

# Quinolinium Structure as Labeled Biomarkers

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Received 12 May 2014; revised 13 June 2014; accepted 27 June 2014

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## Abstract

In this study the compatible chemical and biological investigations of several N-phenylquinolinium derivatives have been carried out in order to find the most perspective quinolinium structures for the nuclear-chemical synthesis of tritium labeled biomarkers.

## Keywords

N-Phenylquinolinium Compounds, Biological Activity, Antimicrobial and Antimycotic Action, Perspective Tritium Labeled Biomarkers, Nuclear-Chemical Synthesis

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## 1. Introduction

It is well-known that nitrogen-containing six-membered heterocycles are extremely important ring systems of biological investigations [1] [2]. Drug efficiency greatly depends on aqueous solubility and permeability through the cell membranes and this results in extensive application of lipophilic quaternary pyridinium derivatives in medicine [3]-[5]. Biological investigations reveal that the activity of pyridinium derivatives mainly depended on the structure of substituents on the heterocyclic ring [6]-[8]. Derivatives of benzopyridine—quinolinium salts open new horizons in drug chemistry [9]-[13]. In the 21st century, interest in new synthesis of perspective biologically active quinolinium structures even will increase [14]-[20].

N-phenyl substituted quinolinium and quinaldinium salts due to their structure may possess antibacterial, antifungal and anti-cancer activity. Unfortunately synthesis of such derivatives is very difficult and complex due to the lack of direct phenylation of nitrogen atom [21] [22]. Our elaborated nuclear-chemical synthetic method us-

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ing free nucleogenic phenyl cations generated by tritium  $\beta$ -decay allows the one-step synthesis of hardly available N-phenyl-substituted heterocyclic compounds [23]-[27].

In this study we have carried out compatible chemical and biological investigations in order to find the most appropriate structures for the nuclear-chemical synthesis of tritium labeled biomarkers.

## 2. Experimental

### 2.1. Synthesis of Quinolinium Compounds

The specificity of nuclear-chemical synthesis requires availability of unlabeled isotopic carriers. For this purpose we have synthesized different N-phenyl substituted quinolinium derivatives by known literature chemical methods.

All reagents and solvents were obtained from commercial suppliers.

#### 2.1.1. N-Phenylquinaldinium Salts

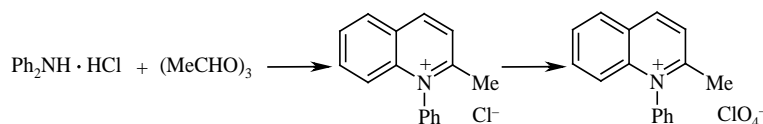
The cyclization of secondary aromatic amines with carbonyl compounds provides a suitable method for the synthesis of N-arylquinolinium salts [28]. Using the Doebner-Miller reaction to diarylamines, Pilyugin and Krainer [29] developed a method for the preparation of quaternary N-phenylquinaldinium salts (**Scheme 1**). Mp 159°C - 160°C (recrystallization from H<sub>2</sub>O) [30].

#### 2.1.2. N-Phenyllepidinium Salts

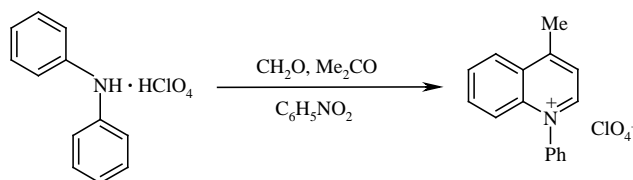
N-Phenyllepidinium perchlorate was obtained by a modification of the Beyer reaction using diphenylamine and formalin in presence of acetone and nitrobenzene according to the procedure by Pilyugin [31] (**Scheme 2**). Recrystallization from water gave almost colorless crystals, mp 172°C - 173°C (mp 172°C) [31].

#### 2.1.3. N-Phenylbenzo[f]quinaldinium Salts

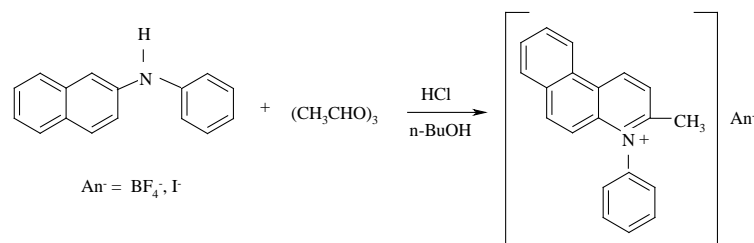
The reference inactive markers N-phenylbenzo[f]quinaldinium salts were synthesized by a modified literature procedure [32] [33] via cyclization of phenyl- $\beta$ -naphthylamine and paraldehyde (**Scheme 3**):



**Scheme 1.** Synthesis of N-phenylquinaldinium perchlorate.



**Scheme 2.** Synthesis of N-phenyllepidinium perchlorate.



**Scheme 3.** Synthesis of N-phenylbenzo[f]quinaldinium salts.

#### 1) N-Phenylbenzo[f]quinaldinium Iodide

Treatment of the reaction mixture with a saturated solution of KI gave N-phenylbenzo[f]quinaldinium iodide. The salt was isolated by crystallization from 50% acetic acid as needle-shaped crystals of mp 196°C - 197°C (mp 196°C - 197°C) [34] [35].

#### 2) N-Phenylbenzo[f]quinaldinium Tetrafluoroborate

N-Phenylbenzo[f]quinaldinium tetrafluoroborate was prepared analogously only using tetrafluoroboric acid (40%) or saturated sodium tetrafluoroborate solution as the precipitant, mp 135°C - 136°C.

## 2.2. General Procedure for the Nuclear-Chemical Synthesis

Nuclear-chemical synthesis was carried out in sealed glass ampoules containing the source of the phenyl cations (tritiated benzene), the nucleophile of interest and with an inorganic salt to serve as a stabilizing anion (KBF<sub>4</sub>, KI, KClO<sub>4</sub>). The benzene/substrate ratio was not less than 1:10<sup>3</sup>, in order to reduce the amount of the side products. The ampule was sealed and maintained for 1 - 2 months to accumulate the nuclear-chemical synthesis products. Since the radioactive decay does not depend on the reaction conditions such as temperature and pressure, the accumulation of tritium-labeled compounds was carried out at -18°C in order to prevent the formation of thermal decomposition products. The ampule was opened and the contents were transferred to a special flask. Benzene (0.5 ml) and acetone solution of inactive N-phenylsubstituted onium carrier (0.5 ml, 1 mg/ml) were then added. Unreacted tritiated benzene was distilled off in vacuum. Then acetone (0.5 ml) was added to the dry residue and 5-μl samples were taken for thin-layer chromatographic separation of the labeled products. Radioactivity of the sorbent layer was measured using a scintillation spectrometer RackBeta 1215 (LKB Wallac, Finland).

## 2.3. Synthesis of Ditriliated Benzene

The synthesis of double tritium labeled benzene was carried out in a vacuum by catalytic dehalogenation of *p*-dibromobenzene with gaseous tritium [36]. The purity of the prepared benzene sample was at least 99%. The specific activity of double tritiated benzene was 58 Ci/mmol. A hexane solution of the tritium-labeled benzene was used without a carrier. The bulk specific activity of the hexane solution was 1 Ci/ml. Such dilution was important to prevent radiolysis and the formation of side products.

## 2.4. Biological Research

Antimicrobial and antifungal activity was studied by the double serial dilution method [37]. The opportunistic pathogenic cultures were strains of *Staphylococcus aureus*-906, *Escherichia coli*-1257 and fungus *Candida albicans*-264/624 from L. A. Tarasevich GOSNII for Standardization and Control of Medical and Biological Preparations (Moscow). *Candida krusei* were the hospital strains, isolated from the hospital environment. Initial dilutions of the pathogens were prepared using an optical standard from a daily agar culture. The microbe loading was 2.5×10<sup>5</sup> microbes per ml. The combined microbe suspension and drug solution (diluted in DMSO) was placed in the growth medium. Results were recorded after 20 hours for bacteria or 48 hours for fungus (MIC) and 7 days (MBC) at 37°C (thermostat). Antimicrobial bacteriostatic (MIC) and bactericidal (MBC) activities were estimated from the minimum active concentration. The maximum test concentration of a new compound was 1.000 mg/ml.

## 3. Results and Discussion

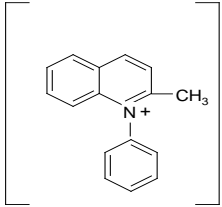
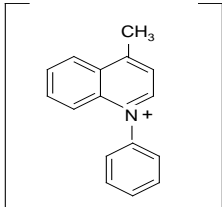
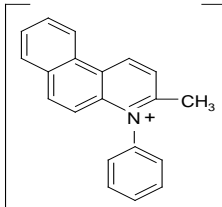
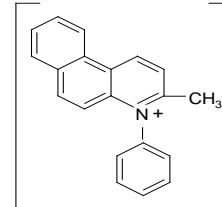
The results of biological activity of the investigated N-phenylquinaldinium derivatives are presented in the **Table 1**.

Carried out biological investigations revealed that a substituent (methyl group and annelated benzene ring in the quinolinium compound) together with the stabilizing anion had high influence on the level of obtained biological activity (both on MIC and MBC). The most active was N-phenylbenzo[f]quinaldinium tetrafluoroborate that showed high antimicrobial activity towards *St. Aureus* and antimycotic action against *Candida* sorts.

## 4. Conclusion

Further research in the field of nuclear-chemical synthetic methods will be focused on the preparation of tritium

**Table 1.** Results of biological activity of the investigated N-phenylquinaldinium derivatives.

Compound	<i>E. coli</i>		<i>St. aureus</i>		<i>Candida albicans</i>		<i>Candida crusei</i>	
	MIC (mcg/ml)	MBC (mcg/ml)	MIC (mcg/ml)	MBC (mcg/ml)	MIC (mcg/ml)	MBC (mcg/ml)	MIC (mcg/ml)	MBC (mcg/ml)
 N-phenylquinaldinium perchlorate	>500.0 <1000.0	1000.0	500.0	1000.0	>250.0 <500.0	1000.0	>500.0 <1000.0	1000.0
 N-phenyllepidinium perchlorate	>250.0 <1000.0	1000.0	250.0	1000.0	>125.0 <250.0	250.0	>500.0 <1000.0	1000.0
 N-phenylbenzo[f]quinaldinium tetrafluoroborate	>125.0 <250.0	250.0	>15.6 <31.2	31.2	7.8	15.6	250.0	1000.0
 N-phenylbenzo[f]quinaldinium iodide	>500.0 <1000.0	1000.0	250.0	500.0	500.0	1000.0	250.0	500.0
Standard chinosolum (8-oxiquinoline sulphate)	<500.0	500.0	<1000.0	1000.0				
Standard nitroxolinum (5-nitro-8-oxiquinoline)						5000.0		

labeled N-phenylbenzo[f]quinaldinium structures as effective radio markers and also investigations on mechanism of drug action.

## Acknowledgements

This work was carried out under the support of grant (basic part) of Russian Ministry of Science and Education.

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