Summary

Introduction

Quinuclidine is a well established pharmacophoric element in which basic nitrogen, occupying a bridgehead position within an azabicyclic system, allocates maximal electrostatic interaction combined with minimal steric demand. Quinuclidine ring system is found in both naturally occurring (e.g., quinine) and synthetic drugs (e.g., azasetron, benzoclidine, palonosetron, solifenacin, cevimeline, quinupramine) **(Figure 1)**

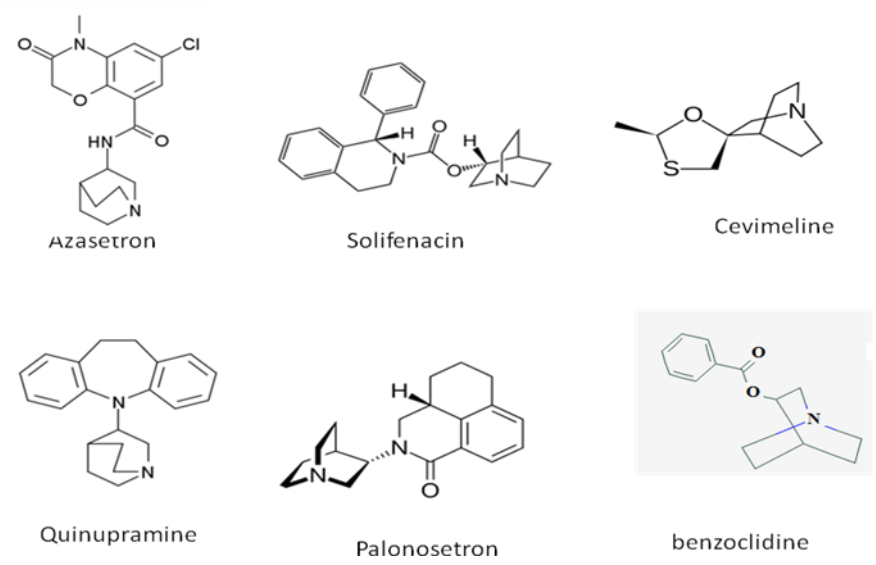


Figure 1: Quinuclidine based drugs

Literature survey revealed that several derivatives of Quinuclidinone have wide range of biological activity. They can be used for treatment of schizophrenia, chronic and neuropathic pain & Alzheimer's disease. It acts as muscarinic agonist and found an important place in the field of Neuropharmacology. Several quinuclidinone were reported as good anti-depressant, Anti-histamine-Broncho dilating agents, anti-histaminic, anti-depressant and anti-cancer agents.

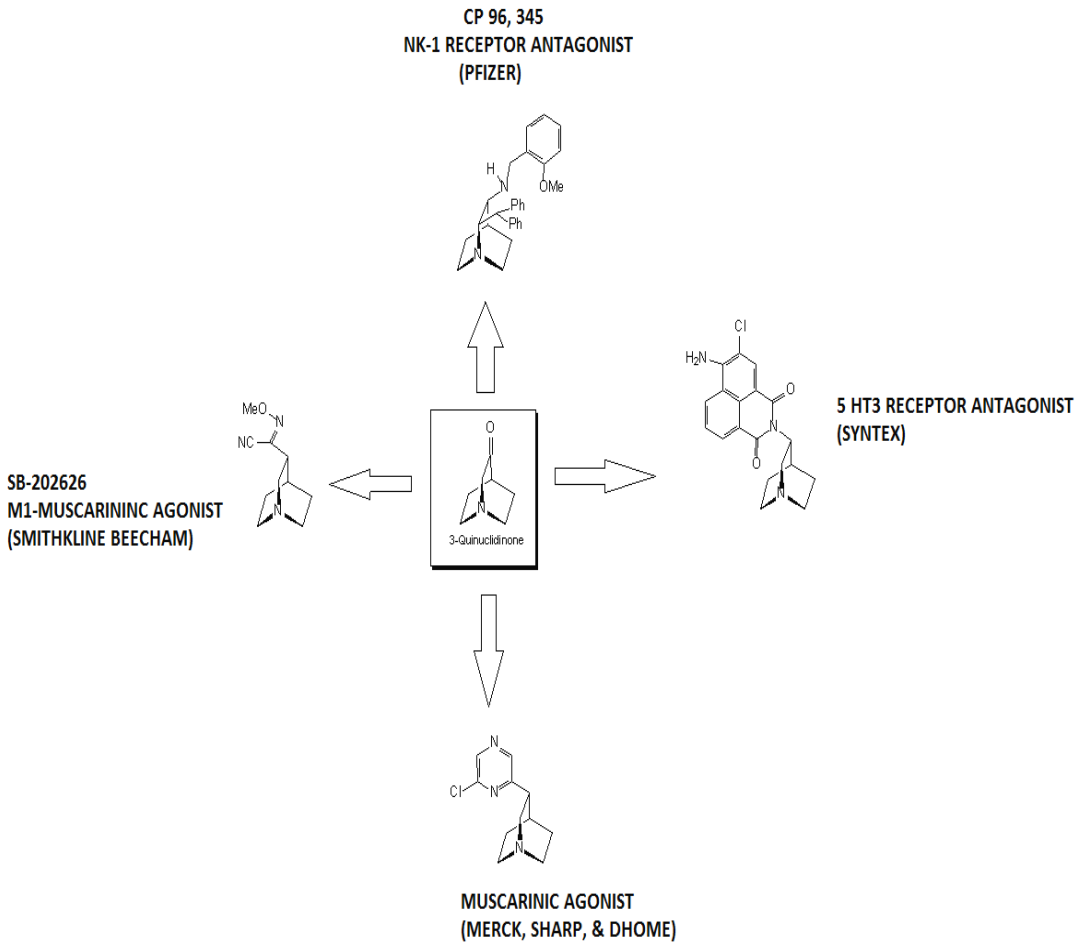
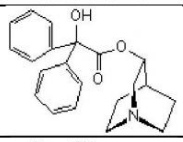
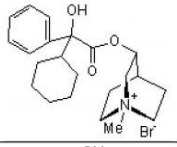
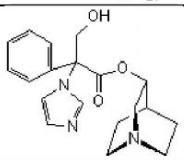
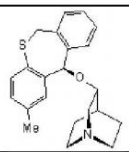
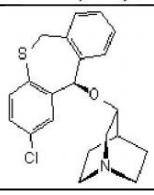


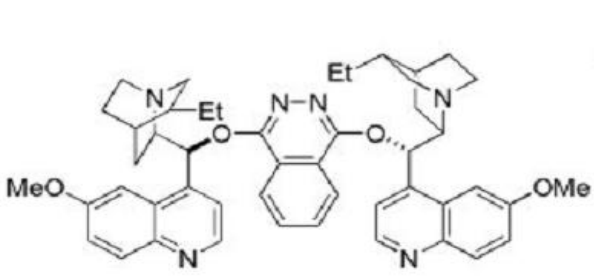
Figure 2: Quinuclidinone derivatives, their receptor and manufacture

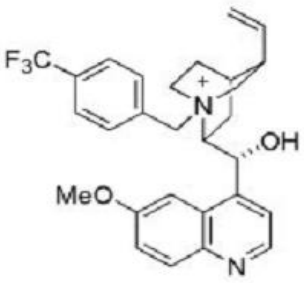
Table 1: Quinuclidinone derivatives and their use in different diseaces

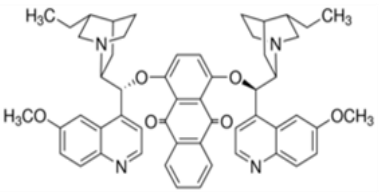
Entry no	Structure	Name	Use
1		3-Quinuclidinyl benzilat (code-name US-Army: BZ)	Incapacitating agent in chemical warfare, desorientation, hallucination, confused state, high-affinity muscarinic antagonist
2		N-methyl-3-[(Cyclohexylhydroxy -phenylacetyl)oxy] quinuclidinium bromide	anticholinergic, antiulcer agent
3		[R-(R*,R*)]alpha-(Hydroxymethyl)-alpha-phenyl-1H-Imidazole-1-acetic acid quinuclidine-3-yl ester	bronchodilator, antichonlinergic
4		(R*,R*)-(±)-3-[6,11-Dihydro-2-methyl-benzo[b,e] thiepin-11-yl)oxy] quinuclidine	antihistaminic
5		(R*,R*)-(±)-3-[2-Chloro-6,11-dihydrobenzo [b,e]thiepin-11-yl)oxy] quinuclidine	antidepressant

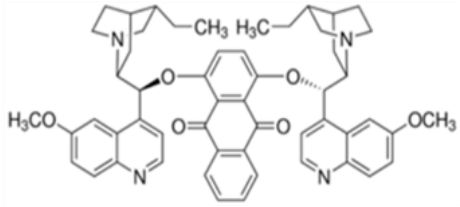
Quinuclidine ring system is also an important part of some catalysts. It is used in preparation of some catalysts for various asymmetric reactions such as Aldol, Henry, Aza-Henry, Diels-Alder, & Mannich reactions **(Figure 3)**

Considering the importance of Quinuclidinone ring system it was decided to synthesis some novel biologically active derivatives of this compound.





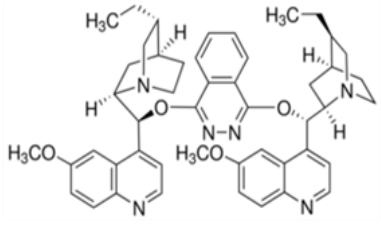


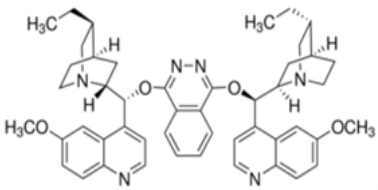


$(DHQ)_2AQN$

$(DHQD)_2AQN$

(Hydroquinine anthraquinone-1,4-diyl diether) (Hydroquinidine (anthraquinone-1,4-diyl) diether)





$(DHQD)_2PHAL$

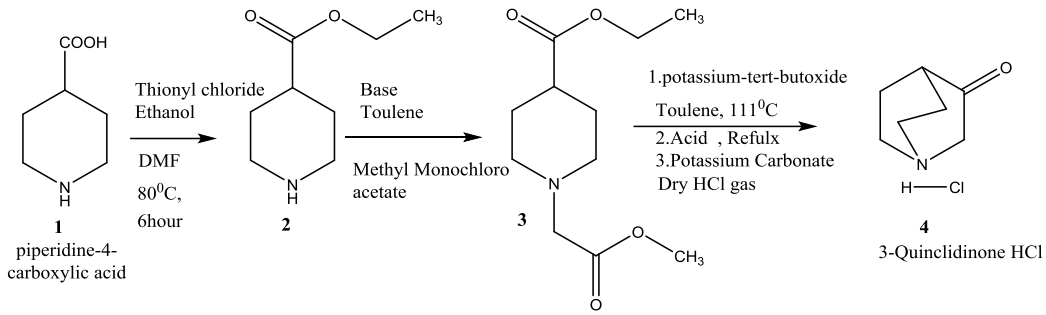
$(DHQ)_2PHAL$

(Hydroquinidine 1,4-phthalazinediyl diether) (Hydroquinine 1,4-phthalazinediyl diether)

Figure 3: Quinuclidine based commercially are available catalyst

Chapter: 2 An improved facile synthesis of 3-quinuclidinone hydrochloride

The most useful approach for the construction of quinuclidinone ring is Dieckmann cyclization. Earlier reported synthesis of quinuclidinone hydrochloride involves multistep organic synthesis. So, we decided to design relatively simple and safer route for the synthesis of 3-quinuclidinone hydrochloride from isonipecotic acid.



Scheme 1: Synthesis of 3-quinuclidione hydrochloride form piperidine-4-carboxylic acid

The present method utilizes commercially available piperidine-4-carboxylic acid as the starting material so that the hydrogenation step and use of expensive catalyst can be avoided. This method has advantage of being cost effective, facile and safe for the synthesis of 3-quinuclidinone hydrochloride. The process is easy to scale up and can be used for large scale synthesis of quinuclidinone hydrochloride.

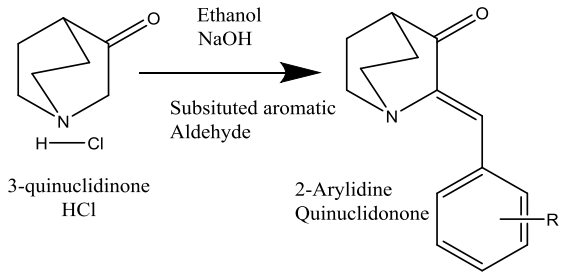
Chapter: 3 Anti-cancer applications of quinuclidinone derivatives

Quinuclidinone hydrochloride possesses wide range of biological activity. According to Literature analogue of quinuclidinone can provide an excellent scaffold for novel anti-cancer agents with improved safety profile. Quinuclidinone derivatives induce apoptosis in human breast cancer cells via reduced expression level of Bcl-2, Bcl-XL and increased

mitochondrial apoptotic pathways by activating the release of cytochrome C. substituted Acetals and di-acetates derivatives which were found to be potent in decreasing cancer cell viability, while parent compound showed no activity in MTT assay. This synthesis of quinuclidinone derivative leads to highly potent and selective newer anticancer agents

Chapter: 3A Synthesis characterization and cytotoxicity evaluation of 2-arylidine quinuclidinones

Several derivatives of quinuclidinone were also reported as a good anti-cancer agent. Many 2- substituted benzyl quinuclidin-3-one derivatives are reported in literature to show inhibitory activityagainst human α7 and α4β2 nicotinic receptors. Hence we synthesized some2-arylidine quinuclidin-3-onesderivativeswhich were screened for their anti-cancer activity using lung carcinoma cell(A 549) and normal cell (L132). IC_{50} values were determined and Structure Activity Relationship (SAR) was established. The results have been presented in Table1.

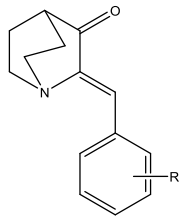


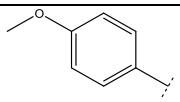
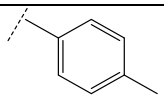
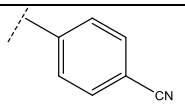
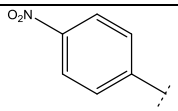
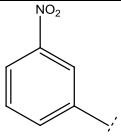
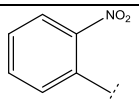
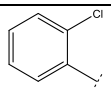
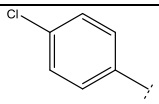
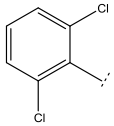
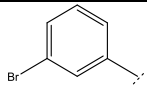
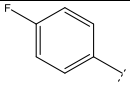
(Where R= -OCH_3, -NO_2 , -CH_3 , -CN, -F, -Br, -Cl)

Scheme 2: Synthesis of 2-arylidine quinuclidinones

The results of *in-vitro* cytotoxicity evaluation showed that all compounds induced apoptosis of lung carcinoma cells with minimal damage to the normal lung cells in dose dependent manner. It was observed that among all derivative 3-nitro and 3-bromo were found to be more potent while 4-methoxyderivative was least potent.

Table 2 Melting point, yield and IC_{50} values of 2-arylidine quinuclidinones



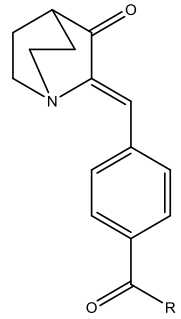
Compound	M.P. (˚C)	Yield (%)	IC_{50}(μM)
	105-108 ˚C	78 %	3.79
	128-130 ˚C	73 %	1.16
	112-114 ˚C	82 %	2.04
	137-139 ˚C	48 %	1.66
	123-125 ˚C	44 %	0.07
	120-122 ˚C	38 %	1.550
	134-136˚C	63 %	1.78
	114-116˚C	83 %	3.96
	121-123 ˚C	81 %	1.38
	138-140 ˚C	79 %	0.01
	117-118˚C	81 %	1.16

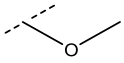
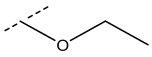
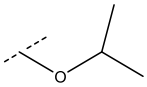
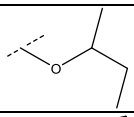
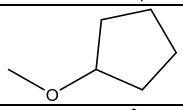
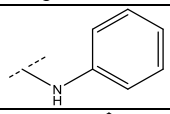
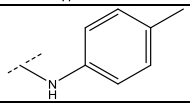
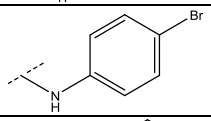
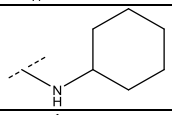
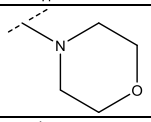
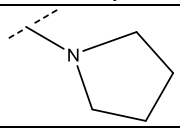
Chapter 3B: Synthesis, characterization and evaluation of 2-arylidine quinuclidinonesester amide derivatives as anti-proliferative agents.

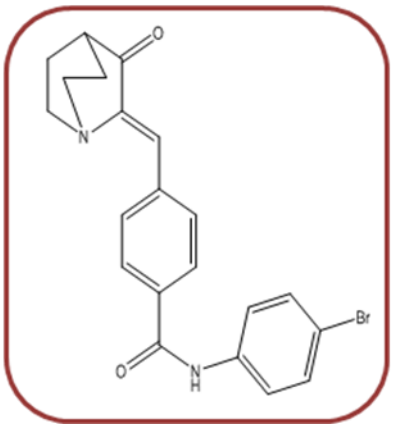
Analogues of quinuclidinoneinduce apoptosis in human breast cancer cells via reduced expression level of Bcl-2, Bcl-XL and increased mitochondrial apoptotic pathways by activating the release of cytochrome C.We synthesised series of novel quinuclidinone based amides and esters. The structures of title compounds are well supported by spectroscopic data and elemental analysis.Test compounds (**E3**, **E4**, **Am3**, **Am5**) were able to induce apoptosis of lung carcinoma cells with minimal damage to the normal lung cells and human RBCs.DNA fragmentation suggests that the cytotoxic effect of the compound is selectively mediated through the induction of apoptosis.The results of *in-vitro* cytotoxicity evaluation showed that all compounds induced apoptosis of lung carcinoma cells with minimal damage to the normal lung cells in dose dependent manner. IC_{50} values were determined and SAR was established.The results have been presented in Table 2.

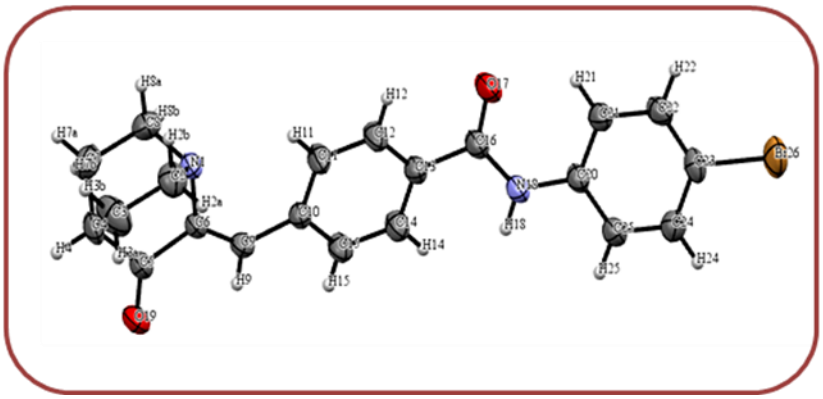
It is seen that in ester series the derivative with isopropyl group **(E3)** was found to be most active while derivative having methyl group **(E1)** was found least potent. The amide derivative with*para*bromophenyl group **(Am3)** was found as most potent one while derivative having phenyl ring (**Am1**) was found to be least active among all amides.

Table 3 Melting point, yield and IC_{50} values of 2-arylidine quinuclidinonesester amide derivatives



Compound	M.P. (˚C)	Yield (%)	IC_{50}(μM)
	142-143 ˚C	70%	9.22
	112-115 ˚C	68%	8.77
	96-97 ˚C	56%	1.5
	100-102 ˚C	72%	5.74
	140-141 ˚C	43%	6.15
	130-133 ˚C	60%	3.26
	>250 ˚C	58%	1.24
	>250 ˚C	69%	0.225
	>230 ˚C	73%	1.301
	155-156 ˚C	55%	0.665
	170-172 ˚C	49%	1.24





Structural formula of compound **A1**

Single crystal Data of derivative **A1**

4’,6-diamido-2-phenylindol (**DAPI)** nuclear Staining

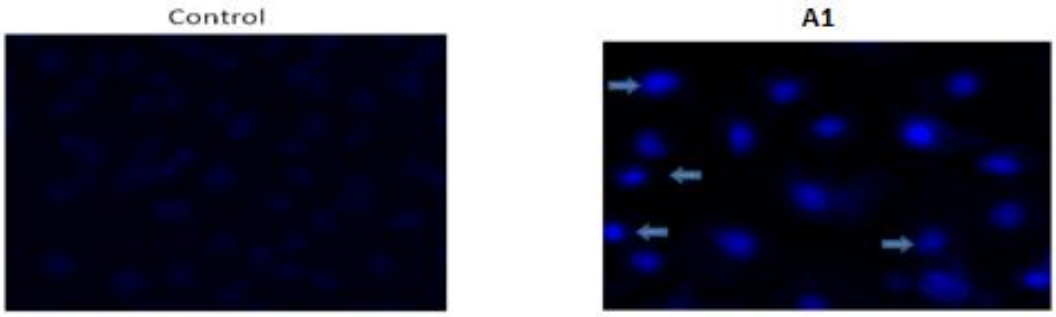


Figure 4: A-549 cells stained with DAPI indicate nuclear condensation in treatment group A1.

Arrows indicate condensation/fragmentation/distortion of nuclei as compared to the control

Acridine orange/Ethidium bromide **(AO/EB) staining**

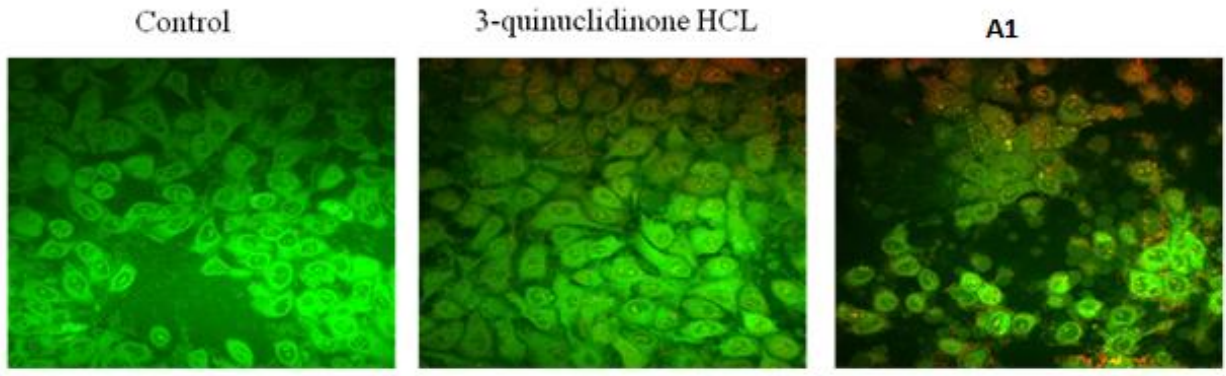


Figure 5: A-549 cells control and treated were stained with Acridine orange/Ethidium bromide showing viable nucleus as green and late apoptotic cells as orange to red.

Chapter: 4 Synthesis, characterization and evaluation of pyrazolylpiperidine derivatives as anti-platelet agents.

Atherothrombotic coronary artery diseasegives rise to a number of cardiocirculatory disorderssuch as myocardial infarction (MI), unstable angina (UA), or acute stroke associated with deep vein thrombosis (DVT). Hence it is one of the most common causes of death worldwide. The abnormal formation of intravascular occlusions is the main cause of these diseases. The prevention of thrombogenesis has become a vital target in the prophylaxis and therapy of cardio circulatory disorders with thromboembolic complications. Thrombosis may occur when the haemostatic becomes unregulated. Important predisposing conditions to thrombosis are disturbed blood flow, hypercoagulation, and altered vessel wall. Clinical studies have demonstrated anti-platelet agent can minimizeischemic recurrences and preventionof thromboembolic disease but accompanied by side effects such as gastrointestinal toxicity due to aspirin including nausea, vomiting, dyspepsia, heartburn, gastrointestinal ulceration etc. In recent years, the issue of resistance to anti-platelet agents, in particular aspirin and thienopyridines, has been highlighted in the medical literature. Novel anti-platelet agents with fast onset of action less inter individual variability and low risks of bleeding are still needed.

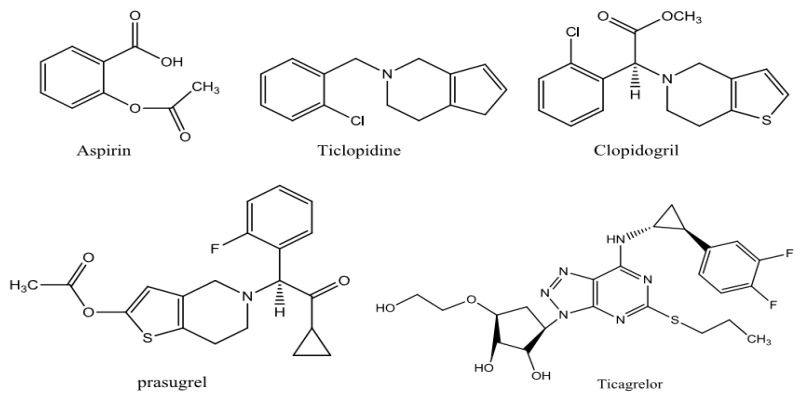
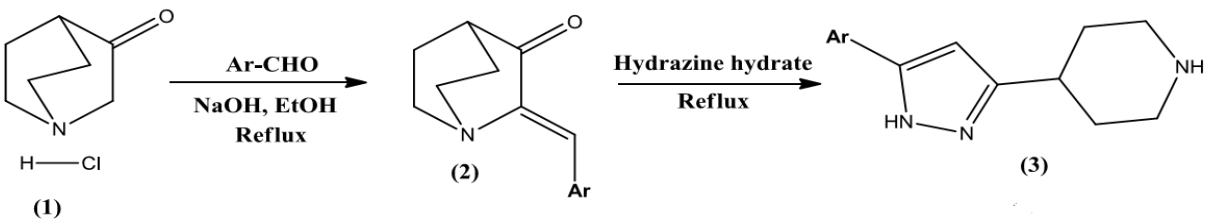


Figure 6: Currently approved Anti-platelet drugs

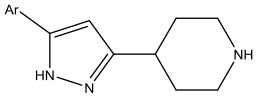


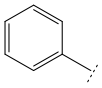
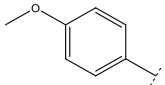
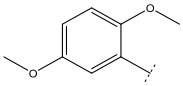
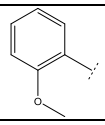
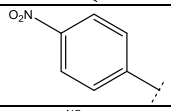
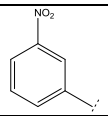
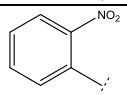
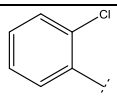
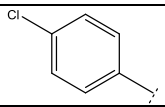
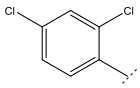
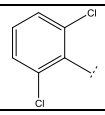
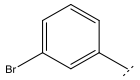
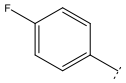
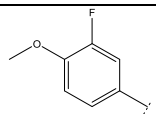
Scheme 3 Synthesis of pyrazolylpiperidine derivative

Pyrazolylpiperidine derivatives are potential anti-platelet compounds.Hence we synthesized some pyrazolylpiperidine derivatives which were screened for their anti-platelet activity.IC_{50} values were determined and SAR was established.These compounds were significant inhibitors (IC_{50}< 20 μM) and anti-platelet profile of it offered promising results when compared to standard aspirin (IC_{50} 16.5±0.2 μM)The results have been presented in Table 3.

The results showed that among all derivatives 2,5dimethoxy was found to most potent while derivatives having nitro group were found to be least active.

Table 4 Melting point, yield and IC_{50} values of pyrazolylpiperidine derivatives



Compound	M.P. (˚C)	Yield (%)	IC_{50}(μM)
	180-181 ˚C	69%	2.476
	180-182 ˚C	74%	1.563 ±0.0366
	158-160 ˚C	89%	1.435
	171-172 ˚C	64%	8.495
	164-166 ˚C	78%	24.07
	158-161 ˚C	65%	0
	140-142 ˚C	67%	0
	162-165 ˚C	91%	4.568
	185-187 ˚C	83%	14.78
	206-208 ˚C	88%	3.418
	148-152 ˚C	84%	3.786
	156-157 ˚C	62 %	16.46
	181-184 ˚C	67%	4.988
	142-144 ˚C	74%	4.771

Summary

Quinuclidinone is well known pharmacophorepresent in currently approved FDA drugs. The present work provides an extensive overview on the synthesis of quinuclidinone hydrochloride and series of its derivatives as well as biological activity thereof. There is always a demand for highly potent compounds with high activity and minimum side effects. **2-arylidine quinuclidinone** derivatives exhibited good anti-cancer activity with minimum effect on normal cells. While pyrazolylpiperidine derivatives were found to show good antiplatelet activity.