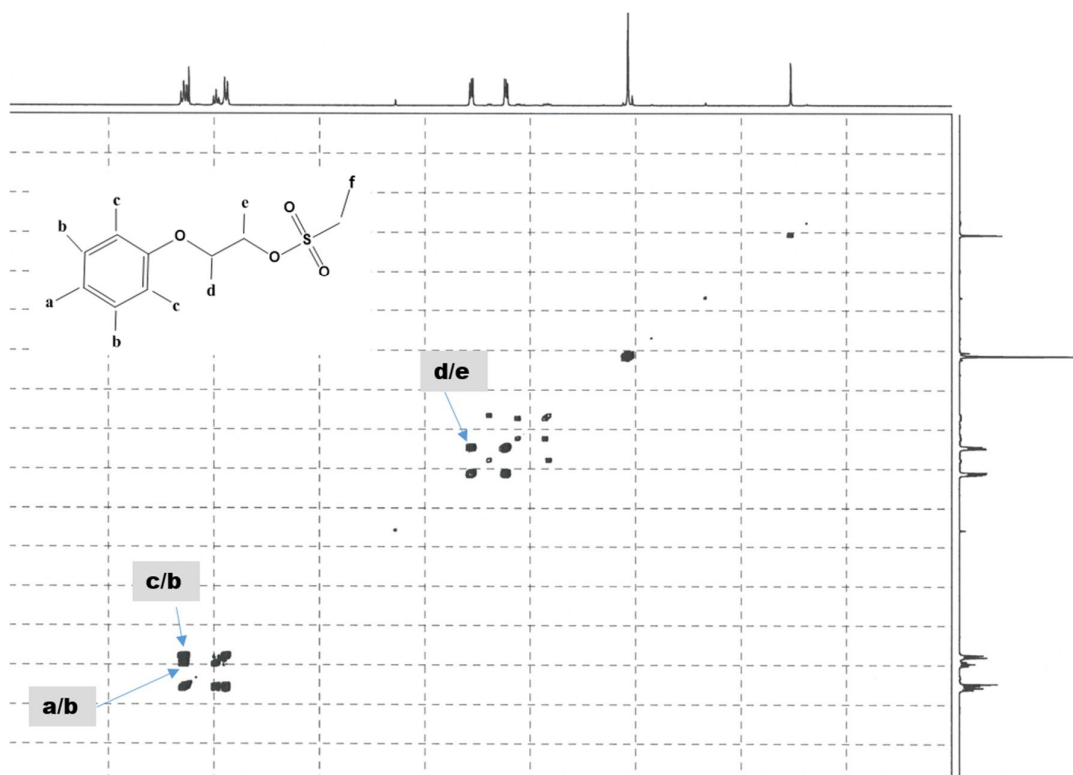
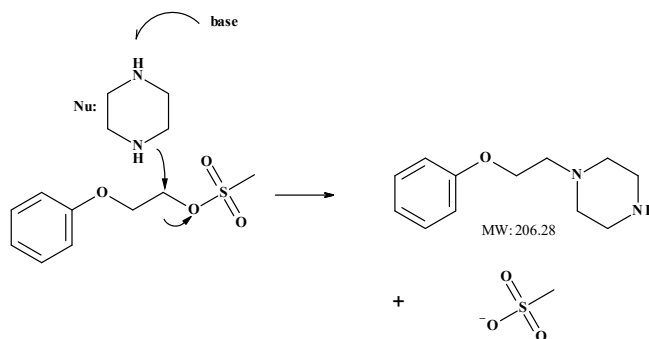


Fig. 10. COSY NMR spectra of 2-phenoxyethyl methanesulfonate

Previously obtained mesylate was substituted with piperazine in the 2. step of nucleophilic substitution using TEA as a base in DMF as solvent.



Scheme 2. Synthesis of intermediate and proposed mechanism of substitution of OMs with piperazine.

Reaction mixture and formation of the desired product was determined by UPLC-UV-MS chromatography (Fig. 11) at basic pH. Desired product was observed at $R_t=0.84$ min, unreacted mesylated alcohol remained at $R_t=0.95$ min and new byproduct was observed at 1.20 min (proposed structure of dimmer is on the Fig. 11). Due to formation of the byproduct reaction

was stopped after stirring at room temperature overnight. Desired product showed ionisation by MS detector (Fig. 12) at $R_t = 0.84$ min, $m/z = 207.18$ ($M+H$)⁺. Proposed dimmer showed ionisation by MS detector (Fig.13) at $R_t = 1.19$ min, $m/z = 327.22$ ($M+H$)⁺.

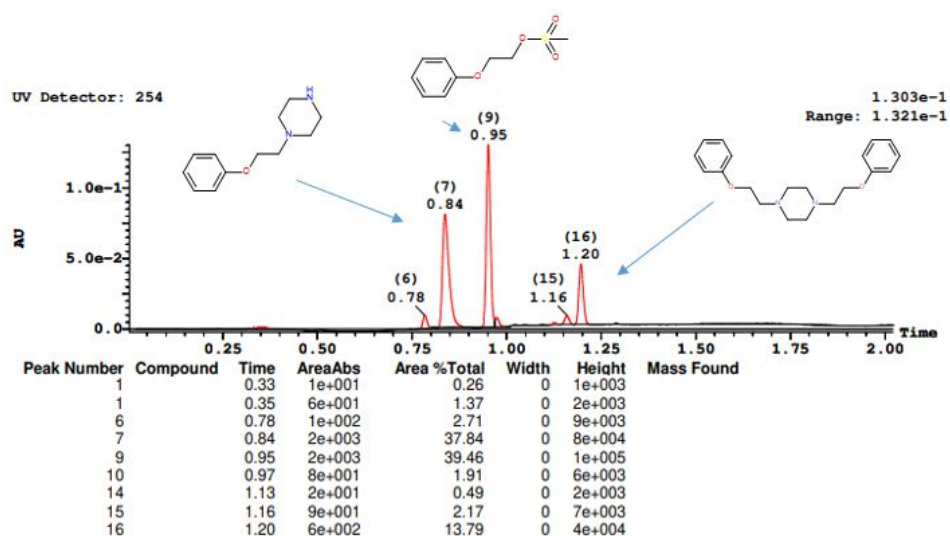


Fig. 11. UPLC-UV of reaction mixture nucleophilic substitution with piperazine.

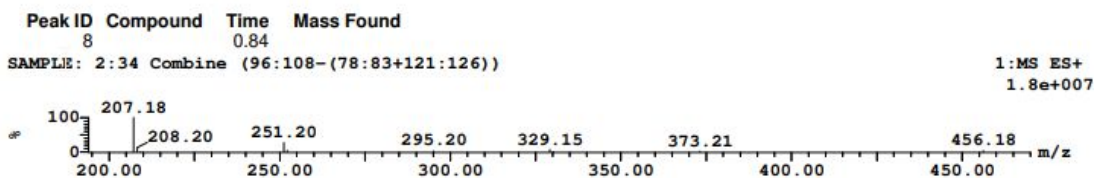


Fig. 12. MS spectra of desired product.

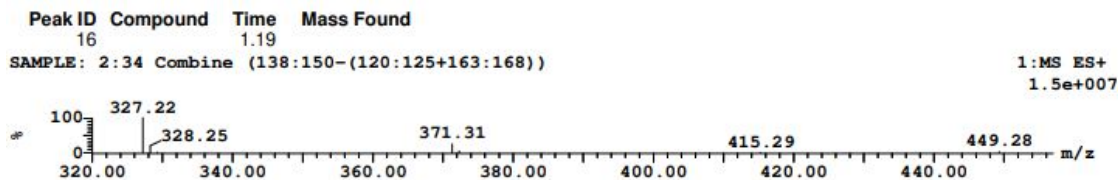


Fig. 13. MS spectra of by-product dimmer.

After work-up of the reaction mixture, desired product remained in the aqueous phase (pH measured 9.6). According to the Percepta software calculations (Fig 14.) pH should be adjusted > 11 in order to extract the desired product into organic phase.

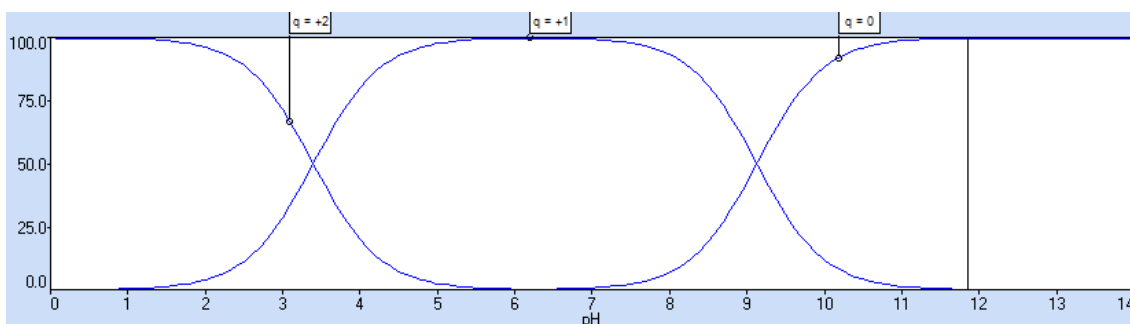


Fig. 14. Percepta calculations of charges distribution vs pH for 2-phenoxyethylpiperazine

By adjusting pH as calculated desired product was isolated, purity determined by UPLC-UV and mol mass by MS detector (Fig. 15.)

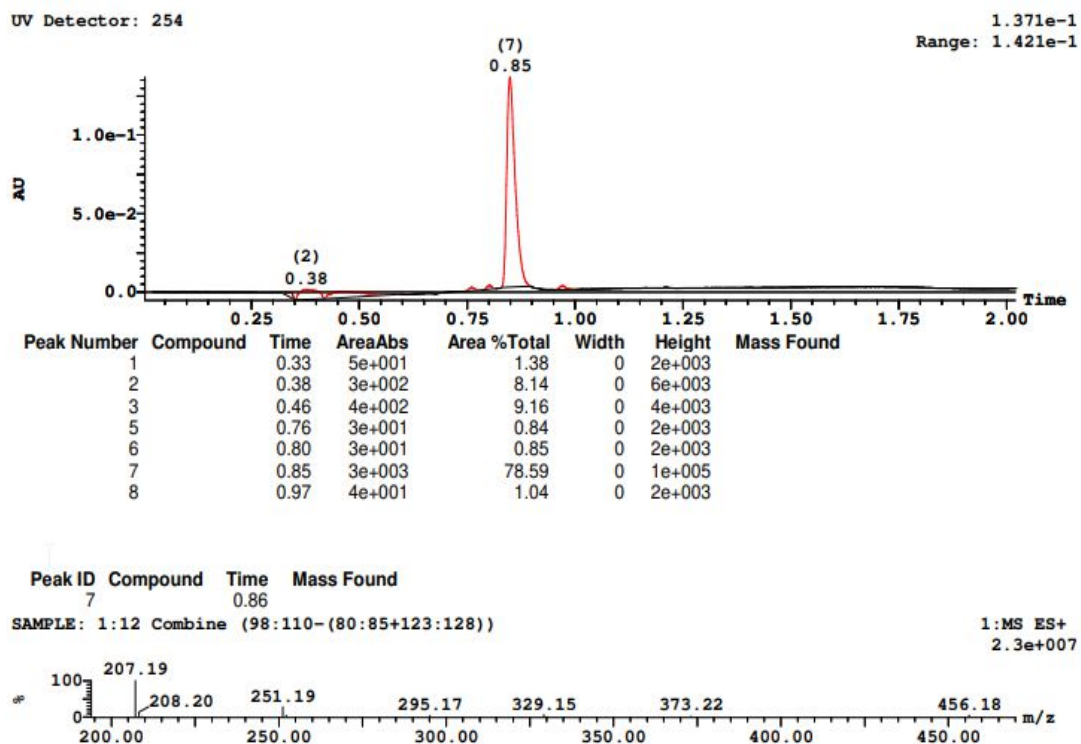


Fig. 15. UPLC-UV-MS of 2-phenoxyethylpiperazine

¹H NMR of desired product showed presence of residual DMF and piperazine. Nevertheless, product will be used in the next step without additional purification (Fig. 16).

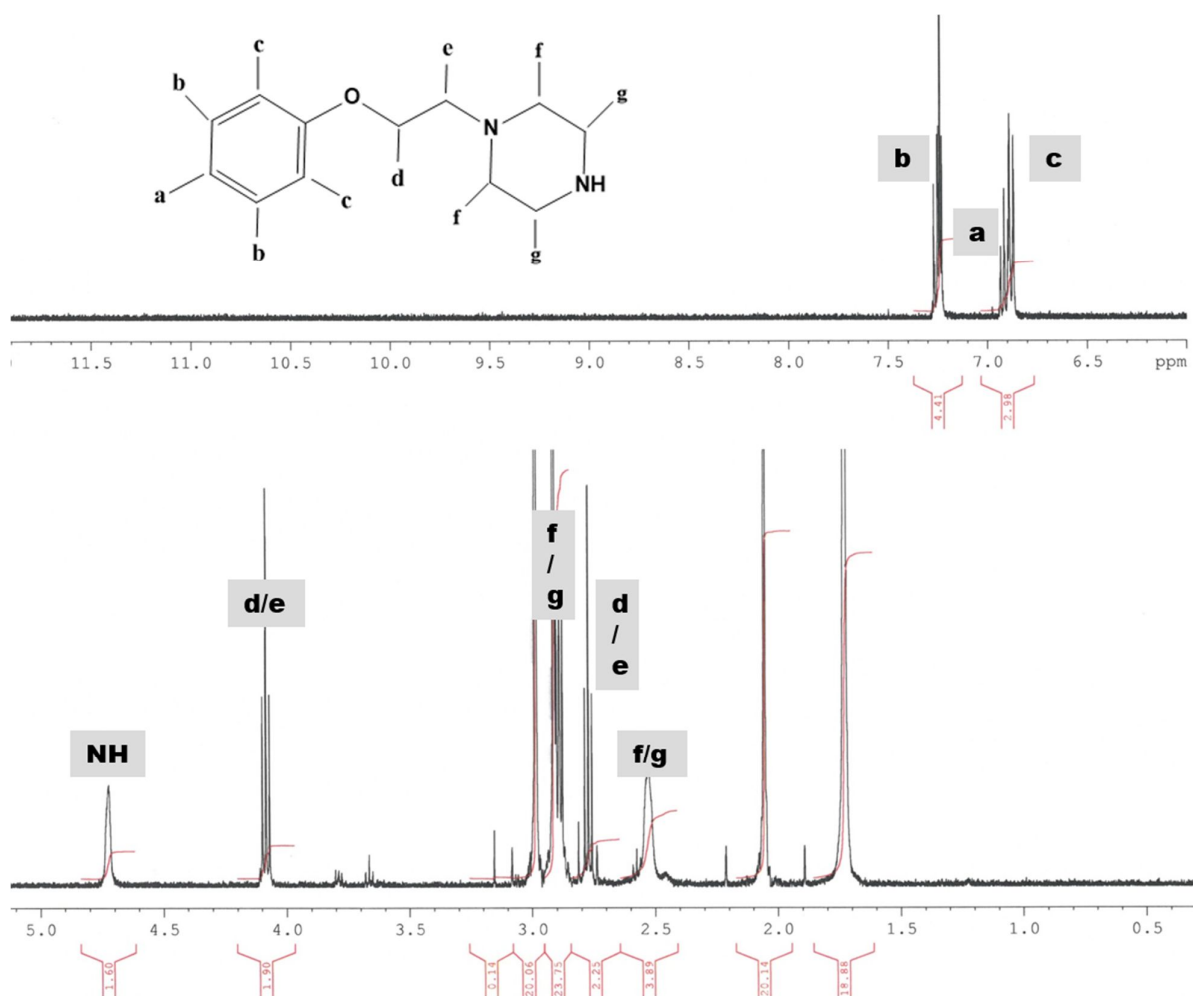
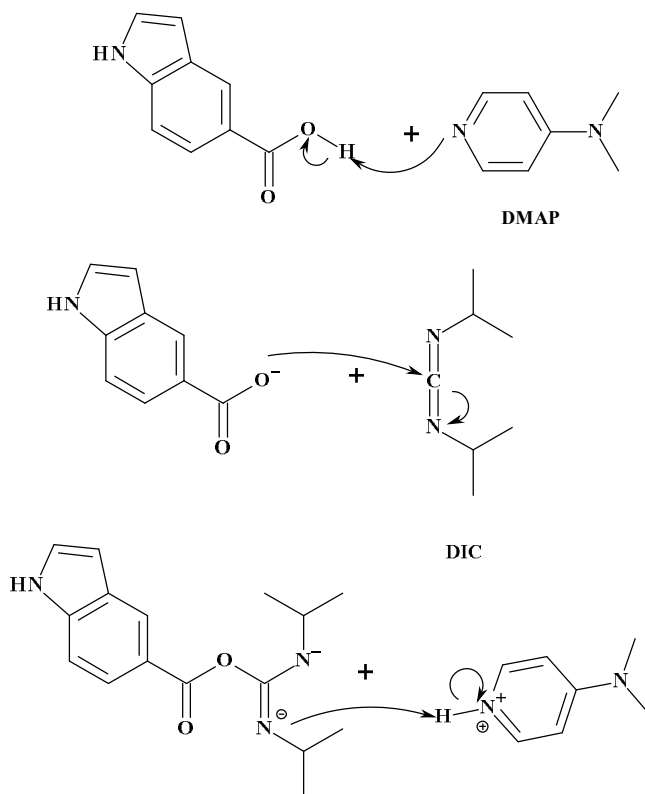


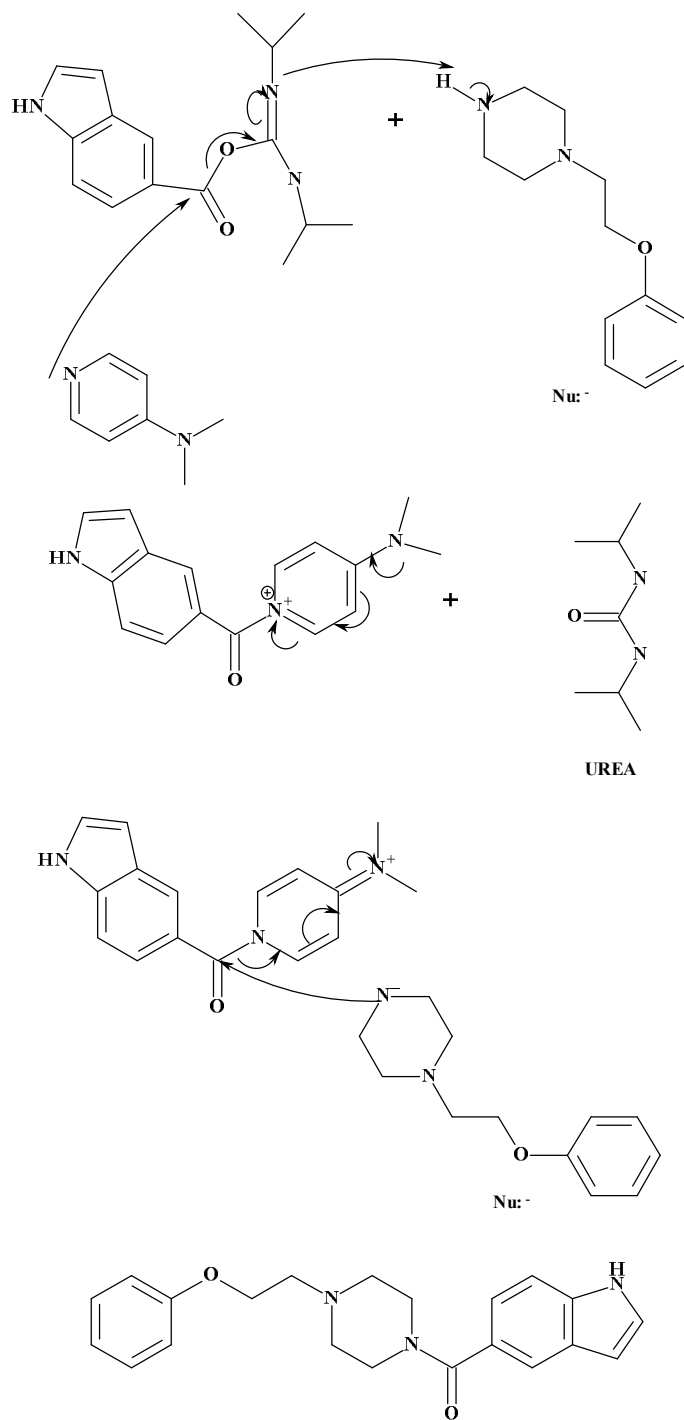
Fig. 16. ¹H NMR spectra of 2-phenoxyethylpiperazine

NMR spectra of 2-phenoxyethylpiperazine shows 5 phenyl hydrogens (Ha, 2Hb, 2Hc) at characteristic chemical shifts between 6.8 and 7.5 ppm, and two triplets with coupling constants of 12 Hz for He and Hd protons at 4.09 and 2.77 ppm. NH proton is visible as a broad singlet at 4.72 ppm. Two multiplets are representing the piperazine protons (2Hf, 2Hg) at higher field, at 2.90 and 2.50 ppm.

Final synthetic step towards targeted molecule 1H-indole-5-yl(4-(2-phenoxyethyl)-piperazine-1-yl)methanone includes Steglich amidation starting from 1H-indole-5-carboxylic acid and piperazine intermediate obtained in previous step. Acid was activated by carbodiimide reagent (DIC) and DMAP as the catalyst/base followed by the formation of an amide bond with the nucleophile (Scheme 3).

In the first step, the nitrogen in DMAP attacks the hydrogen from the indole-5-carboxylic acid. That allows the negatively charged oxygen to attack the carbon in DIC and form a complex as an intermediate to the product. Afterwards the complex reacts with DMAP and takes the hydrogen to gain neutrality and stability. After that the other nitrogen in DIC complex that is double bonded attacks the hydrogen bonded to the piperazine while DMAP attacks the carbon in carboxylic group replacing the oxygen which departs with what was formerly DIC forming a new compound urea. In the final step of amidation, nitrogen from the nucleophile reacts with the carboxylic group, forming an amide bond with departure of chemically unchanged DMAP (Scheme 3.).





Scheme 3. Proposed mechanism of the Steglich amidation.

1H-indole-5-carboxylic acid was dissolved in DMF, activated with DIC and DMAP by stirring 10 minutes at room temperature, followed by addition of piperazine intermediate. Reaction

mixture was continued with stirring 72 hours at room temperature and completed while 2-phenoxyethylpiperazine starting material was consumed.

Reaction mixture and formation of the desired product was determined by UPLC-UV-MS chromatography (Fig. 17) at basic pH. Desired product was observed at $R_t=1.18$ min, DMAP remained at $R_t=0.97$ min and urea intermediate was observed at 1.08 min (proposed structure of intermediate is on the Fig. 17). Desired product showed ionisation by MS detector (Fig. 18) at $R_t=1.19$ min, $m/z=350.23$ ($M+H$)⁺. Proposed urea intermediate showed ionisation by MS detector (Fig. 19) at $R_t=1.09$ min, $m/z=288.14$ ($M+H$)⁺.

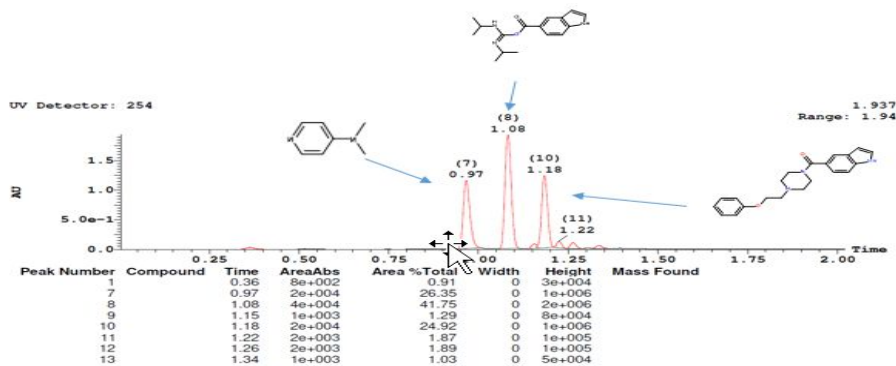


Fig. 17. UPLC-UV of reaction mixture of amidation.

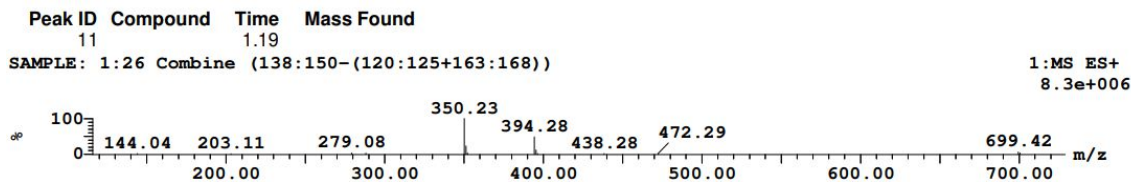


Fig. 18. UPLC-UV-MS of 1H-indole-5-yl(4-(2-phenoxyethyl)piperazin-1-yl)methanone.

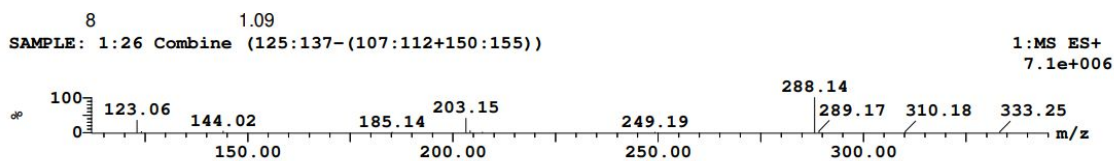


Fig. 19. UPLC-UV-MS of urea intermediate.

After work-up of the reaction mixture obtained residue was purified by automated flash chromatography using Si column. Prior to the purification, screening of solvent systems by thin layer chromatography was explored (Fig. 20.). Ideally, R_f value of desired product should be

0.5, therefore solvent system dichloromethane: methanol = 10:0.5 (DCM:MeOH) was selected for automated purification. Desired product was successfully purified yielding white solid of high purity >99% required for *in vitro* testing.

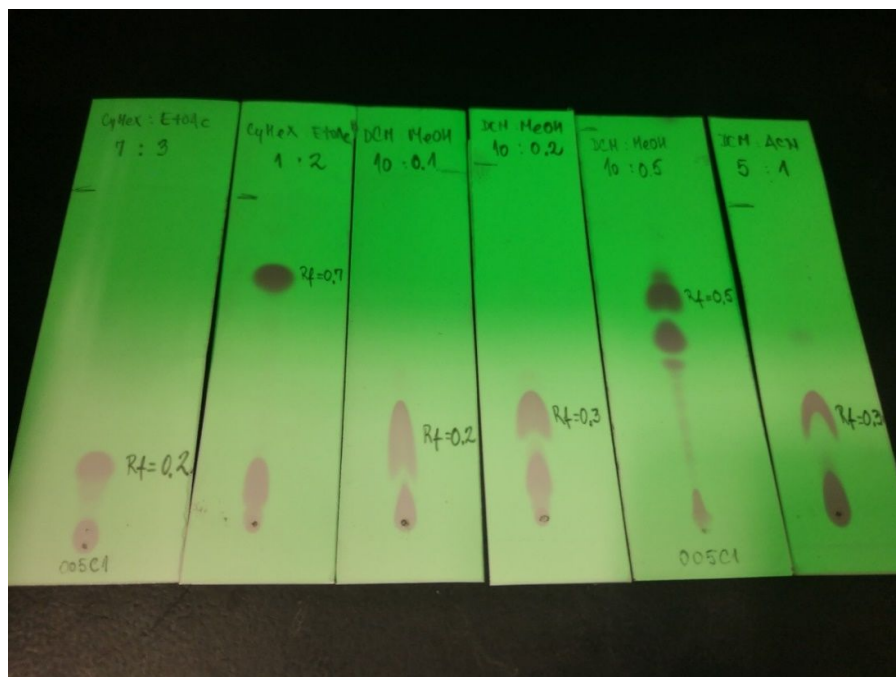


Fig. 20. Screening of solvent systems by TLC.

Characterisation of the final product was obtained by UPLC-UV-MS, 1D and 2D NMR spectroscopy.

UPLC-UV-MS (2 min, high pH)-FID21-005A3; purity (DAD-210-400 nm) of 99.39%; $R_t = 0.98$ min; $m/z = 350.26$ ($M+H$)⁺. (Fig. 21)

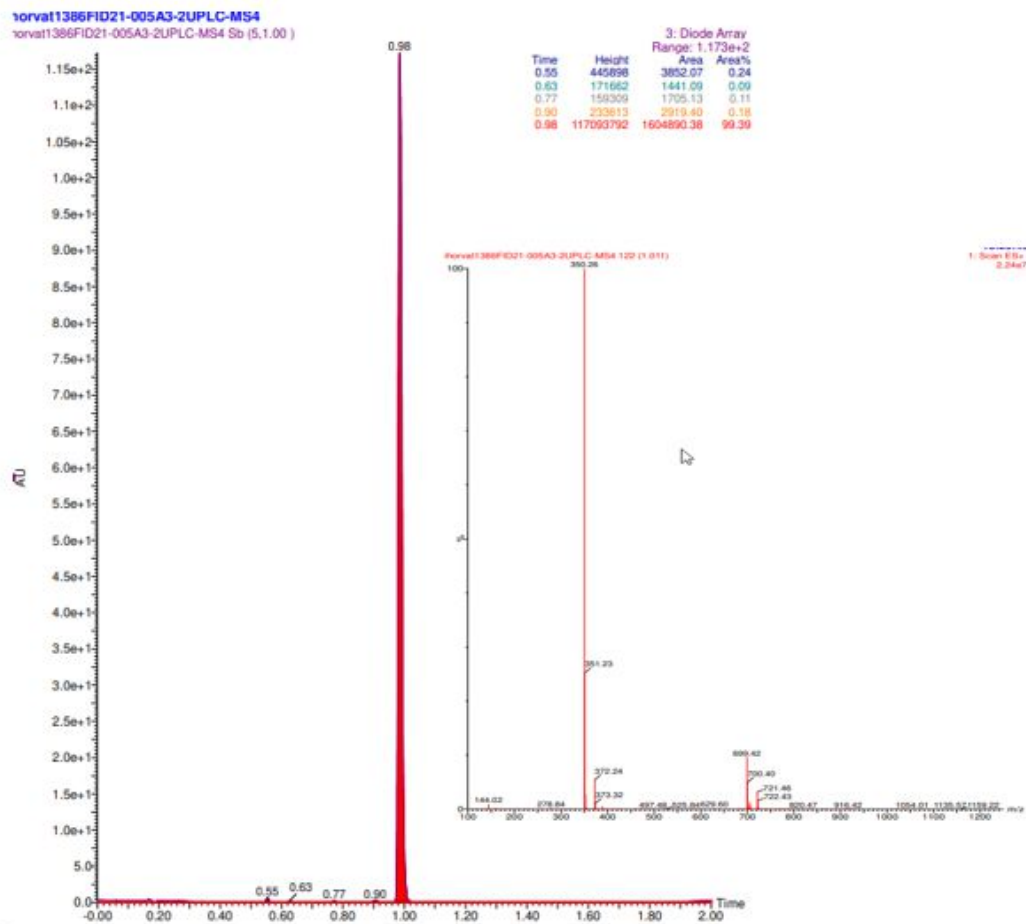


Fig. 21. UPLC-UV-MS of targeted molecule.

For structure confirmation, ^1H NMR, ^{13}C NMR and COSY spectra were recorded (Fig. 22, 23 & 24).

As in the previous product, phenyl protons (2a, 2b and c) show signals between 6.8 and 7.5 ppm. Piperazine hydrogens (2Hf, 2Hg) are visible as broad singlets at 3.66 and 2.62 ppm. Protons Hd and He show two triplets at 4.12 ppm with the coupling constant of 5.8 Hz and 2.85 ppm with coupling constant of 5.2 Hz. The broad singlet at 8.35 ppm represents NH proton, while the rest signals indole protons are between 6.5 and 8 ppm.

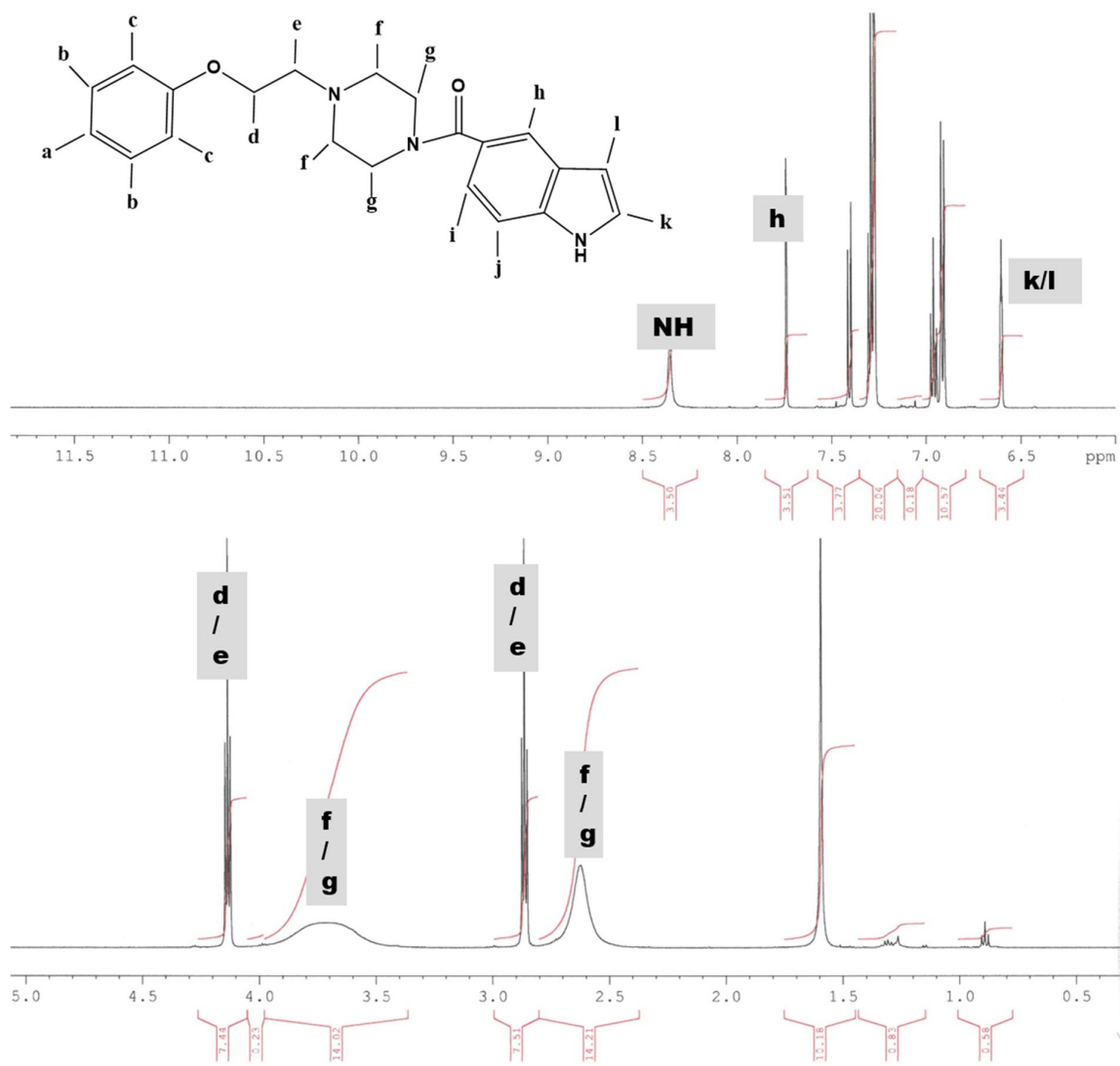


Fig. 22. ¹H NMR spectra of targeted molecule.

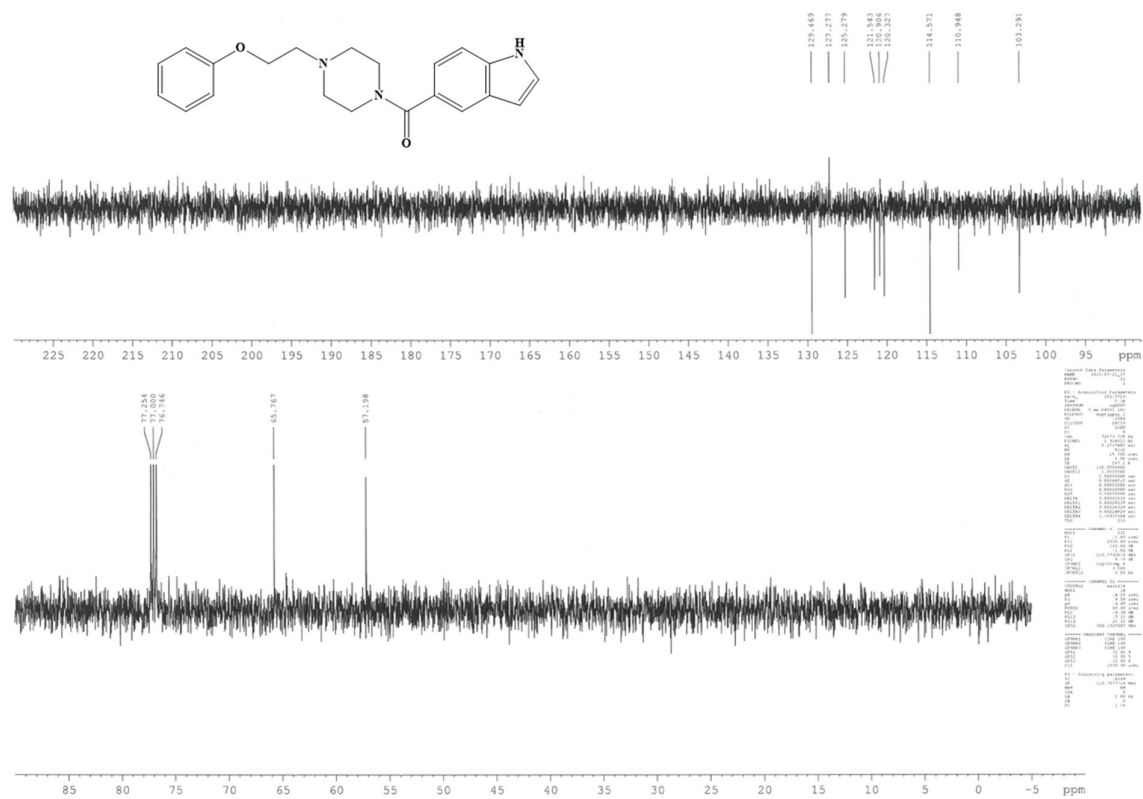


Fig. 23. ¹³C NMR spectra of targeted molecule.

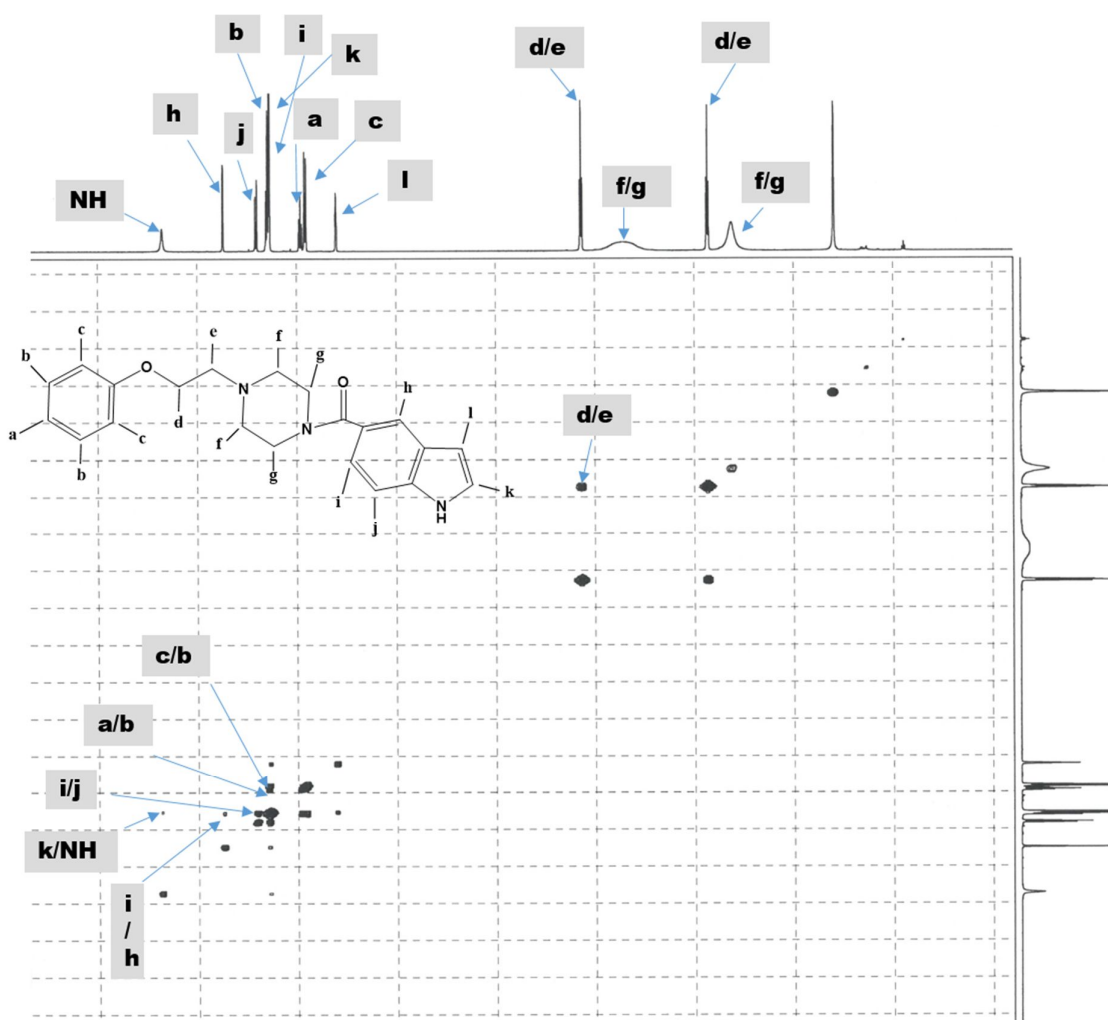


Fig. 24. COSY NMR spectra of targeted molecule.

3.1.2. Prediction of drug-like properties of 1H-indol-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone

ACD Percepta software was also used to predict physico-chemical properties of the synthesized molecule and drug-like properties according to the Lipinski “rule of five” (RO5). According to the calculations, final molecule satisfies all rules by Lipinski and could therefore be of use in further *in vitro* profiling as a drug-like molecule as well as starting point for synthesis of new analogues.

PhysChem Profiling		
LogP	3.20	Optimal
MW	349.43	Good
H-Donors	1	Good
H-Acceptors	5	Good
Rot. Bonds	5	Good
Rings	4	Good
Lipinski	0 violations	Good
Lead-like	0 violations	Good
Solubility	0.27 mg/ml	Soluble

Fig. 25. PhysChem profiling of targeted molecule by Percepta.

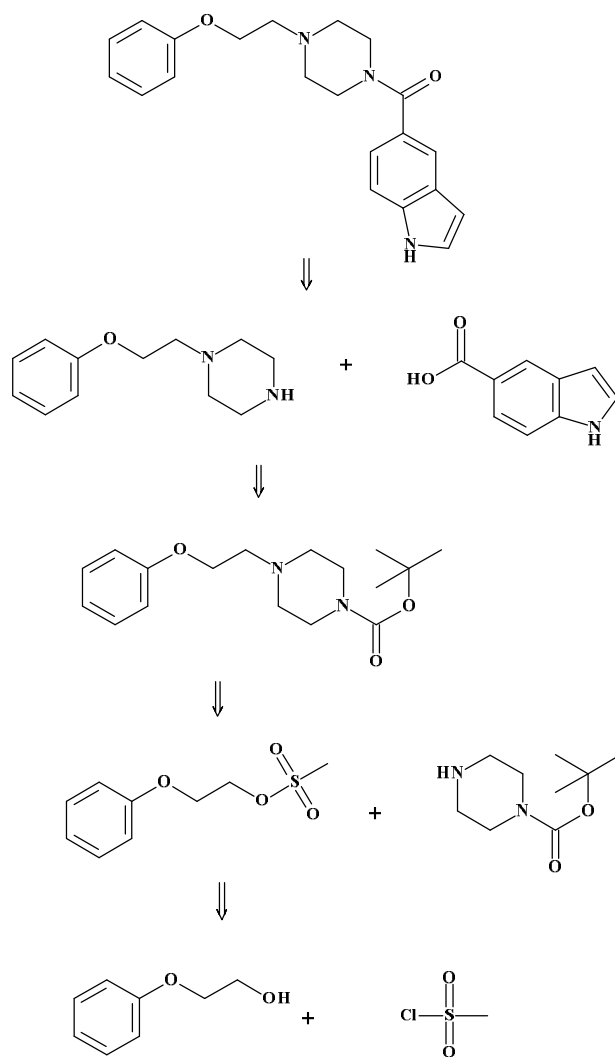
In addition ADME profiling was performed by Percepta (Fig. 26) in order to determine whether molecule could be considered permeable and consequently bioavailable in *in vivo* system. According to the calculated values targeted molecule is permeable which is beneficial, although drawback is high PPB value and potential CNS penetration that could be addressed by further modification of the structure.

ADME Profiling		
Caco-2	Pe = 230E-6 cm/s	Highly permeable
PPB	92%	Extensively bound
CNS	Score = -2.41	Penetrant
HIA	100%	Highly absorbed
Metabolic Stability	0.60	Undefined

Fig. 26. ADME profiling by Percepta.

3.1.3. Alternative synthetic pathway for 1H-indol-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone

In order to increase existing overall moderate yield in the linear 3 steps synthesis due to formation of byproducts, for further exploration and possibility of performing synthesis in scale-up, new synthetic pathway was proposed in 4 steps. Retrosynthetic analysis was described on Scheme 4.



Scheme 4. Retrosynthetic analysis of alternative pathway.

4. EXPERIMENTAL SECTION

4.1. General information

The reactions were carried out on a magnetic stirrer with a heating body, while the part of amidation reactions were carried out on a shaker with a heating body. Chromatographic separations were performed on a Biotage instrument using puriFlash silica gel-filled columns (4g, 15 μ m and 12g, 15 μ m). All used laboratory equipment is property of Selvita Ltd.

All products were identified and characterized by UPLC with mass and UV detectors and by NMR ^1H , ^{13}C and COSY (1H-1H) NMR spectra recorded on Bruker Ultra Shield 300, 400 and 500 depending on the amount of compound, using CDCl_3 as the solvent with TMS as the reference. Waters Acquity UPLC with an SQD mass spectrometer was used for determining the purity of the final product. UPLC method with duration of 2 minutes in basic conditions (0.05% NH_3 in H_2O / NH_3 in MeCN) was used to monitor the range of reactions.

Chemicals used: 2-PhEtOH, DCM, DMF, MsCl, TEA, toluene, $\text{NaCl}_{(\text{aq})}$, piperazine, EtOAc, DMAP, DIC, 1H-indole-6-carboxylic acid, 5% LiCl, MeOH, CDCl_3

4.2. Synthesis of 2-phenoxyethyl methanesulfonate

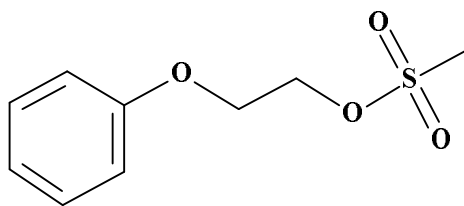


Fig. 27. 2-phenoxyethyl methanesulfonate

2-Phenoxyethanol (CAS No.:122-99-6), (408 μL ; 3.26 mmol) was dissolved in dry toluene (5 mL) at rt. Reaction mixture was cooled to 0 $^\circ\text{C}$ in ice bath, followed by addition of triethylamine (7.5 mmol; 1.04 mL) and methanesulfonyl chloride (324 μL ; 4.24 mmol). Reaction mixture was left with stirring at rt for 6 hours. The reaction mixture was concentrated *in vacuo* to obtain residue. To the residue DCM and water were added, layers were separated upon extraction. Organic phase was additionally washed with $\text{NaCl}_{(\text{aq})}$. Organic phase was

filtered through phase separator and evaporated *in vacuo* to yield 770 mg of brownish crude product (purity 89 %, yield: 97.3%).

UPLC-UV-MS (2 min, high pH): $R_t = 0.94$ min.

$^1\text{H-NMR}$ (300 MHz, CDCl_3) δ [ppm] = 7.31 (t, 2H, $J = 7.5$ Hz), 6.90 (t, 1H, $J = 7.5$ Hz), 6.89 (d, $J = 8.1$ Hz, 2H), 4.58 (t, 2H, $J = 9.0$ Hz), 4.25 (t, 2H, $J = 9.0$ Hz), 3.09 (s, 3H);

4.3. Synthesis of 2-phenoxyethylpiperazine

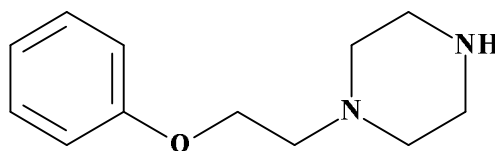


Fig. 28. 2-phenoxyethylpiperazine

2-Phenoxyethyl methanesulfonate (770 mg; 3.17 mmol) was dissolved in DMF (5 ml) at rt with stirring. To the reaction mixture, piperazine (CAS No.: 110-85-0), (0.3 g; 3.49 mmol) was added and the mixture was stirred at rt for 30 minutes. UPLC-UV-MS revealed only 6.5 % of the product, therefore trimethylamine (1.36 mL, 9.83 mmol) was added and reaction mixture continued to stir at room temperature for 24 hours. To the reaction mixture water and ethyl acetate were added, layers were separated. According to the UPLC-MS analysis, desired product remained in the water. pH of water layer was adjusted to pH 11.3 with 1.0 M $\text{NaOH}_{(\text{aq})}$, EtOAc was added, upon extraction layers were separated and organic phase evaporated *in vacuo* to yield 230 mg of transparent oily product (79 % purity acc. to UV). $^1\text{HNMR}$ analysis revealed presence of DMF and piperazine in the sample which will be used in the next synthetic step without additional purification. Due to the presence of solvent and starting reagent, final purity is approximated to 50%.

UPLC-UV-MS (2 min, high pH): $R_t = 0.85$ min; $m/z = 207.18$ ($\text{M}+\text{H}$) $^+$

$^1\text{H-NMR}$ (400 MHz, CDCl_3) δ [ppm] = 7.26-7.23 (m, 2H), 6.93-6.87 (m, 3H), 4.72 (bs, 1H, NH), 4.09 (t, 2H, $J = 12.0$ Hz), 2.95-2.80 (m, 4H), 2.77 (t, 2H, $J = 12.0$ Hz), 2.55-2.45 (m, 4H);

4.4. Synthesis of 1H-indole-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone

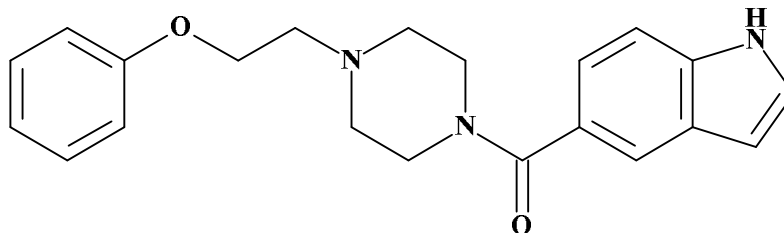


Fig 29. 1H-indole-5-yl(4-(2-phenoxyethyl)piperazine-1-yl)methanone

To the solution of 1H-indole-5-carboxylic acid (CAS No. : 1670-81-1), (57.9 mg; 0.36 mmol) in dry DMF (1 mL) at rt, DMAP (4.4 mg; 0.04 mmol) and DIC (47.6 mg; 0.38 mmol; 58 μ L) were added and the reaction was left with stirring for 10 min at rt upon which solution of 2-phenoxyethylpiperazine (88.9 mg; 0.43 mmol) in DMF (1 mL) was added. The reaction was left with stirring for 72 hours. To the reaction mixture H₂O (20 mL) and EtOAc (20 mL) were added, upon extraction layers were separated. Organic phase was extracted with 5% LiCl(aq) solution, separated and dried over sodium sulfate, filtered and evaporated *in vacuo* to yield the crude. The residue was purified by flash chromatography on Biotage system preadsorbed on silica on the 12 g column (flow 6 mL/min, 50 μ M silica) (mobile phases: DCM: (DCM:MeOH = 10:0.5) desired product was eluted at 60-70% of DCM/MeOH= 10:0.5. Fractions were collected and evaporated to yield 36 mg of desired product as white solid (purity 99.4%).

UPLC-UV-MS (2 min, high pH): Rt = 0.98 min; m/z = 350.26 (M+H)⁺

¹H-NMR (500 MHz, CDCl₃) δ [ppm] = 8.35 (broad s, 1H, NH), 7.73 (s, 1H), 7.40 (d, J = 7.1 Hz, 1H), 7.30-7.26 (m, 4H), 6.95 (t, J = 7.3 Hz, 1H), 6.90 (d, J = 7.9 Hz, 2H), 6.59 (t, J = 2.2 Hz, 1H), 4.12 (t, J = 5.8 Hz, 2H), 3.96-3.36 (broad s, 4H), 2.85 (t, J = 5.2 Hz, 2H), 2.75-2.48 (broad s, 4H);

¹³C-NMR (DEPT, 500 MHz, CDCl₃) δ [ppm] = 129.5, 127.3, 125.3, 121.5, 120.9, 120.3, 114.6, 110.9, 103.3, 65.8, 57.2;

5. CONCLUSION

Targeted compound 1H-indol-5-yl(4-(2-phenoxyethyl)piperazin-1-yl)methanone of high quality with purity > 99 % was synthesised in three synthetic steps starting from commercially available 2-phenoxyethanol. Synthesis included nucleophilic substitution with mesyl-chloride, followed by substitution with piperazine that is used in final amide coupling with 1H-indole-5-carboxylic acid by Steglich conditions to yield desired product. Alternative pathway was proposed in order to avoid by-product formed in the second step. Therefore, BOC protected piperazine would be better option for potential scale up or design of libraries of compounds from common intermediate where varying of acids as coupling partners in amidation could be explored.

Obtained compound was designed and synthesised with the aim of exploring activity in *in vitro* assay on Kv1.5 potassium ion channel as validated biological target in the treatment of arrhythmia. Advantages of piperazine ring as well indole as fragments in drug discovery were incorporated in the structure including alkoxyl chain which contributes to the better solubility of the molecule.

In addition, physico-chemical properties of the synthesised molecule and drug-like properties according to the Lipinski “rule of five” (RO5) were calculated. According to the calculations, final molecule satisfies all rules by Lipinski and could therefore be of use in further in *in vitro* profiling as a drug-like molecule as well as starting point for synthesis of new analogues.

In order to determine whether molecule could be considered permeable and consequently bioavailable in *in vivo* system ADME properties were predicted. According to the calculated values targeted molecule is permeable which is beneficial, although drawback is high PPB value and potential CNS penetration that could be addressed by further modification of the structure.

6. LITERATURE

- [1] Feigin, V.L.; Stark, B.A.; Johnson, C.O.; Roth, G.A.; Bisignano, C.; Abady, G.G.; Abbasifard, M.; Abbasi-Kangevari, M.; Abd-Allah, F.; Abdi, V.; et al. Global, regional, and national burden of stroke and its risk factors, 1990–2019: A systematic analysis for the Global Burden of Disease Study 2019. *Lancet Neurol.* **2021**, *20*, 795–820.
- [2] Humphries, E.S.A.; Dart, C. Neuronal and cardiovascular potassium channels as therapeutic drug targets: Promise and pitfalls. *J. Biomol. Screen.* **2015**, *20*, 1055–1073.
- [3] CDC, What is atrial fibrillation? https://www.cdc.gov/heartdisease/atrial_fibrillation.htm (accessed on January 20th, 2022.)
- [4] Lafuente-Lafuente, C. , Mouly, S. , Longás-Tejero, M. A. , Mahé, I. & Bergmann, J. (2006). Antiarrhythmic Drugs for Maintaining Sinus Rhythm After Cardioversion of Atrial Fibrillation. *Archives of Internal Medicine*, 166 (7), 719-728.
- [5] Vassallo P, Trohman RG. Prescribing amiodarone: an evidence-based review of clinical indications. *JAMA*. 2007 Sep 19;298(11):1312-22.
- [6] Zimetbaum, P.; Antiarrhythmic drug therapy for atrial fibrillation, *Circulation* 125 (2012) 381-389.
- [7] Grandi E.; Sanguinetti C. M.; Bartos D. C.; Bers D. M.; Chen-Izu Ye; Chiamvimonvat N.; Colecraft H. M.; Delisle B. P; et. al. *J Physiol*, volume 595, issue 7, 2016.
- [8] Bartos, D.C.; Grandi, E. & Ripplinger, C.M. (2015). Ion channels in the heart. *Compr Physiol* **5**, 1423–1464.
- [9] Xiaoke Guo; Xianglei Ma; Qian Yang; Jing Xu; Lu Huang; Jianmin Jia; Jiaojiao Shan; Li Liu; et. al. Discovery of 1-aryloxyethyl piperazine derivatives as Kv1.5 potassium channel inhibitors (part I). *European Journal of Medicinal Chemistry*, volume 81, 89-94, 2014.
- [10] Snyders D. J.; Structure and function of cardiac potassium channels, *Cardiovascular Research*, Volume 42, Issue 2, May 1999, Pages 377–390.
- [11] Wang, Z.; Fermini, B.; Nattel, S. Sustained depolarization-induced outward current in human atrial myocytes. Evidence for a novel delayed rectifier K⁺ current similar to Kv1.5 cloned channel currents. *Circulation Research*. 1993. 73; 1061–1076.
- [12] <https://www.acdlabs.com>
- [12] de Haan S., Greiser M., Harks E., et al. AVE0118, Blocker of the transient outward current (I_{to}) and ultrarapid delayed rectifier current (I_{Kur}), fully restores atrial contractility after cardioversion of atrial fibrillation in the goat. *Circulation*. 2006; 114; 1234-1242.

- [13] Alexander, S.P.; Catterall, W.A.; Kelly, E.; Marrion, N.; Peters, J.A.; Benson, H.E.; Faccenda, E.; Pawson, A.J.; Sharman, J.L.; Southan, C.; et al. The Concise Guide to PHARMACOLOGY 2015/16: Voltage-gated ion channels. *Br. J. Pharmacol.* 2015, 172, 5904–5941.
- [14] Yellen, G. The moving parts of voltage-gated ion channels. *Q. Rev. Biophys.* 1998, 31, 239–295.
- [15] Swartz, K.J. Sensing voltage across lipid membranes. *Nature* 2008, 456, 891–897.
- [16] Borrego, J.; Feher, A.; Jost, N.; Panyi, G.; Varga, Z.; Papp, F. Peptide Inhibitors of Kv1.5: An Option for the Treatment of Atrial Fibrillation. *Pharmaceuticals* **2021**, 14, 1303.
- [17] Snyders D. J.; Tamkun M. M.; Bennett P. B. A rapidly activating and slowly inactivating potassium channel cloned from human heart. Functional analysis after stable mammalian cell culture expression *J Gen, Physiol*, 1993. 101, 513-543.
- [18] Fedida, D.; Wible, B.; Wang, Z.; Fermini, B.; Faust, F.; Nattel, S.; Brown, A.M. Identity of a novel delayed rectifier current from human heart with a cloned K⁺ channel current. *Circ. Res.* **1993**, 73, 210–216.
- [19] Brunner, M.; Kodirov, S.; Mitchell, G.F.; Buckett, P.D.; Shibata, K.; Folco, E.J.; Baker, L.; Salama, G.; Chan, D.P.; Zhou, J.; et al. In vivo gene transfer of Kv1.5 normalizes action potential duration and shortens QT interval in mice with long QT phenotype. *Am. J. Physiol. Circ. Physiol.* **2003**, 285, H194–H203.
- [20] Ford, J.W.; Milnes, J.T. New drugs targeting the cardiac ultra-rapid delayed-rectifier current (I_{Kur}): Rationale, pharmacology and evidence for potential therapeutic value. *J. Cardiovasc. Pharmacol.* **2008**, 52, 105–120.
- [21] Milnes, J.T.; Madge, D.J.; Ford, J.W. New pharmacological approaches to atrial fibrillation. *Drug Discov. Today* 2012, 17, 654–659.
- [22] Gutman, G.A.; Chandy, K.G.; Grissmer, S.; Lazdunski, M.; McKinnon, D.; Pardo, L.; Robertson, G.A.; Rudy, B.; Sanguinetti, M.C.; Stühmer, W.; et al. International Union of Pharmacology. LIII. Nomenclature and Molecular Relationships of Voltage-Gated Potassium Channels. *Pharmacol. Rev.* **2005**, 57, 473–508.
- [23] Kamallesh M.; Copeland T. B.; Sawada S. Effect of inotropic stimulation on left atrial appendage function in atrial myopathy of chronic atrial fibrillation. *Echocardiography*. 2000; 17: 313–318.
- [24] Schotten U.; Greiser M.; Bodewick E.; Blaauw Y.; Goergelein H.; Allesie M. Restoration of atrial contractile force by the atrial K⁺ channel blocker AVE0118 in isolated atrial myocardium of patients with chronic atrial fibrillation. *Heart Rhythm*. 2004; 1: S93.

- [25] Holmes, T. C., Fadoo, D. A., Ren, R., & Levitan, I. B. (1996). Association of Src tyrosine kinase with a human potassium channel mediated by SH3 domain. *Science*, 274(5295), 2089-2091.
- [26] Patel R.V., Park S.W. An evolving role of piperazine moieties in drug design and discovery. *Mini Rev Med Chem*. 2013; 13(11); 1579-1601.
- [27] www.drugbanck.ca
- [28] Alexander, A. Kirichock at al., Synthesis of multifunctional spirocyclic azetidines and their application in drug discivery, *Chem, Eur. J.* 24 (2018) 5444.
- [29] Sean, W. Reilly at al., Examination of diazaspino cores as piperazine bioisosters in the Olaparib framework shows reduced DNA damage and cytotoxicity, *J. Med. Chem.* 61 (12) (2018) 5367-5379.
- [30] T.V. Sravanthi, S.L. Manju, Indoles-a promissing scaffold for drug development, *European Journal of Pharmaceutical Sciences* 91 (2016) 1–10.
- [31] Mack, D.J., Weinrich, M.L., Vitaku, E., Njardarson, J.T., 2010. Top 200 Brand Name Drugs by US Retail Sales in 2010. <http://cbc.arizona.edu/njardarson/group/top-pharmaceuticals-poster>
- [32] Alvarez-Builla, J., Vaquero, J.J., Barluenga, J., 2011. *Modern Heterocyclic Chemistry: Chapter – 5:Five-Membered Heterocycles: Indole and Related Systems*. 5. Wiley-VCH Verlag GmbH & Co. KGaA, Germany, p.377.
- [33] Christopher A. Lipinski, Franco Lombardo, Beryl W.Dominy, Paul J.Feeney; Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, *Advanced Drug Delivery Reviews* 46 (2001) 3-26.
- [34] Doak BC, Over B, Giordanetto F, Kihlberg, J.; Oral druggable space beyond the rule of 5 insights from drugs and clinical candidates; 21 (9) (2016) 1115-42.
- [35] Chemistry Steps, Reactions of Alcohols, <https://www.chemistrysteps.com/alcohols-substitution-reactions/> (accessed on February 16th, 2022.)

7. SYMBOLS

acc. – according

AF - Atrial fibrillation

AP - Action potential

APD – Action potential duration

CDCl_3 - Deuterated chloroform

DCM - Dichloromethane

DIC - N, N-Diisopropylcarbodiimide

DMAP - 4-Dimethylaminopyridine

DMF - Dimethylformamide

EtOAc - Ethyl acetate

HBAs – Hydrogen bond acceptors

HBDs – Hydrogen bond donors

hKv1.5 - Human Kv1.5 potassium channel

I_K - Delayed rectifier potassium channel currents

I_{Ks} - Slow delayed rectifier current

I_{Kr} - Rapid delayed rectifier current

I_{Kur} - Ultra-rapid delayed rectifier current

I_{TO} - Transient outward potassium current

Kv - Voltage-gated potassium channel

Kv1.5 - Voltage-gated potassium channel 1.5

LogP – Indication of lipophilicity

MeCN - Acetonitrile

MeOH - Methanol

MsCl - Methanesulfonyl chloride

MsO – Methanesulfonate

MW – Molecular weight

NMR - Nuclear magnetic resonance

on – overnight

PhEtOH - 2-phenoxyethanol

PSA – Polar surface area

Rot B – Number of rotatable bonds

rt - room temperature

SH3 - Src homology 3

S_N – Nucleophilic substitution

TEA - Triethylamine

TIC - Total ion chromatogram


TMS - Tetramethylsilane

UPLC-UV-MS - Ultra pressure liquid chromatography – ultra violet – mass spectrometer

VGICs - Voltage-gated ion channels

VSD - Voltage-sensing domain

CURRICULUM VITAE

 I went to Elementary School Pavleka Miškine during which I practised handball since the second grade. In 2015. I started education at the 10th Gymnasium in Zagreb (Ivan Supek) which I graduated from in 2019. and have been training middle distance running in Zagreb since 2016. until 2020. I have been awarded the Scholarship of the City of Zagreb for athletic and academic achievements since my senior year in high school lasting until the second year of college. Since 2019. to the present day I am a student of Environmental Engineering at Faculty of Chemical Engineering and Technology in Zagreb, during which I had the honor of working on my undergraduate thesis at Selvita Ltd. Also, in 2013. I finished a diving course and acquired Open Water Diver certificate. I own a driving licence for vehicles of B, AM, F and G categories with driving experience in Croatia and parts of Europe.